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Digestive System and Behavior Disorders Related to Weaning in Mammals

Mariem Chaâbane*, Imen Ghorbel, Awatef Elwej, Najiba Zeghal, Nejla Soudani

Department of Life Sciences, Sciences Faculty of Sfax, Sfax, Tunisia

Abstract

Weaning is a natural and inevitable phase of mammalian development. In both animals and humans, this process represents a transition from a milk based diet (low carbohydrate content) to a solid diet (relatively rich in carbohydrates). Both, the dietary change and the decline of the mother's attention during this critical period cause a state of stress in the offspring. The present review was designed to analyze the impacts of the weaning procedure on the digestive system and the behavior of mammals such as humans, rodents and pigs. Based on literature data, it appears that weaning is a complex process that requires physiological, microbiological and immunological adjustments in the digestive tract. The physiological changes are manifested by alterations of the intestinal anatomy, the activity of the digestive enzymes and the function of intestinal absorption. The composition of the intestinal flora undergoes changes depending of food and the environment. Weaning results also in the activation of the intestinal immune system following the introduction of new food and microbiological antigens. It involves also behavioral changes which depend largely on the age at which this process is performed. Thus, these data highlight the influence of diet on one of the early stages of mammalian development that may have an effect on the health during adulthood.

Keywords

Weaning, Mammals, Diet, Digestive Tract, Behavior

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1. Introduction

Weaning is one of the most important events occurring during the early stages of mammalian life. In animals as well as in humans, this process represents a transition from a milk based diet (breast milk) to a solid diet. Indeed, after birth, breastfeeding ensures the nutrition of the newborn. Approximately 69.8% of the newborn's calories come from fat while only 6.8% of its calories come from carbohydrates [1]. At the end of the lactation period, the newborn starts the intake of solid food but continues to consume breast milk until complete weaning, where it becomes nutritionally independent. Thus, weaning cannot be considered as an abrupt process; but rather a gradual process occurring during several days with physiological consequences for offspring [2]. In fact, as a result of the shift from milk to solid diet, having a different composition from that of breast milk, most of the physiological functions are modified in correlation with an important maturation of the organs. In this respect, several studies have shown that the gastrointestinal system is particularly influenced by dietary changes during weaning [3, 4]. Indeed, at birth, the gastrointestinal tract of the newborn is functionally immature. When weaning starts, the digestive capacity undergoes extensive changes to become operational. Thus, the weaning process involves a reorganization of the gastrointestinal system, affecting its physiological, microbiological and immunological aspects [5].

In addition to that, evidence from behavioral studies has

* Corresponding author
E-mail address: maryame.ch@yahoo.com (M. Chaâbane)

reported that the weaning process may also lead to impaired behavioral responses in the offspring [6, 7]. Indeed, during the breastfeeding period, a significant neuro-behavioral development occurs and the mother-offspring interaction is strong. After weaning, the offspring becomes independent of the mother from a behavioral point of view and encounters difficulties in its adaptation to the new conditions of life due to the reduced maternal care. Moreover, it does not yet have the cognitive skills and physical abilities required to receive and accept the solid food. This can have a profound impact on the development, structure and function of its nervous system affecting its behavior [3].

Based on the literature data, the present review was designed to describe the physiological, microbiological and immunological changes in the digestive tract as well as behavior disorders associated with weaning in mammals such as humans, rodents and pigs.

2. Literature Review

2.1. Effect of Weaning on the Digestive Tract

2.1.1. Weaning and Morphological Changes in the Gastrointestinal Tract

Studies in laboratory rats and human babies have shown that weaning is associated with an accelerated growth of the epithelial cells lining the small intestine along with villous and crypt hyperplasia [8, 9]. Such structural modifications reflect probably a preparation of the intestinal mucosa to the nutrient load introduced during weaning.

In the case of pigs, the morphological changes of the intestine, occurring after the weaning process, are manifested by a reduction in villous height [10, 11] and an increase in crypt depths [11, 12]. Villous atrophy observed in weaned piglets may be due to a significant loss of enterocytes or to a low renewal rate of these cells [12, 13]. It is widely accepted that the increased crypt depth is an indication of the enhanced cell production. Indeed, Hedemann et al. [14] showed a good correlation between crypt depth and crypt mitotic counts after 9 days of weaning in piglets. So, while the crypt epithelium responds to counteract these dramatic changes, recovery of the villous size to a pre-weaning status may be slow. Moreover, when the animal is early weaned, villous atrophy and recovery time are of great importance.

The morphological changes during weaning observed at the intestinal level are associated with functional modifications.

