I will talk about the physiology of breast-fed infants. There is growing evidence that nutrition during pregnancy and during the first months after birth has long-term consequences for health and well being later in life. And it is important to understand how early diet can influence the development of risk factors of later diseases.

Evidence emerged from animal studies that early life environment affects food intake, energy intake, food preference, and overall longevity. Also human studies have shown that there is a link between fetal and early life growth and conditions and risk of obesity in later life.

In 1974, Dörner introduced the term "programming" into the scientific literature. He proposed that hormones, metabolites, neurotransmitters during critical windows in early development pre-program brain development, functional disorders, disease risk and body functions in human adulthood. 40 years of animal and human studies have shown that early nutrition is a key factor for health with major biological and social implications.

Human epidemiological studies have shown relationships between low birth weight, low weight at 1 year and catch up growth in childhood and increased risk of adverse outcomes through altered body composition in later life: cardiovascular diseases, type 2 diabetes and obesity. Experimental studies on preterm and term infants have been conducted to test the nutritional programming hypothesis.

Human milk intake was significantly associated with lower leptin concentrations relative to fat mass in adolescence independent of potential confounding factors.

From animal studies it emerges that early life environment affects food intake, energy intake, food preference and overall longevity.

Human studies have shown that there is a link between fetal and early life growth and conditions and risk of obesity in later life.

**Fetal and early postnatal origins of adult disease**

Humans develop in a complex environment. The intrauterine environment is controlled by the mother but is also influenced by the father and the placenta. The developing fetus is not a passive recipient of the environment but is actively responsive to it. The newborn is not a mere extension of the mother, but a unique organism with a life of its own. The transition from intrauterine to extrauterine life is marked by a number of changes that are necessary for survival. These changes include the development of the central nervous system, the respiratory system, the gastrointestinal tract, and the immune system. The newborn is a vulnerable organism with a high risk of developing disease. The risk of developing disease is determined by a number of factors, including the genetic makeup of the newborn, the environment in which the newborn is born, and the care that the newborn receives in the neonatal intensive care unit.
Milk, Hormones & Human Health

**Why did we focus on ghrelin, leptin and IGF-1?**

- They act as mediators between the gastrointestinal tract, adipose tissue and brain in energy balance regulation.
- Leptin may prime or set the endocrinial system at a different homeostatic energy regulation balance.
- Programming of relative leptin concentrations by early diet may be one mechanism that links early nutrition with later obesity.

Possible biological mechanisms for storing throughout life the “memory” of early nutritional experience and its expression in adulthood.

**LEPTIN**

- From Greek leptos, meaning thin.
- Discovered by Zhang et al. in 1994, when the gene responsible for obesity in the ob/ob mouse was positionally cloned.
- Product of the obese gene (ob), localized on chromosome 7q31.3.
- 146 aminoacid peptide hormone (16 Kda)

mainly produced by white adipose tissue, but also by hypothalamus, pituitary, skeletal muscle, stomach, liver, placenta, and mammary gland.


Leptin is a hormone discovered in 1994. It is produced by the ob gene, and is mainly expressed in white adipose tissue, but also in the hypothalamus, pituitary, skeletal muscle, stomach, liver, placenta and mammary gland.

**Leptin receptors**

- Belong to the family of class I cytokine receptors.
- Five alternatively spliced forms, with transmembrane domains (Ob-Ra; Ob-Rb; Ob-Rc; Ob-Rd; Ob-Rf)
  - Only Ob–Rb, the long isoform, has all intracellular protein motifs necessary for signaling via the Jak-STAT signal transduction pathway.

Leptin circulates in the plasma as a free form or bound to leptin-binding proteins (LBP), that are likely to include a soluble form of leptin receptor.

There are five alternatively spliced forms of the receptor, but only Ob-Rb, the long isoform, has all the intracellular protein motifs necessary for signaling via the signal transduction pathway. So leptin circulates in the plasma as a free form or bound to leptin-binding proteins that are likely to include a soluble form of the leptin receptor.

