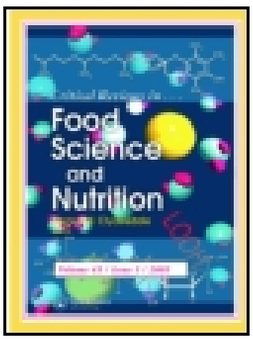

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The vicious cycle of vitamin A deficiency: a review

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ABSTRACT

Vitamin A deficiency (VAD) is a serious and widespread public health problem and the leading cause of preventable blindness in young children. It is also associated with increased rates of death from severe infections, especially in developing countries. Over the past 35 years, researchers have examined the numerous activities of vitamin A in different tissues of the human body. VAD can lead to a series of ocular symptoms, anemia and weak resistance to infection, which can increase the severity of infectious diseases and the risk of death. Cell development, vision, growth and normal metabolism are among the vital processes that are insufficiently

supported in the presence of VAD. VAD leads to impaired tissue function especially during the developmental periods of infancy, childhood, pregnancy and lactation.

We describe a multidirectional model of VAD that demonstrates how VAD can have progressive, negative effects on vital processes of the human body throughout the life cycle. This model starts with impaired intake and its link to decreased absorption and digestion and includes outcomes such as malnutrition, inflammation, and improper growth processes, including possible mechanisms. Together, these clinical and biochemical manifestations contribute to the vicious cycle of VAD.

Abbreviations

atRA all-trans retinoic acid

DC dendritic cell

DHA docosahexaenoic acid

FGF fibroblast growth factor

GH growth hormone

ILC innate lymphoid cells

IRP iron regulatory protein

MUC mucin

RA retinoic acid

RALDH retinaldehyde dehydrogenase

RAR retinoic acid-receptor

RBP retinol binding protein

RXR retinoic X-receptor

sIgA secretory immunoglobulin A

TLR toll-like receptor

VAD vitamin A deficiency

1. INTRODUCTION

Since the late 1980s, there has been an explosion of information on the various functions of vitamin A. The clinical importance of vitamin A as an essential nutrient has become increasingly clear. Adequate vitamin A is required for normal organogenesis, proper growth, immune competence, tissue differentiation, proper gastrointestinal activity and vision. It is also associated with a lower rate of overall morbidity among children (Hall et al., 2011; Sommer and Vyas, 2012).

While the retinol and retinal forms of vitamin A are responsible for normal vision and reproductive functions, the active form of vitamin A, retinoic acid (RA), plays a role in growth and development (Reifen and Wasantwisut, 1998). RA is transported into the cell nucleus where it attaches to retinoic acid-receptor (RAR) and retinoic X-receptor (RXR) heterodimers, which bind RA-response elements (RARE) and regulate the transcription of variety of human genes.

Vitamin A deficiency (VAD) is a serious and widespread public health problem and the leading cause of preventable blindness in young children. It is also associated with increased risk of death from severe infections, especially in developing countries. An estimated 250 million preschool children suffer from VAD globally; 40 to 60% of African children suffer from VAD (Wassef et al., 2014; WHO, 2014). In low-income countries, 10 to 20% of pregnant women develop VAD (Bailey et al., 2015). In South Asia, the prevalence of VAD seems to be determined by economic constraints, sociocultural limitations, insufficient dietary intake and poor absorption, leading to depleted vitamin A stores in the body (Akhtar et al., 2013). UNICEF

has estimated that vitamin A intervention programs, principally periodic high-dose supplementation, save the lives of more than 350,000 children each year. Yet, that still leaves at least twice that many who die unnecessarily from VAD (Sommer and Vyas, 2012).

VAD is associated with specific biochemical and clinical indicators: clinically assessed signs of disease in the eye and particular concentrations of plasma retinol, respectively (WHO, 2009). Plasma retinol concentrations of <0.7 μmol/L are considered indicative of VAD; concentrations of <0.35 μmol/L are considered indicative of severe VAD (Awasthi et al., 2013). For pregnant and lactating women, plasma concentrations of <1.05 μmol/l are considered low (West, 2002).

