



## ADVANCES ON HUMAN MILK HORMONES AND PROTECTION AGAINST OBESITY

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### Abstract

Extensive research shows that breast milk could have positive health effects not limited to infancy, but extend into childhood and adulthood. Recently many studies have provided new evidence on the long-term positive effects of breastfeeding, in particular protection against obesity and type 2 diabetes, suggesting that breast milk may have a role in the programming of later metabolic diseases. The mechanism throughout breastfeeding that exerts these effects has been a major focus of interest for researchers and it is still not completely known. There are some hints for biological plausibility of beneficial effects of breastfeeding including macronutrient intake, hormonal and behavioural mechanisms related to breast milk composition. Breast milk biochemical components, such as protein quantity and quality, polyunsaturated fatty acids, oligosaccharides, cytokines and hormones, in particular leptin, adiponectin and resistin together with the breastfeeding practice itself can influence infants feeding behaviour and regulation of growth and appetite control later in life. Further research is needed to confirm the possibility that hormones present in breast milk exert a metabolic and beneficial effects.

**Key words:** Breast milk, obesity, leptin, adiponectin, resistin, ghrelin, obestatin.

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### INTRODUCTION

The importance of infant feeding practices for short- and long-term children's health outcomes has been documented by a large and growing body of scientific literature (43,92). Extensive research in developed and developing countries provides evidence of health, nutritional, immunologic, developmental, psychological, social and economic benefits of breastfeeding (42).

Breast milk (BM) represents the natural and best nutrition for all infants, for its unique nutritional properties, that changes over the lactation period, and for the biological activities of some specific components. BM is species-specific, not entirely replicable in its biochemical characteristics, in particular in protein quantity and quality, carbohydrate content (lactose, oligosaccharides), lipids (long chain polyunsaturated fatty acids (LC-PUFA) and cholesterol), growth factors, hormones (such as adiponectin, leptin, resistin, ghrelin and obestatin) (table 1) and immunological factors (lymphocytes, macrophages, neutrophils, IgA and immune-related microRNAs, post-transcriptional regulators of gene expression, recently discovered in BM, which seem to be involved in the development of infant immune system) (6, 119). BM contains a wide array of proteins that provide adequate nutrition to breast-fed (BF) infants and simultaneously aid in the defence against infections and in the development of important physiologic functions (56). Many of these proteins represent an important source of amino acids to sustain growth, some play a role in the digestion and utilization of nutrients, others exert antimicrobial action or immune-modulatory activities, others are growth factors involved in the gut maturation of newborns (110).

In addition to providing essential nutrients and to mediate these effects, BM has positive health effects, which are not limited to infancy, but extend into childhood and even into adulthood (92).

It is interesting to note that hormones, such as leptin, adiponectin and ghrelin, provided by the mothers to BM, may help determine long-term appetite signalling (86) and may be involved in protection of children against obesity. Experimental evidence suggests that mutation in these hormones or their receptors or changes in their circadian rhythms, acting on brain pathways and regulating sensation of satiety, are involved in pathogenesis of obesity and metabolic syndrome (17, 115).

### EVIDENCE OF ASSOCIATION BETWEEN BREASTFEEDING AND OBESITY

The effect of breastfeeding on future body composition and later risk of obesity has been extensively studied, even though findings are controversial.

A case-control study by Kramer was the first to report an association between breastfeeding and reduced risk of overweight and obesity in 12 to 18-year-olds, with a dose effect by duration of breastfeeding (51). Armstrong et al. in a large cohort study of Scottish 3-year-old children suggested that breastfeeding was associated with a reduction in obesity risk at this age (3). This association has been reported also during adolescence (28). Multiple large observational studies published from the mid-1990s to mid-2000s investigated the relationship between breastfeeding and later obesity risk, finding contradictory results, as some studies showed protection against obesity, while others failed to do so (9). Three meta-analyses have shown that breastfeeding is associated with a reduced risk of childhood obesity (2, 72), with the level of protection rising with duration of breastfeeding (35). Differently to these findings, a fourth meta-analysis concluded that future body mass index (BMI) is not reduced in BF compared with formula-fed (FF) infants (73). Recently McCrory et al., in a large cohort study in the Republic of Ireland, found an association between the duration of the breastfeeding

