VU VRIJE UNIVERSITEIT AMSTERDAM

VU Research Portal

Early-life endocrine regulation and neurodevelopmental outcomes

Hollanders, J.J.

2020

document version
Publisher's PDF, also known as Version of record

Link to publication in VU Research Portal

citation for published version (APA)
Hollanders, J. J. (2020). *Early-life endocrine regulation and neurodevelopmental outcomes*. [PhD-Thesis - Research and graduation internal, Vrije Universiteit Amsterdam].

General rights
Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

Take down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

E-mail address:
vuresearchportal.ub@vu.nl

Download date: 05. Oct. 2024

4

Nutritional programming by glucocorticoids in breast milk: Targets, mechanisms and possible implications

Jonneke J. Hollanders,
Annemieke C. Heijboer,
Bibian van der Voorn,
Joost Rotteveel,
Martijn J.J. Finken

Best Practice & Research Clinical Endocrinology & Metabolism 2017 Aug; 31(4): 397-408.

ABSTRACT

Vertical transmission of glucocorticoids via breast milk might pose a mechanism through which lactating women could prepare their infants for the postnatal environment. The primary source of breast-milk glucocorticoids is probably the systemic circulation. Research from our group showed that milk cortisol and cortisone concentrations follow the diurnal rhythm of maternal hypothalamus-pituitary-adrenal axis activity, with a higher abundance of cortisone compared to cortisol. Measurement of breast-milk glucocorticoid concentrations is challenging due to possible cross-reactivity with progestagens and sex steroids, which are severely elevated during pregnancy and after parturition. This requires precise methods that are not hindered by cross reactivity, such as LC-MS/MS. There are some data suggesting that breast-milk glucocorticoids could promote intestinal maturation, either locally or after absorption into the systemic circulation. Breast-milk glucocorticoids might also have an effect on the intestinal microbiome, although this has not been studied thus far. Findings from studies investigating the systemic effects of breast-milk glucocorticoids are difficult to interpret, since none took the diurnal rhythm of glucocorticoids in breast milk into consideration, and various analytical methods were used. Nevertheless, glucocorticoids in breast milk might offer a novel potential pathway for signal transmission from mothers to their infants.

INTRODUCTION

Numerous studies suggest that adversities occurring in early life could predispose to later diseases such as cardiovascular diseases, type 2 diabetes mellitus and neuropsychiatric diseases. The mechanisms that could explain these associations relate to the concept of early-life programming, stating that insults early in life could persistently alter the body's structure and/or function. These alterations, although adaptive in nature, might become deleterious with age.[1]

Glucocorticoids are known for their programming effects on metabolism and the brain.[2] The fetal cortisol hypothesis postulates that a lower activity of the placental barrier enzyme 11β-hydroxysteroid dehydrogenase (11β-HSD) type 2 allows a larger proportion of maternal cortisol to reach the fetus, leading to permanent alterations in hypothalamus-pituitary-adrenal (HPA) axis settings, and, hence, predisposition to cardiometabolic and neuropsychiatric diseases in offspring.[3]

Emerging data suggest that postnatal nutrition could play a role in early-life programming.[4] Breast feeding has been associated with improved health outcomes, including reduced risks of infections and obesity.[4,5] Although glucocorticoids were recovered in breast milk already in the early 1970s,[6] only few studies have addressed their effects in offspring. The recent discovery of a diurnal rhythm in the secretion of glucocorticoids into breast milk has opened new avenues for the study of the postnatal programming effects of maternal glucocorticoids.

GLUCOCORTICOIDS, PREGNANCY AND THE MAMMARY GLAND

Cortisol is produced by the zona fasciculata of the adrenal cortex. Its synthesis is regulated by adrenocorticotropin hormone (ACTH) from the anterior pituitary gland. The release of ACTH, in turn, is under the control of corticotropin releasing hormone (CRH) from the hypothalamus. When cortisol is present in adequate amounts, a negative feedback system operates on the pituitary gland and hypothalamus. The hypothalamus also receives input from multiple brain areas involved in the stress response. In healthy individuals, the secretion of cortisol follows a diurnal rhythm, with a peak in the early morning, followed by gradual decline over the day, and a nadir at midnight.

Pregnancy-induced changes in HPA axis activity

Maternal cortisol increases sharply in the last part of gestation due to an exponential rise in the secretion of CRH from placental origin, which stimulates the release of ACTH from the pituitary.[7] In contrast to the inhibitory effect of glucocorticoids on the secretion of CRH by the hypothalamus, glucocorticoids stimulated the expression of the CRH

gene in cultures of human placenta.[8] In the fetal compartment a similar rise in cortisol occurs, which is necessary for the maturation of several organs, such as the lungs and the liver.[9] The cortisol rise has also been implicated to play a pivotal role in the onset of parturition.[9]

Glucocorticoids as hormonal regulators of lactation

Glucocorticoids seem to be involved in the lobulo-alveolar development of the mammary gland during the last stage of pregnancy.[10] Although glucocorticoid receptors were detectable in the lactating mouse mammary gland,[11] the increase in circulating glucocorticoids at parturition is probably not the primary trigger of lactogenesis .[12] The effects of glucocorticoids on lactogenesis are probably more permissive, by enhancing the effects of prolactin and spermidine on the synthesis of α-lactalbumin and casein.[12] However, in bovine mammary epithelium glucocorticoids were found to have direct effects on the sodium transport.[13] Progesterone could antagonize the actions of glucocorticoids at the level of the glucocorticoid receptor.[14] Therefore, the effects of glucocorticoids on the mammary gland may become more evident after parturition, when progesterone levels fall.