2.1.2. Weaning and Functional Changes

The digestive tract is a vital organ which plays a major role in the digestion and absorption of nutrients. Following weaning, these two functions undergo modifications to respond to the metabolic changes imposed by the new diet. In this section, we will study the impact of weaning on digestive enzyme activities and intestinal absorption.

Weaning and digestive enzyme activities

Changes in the enzymatic spectrum of the small intestine during the weaning process are related to the need of assimilating the new nutritional compounds. Indeed, during the weaning period, the diet composition is modified due to the switch from a milk based diet with low content in carbohydrates to a solid diet relatively rich in carbohydrates [15]. Thus lactose, the main source of carbohydrates, is replaced by a more varied mixture of sugars such as sucrose and starch.

Newborns as well as adults are unable to absorb dietary disaccharides. Brush border disaccharidases are therefore required to hydrolyze these molecules into absorbable monosaccharides. Considering these facts, it is conceivable from the physiological point of view that the intestine of the newborn presents a high lactase activity against low sucrase and maltase activities. During the weaning period, these activities are reversed. In fact, activity of lactase decreases strongly, while those of sucrase and maltase increase.

Lactase, the enzyme responsible for lactose hydrolysis, is expressed only at enterocytes lining the small intestine, including the duodenum, jejunum and ileum. Most mammals have a temporary and programmed ability to digest lactose. This substrate represents the main carbohydrate of milk and the first food of all mammals. It forms, together with the fat fraction of milk, the main source of energy for the growing neonate. The activity of intestinal lactase is maximal directly after birth and decreases rapidly upon weaning [16] Indeed, milk is normally the only source of dietary lactose [17]. Only in humans, intestinal lactase activity is maintained functional after weaning, which is consistent with the continuous consumption of lactose from dairy products [18].

In rats, studies have shown that transition from milk to solid diet occurs gradually between days 18 and 30 of postnatal life, suggesting that the functional activity of the lactase decreases gradually in the course of natural weaning [19].

Similarly, a decreased lactase activity followed by an increased maltase and sucrase activities have been also reported during the weaning period in pigs [20], reflecting a maturation of the intestinal function.

It is of note that, during the weaning, the enzymatic spectrum of the small intestine is affected by exogenous (type of nutrition) and endogenous (hormonal) factors. The nutritional factor in relation with the dietary change influences the induction and repression of genes responsible for the

reconstruction of intestinal enzymes. On the other hand, endogenous factors of hormonal nature, namely glucocorticoids, are attractive candidates for the regulation of intestinal development during early separation of the neonates from their mothers, as reported by Henning [21]. In rats, stress resulting from early weaning causes a sufficient rise in plasma levels of corticosterone, the main glucocorticoid secreted by the adrenal gland [22, 23], inducing a precocious maturation of the intestinal enzymes. In this regard, Boyle and Koldovsky [24] have reported an increased jejunal sucrase activity following early weaning and elevated plasma glucocorticoid levels. A decrease in lactase activity and an increase in maltase activity have been also observed in early-weaned rats [25].

Changes in digestive enzymes during weaning may have the important implications on the intestinal absorption.

Effect of weaning on intestinal absorption

Weaning, considered as a critical period in the development of mammals, represents a progressive preparation of the digestive tract to receive solid food. According to Pacha et al. [26], the stimuli regulating the adaptation of the small intestine to the absorption of solid food during the weaning period are complicated. For example, changes in phospholipids of the apical membrane may affect nutrient absorption [27, 28]. It has also been shown that fructose absorption increases dramatically in the small intestine during the weaning period in rats and to a lesser extent in pigs [29, 30]. A similar trend was also observed in the expression of the GLUT5 gene. The latter is a specific transporter which facilitates the absorption of fructose. In the small intestine, GLUT5 is found in the absorptive epithelial cells, where it is localized along the apical brush border. In humans and rats' intestine, the expression of this gene is very low during breastfeeding, whereas it increases strongly during weaning [31].

The effect of weaning on intestinal absorption of glucose has been well described in pigs. A study carried out by Boudry et al. [32] on piglets showed that the Na^+-dependent glucose absorption increased in the proximal part of the jejunum 2 days after weaning and decreased 15 days after, in comparison with its absorption during pre-weaning.

In another study, Boudry et al. [33] changed the diet of piglets from a milk to a barley or wheat based diet, 4 to 6 weeks after weaning. After 4 days of this dietary change, these authors observed that the Na^+-dependent glucose absorption in the proximal jejunum is greater. This result indicates that after weaning, shifting from a milk-based diet to a cereal-based diet leads to an increased active glucose absorption in the small intestine.