**Table 1.** Hormones in breast milk (BM).

Hormone	Year of discovery	Main functions	Year of discovery in BM	Values in BM	Median (interquartile range) or means $\pm$ SD	Detection in BM
<b>Leptin</b>	1994	anorexigenic effect, inhibits the synthesis of fatty acids and triglycerides, increases the oxidation of fatty acids	1997	2.34 (5.73) ng/ml	Median (interquartile range)	(14)
<b>Adiponectin</b>	1995	improves insulin sensitivity and fatty acid oxidation and inhibits hepatic glucose production; exerts anti-inflammatory and anti-atherogenic effects	2006	9.99 (17.05) ng/ml	Median (interquartile range)	(12)
<b>Resistin</b>	2001	antagonizes insulin action, inhibits adipocyte differentiation.	2008	0.18 (0.44) ng/ml	Median (interquartile range)	(40)
<b>Ghrelin</b>	1999	orexigenic effect, stimulates GH secretion, inhibits lipolysis promotes adipogenesis, modulates insulin secretion and gastrointestinal motility.	2006	828.17 $\pm$ 323.32 pg/ml	means $\pm$ standard deviation	(4)
<b>Obestatin</b>	2005	anorexigenic effect?	2008	528.53 $\pm$ 39.00 pg/ml	means $\pm$ standard deviation	(5)

period and the reduction of risk of obesity at 9 years of age after controlling for a wide range of potential confounding variables. In particular, breastfeeding for a period between 13 and 25 weeks was associated with a 38% reduction in the risk of obesity, while for longer than 26 weeks with a 51% reduction in risk of obesity (62).

The relationship between breastfeeding and later health has been investigated also in two randomized control trials, considered the 'gold standard' in establishing causal relationships between interventions and outcomes (25). In one randomized control trial, preterm infants randomized to receive banked donor BM had significantly lower blood pressure, more favourable plasma lipid profile and reduced leptin resistance at age of 13-15 years compared with those who were fed preterm formula, with a dose-response relationship between the proportion of human milk and later outcomes (96). In contrast, a cluster-randomised controlled trial of a breastfeeding promotion intervention in healthy term infants (Promotion of Breastfeeding Intervention Trial study) found no effect of the intervention on adiposity or blood pressure at 6 years, despite increased incidence, duration and exclusivity of breastfeeding (52).

The role of breastfeeding in the protection against the development of obesity may be attributed to different fac-

tors (8). One possible explanation is represented by behavioural differences in mother-infant dyads in relation to breastfeeding or formula-feeding. Several research demonstrate that infants have some ability to regulate milk intake early in life, responding both to characteristics of BM and/or formula (e.g., energy density, supply) as well as to characteristics of the environment (e.g., feeding and sleeping schedule). In particular the mode of feeding and mother-infant interaction during lactation differ between breastfeeding and formula-feeding and can directly influence the infant's ability to self-regulate milk intake and the infant's growth, promoting the development of long-term differences between BF and FF infants in the control of food intake and energy balance regulation. In fact, breastfeeding mothers cannot visually monitor how much infants are consuming, conflicting to bottle-feeding, therefore relying on other information, such as satiety cues, from the infant. Breastfeeding mothers are more likely to trust infant's ability to self regulate milk intake and be attentive to the infant's cues of satiation instead of encouraging over-consumption (113). Recently it has been reported that BF infants learn and develop better control of their milk intake, whereas bottle-feeding may limit infants' ability to self-regulate milk intake (55).

Differences in the composition of BM and formula have also been implicated in explaining the association between breastfeeding and later weight status. Protein content seems to play a main role in this association, as it may exert influences on growth independently of caloric intake. The higher protein content of formulas could promote a faster growth in FF infants, who show an earlier age of adiposity rebound (105) and consequently a higher risk of later obesity (36). Koletzko *et al.* proposed “The early protein hypothesis” showing that infants fed, in the first year of life, with formulas with lower protein content, compared to those fed with higher protein content formulas, had a growth similar to the BF reference group at follow up of 2 years of age (49). In addition, differences in the fatty acid profile of BM versus formula may contribute to differential obesity risk. It has been observed that omega-6/omega-3 ratio found in formula may stimulate adipocyte proliferation and differentiation and promote inflammation, which seems to play a role in the development of obesity early in life. BM omega-3 fatty acids decreases inflammation and reduces the risk of later obesity by acting on regulators of food intake at the central nervous system level, as well as peripherally in the regulation of metabolism (1).