Determinants of breast-milk glucocorticoid concentrations

Owing to their lipophilic structure, glucocorticoids are able to cross mammary epithelia through simple diffusion in the direction of their concentration gradient. The primary source of breast-milk glucocorticoids is probably the systemic circulation. This assumption is based on observations showing that breast-milk cortisol was strongly correlated with plasma cortisol,[15] with the concentration in breast milk being about 1-13% of the circulating level.[15-17] Another possible source of breast-milk glucocorticoids is the skin, which was recently shown to be capable of glucosteroidogenesis,[18] although it is currently unclear whether dermal glucocorticoids could penetrate the mammary gland in significant amounts.

The concentration of breast-milk cortisol was relatively high in the last part of gestation, compatible with the rise in plasma cortisol, followed by a >50% decline within 2 days after delivery.[16] Mothers who delivered prematurely had lower levels of breast-milk glucocorticoids,[19] either due to an earlier disruption of the positive feedback loop in the secretion of placental CRH or immaturity of the mammary gland.

We studied lactating women who provided frequent sample collections over a 24-hr period, when their children had reached the age of 1 month, and found that levels of cortisol and cortisone in breast milk peaked at 7:00 am, mirroring those in saliva obtained at the same time.[19] The early-morning peak was approximately five times as high as the nadir. This typical diurnal rhythm was replicated by another group.[20] We also found that cortisone was much more abundant in breast milk than cortisol, despite the observation that serum cortisone constitutes only a minor fraction of circulating glucocorticoids.

These patterns could probably be attributed to a high expression of 11β-HSD type 2 in the mammary gland, analogous to the salivary gland.[21]

MEASUREMENT OF GLUCOCORTICOID CONCENTRATIONS IN BREAST MILK

Immunoassay versus LC-MS/MS

For the study of glucocorticoids in breast milk, reliable analytical methods are necessary. Steroid hormones can be measured with immunoassays or chromatography, possibly coupled to mass spectrometry. In general, immunoassays are prone to cross reactivity with compounds that share the general structure with the hormone of interest, necessitating the use of more specific analytical methods.[22] This is especially important during pregnancy and after parturition, when concentrations of steroid hormones like progestagens and sex steroids, as well as their precursors and metabolites, are severely elevated. The most specific method today is chromatography coupled to mass spectrometry, with liquid chromatography tandem mass spectrometry (LC-MS/MS) being regarded as the method of choice. Furthermore, LC-MS/MS analysis carries the advantage of multiple steroid hormone measurements during the same run, enabling the simultaneous measurement of cortisol and cortisone.[23]

Binding to proteins

In serum or plasma, glucocorticoids are for >90% bound to corticosteroid binding globulin (CBG) or albumin.[24] However, albumin-bound and free fractions increase, whereas the CBG-bound fraction decrease, at increasing levels of total cortisol.[25] Steroids in breast milk are also protein-bound, with CBG or a CBG-like protein having been identified in human breast milk already in 1976.[26] This protein was found to decline rapidly after parturition.[26] The presence of corticosteroid binding proteins in breast milk has implications for the choice of the analytical method. Measurement of the total cortisol concentration requires that cortisol is displaced from CBG and other corticosteroid binding proteins. Many immunoassays suffer from ineffective displacement of cortisol from its binding protein, leading to falsely lower cortisol concentrations in the presence of higher CBG concentrations.[27] The improper release of a hormone from its binding protein has also been identified in immunoassays for testosterone and 25-hydroxy-vitamin D3,[28,29] illustrating that this is a widespread analytical problem. Underestimation of the total cortisol concentration is unlikely to occur with LC-MS/MS analysis, where the organic solvents used allow a proper release of cortisol from its binding protein. Alternatively, free glucocorticoid concentrations in breast milk could be measured, yet this requires even more sophisticated methods, such as equilibrium dialysis or ultrafiltration, prior to

LC-MS/MS analysis. Moreover, the very low concentrations might challenge the sensitivity of current LC-MS/MS analyzers.

Steroid conjugates

Many steroid hormones form conjugates with sulfate or glucuronic acid, which renders them inactive. Ninety-five percent of steroids recovered from breast milk were conjugated, predominantly to sulfate.[17] Steroid conjugates cannot be absorbed by the intestines, which requires hydrolysis by sulfatases or glucuronidases.[30] In the human gut, these enzymes are absent at birth and they increase with age due to bacterial colonization.[30] Therefore, incubation of the breast milk with conjugate enzymes could yield much higher levels that do not reflect the biologically available, clinically relevant fraction.

Comparisons between studies

In view of these analytical issues, comparing results of studies concerning breast-milk glucocorticoids is difficult. In addition, differences in standardization between methods used for steroid hormone analysis may contribute to these issues.[31-33] We have previously reviewed human breast-milk cortisol concentrations as reported in the literature, and found a wide range of concentrations, varying from 0 to 1,700 nmol/L.[23] A large variety of methods was used by these studies, the majority of which used immunoassays, sometimes preceded by a deconjugation step. However, the wide range of concentrations, notably those reported by Groer et al,[34] could not wholly be explained by the variety in methods.

Given all these uncertainties, it is highly important for future studies to carefully select and validate the method that will be used so that proper conclusions are drawn. We have developed and extensively validated an isotope-diluted LC/MS-MS method for the measurement of total cortisol and cortisone in human breast milk without use of enzymatic deconjugation.[23] Reference ranges of our assay are reported in Table 1.

Table 1: Reference ranges of breast-milk cortisol and cortisone measured with our LC-MS/MS assay.

Sampling time (hrs)	N	Cortisol (nmol/L)	Cortisone (nmolL)
0:00-6:00	46	3.3 (1.4-6.8)	17.6 (10.8-23.6)
6:00-12:00	64	8.3 (5.1-12.2)	29.6 (24.1-35.0)
12:00-18:00	63	2.8 (2.1-4.5)	18.9 (14.9-31.3)
18:00-24:00	64	1.1 (0.8-1.9)	10.1 (6.8-12.7)

Concentrations are reported as median (interquartile range).

