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The Importance of Exclusive Breastfeeding and Melatonin on the GIT of Wistar Rats' Offspring Submitted to Early Weaning

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ABSTRACT

Introduction: the small intestine is the main portion of the digestive tract responsible for the process of digestion and absorption of food. Early weaning directly affects the intestinal mucosa through atrophy of intestinal crypts, reduction of villi or increasing the activity of enzymes such as ornithine decarboxylase, reflecting on absorption, since its absence will cause morphophysiological changes as well as organ growth. Melatonin is a hormone produced by the pineal gland and found in breast milk. As it has an amphiphilic characteristic, it passes easily through the cell membrane and has antioxidant activity, being used as a therapy against numerous pathologies of the digestive tract such as esophagitis, peptic ulcer, ulcerative colitis, intestinal ischemia/reperfusion and liver cirrhosis. Recently it was seen that exogenous melatonin reversed some morphological damage from EW such as presence of picnotic nucleus, villus area and crypt area for example. The present review also highlights the importance of exclusive breastfeeding during the first six months of life of the newborn.

Introduction

Breastfeeding is a practice that provides benefits for both mother and child, and should be exclusive in the first six months of life according to the World Health Organization. This strategy aims to reduce the morbidity and mortality of neonates[1,2]. Breastfeeding is a critical and extremely important period for the morphophysiological maturation of the organism, which may be reflected in child growth and development[1].

Breast milk is considered a complete food supplying the needs of the developing organism, containing in its composition carbohydrates, proteins, lipids, growth factors and others. The abrupt interruption of exclusive breastfeeding is called early weaning and is a topic of worldwide concern. Early weaning can be reflected in multiple systems, including the digestive system, which is responsible for the breakdown and absorption of proteins, lipids, vitamins and minerals[1, 3].

The small intestine is the main portion of the digestive tract responsible for the process of digestion and absorption of food[4]. Early weaning directly affects the intestinal mucosa through atrophy of intestinal crypts, reduction of villi or increasing the activity of enzymes such as ornithine decarboxylase, reflecting on absorption, since its absence will cause morphophysiological changes as well as organ growth[5, 6, 7].

Melatonin is a hormone produced by the pineal gland and found in breast milk. This is only produced at night, during the light-dark cycle, since the presence of light inhibits its production[8,9,10]. As it has an amphiphilic characteristic, it passes easily through the cell membrane and has antioxidant activity, being used as a therapy against numerous pathologies of the digestive tract such as esophagitis, peptic ulcer, ulcerative colitis, intestinal ischemia/reperfusion and liver cirrhosis[11,12,13].

It's necessar to understand the action of melatonin in individuals who have eating disorders during growth. The importance of breastfeeding is already highlighted annually in campaigns in the country, and the results of this proposal can corroborate and expand the set of information for dissemination and awareness of the population. Thus, the objective of this review is to investigate the effect of exogenous melatonin in the duodenum of offspring submitted to early weaning.

Breastfeeding

Breastfeeding goes far beyond newborn nutrition. It's a profound interaction between the binomial (mother and child) with repercussions on physiology, nutritional status, defense against infections, cognitive and emotional development, and the long-term health of the child, in addition to having implications for the physical and psychological health of the mother[14]. Despite all the scientific evidence proving the importance and superiority of breastfeeding over other forms of feeding for the child, as well as national and international campaigns, the prevalence of breastfeeding (BF) in Brazil, especially exclusive breastfeeding, is far below the recommendations[14,15].

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BF should be exclusive for up to six months, after which it is supplemented with any solid or semi-solid food to complement it, not replace it[4,16]. The WHO emphasizes that there are no advantages to introducing solid foods before six months of age, which can cause damage to the child's health such as: diarrhea, respiratory problems, malnutrition, lower absorption of nutrients rich in milk, for example, iron and zinc[14].

Human milk has 68 calories per 100ml, consisting of 1.39g protein, 6.13g sugars, 4.20g fat, minerals and vitamins, amounts according to the Food Composition Table (TBCA/USP)[17]. Several bioactive substances such as growth factors, stem cells and leukocytes are present. Melatonin, insulin, ghrelin, leptin, adiponectin, cortisol, T3, and T4 are among the hormones found in breast milk[18,19,20,21]. Accordingly, the literature reports the presence of concentrations of iron, copper, zinc, manganese, calcium and magnesium, as well as proteins, carbohydrates and fats in the breast milk of rats[22]. In line with the literature, rat breast milk has carbohydrates, lipids and numerous hormones such as prolactin, melatonin, somatostatin, GnRH, IGF-1, among others in its composition[23,24].