Apart from differences in macronutrient profiles, BM and formula milk differ in the presence in BM, of bioactive factors that regulate growth and energy balance. The recent identification in BM of adipokines (leptin, adiponectin and resistin) and other hormones, such as ghrelin and obestatin, involved in food intake and energy balance regulation, add a new factor in the explanation of the protective role of breastfeeding against obesity, as this condition results from an altered energy homeostasis (87). Diet-related differences have been observed during infancy in serum levels of some of these factors involved in energy metabolism, in particular leptin and ghrelin, which have been reported to be respectively higher or lower in BF infants than in FF ones (83, 88). These findings may explain growth differences and differences in dietary habits between BF and FF infants, which may have long-term health consequences, in particular on the development of obesity.

Differences in growth patterns between BF and FF infants begin with the first days of life, as exclusively BF newborns lose more weight and take longer to regain their birth weight as compared to FF newborns (57). After the first week of life and up to 3 months of age growth patterns appear to be similar between BF and FF infants, or show little difference in weight and length. After about 3 months of age, growth differs markedly between BF and FF infants, in particular as concerns weight gain, which is higher in FF infants, while differences in length gain are less pronounced (20). Butte *et al.* reported that early feeding mode also affects body composition in infancy, with BF infants having higher fat mass than FF infants in the first months of life (13). However, more recent studies showed that there are no consistent differences in adiposity between BF and FF infants during the first 4-5 months of life, and the preponderance of the evidence suggests that during the later part of the first year of life BF infants are leaner than FF infants (21, 121). Recently Gale *et al.* in a systematic review and meta-analysis, highlight an altered body composition during infancy in FF infants compared to BF infants. In particular the authors showed that FF infants, compared to BF infants, had lower fat mass at 3–4 and 6 months, but this trend is inverted at 12 months with

higher fat mass in FF infants than BF ones (26).

Recent research indicate that the way in which elements in the breast milk may influence the risk of overweight and obesity later in life includes epigenetic regulation; thus early environmental influences, such as nutritional elements and dietary compositions during pregnancy and the postnatal period, affect gene expression and ultimately growth, development and risk for disease (77).

In conclusion, hormones present in BM may play a role in determining differences between BF and FF infants in growth and body composition during infancy, in particular promoting slower growth and lower body fat levels in BF infants, and in the reduction of the risk of overweight and obesity later in life related to breastfeeding (97).

## LEPTIN

Leptin, is an adipocytokine, belonging to the family of cytokines, discovered in 1994. It is a 167 amino acid peptide and is encoded by the obesity (*ob*) gene localized on chromosome 7q31.3 in humans (117). Leptin is mainly synthesized by the white adipose tissue proportionally to the amount of body fat mass, but it is also produced by human placenta, playing potentially a role in fetal growth (78), and by the mammary gland, where it is secreted by epithelial cells in milk fat globules (98).

Leptin plays an important role in the central regulation of energy balance, acting both at the level of the hypothalamus, inhibiting the hunger center with an anorexigenic effect (19), and at the peripheral level, inhibiting the synthesis of fatty acids and triglycerides and increasing the oxidation of fatty acids (37). Leptin seems to be involved in the neonatal development of neuronal connection pathways in the arcuate nucleus of the hypothalamus that are permanently disrupted in leptin-deficient mice; whereas, its administration during the perinatal period restores the density of neuronal projections (11). In humans, genetic leptin deficiency is associated with congenital obesity and endocrine abnormalities (64). Considering its role on fetal growth, Koistinen *et al.* demonstrated a link between leptin levels in cord blood and intrauterine growth: small-for-gestational age (SGA) neonates have lower leptin levels at birth than appropriate-for-gestational age (AGA) ones, and large-for gestational age (LGA) neonates have higher levels than the other infants (47). Cord blood leptin seems to be a predictor of weight gain also in later life; in fact lower cord blood levels have been observed to be associated with a lower weight at birth, but with a more pronounced weight gain in the first 6 months of life and a higher BMI at 3 years of age (59). Moreover, evidence shows that serum leptin concentration reflects body fat mass during foetal life (106), in infancy (84), in children and in adults.