Based on these literature data, it appears that the active absorption after weaning is influenced by the composition of the new diet and the age at which animals are weaned.

Unlike active absorption, the passive absorption is not affected by these two factors [12, 34] and appears to decrease after weaning. For example, several studies on pigs have reported that the absorption of D-xylose decreases by approximately 50% of the pre-weaning level at 7 days after weaning. Wijjten et al. [35] suppose that the reduction in passive transcellular absorption following weaning represents a defense mechanism that prevents uncontrolled entry of potentially harmful agents to the body.

Weaning induces not only functional changes in the gastrointestinal tract but modifies also the microbiological composition.

Weaning and microbiology of the intestinal tract

The developmental process of the intestinal microbiota is similar in humans and most animals [36]. The first sources of colonization are formed from maternal and environmental microbes, which are transferred to the intestine of the newborn through several processes, such as suckling. During the weaning period, the solid food introduced to the neonate's diet is one of the most important external factors affecting its intestinal microbiota, whose composition changes rapidly [37]. The latter becomes more developed and the stability of the ecosystem is reached at around the age of 2 years. Bifidobacteria dominate the total intestinal microflora population in healthy breast-fed infants [38]. This prevalence of bifidobacteria provides protection to babies by reducing intestinal pH, which causes concomitant decrease in other potentially dangerous bacterial species. However, the frequency of bifidobacteria decreases following weaning [38]. In addition, bifidobacteria present in the infant's intestine are presumed to metabolize oligosaccharides in breast milk. Among these bifidobacteria, the following species can be mentioned: *Bifidobacterium bifidum* and *Bifidobacterium longum subsp. Infantis* [39, 40]. After weaning, the composition of the bifidobacteria population evolves into species which can adapt to the metabolism of plant sugars. Species such as *Bifidobacterium longum subsp. Longum* and *Bifidobacterium adolescentis* can metabolize such carbohydrates [41]. In addition, a study by Amarri et al. [42] on the composition of the microflora during weaning in humans showed that lactobacilli species and those insensitive to vancomycin initially increase significantly from the age of 120 days to 210 days, and then decrease.

In rats, it was shown that only Gram-positive bacteria are present in the jejunum during the lactation period up to the 18^{th} postnatal day. Then, Gram-negative bacteria and yeasts begin to appear with weaning [43].

In the piglet, the stress condition accompanying the weaning process may result from an imbalance of the gastrointestinal microbiota, allowing opportunistic pathogenic microorganisms to proliferate leading to gastrointestinal disorders. Diarrhea, caused mainly by enterotoxigenic *Escherichia coli* infections, is the most common symptom observed in piglets after weaning [44]. Several factors have been suggested to explain the observed post-weaning disorders of the gastrointestinal tract, such as the accumulation of lactate or succinate in the intestine [45] and the insufficient development of the intestinal microbiota and the mucosal immune system [46]. In addition, Konstantinov et al. [47] have quantified the specific microbial groups present in the gastrointestinal tract of weaned piglets using the real-time PCR technique. These researchers showed that *Lactobacillus sobrius*, *Lactobacillus reuteri* and *Lactobacillus acidophilus* populations, colonizing the piglet intestine during the early postnatal period, declined significantly after weaning. In fact, lactobacilli are well known for their protective effect against colonization and infection caused by pathogens, through the production of antimicrobial compounds such as lactic acid and bacteriocins [48]. Similarly, Su et al. [49] showed a decreased lactobacilli proportion in the jejunum and ileum of weaned piglets. On the other hand, they reported the dominance of potentially harmful *Streptococcus suis* strains. In addition, Su and Zhu [50] found that the pH of the stomach in the piglet increases from 3.0 to 5.0 after weaning. These results indicate that the ability of the stomach to act as a barrier against pathogens may be impaired following weaning.

The new microbiological antigens introduced during weaning lead to an activation of the intestinal immune system.

Weaning and immune function of the digestive tract

At birth, the intestinal immune system is immature and its development is slow. During suckling, breast milk confers to the neonate a protection against infectious agents by the transfer of antibodies such as immunoglobulin G and immunoglobulin A and immune cells such as macrophages [51, 52]. After weaning, this so-called "passive" immunity decreases at a time when the active immunity specific to the neonate is not fully developed. In addition, the weaning period in humans is associated with a progressive exposure to an increasing range of food antigens, primarily from rice flour proteins, vegetable proteins, fruits and later chicken ovalbumin and other egg proteins, gluten, cow's milk and meat proteins. The novel antigens introduced during weaning include also the bacterial populations colonizing the gastro-intestinal tract as a result of dietary change. This increases the risks of allergic reactions and the susceptibility to diseases. Therefore, food and bacterial antigens can initiate an active immune response in the neonate.