VAD can lead to a series of ocular symptoms, anemia and weak resistance to infection, which can increase the severity of infectious diseases and the risk of death. These represent the most compelling consequences of VAD and underlie its significance as a public health problem (Bailey et al., 2015; WHO, 2009). Cellular development, the maintenance of proper vision, growth and normal metabolism are among the vital processes that are insufficiently supported in a person experiencing VAD. VAD leads to impaired tissue function especially during the developmental periods of infancy, childhood, pregnancy and lactation (WHO, 2009).

Here, we review the varied health consequences of VAD from impaired intake and its link with decreased nutrient absorption and digestion to outcomes such as malnutrition, inflammation and improper growth, including possible mechanisms. Together, these clinical and biochemical manifestations contribute to the vicious cycle of VAD.

2. VITAMIN A DEFICIENCY

2.1 IMPAIRED TASTE AND INTAKE

VAD affects the ability to sense flavor. We believe that lack of food sensing via taste and smell can certainly decrease food consumption. Oral taste and odor receptors stimulate the appetite and affect the brain reward circuits that drive eating (Sclafani and Ackroff, 2012). Food palatability plays a central role in nutrient intake (de Araujo et al., 2008), and alterations in how different flavors are sensed can result in poor nutrient intake. Vitamin A is required for normal taste-sensing and its effect is exerted at the receptor level, due to its role in the biosynthesis of the mucopolysaccharides that reside in the pore area of taste buds and are present in the various secretions of the oral cavity (Bernard and Halpern, 1968).

Reduced food consumption and impaired weight gain due to VAD is a well-established phenomenon in animal studies (Reifen et al., 2002). For example, rats with VAD exhibited a decreased preference for sucrose relative to control rats, apparently due to a specific impairment of their ability to taste (Reifen and Agami and et al., 1998). Moreover, in another study, rats with VAD consumed less food and consequently gained less weight, as compared with their vitamin A-sufficient counterparts. This was most likely due to a loss of tasting ability and VAD-induced atrophy of the gustatory apparatus (Zhou et al., 2006).

The results of experiments in humans support the results of animal studies. Those studies have found that among individuals with VAD, initial taste and olfaction mean detection thresholds are substantially higher and less sensitive than those found among non-VAD healthy individuals. Following vitamin A treatment, a significant improvement in the thresholds for perceiving bitter and salty tastes, as well as pyridine olfaction were observed (Garrett-Laster et al., 1984).

Taken together, these findings show that VAD may lead to reduced intake of food secondary to a disturbance in food sensing. This can be aggravated even further when VAD coexists with one of the upper respiratory or gastrointestinal diseases that are often accompanied by depressed appetite (WHO, 2009).

2.2 ABSORPTION AND DIGESTION

Vitamin A derivatives are incorporated into rapidly turning over mucosal tissues and have been shown to regulate the growth and differentiation of gastrointestinal epithelial cells (Amit-Romach et al., 2009; Reifen et al., 2003). In VAD, cell differentiation is altered and there is an increase in keratinizing cells and a decrease in the number of mucous-secreting cells in the intestine (Reifen and Wasantwisut, 1998).

Experimental animals with VAD have shorter villi in their intestines and fewer enterocytes. In addition, activity levels of brush-border enzymes, like disaccharidases and peptidases, are markedly lower among animals with VAD (Kozakova et al., 2003; Reifen and Zaiger and et al., 1998; Reifen and Nyska and et al., 1998). A low supply of vitamin A affects the proliferation and maturation of enterocytes, thereby interfering with normal growth and mucosal activity (Uni et al., 1998; Uni et al., 2000). Another study indicated that VAD inhibits intestinal adaptation not only by reducing the proliferation of crypt cells, but also by enhancing early crypt-cell apoptosis and by reducing enterocyte migration rates, which may be related to changes in the expression of collagen and other extracellular matrix components (Swartz-Basile et al., 2003).

More than a decade ago, several papers were published that described human studies conducted in Gambia, India and South Africa that explored gut health and vitamin A status

(Filteau et al., 2001; Thurnham et al., 2000). In the Gambian study, during seasons in which a principal vitamin A source (such as mango) was abundant, participants exhibited higher gut integrity. The Indian and African studies showed more rapid improvement in gut integrity following vitamin A supplementation than in placebo groups, supporting the hypothesis that VAD may be associated with spoiled gut integrity.