TARGETS OF GLUCOCORTICOIDS IN THE DEVELOPING GUT

Fate of breast-milk glucocorticoids in the neonatal gut

There is some evidence showing that breast-milk glucocorticoids are absorbed by the developing gut into the systemic circulation. Experiments in rats showed that milk-ingested labeled corticosterone – the principal glucocorticoid in rats – was able to cross the pups' intestinal epithelial barrier and was, subsequently, detectable in their plasma and brains.[35] Additionally, corticosterone was detectable in the serum of adrenalectomized pups fed with their own mother's milk.[36] In humans, a 40% higher salivary cortisol level was found among infants who were breastfed during one year in comparison with infants who were formula-fed.[37] Moreover, another study found that salivary cortisol concentration in infants correlated positively with the level in their mothers only in those who were breast-fed.[38]

Effects of breast-milk glucocorticoids on intestinal maturation

There is overwhelming evidence, mostly from studies in rats, indicating that glucocorticoids are important for intestinal maturation. Rat pups can be compared to preterm infants until postnatal day 20, at which stage their development resembles that of term infants. Therefore, rat pups are ideal for the study of gut development. Glucocorticoids administered systemically were found to increase the activities of lactase and sucrase, and fucosylation, and to decrease sialylation,[39] which are all indicative of intestinal maturation. Other studies have assessed the effect of enterally supplemented glucocorticoids on gut maturation. Yeh et al.[36] studied the effect of enterally supplemented corticosterone on the digestive capacity of the intestine of rats that were adrenalectomized at day 12. Adrenalectomized pups were fed with formula supplemented with either 0, 0.1, 0.5, 1.0, 5.0, 10.0 or 50.0 µg/ml corticosterone, or with their own mother's milk. Intact pups, serving as controls, were either mother-fed or fed with unsupplemented formula. They found a dose-dependent increase in the activities of sucrase and maltase with increased glucocorticoid supplementation. In comparison, mother-fed pups, both intact and adrenalectomized, had maltase activity comparable to pups fed with formula supplemented with 0-0.5 µg/ml corticosterone, and undetectable sucrase activity, indicating that the maturational effect of corticosterone might only occur when administered at supraphysiological doses. However, systemic glucocorticoid levels were detectable in mother-fed adrenalectomized pups and all of these pups survived, compared to only 11% of the adrenalectomized pups that did not receive corticosterone-supplemented formula. It appeared, however, that when glucocorticoids were administered systemically, they were more effective in inducing maturation than enterally administered glucocorticoids, and it was thus suggested that enteral glucocorticoids probably influence gut maturation after absorption into the systemic circulation.[36]

Teichberg et al.[40] studied the effect of supplementing formula with corticosterone in early-weaned (i.e., postnatal day 17, whereas normal weaning occurs at age 21 days) rats. They found that supplying formula with corticosterone at levels normally found in the breast milk of dams (i.e., 0.26 µmol/L, by use of a local immunoassay) prevented the delay in jejunal closure to macromolecule uptake usually seen with early weaning. These pups were already relatively mature, and therefore these findings might be extrapolated to term-born infants.[40] However, contrary to rats, normal gut development in humans does not seem to rely on the uptake of macromolecules, and the amount of macromolecule uptake appears to be lower in humans than in rats.[41]

Studies in humans, or in ex-vivo human tissues, were all designed to investigate the effects on the developing gut of glucocorticoid treatment given to mothers presenting with impending preterm delivery. Nanthakumar et al.[42] studied human intestinal xenografts implanted in mice treated with a single injection of cortisone acetate. They found an increase in lactase activity, as well as a decrease in the immune response to both endogenous and exogenous inflammatory stimuli, in immature (20-week-old) intestine. These results were not seen in more mature (30-week-old) intestine. They concluded that glucocorticoids could accelerate intestinal maturation, but that this effect was restricted to earlier pregnancy. Villa et al.[43] studied the effect of hydrocortisone on in vitro cultured human intestine from 10 fetuses with gestational age 14-20 weeks and from one term newborn. Hydrocortisone induced a 2-fold increase in lactase activity after 5 days of culture, although it did not affect lactase mRNA, suggestive of a possible posttranscriptional effect on lactase activity.[43]

Only few in vivo studies have been conducted in humans, none of them studying the effect of enteral glucocorticoids. Costalos et al.[44] addressed the effect of antenatal glucocorticoids on the secretion of gastrointestinal peptides in preterm infants (gestational age <34 weeks), both immediately after birth and after the initiation of enteral feeding. They found higher gastrin levels from birth onwards in infants who had received antenatal glucocorticoids (n=28), and higher motilin levels only after the initiation of enteral feeding compared to infants who did not receive antenatal glucocorticoids (n=17). Antenatal glucocorticoids did not influence vasointestinal peptide. Gastrin promotes gastric secretion, while motilin accelerates gastric emptying, suggesting that antenatal treatment with glucocorticoids might aid in improving digestion. Watkins et al.[45] studied 9 preterm infants (gestational age: 32-36 weeks), of whom four had received dexamethasone (n=3) or phenobarbital (n=1) antenatally. In these infants, the bile salt pool and production were markedly increased, approaching levels of full-term infants, implying a more mature liver and possibly gastrointestinal tract.[45]

To summarize, glucocorticoids, whether administered enterally or systemically, seem to have a trophic effect on the intestine in both animals and humans, although this effect appears to be restricted to a limited time window. Whether cortisol in breast

milk has trophic effects on the intestines of term-born human neonates has yet to be determined.