Leptin is also present in BM (14), while its presence in formula is still controversial. O’Connor *et al.* demonstrated that the RIA-leptin methodology can not be used to determine leptin presence in infant formulas because of the interference with supplemented iron, emulsifiers and other additives (69). Moreover pasteurization methods reduce the amount of detectable leptin; the authors hypothesize that infant formulas do not contain leptin because whey proteins added to formula are isolated from skimmed and bovine milk, and leptin associated with milk fat globules would be removed during the skimming process.

It is produced and secreted by mammary epithelial cells in milk fat globules (98), but other amounts of leptin may be transferred from the blood to milk by secretory epithelial cells (10). Casabiell *et al.* observed that leptin is transferred from the maternal serum to BM and then passes to neonatal blood, suggesting that maternal leptin may exert biological effects on the infant (14). Furthermore leptin receptors have been identified in the absorptive cells of mouse and human small intestine (7), which suggests that oral leptin can be absorbed by the immature stomach, pass into blood circulation and exerts biological effects, down-regulating endogenous leptin production and playing a potential role in the short-term control on food intake during the lactation period (79). Moreover, we demonstrated that leptin concentration is higher in BF infants than in FF ones (84) and that there is a positive correlation between serum leptin levels in BF infants and their growth parameters (weight and BMI) (84).

Schuster *et al.* found a negative correlation between BM leptin values during the first week after delivery and the infant weight gain from the end of the first to the sixth month (95). Other studies show a positive correlation between leptin in BM and in lactating mothers' serum (111), maternal BMI, mother's adiposity (38) and infant plasma leptin (107), while also a negative correlation have been reported between leptin in BM and infant BMI (63). Moreover, in our previously study, we observed a positive correlation between serum leptin concentration in BF infants and maternal BMI (81).

Studies in rodent demonstrated that leptin given during late pregnancy and lactation will prevent the excess obesity induced by feeding a high-fat diet in later life. These findings sustain the hypothesis that leptin present in BM or supplemental leptin in early infancy may be a protective agent in reducing the obesity risk later in life (75,103).

In conclusion, leptin in BM, involved in the regulation of food intake, energy balance and infant's growth and body composition, could be a key molecule in the prevention of overweight and obesity in childhood and adulthood.

## ADIPONECTIN

Adiponectin is an adipocytokine, a hormone secreted by adipose tissue, discovered in 1995 (93). It is a 224 amino acid peptide, encoded by *apM1* gene localized on chromosome 3q27, expressed in adipose tissue.

This protein regulates lipids and glucose metabolism, improving insulin sensitivity and fatty acid oxidation and inhibiting hepatic glucose production; it exerts anti-inflammatory and anti-atherogenic effects (85). In addition, adiponectin is involved in the regulation of energy homeostasis, stimulating food intake and reducing energy expenditure (53). It circulates in very high concentrations in human serum and it is present as three main isomeric forms: trimeric low molecular weight (LMW), hexameric medium molecular weight (MMW) and high molecular weight (HMW) (66). Adiponectin levels are inversely correlated to degree of adiposity and they are reduced in obese compared to normal weight adult subjects. Its secretion is decreased in metabolic disorders, such as type 2 diabetes, insulin resistance and dyslipidaemia (94).

In 2006 adiponectin was discovered in BM and its range of concentration was 4.2–87.9 ng/ml (12, 61), which is higher than those of other hormones present in BM. In

particular BM adiponectin values are around 20 times higher than BM leptin values (88), and around 100 and 20 times higher than respectively ghrelin and obestatin (4, 5). Moreover the most abundant adiponectin form present in human milk is HMW-adiponectin, that is the most active form exerting metabolic functions, suggesting that milk adiponectin could play a significant role in early regulation of infants' growth during lactation (66).

Adiponectin serum concentration in infancy is higher than that found in children and adults (41, 50). A recent longitudinal study showed that adiponectin cord blood values are inversely associated with weight gain in the first 6 months of life, and predict an increase in central adiposity at age 3 years (59). In children aged between 5 and 10 years serum adiponectin levels correlate negatively with the degree of adiposity and low adiponectin concentrations are closely related to the increased concentration of insulin in children (100). Adiponectin in BM seems to be related to lower infant weight in the first 6 months of life in BF infants suggesting a significant role for milk adiponectin in early regulation of neonatal weight gain. In addition, adiponectin in BM, considering its anti-inflammatory functions, may have a role in the attenuation of inflammatory processes, especially those of the intestinal mucosa (66).