**Regulation of leptin expression**

<table>
<thead>
<tr>
<th>Site</th>
<th>Increase</th>
<th>Decrease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adipose tissue</td>
<td>Overfeeding</td>
<td>Fasting</td>
</tr>
<tr>
<td>Liver</td>
<td>Glucose metabolism</td>
<td>Stress</td>
</tr>
<tr>
<td>Liver</td>
<td>Glycerololipids</td>
<td>Sleep deprivation</td>
</tr>
<tr>
<td>Liver</td>
<td>Glycogenolipids</td>
<td>Cold exposure</td>
</tr>
<tr>
<td>Placenta</td>
<td>IGF-1</td>
<td>Hyperactivity</td>
</tr>
<tr>
<td>Skeletal muscle (st)</td>
<td>Glyceroneolipids</td>
<td>Leptin positive</td>
</tr>
<tr>
<td>Stomach fluids (st)</td>
<td>Glyceroneolipids</td>
<td>Leptin negative</td>
</tr>
</tbody>
</table>

Listed here are the different sites of production and the situations that lead to increases and decreases in leptin production. In general, leptin levels in blood correlate with total body fat stores. During fasting or weight loss, leptin decreases while it increases in overfeeding and weight gain.

**Leptin circadian rhythm**

- Leptin levels INCREASE at night, peaking in the middle of the night hours (between 12 p.m and 2 a.m.)
- The diurnal rhythm in circulating leptin level is opposite to that in cortisol level in humans.
- This pattern represents the response to insulin secretion during the feeding cycle.

Leptin circadian rhythm increases at night, peaking in the middle of the night hours. And this pattern may represent the response to insulin secretion during the feeding cycle. Very recently, it has been observed that leptin, acting on the brain during a restricted neonatal critical period, promotes the formation of neural circuits that control food intake and adiposity later in life. So leptin represents a likely hormonal mediator of the environmental nutrient-sensing apparatus that directs metabolic programming.

Plasma leptin concentration correlates positively with total body fat stores at different age periods. At birth, serum leptin concentrations were found decreased in SGA infants and increased in LGA infants compared with AGA ones. Leptin levels on the 30th and 90th days after birth correlate significantly with BMI. In infancy a positive correlation between cord blood leptin levels and BMI has been reported for full term infants and preterm ones. In adults leptin is positively correlated to BMI.

Houseknecht reported that leptin is present in human milk and is related to maternal adiposity. Leptin in breast milk correlates with maternal plasma leptin concentration, even though breast milk leptin concentration is lower than maternal plasma leptin levels. Could leptin be considered a possible link between maternal body composition and neonatal growth and development? Smith-Kirwin showed that leptin is produced by human mammary epithelial cells and is associated with milk fat globules. More recently it has been observed that secretory epithelial cells may transfer leptin from the blood. Very recently, it has been observed that leptin, acting on the brain during a restricted neonatal critical period, promotes the formation of neural circuits that control food intake and adiposity later in life. So leptin represents a likely hormonal mediator of the environmental nutrient-sensing apparatus that directs metabolic programming.

The major function of leptin is to provide a long-term signal to inform the brain about fat storage. Further, there is an action on peripheral organs such as the pancreas, liver and skeletal muscle.

Leptin reduces food intake by regulating the activity of neurons in the arcuate nucleus of the hypothalamus that inhibit food intake and stimulate energy expenditure.

Houseknecht et al. showed that leptin is produced by human mammary epithelial cells and is associated with milk fat globules. More recently it has been observed that secretory epithelial cells may transfer leptin from the blood.

Ghrelin, Leptin and IGF-I Levels in Breast-Fed Infants

Francesco Savino
Casabelli et al. showed that human milk contains immunoreactive leptin, which is identical to intact human leptin. In experimental studies on animals, they demonstrated that leptin is transferred from the circulation to mothers’ milk, to the infant rat’s stomach via the milk and then passes to infant’s blood.

Ucar et al. showed a positive correlation between breast milk leptin levels and infant plasma leptin.

More recently leptin receptors have been identified in gastric epithelial cells and in the absorptive cells of mouse and human small intestine.

** Does infant milk formula contain leptin?

- There was no detectable leptin in milk formula because whey proteins added to formula are isolated from skim, bovine milk and leptin associated with milk fat globules would be removed during the skimming process.
- Lage M et al. using RIA methods detected significant and variable leptin concentrations in edible commercial bovine milk with higher concentration in infant formulas.
- O’Connor D et al. convey that RIA-leptin methodology cannot be used to determine the presence or absence in infant formulas of leptin due to interference with supplemented iron, emulsifiers among other additives.