Effects of breast-milk glucocorticoids on the gut microbiota

Glucocorticoids might play a role in the composition of the microbiome. Although no studies have been conducted aimed at the development of the microbiome after the enteral administration of glucocorticoids, several studies have researched the interaction between HPA axis activity and the microbiome, which is part of the "gut-brain axis hypothesis".[46,47]

Zijlmans et al.[48] studied maternal prenatal stress, defined as elevated basal salivary cortisol or as reported by mothers, and the development of the neonatal microbiome. They found that increased maternal prenatal stress was associated with alterations in the microbiome during the first 110 days after birth, with an increased abundance of Proteobacteria such as Escherichia, and a lower abundance of lactic acid bacteria and Actinobacteria. Moreover, this pattern was associated with an increase in maternally reported gastrointestinal and allergic symptoms.[48] In animals, maternal separation early in life has been shown to alter the diversity and composition of the microbiome,[49] while treatment with Lactobacillus during separation appears to normalize basal corticosterone levels in rats.[50] Additionally, stress induced by maternal separation was associated with increased bacterial translocation in 10-day-old rat pups.[51] The role of the microbiome on HPA axis activity has also been demonstrated in mice. Germ-free mice had an elevated stress response, while mono-colonization with Bifidobacterium infantis was associated with an attenuated stress response and mono-colonization with Escherichia coli with an even stronger response to stressful insults,[52] implicating an effect of the microbiome on HPA axis activity.

Gut bacteria were found to have steroid-converting properties. Not only have they been implicated to play a role in the metabolism of cortisol, they probably also metabolize glucocorticoid precursors and metabolites.[53-57] Some of these compounds enter the enterohepatic circulation, but their biological effect is unknown.

Therefore, although the developmental timing for local, trophic effects of glucocorticoids on the intestine is probably before birth, the interaction between glucocorticoids and the gut microbiome (i.e., the "gut-brain axis") might be a possible mechanism through which glucocorticoids in breast milk could exert systemic effects in the infant.

Table 2: Summary of studies addressing the systemic effects of breast-milk glucocorticoids in offspring.

Study	N	Species	Age at breast-milk sampling	Timing of breast-milk sampling (hrs)	Outcomes	Main results
Primates						
Sullivan et al. (2011)	44	Rhesus monkey	3-4 mo.	NR	Behavioral observations over 25-hr period	Mothers of males had higher cortisol concentrations in milk than did mothers of females, and cortisol concentrations in maternal milk were related to a confident temperament factor in sons, but not daughters.
Hinde et al. (2015)	108	Rhesus monkey	1 mo. and 3.5 mo.	11.30–13.00 after 3.5–4 h of milk accumulation	Behavioral observations over 25-hr period	Milk cortisol was positively associated with a more nervous, less confident temperament in offspring, albeit with few differences between genders. Milk cortisol was positively associated with weight gain in offspring.
Dettmer et al. (2017)	34	Rhesus monkey	7 and 21 d, or 14 and 30 d	13.45-14.00	Social behavior and cognitive function (inhibitory control, impulsivity)	Milk cortisol during the first month post-partum was positively associated with impulsivity on a cognitive task, but not global social behaviors, months later.
Rodents						
Catalani et al. (2000)	NR	Wistar rats (m)	NA	NA	Restraint stress, learning test (conditioned avoidance learning), tests of anxiety (elevated plus-maze test, dark-light test) and hippocampal corticosteroid receptor expression	CORT-exposed pups exhibited a lower adrenocortical response to stress, improvements in learning, reduced fearfulness in anxiogenic situations and greater numbers of hippocampal corticosteroid receptors.
Catalani et al. (2002)	NR	Wistar rats (f)	NA	NA	Restraint stress, learning tests (Morris water maze, aquatic T maze, conditioned avoidance learning), tests of anxiety (elevated plus-maze test, dark-light test, conditioned suppression of drinking) and hippocampal corticosteroid receptor expression	CORT-exposed pups exhibited a lower adrenocortical response to stress, improvements in learning and reduced fearfulness in anxiogenic situations. The numbers of hippocampal corticosteroid receptors was no different from unexposed pups.

Table 2: Summary of studies addressing the systemic effects of breast-milk glucocorticoids in offspring. (continued)

Study	N	Species	Age at breast-milk sampling	Timing of breast-milk sampling (hrs)	Outcomes	Main results
Man						
Hart et al. (2004)	32	-	7-11 d	Mid-morning	Neonatal Behavioral Assessment Scale	Milk cortisol was positively correlated with the Autonomic Stability cluster.
Grey et al. (2013)	52	-	3 mo.	NR	Infant Behavioral Questionnaire	Milk cortisol was positively associated with negative affectivity.
Hahn-Holbrook et al. (2016)	51	-	3 mo.	11.30-16.00	Length and weight until age 2	Milk cortisol was inversely associated with BMI gains in the first 2 years of life.

Abbreviations: CORT = corticosterone; f = female; m = male; NA = not applicable; NR = not reported

SYSTEMIC EFFECTS OF BREAST-MILK GLUCOCORTICOIDS IN OFFSPRING

Surprisingly few studies have investigated associations between breast-milk glucocorticoids and systemic effect in offspring. Here, we review the evidence from studies in both animals and man. The findings from these studies are summarized in Table 2.

Studies in primates

The few animal studies on this topic have nearly all been conducted in Rhesus macaques. The choice for Rhesus monkeys was based on data showing marked variation in behavior and temperament as well as HPA axis vulnerability to early-life experiences in this species.[58]

Sullivan et al.[58] investigated 44 mothers and their offspring at 3-4 months of age. Cortisol was measured in breast milk, and in the plasma of mothers and their infants. Infant temperament was assessed during a 25-hr behavioral observation. Milk cortisol was correlated with maternal plasma cortisol ($r = 0.586$, $P = 0.017$) as well as with protein ($r = 0.441$, $P = 0.003$) and fat contents in milk ($r = 0.398$, $P = 007$). Milk cortisol was higher in the mothers of males ($p = 0.043$). In males, milk cortisol was correlated with confident behavior ($r = 0.669$, $P = 0.002$), but not with the other temperament factors vigilance, gentleness or nervousness. In females, milk cortisol was not correlated with temperament.