VAD may also exacerbate cholestasis, due to excessive intrahepatic bile duct proliferation, which has a negative effect on the digestion of lipids (Weiss et al., 2010). These findings may point to an exacerbating effect of VAD on intestine functionality in terms of both the absorption and the digestion of nutrients, through alterations in cell differentiation, proliferation, maturation and migration, as well as enzymatic processes.

2.3 MALNUTRITION AND INFLAMMATION

The relationship between inflammation and malnutrition is complex. Inflammatory conditions, mediated by a cytokine-driven pathway, may lead to malnutrition through increased requirements for nutrients, while promoting a nutrient-wasting catabolic state such as loss of vitamin A, reduced mucosal barrier function, anorexia, decreased food intake, increased resting energy expenditure and the diversion of nutrients to inflammatory responses. Moreover, the presence of inflammation may limit the effectiveness of nutrition interventions, exacerbating both inflammation and malnutrition (Jensen, 2015; Jones et al., 2014; Mehta et al., 2013).

According to epidemiological evidence, increased susceptibility to infection is associated with malnutrition. One of the frequently described immunologic abnormalities associated with malnutrition is impaired cell-mediated immunity (Jones et al., 2014; Sirisinha, 2015).

Micronutrient deficiencies, such as VAD, often occur as part of a cycle of malnutrition and may be coupled with protein or energy malnutrition (Bailey et al., 2015). In populations in which VAD is widespread, poor diet and inflammatory reactions to microbial invasion are more likely. In those settings, VAD can increase the severity of infection which, in turn, can reduce intake and accelerate the loss of vitamin A from the body, exacerbating the deficiency (WHO, 2009).

2.3.1 Malnutrition

VAD is often associated with malnutrition. In developing countries, poor crop yields and difficulty transporting food may lead to diets low in vitamin A. In addition, malnutrition, especially the low content of lipids in the food in these areas, can impair the absorption of vitamin A, even when normal levels of vitamin A are consumed. On top of these issues, in malnourished children, there is an association between low levels of retinol and low levels of retinol binding protein (RBP) and transthyretin (TTR), which are vitamin A's carrier proteins, implying that VAD may also be related to a deficiency of these carrier proteins (Goetghebuer et al., 1996).

Malnourished children often have defective mucosal immunity in the form of reduced levels of secretory IgA (sIgA). Looking at these malnourished children, the ones with VAD had ever lower sIgA levels as compared to non-deficient children (Sirisinha, 2015).

In addition, most children with low retinol levels experience severely stunted growth (Fuchs et al., 1994; Marasinghe et al., 2015). Children who received a high-dose vitamin A capsule supplement twice a year were protected against malnutrition, diarrhea and acute

respiratory infection more than children who received the supplement only once or not at all (Grubesic, 2004).

2.3.2 VAD and iron deficiency

Iron deficiency is another major micronutrient deficiency (WHO, 2009) that can promote malnutrition by reducing appetite (Stoltzfus et al., 2004). Vitamin A and iron deficiencies interact with each other and affect preschool children, as well as pregnant and lactating women. Vitamin A can interfere at several stages of iron metabolism. First, vitamin A is an important factor for hematopoiesis, a process that requires relatively large amounts of iron because of its requirement for heme synthesis and subsequent incorporation into hemoglobin molecules. Second, vitamin A has a regulatory role in the expression of genes involved in iron metabolism. It also supports the mobilization and transport of iron (West et al., 2007). Iron enters the lumen and is imported into the enterocyte through the apical membrane, facilitated by unique proteins. Inside the cell, iron is driven to the basolateral membrane or stored within ferritin. The iron regulatory protein (IRP)/iron responsive element (IRE) system controls mRNA stability, as well as the translation of proteins involved in iron uptake, export and transport. When ferritin levels are up-regulated in the absence of the IRE/IRP system, iron is mostly stored in enterocytes instead of being delivered to the bloodstream, independent of iron status. The only known iron exporter, ferroportin-1 (Fpn1), is expressed on the basolateral membrane of the enterocyte and enables iron to exit the cell and associate with the circulatory carrier transferrin (Tf). Cellular uptake of the transferrin iron complex is mainly mediated by the transferrin receptor 1 (TfR1) (Silva and Faustino, 2015). Several studies have shown that vitamin A induces the expression of the *Fpn1* gene (Citelli et al., 2012; Katz et al., 2015) and that β-carotene, a precursor of vitamin

A, decreases levels of both light and heavy ferritin and increases Fpn1 levels, which results in the alleviation of induced intracellular iron sequestration (Katz et al., 2015).

