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REVIEW

Association between Bioactive Molecules in Breast Milk and Type 1 Diabetes Mellitus

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العلاقة بين الجزيئات النشطة بيولوجيًا في حليب الثدي وداء السكري من النوع الأول

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ABSTRACT: The association between breastfeeding and type 1 diabetes mellitus (T1DM) is controversial. However, several recent studies have established a link between these two factors, necessitating a need to review this subject to raise public awareness. Current research indicates that breast milk contains a variety of bioactive substances including immunoglobulins, oligosaccharides, insulin, lactoferrin, lysozyme, cytokines, epidermal growth factors, leukocytes, nucleotides, beneficial bacteria and vitamins. Such substances strengthen the breastfeeding infant's immune system, both directly, by increasing gut microbiota diversity and attacking harmful bacteria and pro-inflammatory molecules, and indirectly, by increasing thymus performance. Accordingly, a lack of or inadequate breastfeeding may predispose infants to several autoimmune disorders, including T1DM. Nursing mothers and caregivers are therefore advised to follow optimal breastfeeding practices prior to introducing complementary foods.

Keywords: Breastfeeding; Type 1 Diabetes Mellitus; Autoimmune Diseases; Immunoglobulins; Oligosaccharides; Review Literature.

الملخص: العلاقة بين الرضاعة الطبيعية وداء السكري من النوع الأول مثيرة للجدل. ومع ذلك فقد أثبتت العديد من الدراسات الحديثة وجود صلة بين هذين العاملين مما يستدعي الحاجة إلى مراجعة هذا الموضوع لرفع مستوى الوعي العام. تشير الأبحاث الحالية إلى أن حليب الثدي يحتوي على مجموعة متنوعة من المواد النشطة بيولوجيًا بما في ذلك الغلوبولينات المناعية، السكريات قليلة التعدد، الأنسولين، اللاكتوفيرين، الليزوزيم، السيتوكينات، عوامل نمو البشرة، كريات الدم البيضاء، النيوكليوتيدات، البكتيريا النافعة والفيتامينات. مثل هذه المواد تقوي الجهاز المناعي لدى الرضع المعتمدين على الرضاعة الطبيعية سواء بشكل مباشر وذلك من خلال زيادة تنوع الأحياء المجهرية المعوية ومهاجمة البكتيريا الضارة والجزيئات المسببة للالتهاب او بشكل غير مباشر عن طريق زيادة أداء الغدة الزعترية. وفقًا لذلك قد يؤدي نقص الرضاعة الطبيعية أوعدم كفايتها إلى تعريض الرضع إلى العديد من اضطرابات المناعة الذاتية، بما في ذلك داء السكري من النوع الأول. لذلك تُنصح الأمهات المرضعات ومقدمي الرعاية باتباع الممارسات المُثلى للرضاعة الطبيعية قبل إدخال الأطعمة التكميلية.

الكلمات المفتاحية: رضاعة طبيعية؛ داء السكري من النوع الأول؛ أمراض المناعة الذاتية؛ غلوبولينات مناعية؛ سكريات قليلة التعدد؛ مراجعة الأدبيات.

Type 1 diabetes mellitus (T1DM) is a long-term degenerative disease that begins when the body's defense mechanism starts to destroy its own pancreatic β-cells.[1,2] While the exact aetiology of the disease is unknown, several triggers are thought to play a role in disease causation, including genetic, epigenetic and environmental factors. Previously, T1DM was thought to affect only children and thus termed juvenile or childhood diabetes mellitus; however, as a result of technological advancements in diagnostic tools, it is now understood that it occurs at any age.[1,2] While T1DM affects both genders equally, there are racial disparities in terms of prevalence, with the disease being more common among Caucasian Americans compared to African Americans.[3,4]

Generally, T1DM is considered the most taxing form of diabetes mellitus (DM) and is characterised by severe insulin deficiency and hyperglycaemia.[5] Early symptoms include increased thirst, appetite and weakness, frequent urination and weight loss.[6] Uncontrolled hyperglycaemia may subsequently result in multiorgan damage involving the eyes, kidneys, nerves and heart, among other organs.[7] Unfortunately, there is no cure for the disease yet; as such, affected patients need to self-medicate and take daily doses of insulin for survival.[8] Although type 2 DM (T2DM) accounts for the majority of the overall economic burden of DM due to its higher prevalence, the economic burden is greater on individual patients with T1DM.[9]