Breast milk plays important roles in the growth, differentiation and development of various tissues of the baby, especially in the development of the intestinal mucosa[7,1,25]. In the lumen of the digestive tract, the interaction of the epithelium with nutrients occurs, thus providing proliferation, apoptosis, differentiation and migration of cells[26].

Breastfed babies have smaller villi and crypts than bottle-fed babies, suggesting that crypt fission is related to inhibitory factors present in milk[27]. It was found that rats that did not undergo early weaning remained with the size of the crypt-villus axis, cell proliferation, distribution of goblet cells and the expression of genes involved in intestinal functions and renewal without any change, so breastfeeding contributes to the integrity of the intestinal mucosa[7].

Intestine

The small intestine is the final portion of the digestive tract responsible for the process of digestion and absorption of food as well as the site of endocrine secretions. The nutrients (carbohydrates, lipids, proteins and nucleic acids) present in the food bolus are digested and absorbed by the lining epithelial cells, the enterocytes. The small intestine is a very long organ approximately five meters long and is divided into: duodenum, jejunum and ileum. The latter have structural features in common. It is a hollow tube in which at its center we find a lumen, or lumen, whose diameter is variable and surrounded by a wall formed by several layers: mucosa, submucosa, muscular and adventitia/serosa[28].

The mucous layer is composed of simple cylindrical epithelial tissue, a lamina propria of loose connective tissue and the muscularis mucosa. It is worth mentioning the presence of villi in the mucosa, elongated projections formed by the mucosa and submucosa. In the duodenum it has the shape of leaves taking the form of fingers near the ileum. The villi epithelium is formed by enterocytes (absorptive cells), goblet cells (mucus production) connecting with the crypt epithelium, which has some absorptive, goblet, enteroendocrine, Paneth and stem cells. The crypts are tubular in shape and have a high proliferative rate[7, 28].

Enterocytes have a cylindrical morphology and an oval nucleus in the basal portion. In the apical portion, we find the microvilli, specialization of the membrane, creating a brush border, easily observed in light microscopy. In electron microscopy, the brush border is seen as a set of very close microvilli[28]. The goblet cells are distributed among the enterocytes and their function is to produce mucus to protect and lubricate the intestinal mucosa, a product that is easily stained by Periodic acid–Schiff (PAS)[28]. Paneth cells are found in the most basal layer of the crypts and have a defense function through the release of their eosinophilic granules rich in lysozymes and defensins. And stem cells have the function of giving rise to other cells in the crypts and villi[28,29].

The submucosa is rich in connective tissue, which is rich in blood and lymphatic vessels, and a submucosal plexus (Meissner's plexus). This layer may contain duodenal glands and lymphoid tissue. The muscle layer has smooth muscle cells oriented in two sublayers: circular (inner) or longitudinal (outer). We can see between these two layers the myenteric nervous plexus (Auerbach's plexus). Just below this we can find the serous layer or the adventitia layer. The serosa is a thin layer of loose connective tissue lined by mesothelium (simple flat epithelium). The adventitia formed by connective tissue together with adipose tissue[28].

Early Weaning

The first six months of life are a critical window of vulnerability, where exposure to environmental factors can positively or negatively influence health throughout life. The exclusive intake of breast milk, combined with maternal care, during the first six months of life guarantees a better development of the newborn. However, when weaning occurs abruptly, early weaning (EW), a practice that has been increasing, can cause morphophysiological and behavioral damage to the body[30].

Early weaning is still prevalent in several countries, including Brazil, and is the result of several factors, such as maternal characteristics, working conditions, socioeconomic status, marital status and follow-up[31]. Although most mothers are aware of the importance of exclusive breastfeeding until six months of age and complementary breastfeeding until two years of age, this practice is still small in Brazil. The reasons for stopping breastfeeding early are lack of

time due to work; physical reasons, early introduction of other foods before six months; thinking that your milk is weak due to watery consistency in addition to the small amount; delay in milk let-down; anatomical issues such as the flat or inverted beak; the family's own interference in their decisions, among others[32, 31].

Early weaning vs TGI

By identifying the morphological changes of in gastric tissue, it was found that (EW) increases the mucosal area, thus increasing the area of the glands and mucosal muscle, consecutively increasing stomach acidity. A decrease in the longitudinal and submucosal muscle area was also observed, causing the organism to undergo adaptations for functioning in rats submitted to (EW). Thus, indicating possible damage that the abrupt interruption of breastfeeding can cause to health[33].