VAD can cause anemia, a manifestation of iron deficiency (West et al., 2007). In addition, a recent study found that, in cases of VAD, mRNA levels of IRP2 and ferritin are increased and levels of TfR1 mRNA are decreased. This keeps the iron in the enterocytes and prevents it from reaching the target cells (Jiang et al., 2012). Simultaneous use of iron and vitamin A supplements seems to be more effective for prevent iron deficiency anemia than the use of either of these micronutrients alone (Bloem, 1995; Michelazzo et al., 2013).

VAD affects malnutrition both directly and indirectly, by affecting the bioavailability of ingested nutrients and by interacting with other essential nutrients, such as iron. However, it should also be remembered that malnutrition itself may lower vitamin A levels.

2.3.3 Inflammation

Vitamin A has a role as an anti-inflammatory agent. It plays a major part in regulating the immune response (as will be discussed later in this article) and in repairing protective mucosal epithelium damaged by infection (Pino-Lagos et al., 2010; Reifen, 2002).

Vitamin A can strongly reduce intestinal inflammation and restore antibody responses impaired by VAD (Dong et al., 2010), and may inhibit eosinophilic and neutrophilic infiltration of the lungs through its suppression of the production of TNF-α and eotaxin and the activation of NF-κB (Torii et al., 2004). Conversely, VAD also induces inflammation and aggravates existing inflammation (Reifen, 2002). In animal models, VAD has been shown to cause inflammatory changes in the colon that are similar to processes that occur as part of colitis (Nur et al., 2002).

Moreover, colitis is amplified by VAD through activation of NF-kB and collagen formation, and can be ameliorated by vitamin A supplements (Reifen et al., 2002).

Another study demonstrated that VAD induces a pro-oxidant environment and inflammation in rat aorta. Higher binding activity of NF-kB, increased concentrations of thiobarbituric acid reactive substances (TBARS) and reduced activity of anti-oxidative enzymes such as superoxide dismutase (SOD) 1, glutathione peroxidase (GPx) and chloramphenicol acetyltransferase (CAT) were all observed in rats with VAD, as compared to controls. In addition, NO production increased and there were higher expression of iNOS, eNOS and COX-2 in vitamin A-deficient rats. The incorporation of vitamin A into the diet of VAD rats reversed almost all of these changes (Gatica et al., 2005).

Recently, an irregular surface of the intimal layer in the aorta has been discovered in VAD rats. This abnormality may be associated with oxidative stress and inflammation. These rats also exhibited high levels of TBARS in serum and the aorta, increased expression of inflammation factors such as TNF-α, NOX-2, VCAM-1, and TGF-β1; and low levels of glutathione in the aorta (Gatica et al., 2012).

Researchers have also found that higher levels of RBP are associated with a lower risk for multiple sclerosis. That finding suggests that VAD may be correlated with this inflammatory demyelinating condition (Salzer et al., 2013).

Additional evidence suggests that there may be an association between VAD and asthma, which is fundamentally an inflammatory disorder (Reifen et al., 2015). In studies involving

humans and animal models, serum vitamin A concentration was significantly lower among asthmatics, as compared to the control groups (Riccioni et al., 2007).

It is important to note that optimal vitamin A levels and treatment with supplemental vitamin A have been shown to be beneficial in the context of a number of inflammatory conditions, including skin disorders, bronchopulmonary dysplasia and some precancerous and cancerous states (Reifen, 2002). On the other hand, an underlying infective or inflammatory process may cause low concentrations of serum retinol (Mallett et al., 2015). Infections increase the body's vitamin A requirements, reduce intestinal absorption of vitamin A and decrease liver production of RBP. Indeed, infected children with high CRP serum levels have lower retinol levels than healthy children (Goetghebuer et al., 1996). The effect of vitamin A status on the inflammatory state can most likely be attributed to the relationships between vitamin A and immunity and epithelial integrity.