Hinde et al.[59] assessed associations between milk cortisol, available milk energy (i.e., the product of milk yield and milk energy density), infant temperament and weight gain in 108 mother-infant dyads. Breast milk was analyzed at 1 month and at 3.5 months

post-partum. Milk cortisol was positively associated with milk fat (P = 0.038) and protein contents (P = 0.0064). A higher milk yield was associated with a lower milk cortisol concentration (P = 0.0014), attributable to dilution. Lower-parity mothers produced milk with a higher cortisol concentration. There were no effects of social ranking or age at sampling on the milk cortisol concentration, and the effect of maternal body weight was negligible. Milk cortisol, independent of available milk energy, predicted a more nervous, less confident temperament, with few differences between genders. It was positively associated with infant weight gain.

Dettmer et al.[60] investigated 34 mother-infant dyads from birth to 8 months of age. Milk collections for determination of cortisol occurred twice in the first month post-partum, and the mean concentration was calculated. At 8 months, social behavior and cognitive function were measured. Mean milk cortisol was positively associated with impulsivity on a cognitive task, and, in females only, with total frequency of play.

Studies in rodents

There are few data suggesting that adult offspring from rat mothers who had free access to 200 µg/ml corticosterone hemisuccinate solution from day 1 post-partum until weaning exhibited longer-term sequelae. These sequelae included a better performance during learning tests, reduction of fearfulness in anxiogenic situations, lower stress-induced corticosterone secretion, and, in males only, greater numbers of hippocampal corticosteroid receptors, as compared to unexposed animals.[61,62] Corticosterone was not measured in milk during these experiments, although previous data showed subsequent elevations in maternal plasma and milk as well as in the gastric contents, plasma and brains of offspring.[35]

Studies in man

Hart et al.[63] investigated associations between maternal mood, milk cortisol, and neonatal behavior in 32 mother-infant pairs. Maternal depressive symptoms were assessed at 7-11 days post-partum using the Beck Depression Inventory (BDI), the Profile of Mood States (POMS) and the State Trait Anxiety Inventory (STAI). At the same time, neonatal behavior was assessed using the Neonatal Behavioral Assessment Scale (NBAS). Milk cortisol was correlated with the POMS Hostility scale ($r = 0.35$, $P < 0.05$), suggesting that mothers' greater hostility coincided with higher concentrations of milk cortisol, but not with scores on the other POMS subscales, or on the BDI or the STAI. Milk cortisol was correlated with the Autonomic Stability cluster of the NBAS ($r = 0.40$, $P < 0.05$). Due to lack of follow-up data, the sustainability of these associations could not be tested.

Grey et al.[64] tested whether milk cortisol is associated with infant temperament in 52 mother-infant pairs. Milk cortisol and infant temperament, by maternal report of the Infant Behavior Questionnaire (IBQ), were assessed when the infants had reached the

age of 3 months. Analyses revealed a positive association between milk cortisol and the negative affectivity dimension of the IBQ ($r = 0.37$, $P < 0.01$). No correlation was found between milk cortisol and the surgency/extraversion or the orienting/regulation dimensions of the IBQ. Again, the sustainability of observations was not tested. In the same cohort, Hahn-Holbrook et al.[65] tested associations between milk cortisol and change in body mass index (BMI) across time. They found that a higher milk cortisol was associated with lower gains in BMI in the first 2 years of life ($P = 0.046$).

For several reasons, the findings obtained from these studies were highly contradictory. All of them had measured cortisol (but not cortisone) by use of immunoassays, which are prone to cross reactivity. Moreover, studies differed in sampling procedures. None of them had obtained multiple samples across the day, and only one adjusted the analyses for timing of sampling. However, we have previously argued that, in spite of correction for time of collection, vertical transmission of glucocorticoids cannot be determined from a single milk specimen.[66] This reasoning is based on observations showing that there was a large inter-individual variability in the diurnal rhythmicity of salivary cortisol.[67]Therefore, frequent sample collections over a 24-hr period are necessary to obtain meaningful indices of glucocorticoid exposure, such as peak, nadir, diurnal variability and area under the curve.

CONCLUSIONS AND FUTURE PROSPECTS

Given the methodological shortcomings of the studies conducted thus far, at present there is no compelling evidence for persistent effects of breast-milk glucocorticoids in offspring. Future studies should use sound methodologies to test associations between breast-milk glucocorticoids and infant developmental pathways, including frequent sampling and LC-MS/MS analysis with simultaneous measurement of cortisol and cortisone.

Recent evidence from studies in adults suggests that flattening of HPA axis rhythmicity predicts the onset of type 2 diabetes mellitus and cardiovascular mortality.[68,69] Such patterns have previously been associated with major depressive disorder and other psychopathologies, albeit not unequivocally.[70,71] If associations between flattened HPA axis rhythmicity in breast milk and adverse infant outcomes are demonstrated, lactating women should be requested not to express their milk, or to give their infants expressed milk that was obtained at the same time of the day. Such results could also have implications for donor milk banks, which provide donor human milk to vulnerable infants when own mother's milk is not available. In clinical practice, preterm infants are often fed with pooled donor milk, which lacks a diurnal rhythm. Our group has recently demonstrated

that pasteurization, necessary to secure the safety of donor milk, does not affect glucocorticoid contents in milk.[72]

Future studies should also explore the mechanisms, including possible sexually dimorphic effect as observed in animals, that could underpin associations between breast-milk glucocorticoids and infant outcomes. Studies conducted to date have mainly focused on the effects glucocorticoids might have on intestinal maturation. The effects on the gut microbiota remain to be explored. Gut bacteria were found to be sensitive to disruptions in circadian clock rhythms,[73] but the role of disturbances in HPA axis rhythmicity has never been studied. Furthermore, the steroid-converting properties of gut bacteria require further study. The implication of microbial 11β-reductase activity, if present, is that cortisol could be produced in the gut from inert cortisone. However, the net effect will be unclear, as intestinal epithelia were found to express 11β-HSD type 2, which catalyzes the reverse reaction.[21]

In conclusion, breast-milk glucocorticoids follow the diurnal rhythm of maternal HPA axis activity probably through simple diffusion from the systemic circulation. Breast-milk glucocorticoids might have (direct or indirect) effects in infants, both locally (e.g., intestinal maturation, microbiome) and systemically (e.g., growth, body composition, neurodevelopment). In order to investigate this, the diurnal rhythm of glucocorticoids should be taken into account and the analytical methods should be chosen carefully. Nevertheless, glucocorticoids in breast milk might offer a novel potential pathway for signal transmission from mothers to their infants.