Overall, the prevalence of T1DM is rising steadily, with an annual global increase of over 3%, which is projected to double in the next 20 years.[10] For many European and North American countries, the rising incidence of T1DM began around the mid-20th century, coinciding with increased industrialisation.[11,12] Among other lifestyle modifications, breastfeeding patterns

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changed due to the introduction of infant feeding alternatives such as formula. The loss of immunological function due to inadequate or inappropriate breastfeeding habits may contribute to the rising incidence of T1DM. This review therefore aimed to examine the association between T1DM and the immunological function of bioactive molecules in breast milk.

Association of Bioactive Molecules with Breastfeeding

The role of breast milk in the aetiology and prevention of T1DM is controversial, with certain studies showing a protective effect and others reporting predisposing or no effects at all.[13–15] In a meta-analysis of 25 studies with 226,508 participants from 12 countries, Yan *et al*. found that breastfeeding resulted in a reduced risk of DM triggers.[13] Importantly, 17 of these studies indicated a dose-response relationship between breastfeeding duration and a lowered risk of childhood obesity. In another meta-analysis involving 155,392 Norwegian and Danish children, Lund-Blix *et al*. reported that T1DM was related to the early introduction of infant formula, with non-breastfed children having a two-fold increased risk of disease compared to those who were breastfed.[14] Nevertheless, in a meta-analysis involving 43 observational studies of 9,874 individuals with T1DM, Cardwell *et al*. observed a weak link between exclusive breastfeeding and T1DM.[15] Many factors, particularly the experimental designs of these studies and variations in breastfeeding patterns in different countries, could be responsible for these inconsistent results. However, studies that report weak or negative effects usually monitor breastfeeding to an imprecise degree, without considering whether breastfeeding is exclusive or complementary.

Exclusive breastfeeding without vitamin supplementation may cause vitamin D deficiency in infants, particularly if the mother herself is deficient.[16] It may also result in vitamin E deficiency as vitamin E content in breast milk decreases as *colostrum* matures.[17] Vitamin E scavenges free radicals, blocking infiltrating toxins and cytokines and protecting cells, including the pancreatic islet cells. Deficiencies of both vitamins may play a role in the pathogenesis of T1DM.[18,19] Giulietti *et al*. reported the overexpression of pro-inflammatory cytokines, reduced thymus performance and pancreatic islet dysfunction in non-obese diabetic mice kept away from ultraviolet light and fed with a vitamin D-depleted diet.[20] In a Finnish birth cohort study, Hyppönen *et al*. found that daily supplementation of 200 IU of vitamin D was associated with a lower incidence of T1DM among children.[21] In a prospective clinical study of both T1DM and T2DM patients, vitamin E supplementation decreased blood glucose levels and reduced the progression of the disease.[22]

According to Virtanen *et al*., complementary feeding may yield no effect if this feeding consists of dietary formulas containing certain complex proteins.[23] Similarly, Chia *et al*. showed that dietary A1 β-casein may affect glucose homeostasis and induce progression to T1DM in non-obese diabetic mice.[24] Gluten in cereals is also linked with increased T-cell reactivity, with diabetogenic effects in rodents.[25] Additionally, breastfeeding from containers, which are often coated with preservatives and anti-rust agents such as bisphenol A (BPA), may affect breastfeeding outcomes.[26] In a study of new and used baby bottles in Iran, BPA levels ranged from 0.49–8.58 µg/L and 0.63–2.47 µg/L, respectively.[26] In pregnant rats, BPA doses as low as 0.5 µg/L were found to induce persistent islet insulin hypersecretion for up to one year.[27]

The duration of breastfeeding and the age at which complementary foods are administered may also determine the protective role or otherwise of breast milk on TIDM pathogenesis. Short-term breastfeeding (<3 months) and the early or late introduction of complementary foods (<4 months and ≥6 months, respectively) are risk factors for T1DM.[28] Additionally, the introduction of cereals before three months of age may be related to early β-cell autoimmunity.[29] Furthermore, certain maternal factors and prenatal lifestyle choices such as tobacco smoking, age, mode of birth and psychological stress levels may predispose an infant to T1DM.[30–32] These factors may also be linked to breastfeeding outcomes.