In our recent study we observed positive correlations between adiponectin in mothers, human milk and BF infants (90) suggesting the existence of a metabolic link between nursing mother and infants through milk and assuming adiponectin ability to pass through intestinal mucosa. Adiponectin in BM may be absorbed by the gastrointestinal tract and its levels may influence those in infants' serum. In support of this hypothesis it was demonstrated the presence of adiponectin receptor 1 in the small intestine of mouse embryos, the absorption of adiponectin after its administration into the stomach in infant mouse and its resistance to degradation in the stomach (120).

Recently Woo *et al* hypothesized that adiponectin in BM may influence BF infants' growth beyond the period of exclusively breastfeeding. They observed that infants, exposed at higher adiponectin BM concentration during breastfeeding, had a slower weight gain in first years of life, but they presented a greater weight gain during the second year of life (112). The Authors explain this finding hypothesizing that higher adiponectin level in BM could reflect higher maternal BMI that influence indirectly infants' growth, because some study highlighted an association between adiponectin in BM and maternal BMI (61); another explanation of this finding is that the exposure to high levels of adiponectin in BM during the lactation period, could delay the catch-up growth in the first six months of life, which is associated mainly with an increase in fat mass, postponing weight gain in the second year of life, where it has been observed an increase in lean body mass (109, 112). Thus, it is possible to hypothesize that higher adiponectin value in BM may have a protective role in developing of overweight and obesity in childhood.

These findings and the noted biological properties of adiponectin suggest that adiponectin could play a role in the programming of energy balance in an early critical period for the development of metabolic homeostasis.

## RESISTIN

Resistin is an adipocyte-derived secretory factor, dis-

covered in 2001, belonging to the adipocytokines, together with leptin and adiponectin (102). Resistin is a protein of 12.5 kDa, consisting of 94 amino acids, encoded by RETN gene localized on chromosome 19p13.3. The source of resistin in mice and humans is different: in mice it is expressed, especially, from the adipose tissue while in humans it is found at low levels in adipose tissue and in larger amounts in other tissues, such as bone marrow, lung, circulating mononuclear cells, endothelial cells and vascular smooth muscle, human placenta. Resistin has been reported to antagonize insulin action in cells *in vitro* and *in vivo*; moreover its production is increased with feeding and in genetic models of obesity and insulin resistance, while decreased by PPAR $\gamma$  ligands (76); thus, resistin seems to have a significant role in obesity-induced insulin resistance (101). In addition, in animal models, Kim *et al.* observed an inhibitory effect on adipocyte differentiation hypothesizing a role of this hormone in adipogenesis as an adipose sensor for the nutritional state (45).

Resistin has been detected also in BM in 2008 (40, 91). Its concentration is higher in colostrum and decreases significantly during the breastfeeding period. BM resistin values are lower than in maternal serum with a positive correlation between the two levels. The source of resistin in BM, as for the other adipokines, is not already known, but it is conceivable that resistin is produced and secreted by the mammary epithelial cells and another amount seems to be transferred from circulation into milk (58), as well as for leptin. In BF infants serum resistin concentration was found significantly higher than those in BM and in nurse's serum. Furthermore we recently observed a positive correlation between BM and BF infants serum resistin. These findings suggest that resistin in BM may be absorbed through infants' gut and influence infants' serum levels although metabolic pathways involved in this process are not yet known (18).

Few studies have investigated resistin in prenatal and early postnatal period of life. It has been observed a progressive increase and high levels of resistin in umbilical venous blood of fetus mainly during the last trimester of pregnancy, when there is a rapid accumulation of fetal fat mass: resistin may have a regulatory action on adipogenesis (58), on tissue differentiation and fetal growth controlling body weight (18). Concerning the early postnatal period of life Ng *et al.* observed higher plasma resistin levels in term than in preterm infants and a positive correlation with gestational age and anthropometric parameters. These findings suggested that high resistin level at term gestation, promoting hepatic glucose production, could prevent hypoglycemia after birth (68). We recently have found a positive correlation between serum resistin and leptin in infancy (91), and other studies reported this correlation in cord blood and in newborns (60,114). The detection of resistin in BM and in infants' serum, considering its metabolic function, suggested that resistin may play a role in regulation of metabolic homeostasis and infants growth. Further research is necessary to better define its role in relation to the development of obesity.