A very controversial topic currently is what happens in infant formula? Does infant formula contain leptin? At the moment, there are only a few papers on this topic. Resto reported that there was no detectable leptin in formula because whey proteins added to formula are isolated from skim bovine milk, and since leptin is associated with milk fat globules it would be removed during the skimming process. Meanwhile, Lage, using RIA methods, detected significant and variable leptin concentrations in edible commercial bovine milk with higher concentration in infant formulas. But O’Connor conveys that RIA methodology cannot be used to determine the presence or absence in infant formulas of leptin due to interference with supplemented iron, emulsifiers, and other additives. We are conducting a study in order to clarify this topic. At the moment, it is very controversial.

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We have to take into account that leptin concentrations in breast milk change during lactation. It has been observed that there are higher serum and breast milk leptin levels in term infants than in preterm ones. Also leptin levels decline from the colostrum to the transitional milk.

- Bielicki J et al. observed higher serum and breast milk leptin levels in term infants than in preterm ones; leptin levels declined from colostrum to transitional milk.

** Leptin concentration in breast milk during lactation**


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This is the first study that we published about leptin levels in breast-fed compared with formula-fed infants. You can see that serum leptin levels were significantly higher in breast-fed than in formula-fed infants in the first four months of life. Exclusive breast-feeding is more likely to occur during the first five months. After this period many more confounding factors may be present.
Breast-fed infants have higher leptin values than formula-fed infants in the first four months of life.


With a larger sample, we confirmed that breast-fed infants (n=38) have higher leptin levels than formula-fed ones (n=44).

2005: Petridou E et al. reported that formula-fed newborns have higher serum leptin concentrations than breast-fed ones.


- Difficult to evaluate the influence of the kind of feeding on serum leptin concentration after only a few days of life (up to 5 days).
- As leptin secretion is pulsatile, it is important to determine serum leptin values at the same time in all infants (They did not specify the time of collection of the serum assay).
- Garnett SP et al. “The art and science of regression modelling; methods for building valid models to explore hormone and body composition interactions” Ped Endocrinol Rev 2005 — it is not clear if assumptions for regression are met so that spurious associations are not generated

* Petridou E et al. Clin Endocrinol 2005
** Weigle DS et al. Am j Clin Nutr 2003

But, to our surprise, Petridou and colleagues reported in 2005 that formula-fed newborns have higher serum leptin concentrations than breast-fed ones. We commented on this paper some months later, pointing out that it is difficult to evaluate the influence of the kind of feeding on serum leptin concentration after only a few days of life, up to five days. Further, as leptin secretion is pulsatile, it is important to determine serum leptin values at the same time in all infants. In our study, we performed our evaluation in the fasting period. The Petridou paper did not specify the time of collection of the serum sample. Further, leptin levels do not follow a normal distribution curve, which makes the interpretation more difficult. Our statistical analyses included regression analyses to eliminate spurious associations.

In another study we evaluated the relationship between maternal BMI and serum leptin concentrations in infants in the first year of life. We found a positive correlation between infant serum leptin concentration and maternal BMI in breast-fed infants, but not in formula-fed infants. So maternal adiposity is a factor that might affect the leptin levels in human milk.

Another hormone more recently discovered in 1999 is ghrelin. Human ghrelin stimulates the release of growth hormone from the pituitary working through the G protein coupled receptor. There are two types of receptors.

Ghrelin, Leptin and IGF-I Levels in Breast-Fed Infants

Savino F

Positive correlation between infant serum leptin concentration and maternal BMI in breast-fed infants, but not in FF infants

Maternal adiposity is a factor that might affect leptin levels in human milk.

Ghrelin: “Gh” = growth hormone “relin” = release

- 1999 Kojima et al. purified an endogenous ligand for GHS-R from rat stomach and named it Ghrelin
- Human ghrelin gene is localized on chromosome 3p25-26
- Ghrelin stimulates the release of growth hormone (GH) from the pituitary, working through a G protein coupled receptor (GPCR), the GHS-receptor (GHS-R)

Two types of GHS-R

GHS-R 1a activates the phospholipase C signaling pathway

GHS-R 1b Its functional role remains to be determined

The secretion is mainly produced in the stomach by the enteroendocrine X/A-like cells. There are two forms of human ghrelin, acylated and nonacetylated. The acylated form is the active form that is essential for endocrine activity.