Mechanistic Links

Breast milk has been found to contain various anti-pathogenic and anti-inflammatory bioactive molecules, some of which can confer infants with lifelong immunity against many diseases, including T1DM.[33] Thus, breast milk can be described as a medium through which the maternal defense mechanism trains the immune system of the infant.[34] Established mechanisms through which breast milk prevents T1DM and other autoimmune diseases are outlined in Table 1.[33,35–55]

REDUCED GUT PERMEABILITY AND PRIMING OF THE IMMUNE SYSTEM

The diversity of the gut microbiota—the composition of which is influenced by various environmental factors such as diet and lifestyle—is important in the aetiology and prevention of T1DM. Homeostatically imbalanced microbiota, as characterised by a high preponderance of certain bacteria, increases intestinal permeability, eliciting autoantibodies and causing β-cell auto-

Table 1: Bioactive molecules in breast milk and their role in type 1 diabetes mellitus risk reduction[33,35–55]

Category	Molecule	Potential roles
Adipokines[33,40,43]	• Leptin • Adiponectin	• Antimicrobial function • Immune modulation • Increased β-cell function
Immunoglobulins[33,43,45]	• IgA • IgG	• Antimicrobial function • Anti-inflammatory function • Immune modulation
Hormones[39–41,46,47]	• Insulin • Lactoferrin • Lysozyme • Caseins • Corticosteroids	• Anti-inflammatory function • Strengthened immune tolerance • Increased thymus performance
Maternal immune cells[51,53,55]	• Leukocytes • Stem cells • CD4+ • miRNAs	• Antimicrobial function • Strengthened immunity • Increased thymus performance
Growth factors[50–52]	• EGFs • IGF	• Immune modulation • Increased β-cell mass •Pancreatic morphogenesis
Cytokines[44,49]	• IL-1 • IL-6 • IL-7	• Anti-inflammatory function • Immune modulation • Increased thymus size
Beneficial bacteria[35–38]	• *Lactobacillus* sp. • *Bifidobacterium* spp. • *Firmicutes* spp.	• Anti-infectious function • Increased gut diversity • Strengthened immunity
Nutrients[37,42,48,54]	• Oligosaccharides • Triglycerides • Vitamins • Minerals	• Increased gut diversity • Anti-microbial function • Immune modulation

Ig = immunoglobulin; CD = cluster of differentiation; miRNAs = microribonucleic acids; EGFs = epidermal growth factors; IGF = insulin-like growth factor; IL = interleukin.

immunity, the hallmark of T1DM.[35] Individuals with T1DM have less stable and diverse microbiota compared to non-diabetics.[36,56] The most frequently reported microbiome imbalance in diabetic individuals is a decreased *Firmicutes* population with a corresponding increase in the *Bacteroides* genus, the opposite of which is usual in non-diabetics.[36]

In experimental mice, microbiota imbalance was found to decrease tolerance to food antigens and the proportions of regulatory T-cells (Tregs) in the intestinal *lamina propria*, causing intestinal inflammation.[37] This results in high intestinal permeability, inducing insulitis or allowing more exogenous antigens into the mucosal immune system. Studies of obese and non-obese diabetic mice have observed that this leads to increased cytokine production that may attack and damage pancreatic β-cells.[57–59] Bacterial metabolites may also attack the pancreatic islet directly. This was shown during a study of mice in which *Streptomyces*-derived toxins impaired glucose tolerance, reducing islet size and β-cell mass at low doses via adenosine triphosphatase inhibition.[60]

The innate immune system—the body's first defence mechanism after birth—is weak and lacks important components. In healthy infants, a developing gut microbiome undergoes several stages of maturation in which the ingestion of breast milk is considered the most important factor.[61] This is possible because breast milk contains many beneficial bacteria and bioactive molecules. Diverse groups of beneficial bacteria in breast milk reduce gut permeability, promote gut microbiota diversity and maturation as well as boost immunological and metabolic function.[38]