REFERENCES

1. Gluckman PD, Hanson MA, Pinal C. The developmental origins of adult disease. Matern Child Nutr 2005; 1:130-141
2. Cottrell EC, Seckl JR. Prenatal stress, glucocorticoids and the programming of adult disease. Front Behav Neurosci 2009; 3:19
3. Edwards CR, Benediktsson R, Lindsay RS, Seckl JR. Dysfunction of placental glucocorticoid barrier: link between fetal environment and adult hypertension? Lancet 1993; 341:355-357
4. Patro-Golab B, Zalewski BM, Kolodziej M, Kouwenhoven S, Poston L, Godfrey KM, Koletzko B, van Goudoever JB, Szajewska H. Nutritional interventions or exposures in infants and children aged up to 3 years and their effects on subsequent risk of overweight, obesity and body fat: a systematic review of systematic reviews. Obes Rev 2016; 17:1245-1257
5. Kramer MS, Kakuma R. Optimal duration of exclusive breastfeeding. Cochrane Database Syst Rev 2012:CD003517
6. Tucker HA, Schwalm JW. Glucocorticoids in mammary tissue and milk. J Anim Sci 1977; 45:627-634
7. Glynn LM, Davis EP, Sandman CA. New insights into the role of perinatal HPA-axis dysregulation in postpartum depression. Neuropeptides 2013; 47:363-370
8. Robinson BG, Emanuel RL, Frim DM, Majzoub JA. Glucocorticoid stimulates expression of corticotropin-releasing hormone gene in human placenta. Proc Natl Acad Sci U S A 1988; 85:5244-5248
9. Braun T, Challis JR, Newnham JP, Sloboda DM. Early-life glucocorticoid exposure: the hypothalamic-pituitary-adrenal axis, placental function, and long-term disease risk. Endocr Rev 2013; 34:885-916
10. Topper YJ, Freeman CS. Multiple hormone interactions in the developmental biology of the mammary gland. Physiological reviews 1980; 60:1049-1106
11. Shyamala G. Specific cytoplasmic glucocorticoid hormone receptors in lactating mammary glands. Biochemistry 1973; 12:3085-3090
12. Neville Me. Lactation: Physiology, Nutrition, and Breast-Feeding. New York: Plenum Press.
13. Quesnell RR, Han X, Schultz BD. Glucocorticoids stimulate ENaC upregulation in bovine mammary epithelium. Am J Physiol Cell Physiol 2007; 292:C1739-1745
14. Shyamala G, Dickson C. Relationship between receptor and mammary tumour virus production after stimulation by glucocorticoid. Nature 1976; 262:107-112
15. Patacchioli F, Cigliana G. Maternal plasma and milk free cortisol during the first 3 days of breastfeeding following spontaneous delivery or elective cesarean section. Gynecolog Obstet Invest 1992; 34:159-163
16. Kulski JK, Hartmann PE. Changes in the concentration of cortisol in milk during different stages of human lactation. Aust J Exp Biol Med Sci 1981; 59:769-778
17. Sahlberg BL, Axelson M. Identification and quantitation of free and conjugated steroids in milk from lactating women. Journal of steroid biochemistry 1986; 25:379-391
18. Slominski AT, Manna PR, Tuckey RC. On the role of skin in the regulation of local and systemic steroidogenic activities. Steroids 2015; 103:72-88
19. van der Voorn B, de Waard M, van Goudoever JB, Rotteveel J, Heijboer AC, Finken MJ. Breast-Milk Cortisol and Cortisone Concentrations Follow the Diurnal Rhythm of Maternal Hypothalamus-Pituitary-Adrenal Axis Activity. J Nutr 2016; 146:2174-2179