Ghrelin also is secreted in a pulsatile manner, with a nocturnal increase. The most important factor in the regulation of ghrelin secretion is feeding. Ghrelin increases during fasting, (preprandial rise) and decreases after food intake (postprandial fall).

As concerns appetite regulation, ghrelin stimulates NPY/AgRP neurons in the arcuate nucleus of the hypothalamus. These are implicated in the central control of meal initiation because their expression increases at times of maximal spontaneous feeding. So ghrelin has orexigenic action.

This year two studies were published showing that ghrelin is present in breast milk. Aydin reported the presence of ghrelin in colostrum, transitional and mature milk at levels lower than those typically found in plasma. So milk ghrelin probably comes from the plasma? More recently, Kierson showed that ghrelin levels are higher in whole milk than skim milk and that breast milk ghrelin levels are higher than plasma levels. So ghrelin in breast milk is likely synthesized and secreted from the breast? These data are all that we have currently.

As concerns insulin like growth factor-1, IGF-1 is a hormone produced by the liver. IGF-1 levels decrease with sustained fasting and poor nutritional status but are unaffected by recent food intake.
The IGF-1 receptor plays an important role in both embryonic and postnatal growth. IGF-1 levels in humans correlate with body size.

**IGF-I and milk**

- **Klagsburn** was the first to show that human milk contained growth factors that stimulated the growth of cells in culture.
- **Baxter et al.** demonstrated the presence of IGF-1 in human milk.
- Its levels are several-fold higher in colostrum than in mature milk and they decrease precipitously in the first few days of lactation.
- Also IGFBP have been identified in human milk.

Also, IGF is present in human milk. Klagsburn was the first to show that human milk contained growth factors that stimulated the growth of cells in culture. Baxter demonstrated the presence of IGF-1 in human milk. Its levels are several-fold higher in colostrum than in mature milk and they decrease precipitously in the first few days of lactation. Also, IGF binding proteins (IGFBP) have been identified in human milk.

We performed a cross-sectional study in order to evaluate IGF-1 in breast-fed and formula-fed infants. We found a positive correlation between IGF-1 and zeta (Z) score for weight, BMI, and tricipital skin-fold thickness. So we hypothesize that there is a programming of the IGF-1 axis during infancy. Lower IGF-1 levels in breast-fed infants during the breast-feeding period re-sets the pituitary due to less feedback, resulting in higher IGF-1 levels and thereby higher growth velocity later in childhood. Later speakers will talk about this.

In a more recent paper, we focused our attention on all three hormones, and found that breast-fed infants have higher leptin levels, lower ghrelin levels, and lower IGF-1 levels than formula-fed ones.

Further, we observed a negative correlation between ghrelin and leptin. So a decrease in leptin secretion allows stimulation of NPY neurons by ghrelin to induce feeding.
Ghrelin, motilin, insulin, and energy balance in infants: the chicken or the egg?

Department of Paediatrics, Regina Margherita Children's Hospital, Torino University, Italy

Ghrelin and Motilin

Ghrelin and motilin levels are lower in formula-fed than breast-fed infants age 3 to 9 months.

Table 3. Pearson’s correlation coefficients between hormones and anthropometrics, breast and formulas:

<table>
<thead>
<tr>
<th></th>
<th>Ghrelin (n=62)</th>
<th>Motilin (n=62)</th>
<th>Insulin (n=40)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight</td>
<td>0.51</td>
<td>0.43</td>
<td>0.08</td>
</tr>
<tr>
<td>Age</td>
<td>0.54</td>
<td>0.42</td>
<td>0.36</td>
</tr>
<tr>
<td>BMI</td>
<td>0.50</td>
<td>0.36</td>
<td>0.50</td>
</tr>
<tr>
<td>Head-length</td>
<td>0.51</td>
<td>0.47</td>
<td>0.47</td>
</tr>
<tr>
<td>Length</td>
<td>0.46</td>
<td>0.43</td>
<td>0.42</td>
</tr>
<tr>
<td>Gestation</td>
<td>0.47</td>
<td>0.36</td>
<td>0.47</td>
</tr>
<tr>
<td>Ghrelin</td>
<td>0.33</td>
<td>0.25</td>
<td>0.05</td>
</tr>
<tr>
<td>Motilin</td>
<td>0.27</td>
<td>0.20</td>
<td>0.05</td>
</tr>
</tbody>
</table>
| *Statistical significance at P < 0.05.
| **Statistical significance at P < 0.01.