In this regard, studies in rats have shown that weaning is associated with a strong activation of the intestinal immune system including mucosal mast cells [53] and T cells [54].

Moreover, it has been reported that during weaning, several spatio-temporal changes in cytokine expression occur in piglet intestine. In fact, Pie et al. [55] noted a transient increase in several inflammatory cytokines along the digestive tract in weaned piglets. Kinetically, mRNA levels of IL-1β, IL-6 and TNF-α increase during the first 2 days after weaning in the small intestine and colon. During the next 5 days, IL-12p40 gene expression decreased in the gastrointestinal tract except the jejunum. Finally, expression levels of TNF-α and IL-8 genes also decrease in the proximal parts of the small intestine while they increase in the colon. It is well known that pro-inflammatory cytokines play an important role in intestinal inflammatory processes. Thus, weaning in piglets seems to be accompanied by an early inflammatory process [56].

It is known that the immune system is informed about the cognitive, emotional and physical stimuli integrated by the brain through the autonomic and central nervous systems. In return, the brain receives messages from the immune system via hormonal neuropeptides and cytokines. Therefore, immune system activation following weaning can have neurobehavioral consequences.

2.2. Weaning and Behavior

In rats, spontaneous weaning begins around the third week of postnatal life and continues until the age of 30 days, when pups stop completely the intake of maternal milk [57]. Mother-pup interactions during the end of the lactation period are important for the development of rodent behavior [58]. The decline in mothers' attention during weaning was associated with alterations in behavioral responses [59]. These behavioral disorders depend greatly on the age of weaning. Previous studies have shown that weaning rat pups on postnatal day 21, the standard weaning age, compared to those remaining with their mothers until postnatal day 25, activates the development of delta-opioid receptors population, predominantly in the somatosensory cortical areas of the brain [60]. The development of these receptors, induced by weaning, is dependent on the loss of dietary casein, one of the major proteins in milk which can produce peptides with opioid activity [61].

In addition, recent studies have reported the involvement of oxytocin, which reacts with the opioid receptor, in behavioral changes induced by weaning. Indeed, oxytocin and its receptor play an important role in the regulation of anxiety, stress and social behavior and have also antidepressant effects [62]. It has been demonstrated that weaning can

influence the levels of oxytocin receptors in the ventromedial hypothalamus, which allows the regulation of emotional behaviors during early postnatal development [63].

Previous works on mice have also shown that early weaning can induce neurobehavioral changes. Compared to Balb/cA and ICR mice weaned on day 21 postnatal, mice of the same strains weaned on postnatal day 14 showed an increased aggression [64], anxiety [64, 65] and general activity [58]. According to Ladd et al. [66], these behavioral changes may be due to a hyperactivity of the hypothalamic-pituitary-adrenal axis and an increase in circulating glucocorticoid levels in response to stress. In addition, Dallman et al. [67] showed that high glucocorticoid plasma levels lead to an increased sucrose intake, considered as a compensation form to reduce stress signs.

As in rats and mice, sudden maternal separation induces behavioral alterations in pigs. Besides, the earlier is the weaning age, the higher is the intensity of these alterations [68]. For example, a reduction in cognitive performance was observed by Poletto et al. [69] in early weaned piglets. According to these authors, this can be attributed to an aberrant expression of the enzyme 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) and type 2 (11β-HSD2), which modulates the activity of glucocorticoids, and the receptors for glucocorticoids and mineralocorticoids in the frontal cortex and the hippocampus. Other researchers have reported that early weaned piglets show an increased aggression behavior compared to lately weaned piglets [68, 70].

3. Conclusion

In general, weaning studies on humans, rodents or pigs show that this critical period of development affects various physiological, microbiological and immunological aspects of the digestive tract. Physiologically, deep anatomical alterations of the intestine and changes in digestive enzyme activities and intestinal absorption have been reported. From a microbiological point of view, major changes occur in the intestinal flora composition due to the dietary and environmental changes. Regarding immunity, the novel food or microbiological antigens introduced during weaning lead to the activation of the intestinal immune system. Weaning imposes also behavioral alterations, depending largely on the age at which this process is performed.

These weaning-induced disturbances in the digestive tract and behavior of the offspring would be more important when associated with diseases affecting the mothers such as diabetes and hypothyroidism. This hypothesis could be investigated in future weaning studies.

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