## GHRELIN

Ghrelin is a gut hormone derived from the precursor pre-pro-ghrelin by post-translational processing, discovered in 1999 (48). It is an acylated peptide, encoded by

gene localized on chromosome 3p25-26, consisting of 28 amino acids with an octanoyl group on the serine in position 3, which is crucial for its biological activity (114). This hormone is produced mainly in the oxyntic mucosa of the stomach by enteroendocrine X/A-like cells and, to a lesser extent, by other organs such as the small and large intestine, pancreas, kidney, lung, placenta, ovary, testis, pituitary and hypothalamus (108).

Ghrelin is one of the most important orexigenic peptides and was identified as an endogenous ligand of growth hormone secretagogue receptors (GHS-R). The main function of ghrelin consists in the release of GH by the somatotrope adenohypophysis cells with dose-dependent effect; it is involved in the short-term regulation of appetite and in the long-term regulation of energy homeostasis and body composition, by inhibiting lipolysis and promoting adipogenesis. Moreover this hormone has other several functions such as modulation of insulin secretion, gastrointestinal motility, cardiovascular function, cell proliferation, bone metabolism and reproduction (122).

In 2006 Aydin *et al.* identified ghrelin in colostrum, transitional and mature milk at concentrations lower than those in maternal serum. The ghrelin levels in BM correlated positively with maternal serum concentration. Aydin hypothesized a direct passage of ghrelin from serum to milk but also the possibility of its production by the mammary glands (4, 44). Furthermore the discovery of ghrelin in BM together with the identification of ghrelin receptors in human gastric epithelial cells (104) suggested that ghrelin in BM could be absorbed by infant's gut and thus influences metabolic pathways and growth in infancy in relation to infant's needs (15).

Considering orexigenic function of ghrelin, its presence in BM may influence directly milk intake in BF infants, acting on feeding behavior, and control infants growth in early period of life. Ghrelin concentration in BM increases during lactation and it correlates with serum ghrelin levels in BF infants (39). It has been demonstrated that ghrelin serum concentrations in FF infants are higher than in BF ones in first months of life; moreover ghrelin levels in BM are lower than in artificial milk. According to these results it is possible to assume that FF infants, receiving greater amount of ghrelin, have an increase of appetite more than BF infants with later consequents such as increase in weight gain (89).

Ghrelin role has been evaluated in prenatal and early postnatal period of life. It was detected in placenta (31), cord blood and neonatal serum, suggesting a role of the hormone in fetal and neonatal growth (99). In particular ghrelin in the cord blood correlates negatively with infants' birth weight and BMI (71); its levels in SGA infants was higher than in AGA and LGA ones (24, 82). Hormone values in infancy are higher than in the cord blood and in healthy weight adult (46). Ghrelin seems to be involved in the long-term regulation of body weight, but strong evidences of this association are lacking. It has been showed that infants' serum ghrelin levels correlate positively with age and anthropometric parameters in the first year of life and a negatively with weight gain in infants' group BF for at least four months (80, 82). Others authors found, in a smaller sample, a negative correlation between infant serum ghrelin levels and infants BMI at the first month of life, while they observed a positive correlation between ghrelin in BM and weight gain at fourth month of life (99).

At birth in term infants, serum ghrelin levels are inversely associated to birth weight and body length (67,116); moreover also ghrelin in BM correlates negatively with birth weight and BMI (22). A recent study evidenced that ghrelin in preterm infants were significantly higher than in the term ones with an levels increase in early postnatal life. These evidences suggested an increased ghrelin production in the preterm infants to compensate for the negative energy balance through an adequate stimulus of appetite (16).

All these observations suggest that ghrelin exerts adipogenic activity, is involved in the postnatal growth, in long-term regulation of body weight and in energy homeostasis during the fetal and neonatal life. Further studies are required to clarify how the ghrelin in BM may influence infant feeding behavior and body composition in childhood.

## OBESTATIN

Obestatin is a gut hormone derived from the same precursor of ghrelin, named pre-pro-ghrelin, discovered in 2005 by Zhang *et al.* (118). It is a 23 amino acid peptide, encoded by exon 3 of the gene ghrelin (GHRL), localized on chromosome 3p25-26 (32).