Do postnatal changes in ghrelin levels precede changes in appetite or follow them?

Examination of circulating blood ghrelin concentrations may offer an insight into the fine-tuning of the coordination between dietary intake, energy balance, and growth factor modulation of somatic growth in the first years of life.

Breast-feeding and formula-feeding: effects on growth

- Dewey KG et al. Pediatrics 1992: Breast-fed (BF) and formula-fed (FF) infants have similar weight gain in the first 3 months of life, but BF infants gain less rapidly during the remainder of the first year.
- Butte et al. Pediatrics 2000: early feeding mode affects growth and body composition; they observed lower fat free mass and higher fat mass and percentage of fat mass in BF than in FF infants aged 3-9 months.

But we know that breast-feeding and formula feeding can modify the amount of growth. Dewey reported that breast-fed and formula-fed infants may have similar weight gain in the first three months of life, but the breast-fed infants gained less rapidly during the remainder of the first year. Butte showed that early feeding mode affects growth and body composition. She observed lower fat free mass and higher fat mass and percentage of fat mass in breast-fed than in formula-fed infants age 3 to 9 months.
So formula feeding is associated with a higher weight gain than breastfeeding during infancy, particularly after three months of age. A dose-response relationship between formula intake and both length and weight gain has been demonstrated. The 3 to 6 month interval is when these effects are greatest.

We also have to take into account the protective effect of breastfeeding against obesity. An increasing number of papers have been published about the protective role of breastfeeding against obesity. But there are some limitations in these studies.

Two recent meta-analyses, both independently performed, support a protective effect of breastfeeding as compared with formula feeding on long-term overweight risk in children.

Breast feeding up to 9 months is inversely associated with overweight risk, showing a dose-response relationship. This relationship strongly suggests causality of associations, as it also does in epidemiologic studies.

And the duration is very interesting. Breast-feeding for up to 9 months is inversely associated with overweight risk, showing a dose response relationship. This relationship strongly suggests causality in the associations, as it also does in epidemiologic studies.

In conclusion, we observed that breast-fed infants have different ghrelin, leptin, and IGF-1 levels in comparison with formula-fed ones. These hormones may be involved in the regulation of growth and development in neonatal age and infancy and may influence the programming of energy balance regulation in childhood and adulthood. The question of whether breast milk hormone synthesis and action are reflected by peripheral blood concentrations in the neonate and infant remains open to debate and further investigation.

**DISCUSSION:**

DR. NEVILLE: I have always thought that the decrease in the rate of weight gain after the first three months in breast-fed infants reflected a change in appetite, on the basis of no evidence, but it always seemed like a good idea. The question is, of any of these factors which may or may not be present in formula, if you had to guess, which one would you think might be responsible?
DR. SAVINO: Yes, in fact, it is a very complex topic. More recently, new cytokines, new hormones, new proteins, adiponectin, for example, have been identified in milk that suggest other compounds are responsible. In my opinion, the issue is not that these mediators are present in human milk but rather, the quantity of protein that is present in formula compared with human milk may be determinant of some of these differences.

DR HAN: That was an interesting summary of a lot of thoughts and findings. I just want to make a comment that I think most of us believe that whatever you see in the infant or the child blood in terms of IGF or ghrelin or leptin is probably endogenous and not exogenous, right?

DR. SAVINO: That is a very interesting question. Our data, particularly in the first month of life, are based on similar weight and body mass index among infants. So we have to expect that leptin was the same in the first month if the infant has the same weight and body mass index. However, further analyses among infants with different body composition also don't show any differences in leptin levels. So if there are no differences in leptin levels among different body composition parameters like weight, I hypothesize that the leptin levels observed in the first month come from the breast milk, as well as endogenous sources.