20. Pundir S, Wall CR, Mitchell CJ, Thorstensen EB, Lai CT, Geddes DT, Cameron-Smith D. Variation of Human Milk Glucocorticoids over 24 hour Period. J Mammary Gland Biol Neoplasia 2017; 22:85-92
21. Smith RE, Maguire JA, Stein-Oakley AN, Sasano H, Takahashi K, Fukushima K, Krozowski ZS. Localization of 11 beta-hydroxysteroid dehydrogenase type II in human epithelial tissues. J Clin Endocrinol Metab 1996; 81:3244-3248
22. Ackermans MT, Endert E. LC-MS/MS in endocrinology: what is the profit of the last 5 years? Bioanalysis 2014; 6:43-57
23. van der Voorn B, Martens F, Peppelman NS, Rotteveel J, Blankenstein MA, Finken MJ, Heijboer AC. Determination of cortisol and cortisone in human mother's milk. Clin Chim Acta 2015; 444:154-155
24. Nguyen PT, Lewis JG, Sneyd J, Lee RS, Torpy DJ, Shorten PR. Development of a formula for estimating plasma free cortisol concentration from a measured total cortisol concentration when elastase-cleaved and intact corticosteroid binding globulin coexist. J Steroid Biochem Mol Biol 2014; 141:16-25
25. Dorin RI, Pai HK, Ho JT, Lewis JG, Torpy DJ, Urban FK, 3rd, Qualls CR. Validation of a simple method of estimating plasma free cortisol: role of cortisol binding to albumin. Clin Biochem 2009; 42:64-71
26. Payne DW, Peng LH, Pearlman WH. Corticosteroid-binding proteins in human colostrum and milk and rat milk. J Biol Chem 1976; 251:5272-5279
27. Vos MJ, Bisschop PH, Deckers MML, Endert E. The cortisol-CBG ratio affects cortisol immunoassay bias at elevated CBG concentrations. Clin Chem Lab Med 2017;
28. Heijboer AC, Blankenstein MA, Kema IP, Buijs MM. Accuracy of 6 routine 25-hydroxyvitamin D assays: influence of vitamin D binding protein concentration. Clin Chem 2012; 58:543-548
29. Heijboer AC, Savelkout E, Kruit A, Endert E, Blankenstein MA. Inaccurate First-Generation Testosterone Assays Are Influenced by Sex Hormone–Binding Globulin Concentrations. The Journal of Applied Laboratory Medicine 2016; 2:194-201
30. Kent TH, Fischer LJ, Marr R. Glucuronidase activity in intestinal contents of rat and man and relationship to bacterial flora. Proc Soc Exp Biol Med 1972; 140:590-594
31. Buttler RM, Martens F, Ackermans MT, Davison AS, van Herwaarden AE, Kortz L, Krabbe JG, Lentjes EG, Syme C, Webster R, Blankenstein MA, Heijboer AC. Comparison of eight routine unpublished LC-MS/MS methods for the simultaneous measurement of testosterone and androstenedione in serum. Clin Chim Acta 2016; 454:112-118
32. Buttler RM, Martens F, Fanelli F, Pham HT, Kushnir MM, Janssen MJ, Owen L, Taylor AE, Soeborg T, Blankenstein MA, Heijboer AC. Comparison of 7 Published LC-MS/MS Methods for the Simultaneous Measurement of Testosterone, Androstenedione, and Dehydroepiandrosterone in Serum. Clin Chem 2015; 61:1475-1483
33. El-Farhan N, Pickett A, Ducroq D, Bailey C, Mitchem K, Morgan N, Armston A, Jones L, Evans C, Rees DA. Method-specific serum cortisol responses to the adrenocorticotrophin test: comparison of gas chromatography-mass spectrometry and five automated immunoassays. Clin Endocrinol (Oxf) 2013; 78:673-680
34. Groer MW, Humenick S, Hill PD. Characterizations and psychoneuroimmunologic implications of secretory immunoglobulin A and cortisol in preterm and term breast milk. J Perinat Neonatal Nurs 1994; 7:42-51

35. Angelucci L, Patacchioli FR, Scaccianoce S, Di Sciullo A, Cardillo A, Maccari S. A model for later-life effects of perinatal drug exposure: maternal hormone mediation. Neurobehav Toxicol Teratol 1985; 7:511-517
36. Yeh KY, Yeh M, Holt PR. Induction of intestinal differentiation by systemic and not by luminal corticosterone in adrenalectomized rat pups. Endocrinology 1989; 124:1898-1904
37. Cao Y, Rao SD, Phillips TM, Umbach DM, Bernbaum JC, Archer JI, Rogan WJ. Are breast-fed infants more resilient? Feeding method and cortisol in infants. The Journal of pediatrics 2009; 154:452-454
38. Benjamin Neelon SE, Stroo M, Mayhew M, Maselko J, Hoyo C. Correlation between maternal and infant cortisol varies by breastfeeding status. Infant Behav Dev 2015; 40:252-258
39. Mahmood A, Torres-Pinedo R. Effect of hormone administration on the sialylation and fucosylation of intestinal microvillus membranes of suckling rats. Pediatr Res 1985; 19:899-902
40. Teichberg S, Wapnir RA, Moyse J, Lifshitz F. Development of the neonatal rat small intestinal barrier to nonspecific macromolecular absorption. II. Role of dietary corticosterone. Pediatr Res 1992; 32:50-57
41. Axelsson I, Jakobsson I, Lindberg T, Polberger S, Benediktsson B, Raiha N. Macromolecular absorption in preterm and term infants. Acta Paediatr Scand 1989; 78:532-537
42. Nanthakumar NN, Young C, Ko JS, Meng D, Chen J, Buie T, Walker WA. Glucocorticoid responsiveness in developing human intestine: possible role in prevention of necrotizing enterocolitis. Am J Physiol Gastrointest Liver Physiol 2005; 288:G85-92
43. Villa M, Menard D, Semenza G, Mantei N. The expression of lactase enzymatic activity and mRNA in human fetal jejunum. Effect of organ culture and of treatment with hydrocortisone. FEBS Lett 1992; 301:202-206
44. Costalos C, Gounaris A, Sevastiadou S, Hatzistamatiou Z, Theodoraki M, Alexiou EN, Constandellou E. The effect of antenatal corticosteroids on gut peptides of preterm infants--a matched group comparison: corticosteroids and gut development. Early Hum Dev 2003; 74:83-88
45. Watkins JB, Szczepanik P, Gould JB, Klein P, Lester R. Bile salt metabolism in the human premature infant. Preliminary observations of pool size and synthesis rate following prenatal administration of dexamethasone and phenobarbital. Gastroenterology 1975; 69:706-713
46. Cryan JF, Dinan TG. Mind-altering microorganisms: the impact of the gut microbiota on brain and behaviour. Nat Rev Neurosci 2012; 13:701-712
47. Foster JA, McVey Neufeld KA. Gut-brain axis: how the microbiome influences anxiety and depression. Trends Neurosci 2013; 36:305-312
48. Zijlmans MA, Korpela K, Riksen-Walraven JM, de Vos WM, de Weerth C. Maternal prenatal stress is associated with the infant intestinal microbiota. Psychoneuroendocrinology 2015; 53:233-245
49. O'Mahony SM, Marchesi JR, Scully P, Codling C, Ceolho AM, Quigley EM, Cryan JF, Dinan TG. Early life stress alters behavior, immunity, and microbiota in rats: implications for irritable bowel syndrome and psychiatric illnesses. Biol Psychiatry 2009; 65:263-267
50. Gareau MG, Jury J, MacQueen G, Sherman PM, Perdue MH. Probiotic treatment of rat pups normalises corticosterone release and ameliorates colonic dysfunction induced by maternal separation. Gut 2007; 56:1522-1528
51. Moussaoui N, Braniste V, Ait-Belgnaoui A, Gabanou M, Sekkal S, Olier M, Theodorou V, Martin PG, Houdeau E. Changes in intestinal glucocorticoid sensitivity in early life shape the risk of epithelial barrier defect in maternal-deprived rats. PLoS One 2014; 9:e88382