Breast milk contains high levels of *Lactobacillus* and *Bifidobacterium* species, which promote the growth of *Firmicutes* bacteria.[61,62] *Firmicutes* deficiency has been reported in individuals with T1DM.[36,56] *Bacteroides* species are also present in breast milk and increase gut diversity and maturation.[61] Gut bacteria achieve these functions *in vitro* by either naturally killing pathogenic bacteria during the competition for food and survival or by producing antimicrobial effects.[63,64]

Insulin is another bioactive molecule found in breast milk; this hormone enhances gut maturation and reduces gut permeability to macromolecules.[39] In addition, insulin in breast milk may induce tolerance to blood insulin and prevent T1DM pathogenesis.[39] Breast milk insulin enhances the diversity of the gut microbiota by boosting the growth of some members of the *Gammaproteobacteria* family and reducing the *Streptococcaceae* population.[40] *Gammaproteobacteria* are involved in the maturation of infant gut microbiota, primarily in the first week after birth.[41] Insulin and leptin, another hormone in breast milk, also influence gut microbiota diversity by suppressing certain microbial metabolic pathways associated with intestinal inflammation while promoting beneficial ones.[40]

Oligosaccharides are non-digestible sugars in breast milk that promote the growth of protective bacteria in the colon.[33] The non-digestible properties of oligosaccharides allow these molecules to escape the acidic medium of the small intestine into the colon

where they produce short-chain fatty acids.[42] These fatty acids enhance the growth of probiotic species, including *Lactobacillus* and *Bifidobacterium*, resulting in a balanced microbiome.[43] In an *in vitro* study, oligosaccharides were found to inhibit and block harmful intestinal microorganisms from binding to their normal targets in the epithelial cells, thus reducing their population.[44] Oligosaccharides were also reported to confer a protective effect in a murine model of T1DM.[65] The administration of oligosaccharides was shown to influence microbiota diversity and produce short-chain fatty acids in non-obese diabetic mice, reversing DM progression.[66,67]

Breast milk also contains large quantities of secretory immunoglobulin (Ig) A, which accounts for the majority of the Igs in human breast milk.[33,43] Besides IgA, there are four other types of Igs in breast milk: IgE, IgG, IgM and IgD.[45] Breast milk secretory IgA helps train the immune system of newborns against enteric pathogens acquired through maternal exposure.[33,43] Breast milk also contains certain bioactive substances capable of stimulating IgA secretion in infants.[43] Secretory IgA can neutralise infectious agents and reduce the inflammatory effects of other antibodies.[68] Some children with DM are both IgA- and IgG-deficient.[69]

Lactoferrin, an iron-binding glycoprotein, possesses several anti-infective properties that form part of the innate defense mechanism conferred by mature human breast milk.[46] Lactoferrin has a high binding affinity for iron, thus limiting its availability to bacteria and other microorganisms.[43] In the intestine, lactoferrin may bind to certain receptors, such as toll-like receptors (TLRs) and the *cluster of differentiation* (*CD*)*14* gene, thereby blocking the attachment of pathogens to the intestinal epithelium.[47] In the stomach, lactoferrin combines with pepsin to form lactoferrincin, an antimicrobial agent powerful enough to damage the cell membrane of Gram-negative bacteria.[70,71] Lactoferrin also strengthens neonate immunity by inhibiting tumour necrosis factor-α and interleukin (IL)-1β, as well as initiating the maturation of lymphocytes and assisting antioxidation processes.[72] Adequate levels of lactoferrin have been reported to improve diabetic conditions, while their decline in obese individuals has been linked to insulin resistance.[73,74]

Other molecules present in breast milk include lysozyme, caseins, cytokines, epidermal growth factors (EGFs), leukocytes and nucleotides. Lysozyme is an antibacterial enzyme that works together with lactoferrin in the stomach to destroy Gram-negative bacteria.[75] Lysozyme can also degrade bacteria independently by breaking β-glycoside chains in the bacterial cell wall.[48] Caseins are a family of highly glycosylated proteins; they account for up to 40% of the proteins present in breast milk (mainly β-casein) and functionally boost newborn immunity.[43] A minor subunit known as κ-casein mimics a receptor analogue, preventing the attachment of bacteria to the mucosal epithelium.[76] Cytokines are signalling molecules that mediate, regulate and modulate immune responses, with one example being the anti-inflammatory cytokine IL-10.[44,49] While many growth factors are present in breast milk, EGFs are of particular importance, as these factors aid in the healing and maturation of the intestinal mucosa, nervous system and endocrine system, among others.[50]