Differentiation of gastric epithelial cells ends at the time of weaning. However, when weaning occurs early, this differentiation is affected. In the study to investigate the role of corticosterone on the gastric mucosa of rats submitted to early weaning, Zulian *et al.*,[34], found that (EW) increases the expression of mucins, Mist1 and pepsinogen C in mRNA and protein levels, and changed the number of colonic mucosal cells (CMC) and zymogenic cells (ZC). Corticosterone, on the other hand, regulated the expression of pepsinogen C and the distributions of CMC and ZC. Thus, proving the importance of corticosterone in gastric cell maturation. Corroborating this study, when comparing the immediate and long-term effects of early weaning on gastric mucosa cell differentiation, Teles Silva *et al.*,[26], observed that (EW) transiently affects the expression of genes related to differentiation (Atp4b, Bhlha15 and Pgc) as well as increasing the population of (ZC).

Based on previous studies on how (EW) affects the distribution of gastric glands in rats, Bittar *et al.*,[35] investigated the distribution of ghrelin and its receptor in the gastric epithelium of rats during the postnatal period. Among the findings we have that (EW) increased the distribution of ghrelin-secreting cells. The ghrelin receptor is found in the neck area of the glands, but there is no change in its levels. Therefore, ghrelin and its receptor are involved in somatic growth of the stomach during early weaning.

Non-pharmacological early weaning also affects the liver of adult male rats. A study carried out by Bertasso *et al.*,[36], evaluated between two experimental models, non-pharmacological and pharmacological early weaning (depriving milk production with bromocriptine), lipogenesis, β-oxidation, very low-density lipoprotein (LDLP) and gluconeogenesis in both sexes in adult Wistar rats. Male rats submitted to non-pharmacological early weaning showed elevated plasma triglycerides, hepatic triglycerides and cholesterol by lipogenesis. The females of both groups did not have any changes, thus showing normal plasma levels and preserved liver cytoarchitecture.

Studies have linked early weaning to the development of obesity and type 2 diabetes in adulthood. In view of this, it was investigated whether (EW) could affect the pancreatic islets, since research on this topic is rare. Early weaning has been shown to lead to increased insulin secretion in adolescent males and reduced insulin secretion in adult offspring[2]

Early Weaning vs Intestine

When evaluating the late effects of early weaning in rats through histomorphometry, Barbosa *et al.*,[6] observed that in the small intestine, EW caused atrophy of the intestinal crypts. Corroborating this, Lemos, *et al.*,[1], found that the (EW) group showed significantly ($P<0.001$) a reduction in the depth of the Lieberkuhn crypts, compared to the control group.

Studying the size of the crypt-villus axis, cell proliferation, the distribution of goblet cells, and the expression of genes involved in intestinal functions and jejunal renewal, da Costa *et al.*,[7] observed that villus height decreased significantly. by (EW) at 18 days, showing an evident atrophy. However, at 60 days there was no difference between the control group and the early weaned group. However, there was no recovery of the number of goblet cells at 60 days in the rats that were submitted to (EW) on the 18th day. The cell proliferation index decreased in pups (EW) (18 days), and the effect was maintained in adults (60 days). This suggests that early weaning affects cell proliferation, gene expression and the number of goblet cells in rats subjected to weaning on the 18th day and that these effects last until adulthood. It can thus affect the physiology of the intestine, such as the absorption of nutrients[7].

By evaluating the effects of weaning age on morphological changes that occur in the intestine (jejunum and ileum) of rats, through intestinal histomorphometry and somatic growth, in 21-day-old pups and 90-day-old mature rats that were weaned early (day 16), it was observed that early weaning resulted in deeper crypts, a lower villus/crypt ratio, and a smaller villus area on day 21. At 90 days, early weaned animals had shallower crypts, a higher villus/crypt smaller villosa compared to normally weaned animals. Thus, stating that early weaning affects the intestinal mucosa, which can cause damage to food absorption and concomitantly in the growth of the organ[38]. Rojas Castañeda *et al.*,[39] observed, in a morphometric study in rats, that (EW) increased villi size, depth and number of crypts in the duodenum and jejunum, while the number of villi decreased. However, the ileum showed no changes with early weaning.