52. Sudo N, Chida Y, Aiba Y, Sonoda J, Oyama N, Yu XN, Kubo C, Koga Y. Postnatal microbial colonization programs the hypothalamic-pituitary-adrenal system for stress response in mice. J Physiol 2004; 558:263-275
53. Bokkenheuser VD, Winter J, Dehazya P, de Leon O, Kelly WG. Formation and metabolism of tetrahydrodeoxycorticosterone by human fecal flora. J Steroid Biochem 1976; 7:837-843
54. Feighner SD, Hylemon PB. Characterization of a corticosteroid 21-dehydroxylase from the intestinal anaerobic bacterium, Eubacterium lentum. J Lipid Res 1980; 21:585-593
55. Winter J, Bokkenheuser VD. 21-dehydroxylation of corticoids by anaerobic bacteria isolated from human fecal flora. J Steroid Biochem 1978; 9:379-384
56. Winter J, Bokkenheuser VD, Ponticorvo L. Bacterial metabolism of corticoids with particular reference to the 21-dehydroxylation. J Biol Chem 1979; 254:2626-2629
57. Winter J, Cerone-McLernon A, O'Rourke S, Ponticorvo L, Bokkenheuser VD. Formation of 20 beta-dihydrosteroids by anaerobic bacteria. J Steroid Biochem 1982; 17:661-667
58. Sullivan EC, Hinde K, Mendoza SP, Capitanio JP. Cortisol concentrations in the milk of rhesus monkey mothers are associated with confident temperament in sons, but not daughters. Dev Psychobiol 2011; 53:96-104
59. Hinde K, Skibiel AL, Foster AB, Del Rosso L, Mendoza SP, Capitanio JP. Cortisol in mother's milk across lactation reflects maternal life history and predicts infant temperament. Behav Ecol 2015; 26:269-281
60. Dettmer AM, Murphy AM, Guitarra D, Slonecker E, Suomi SJ, Rosenberg KL, Novak MA, Meyer JS, Hinde K. Cortisol in Neonatal Mother's Milk Predicts Later Infant Social and Cognitive Functioning in Rhesus Monkeys. Child Dev 2017;
61. Catalani A, Casolini P, Cigliana G, Scaccianoce S, Consoli C, Cinque C, Zuena AR, Angelucci L. Maternal corticosterone influences behavior, stress response and corticosteroid receptors in the female rat. Pharmacol Biochem Behav 2002; 73:105-114
62. Catalani A, Casolini P, Scaccianoce S, Patacchioli FR, Spinozzi P, Angelucci L. Maternal corticosterone during lactation permanently affects brain corticosteroid receptors, stress response and behaviour in rat progeny. Neuroscience 2000; 100:319-325
63. Hart S, Boylan LM, Border B, Carroll SR, McGunegle D, Lampe RM. Breast milk levels of cortisol and Secretory Immunoglobulin A (SIgA) differ with maternal mood and infant neuro-behavioral functioning. Infant Behavior & Development 2004; 27:101-106
64. Grey KR, Davis EP, Sandman CA, Glynn LM. Human milk cortisol is associated with infant temperament. Psychoneuroendocrinology 2013; 38:1178-1185
65. Hahn-Holbrook J, Le TB, Chung A, Davis EP, Glynn LM. Cortisol in human milk predicts child BMI. Obesity (Silver Spring) 2016; 24:2471-2474
66. Finken MJJ, van der Voorn B, Hollanders JJ, Dijkstra LR, Toorop AA, Rotteveel J. Cortisol in human milk: The good, the bad, or the ugly? Obesity (Silver Spring) 2017; 25:1153
67. Stone AA, Schwartz JE, Smyth J, Kirschbaum C, Cohen S, Hellhammer D, Grossman S. Individual differences in the diurnal cycle of salivary free cortisol: a replication of flattened cycles for some individuals. Psychoneuroendocrinology 2001; 26:295-306
68. Hackett RA, Kivimaki M, Kumari M, Steptoe A. Diurnal Cortisol Patterns, Future Diabetes, and Impaired Glucose Metabolism in the Whitehall II Cohort Study. J Clin Endocrinol Metab 2016; 101:619-625
69. Kumari M, Shipley M, Stafford M, Kivimaki M. Association of diurnal patterns in salivary cortisol with all-cause and cardiovascular mortality: findings from the Whitehall II study. J Clin Endocrinol Metab 2011; 96:1478-1485

70. Heim C, Ehlert U, Hellhammer DH. The potential role of hypocortisolism in the pathophysiology of stress-related bodily disorders. Psychoneuroendocrinology 2000; 25:1-35
71. Zorn JV, Schur RR, Boks MP, Kahn RS, Joels M, Vinkers CH. Cortisol stress reactivity across psychiatric disorders: A systematic review and meta-analysis. Psychoneuroendocrinology 2017; 77:25-36
72. van der Voorn B, de Waard M, Dijkstra LR, Heijboer AC, Rotteveel J, van Goudoever JB, Finken MJJ. Stability of Cortisol and Cortisone in Human Breast Milk During Holder Pasteurization. J Pediatr Gastroenterol Nutr 2017;
73. Oster H, Challet E, Ott V, Arvat E, Ronald de Kloet E, Dijk DJ, Lightman S, Vgontzas A, Van Cauter E. The Functional and Clinical Significance of the 24-Hour Rhythm of Circulating Glucocorticoids. Endocr Rev 2017; 38:3-45