Breast milk, particularly *colostrum*, contains high concentrations of leukocytes, of which approximately 10% are lymphocytes, T-cells, macrophages, neutrophils and antibody-producing B-cells.[51] These cells survive passage into the newborn intestines, where they phagocytise microbial pathogens and strengthen the infant's immune response. Triglycerides are also an important component of breast milk. The newborn stomach digests triglycerides using lingual and gastric lipases, releasing free fatty acids and monoglycerides. These two products strengthen the immune system and—through their lytic activities—protect the newborn from various viruses, bacteria and some protozoa, specifically those of the *Giardia* family.[52] Nucleotides are also present in human breast milk and enhance immune function in infants.[53] Finally, apart from vitamin D, breast milk contains sufficient quantities of all vitamins essential for normal growth in children.[54]

ENHANCED THYMUS SIZE AND FUNCTION

During both the fetal and neonatal periods, the primary function of the thymus is to assist in the development and maturation of T-lymphocytes or T-cells.[77] This specific type of white blood cell protects the body from microbial infections and other risks by either controlling immune reactions or directly attacking infected or cancerous cells.[77,78] After puberty, the thymus reduces its function and slowly decreases in size until it is replaced by fat at about 75 years of age.[77,79] Nevertheless, during its active phase, the thymus produces enough T-cells to protect the body from autoimmunity for life.[77]

Due to its role in maintaining a strong neonate defence mechanism, loss of thymus function may be implicated in the pathogenesis of several diseases. For instance, autoimmune-mediated DM begins with the failure of the thymus to develop a normal β-cell self-tolerance.[80] Dysfunctional thymic activity along with expression of insulin-like growth factor 2 is also suspected in certain cases of insulin resistance.[80] One animal study noted the development of autoimmune

diseases in mice whose thymuses were removed.[81] While scientists are still skeptical about the role of thymus size in its performance, the fact that the organ is largest during childhood when an individual is most prone to immunological threats seems to indicate that increased thymus size is associated with better immunity.[77]

Several factors may influence thymus size and activity, potentially leading to the prevention of autoimmune diseases such as T1DM. Some studies show that breast milk may enlarge the thymus gland, either directly via certain components in breast milk or indirectly as a result of the effects of breast milk on infant gut microbiota.[82,83] Breast milk contains CD4+ T-cells, which are passed into the thymus of the infant where they help train CD8+ T-cells to fight pathogens.[55] Breast milk also contains maternal cytotoxic T-lymphocytes which pass into the thymus of infants, after which they find their way to the lymphatic tissues in the ileum where they prevent the growth of harmful bacteria.[84]

Microribonucleic acids (miRNAs) that regulate post-transcription immune cell activity are also present in breast milk.[85] Notably, miRNA-155 plays a key role in regulating thymus Treg and T-helper-2 cell development in humans and animals.[86,87] In mice, miRNA-449a regulates thymus medullary epithelial cell development.[88] Certain hormones involved in thymus development are also present in breast milk. Among these are corticosteroids, which control thymus epithelial differentiation and the production of thymus corpuscles.[89,90] In addition, IL-7 is also present in breast milk and regulates thymus size. Collinson *et al*. reported that the children of malnourished mothers have small thymuses, with small thymus size linked with low IL-7 levels in maternal breast milk.[91]

Conclusion

Breast milk contains a wide variety of bioactive substances which functionally protect the infant's immune system from autoimmune disorders by increasing the population of beneficial bacteria in the gut, attacking pathogenic bacteria and pro-inflammatory molecules as well as increasing thymus size and performance. As such, inadequate breastfeeding may predispose an infant to autoimmune diseases, including T1DM. Nursing mothers and caregivers are therefore advised to follow optimal breastfeeding practices. For babies unable to be breastfed, perhaps due to maternal death or the risk of vertical disease transmission, the bioactive substances mentioned in breast milk can be used to formulate infant feeding formula for such babies.

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