It has been demonstrated that obestatin tissue distribution is strictly co-localized with ghrelin and it is present in the gastrointestinal tract (54), spleen, pancreas, salivary gland, ductal epithelium of the mammary glands, plasma and to a lesser extent in other human tissues such as pituitary, prostate, testis, placenta, ovary, thyroid and parathyroid.

Obestatin was initially identified as an anorectic peptide, with an opposite action to ghrelin, although the results of the studies about this topic are still narrow and conflicting. It appears to decrease food intake, slow gastric emptying, suppress intestinal motility and reduce body weight gain; moreover obestatin seems to have other effects such as inhibition of thirst and anxiety, improving of memory, regulation of sleep, stimulation of cell proliferation and increasing of exocrine pancreatic secretion inhibiting glucose-induced insulin secretion (54).

Considering the obestatin effects on energy homeostasis and on appetite regulation, its role has been investigated in diseases such as anorexia nervosa, obesity, glucose intolerance and diabetes mellitus type 2. The observation of elevated levels of ghrelin and obestatin in patients with anorexia nervosa has suggested that, in these subjects, despite the increase in the sense of hunger, there is a reduced motivation to feed themselves (34). Obestatin levels have been found lower in obese subjects compared to the control group; moreover the preprandial ratio ghrelin / obestatin was increased in obese subjects and correlated positively with BMI (33). In addition, overweight and obese patients have a reduced expression of obestatin in the gastric mucosa, which correlates with its lowest level in plasma. Therefore, the reduced gastric expression of the hormone may affect its plasma concentrations and consequently influence the accumulation of weight in these patients (27). These findings may suggest that obestatin is involved in the regulation of body weight in the long term and that the balance between ghrelin and obestatin is essential to modulate energy homeostasis and the body adaptive responses to acute and chronic changes in nutritional status. Nakahara *et al.* demonstrated that obestatin plasma levels

correlated negatively with BMI, leptin, insulin and glucose in patients with anorexia nervosa and obesity (65).

Obestatin is involved in glucose homeostasis and the development of type 2 diabetes mellitus: It seems that obestatin promotes the insulin response at a low glucose concentration, while inhibits the insulin release at high glucose concentration (23). Moreover Granata *et al.* showed obestatin protective role in proliferation and survival of pancreatic  $\beta$ -cells (29).

With regard to childhood obesity, Zou *et al.* observed that ghrelin/obestatin ratio was significantly lower in the obese group compared to controls; furthermore ghrelin, obestatin ratio increased after weight reduction and was associated with a decrement in insulin resistance (123).

In 2008 Aydin *et al.* identified obestatin in colostrum and mature milk assuming a secretion by ductal epithelium of the mammary gland or a direct passage from serum into BM. Its concentrations in BM is greater than seen in nurses' serum and with values slightly reduced in mature milk than in colostrum. These findings may be a benefit for infants, because obestatin could be associated with less overfeeding, in the early stages of breastfeeding, suppressing newborns' appetite to prepare the gastrointestinal tract to receive the milk (5). Obestatin in BM could exert its action on the energy balance and maturation of the gastrointestinal tract in BF infants.

## CONCLUSIONS

Evidence showing that breastfeeding is associated with positive health outcomes compared to formula feeding is growing. In particular some researchers support the role of a longer duration of breastfeeding in its protection against later obesity risk, a dose-dependent relationship, with a decrease in risk of overweight later in life (35). The mechanisms through which breastfeeding exerts this effect have been largely investigated and are based on the unique composition of human milk and the metabolic and physiological responses to it (70).

BM biochemical components, such as protein quantity and quality, polyunsaturated fatty acids, oligosaccharides, cytokines and hormones, in particular leptin, adiponectin, resistin, ghrelin and obestatin, together with breastfeeding practice itself can influence infants feeding behaviour and regulation of growth and appetite control later in life (6). These findings suggests that BM is a source of critical components for the metabolic development of the infants; even though a clear metabolic effect of these BM hormones, in infancy, still needs to be documented.

They may be involved in growth and appetite control in the neonatal period and infancy, affecting the programming of energy balance regulation both in childhood and adulthood (87). In the present review we summarized the properties of hormones present in BM, highlighting their actions on infant growth and energy balance regulation. Further researches are needed in order to investigate the relationships between BM components and obesity risk, including hormonal and behavioural mechanisms related to BM macronutrients.

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