Lin *et al.*[5] investigated whether ornithine decarboxylase (ODC) activity is involved in intestinal (jejunal) growth after early weaning, as the enzyme activity is associated with rapid cell proliferation in

many cell types through from immunohistochemistry to cell proliferation. It was seen that EW increased ODC activity in the jejunal mucosa, peaking on the third day of weaning (18th day), compared to the breastfed group. The sequence of increased ODC activity suggests that it can be used as a marker of mucosal maturation in the face of early weaning. In neonatal rats, early introduction of fructose into the diet significantly increases brush-edge fructose transport rates and GLUT-5 mRNA levels during early weaning, thus confirming gut adaptation to diet[40].

Pups weaned on day 17 showed an immediate increase in intestinal length, a decrease in lactase, and an early increase in sucrose and maltase[41]. Sucrase activity did not depend on the weaning period in the study by Shinder *et al.*,[42] in suckling rats weaned prematurely on the 15th, 17th, 19th or 21st day of life. Weaning on the 15th or 17th day of life considerably delayed the decrease in lactase activity[42]. The delay in body weight gain and small bowel weight was greater in infants who were weaned early[42]. Changes in segmental mucosal weight, DNA and protein content, however, paralleled those of controls. Enterokinase and leucine aminopeptidase showed little change, regardless of dietary changes. At 19 days, early weaned pups had serum levels of corticosteroids about three times those of control or prolonged pups[41]. Thus, proving that the change in the diet causes a change in the levels of enzymes, for the latter to act against the dietary pattern that the animal is subjected to.

Melatonin

Melatonin (MEL) is a hormone produced in many cells and tissues, but it is synthesized in high amounts by the pineal gland, especially in mammals, during the night period of the light-dark cycle, since the presence of light inhibits its production[8,9,10]. The synthesis of melatonin by the pinealocytes in the pineal gland is initiated with tryptophan which, under the action of tryptophan hydroxylase, is transformed into 5-hydroxytryptophan which, being converted into serotonin, which is acetylated by arylalkylamine N-acetyltransferase (AANAT) into N-acetylserotonin (NAS) which is converted to melatonin by acetylserotonin O-methyltransferase (ASMT). The three enzymes above are under the control of the neural and endocrine systems that regulate the timing, duration and amount of melatonin produced[43,8].

The gastrointestinal tract (GIT) in mammals is the most abundant extrapineal source of MEL, with mucosal concentrations exceeding blood plasma levels by 100-400 times. This source is responsible for concentrations in peripheral blood during the day. In rats, intestinal MEL concentration varies with age, peaking at birth and decreasing with age. In the jejunum, ileum and colon, the decline in MEL is greater compared to the stomach. Later in life, the concentration of MEL in the mucosa of the ileum and in the distal colon is 126% higher in older rats compared to younger rats[44,45,46,47] 2017. MEL biosynthesis in the GIT is not dependent on the pineal gland, as was seen in pinealectomized rats[48]. The main production of honey in the GIT occurs during the day and is related to the frequency of food intake. Through HPLC-validated immunohistochemistry and radioimmunoassay studies, the presence of MEL in the GIT mucosa was confirmed, as well as the identification of enterochromaffin cells as the main source of MEL in the GIT[49]. Messner *et al.*[50] found high concentrations of MEL in gastric, duodenal and colonic mucosa.

MEL plays an important role in the GIT by acting in the regulation of gastrointestinal motility, free radical scavenging, local anti-inflammatory activity, thus being a potential therapeutic target in the treatment of intestinal diseases12,13. Wu *et al.*,[51] highlighted the adjuvant therapeutic potency of melatonin against colorectal cancer, as it activates apoptosis and immunity to colon cancer, concomitantly reducing proliferation, autophagy, metastasis and angiogenesis, thus exerting its anticancer effects. The results of Lin *et al.*,[52] suggest that MEL significantly attenuates psychological stress-induced injury to the intestinal mucosa. Melatonin treatment restored colonic melatonin concentration and modulated gut microbiota dysbiosis[53]. Melatonin supplementation increased body weight and preserved intestinal morphology, having little effect on cell proliferation and apoptosis, as well as on goblet cells and Paneth cells in the ileum of weaned mice[3].

Recent Findings

Recently it was seen by Costa, MAS *et al*, 2021, that exogenous melatonin reversed some morphological damage from EW such as presence of picnotic nucleus, villus area and crypt area for example. The study also highlights the importance of exclusive breastfeeding during the first six months of life of the newborn.

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