2.4 ALTERATIONS IN THE IMMUNE SYSTEM

Interest in vitamin A as a regulator of immune function goes back to the beginning of the 20th century. Vitamin A provided to offspring in gestation is required for the development of full-size lymph nodes (Eberl, 2014). Moreover, gestational or early life VAD decreases the number of immune cells in the offspring, which may suppress the activities of the mucosal immune responses in the intestine (Liu et al., 2014).

Vitamin A interacts with both the innate immune system and the adaptive immune system, and improves the host's defenses against infections. A study conducted in Brazil reported fewer parasitic infections and *Giardia* infections, in particular, among children treated

with vitamin A than among children who received a placebo (Lima et al., 2010). In addition, in several studies conducted in developing countries, vitamin A treatments given to children with measles were associated with improved health and survival (AAP, 1993).

VAD is known to be associated with increased susceptibility to infection, especially with regard to disturbances in the integrity of the gastrointestinal mucosal barrier. This includes changes in the mucin (MUC) dynamic (decreased MUC2 mRNA expression and increased MUC3 mRNA expression), down-regulation of the expression of *defensin6* mRNA and up-regulation of toll-like receptors 2 (TLR2) and toll-like receptors 5 (TLR5) mRNA expressions (Amit-Romach et al., 2009). VAD aggravates the course of rotavirus infection through intestinal damage. The colons of infected mice that are also suffering from VAD have smaller glandular areas and higher levels of MUC. These mice also have more viral excretion in their stool than control mice (Reifen et al., 2004). Hosts with VAD also become non-symptomatic reservoirs of *Escherichia coli*-like enteric infections. Improvements in vitamin A status might decrease susceptibility to enteric pathogens and prevent the spread of infection (McDaniel et al., 2015).

Vitamin A is essential for transport and secretion of sIgA across the mucosa. In vitamin A-deficient animals, the secretory component of sIgA is adversely affected (Sirisinha, 2015). In addition, VAD also impairs induction of oral tolerance, a systemic induction of unresponsiveness to antigens ingested by the oral route and loss of immune response (Nakamoto et al., 2015).

The induction of mucosal homing receptors on T and B cells by mucosal dendritic cells (DC) depends on the presence of vitamin A. Recent studies have indicated that the differentiation of

certain DC subsets in the mucosa, as well as in the spleen also depends on vitamin A signaling (Beijer et al., 2014).

RA regulates adaptive T cell responses in two different ways. On the one hand, it promotes the initiation of effector T cell differentiation and, on the other, it restrains the inflammatory T cell responses in tissues (Kau et al., 2011). Recently, several lines of evidence have converged to show that RA plays a key role in the differentiation and migration of T cell subsets, as well as the proper development of T cell-dependent antibody responses. Studies have shown that RA helps to suppress inflammatory reactions by promoting the differentiation of regulatory T cells, which modulate T cell activation and control cell trafficking. Conversely, in a state of VAD, inflammatory T cell reactions may be inadequately opposed and become dominant (Ross, 2012). RA inhibits the IL-6-driven induction of pro-inflammatory Th17 cells and promotes anti-inflammatory regulatory T cell generation in the presence of TGF-β (Hall et al., 2011). VAD has been shown to inhibit Th17 cell differentiation and lead to low expression of the mRNA sequences associated with IL-17 and IFN regulatory factors 4, IL-21, IL-22, and IL-23 in the small intestines of mice (Cha et al., 2010)**.** The acute Th1 cell response, which drives antiviral responses, and the emergence of Th2 cells, which protect against parasitic worms, are substantially impaired in mice with VAD. RA promotes differentiation of Th2-cells over Th1-cells (Kau et al., 2011). Furthermore, RA serves as a cofactor in the development of $Foxp3^+$ -inducible Treg (iTreg) cells (Hall et al., 2011). Overall, RA contributes to intestinal immune balance by promoting T-cell homing, proliferation and differentiation, as well as the production of Treg cells (Kau et al., 2011).

Innate lymphoid cells (ILCs) respond directly to cues from signaling molecules that are produced in infected tissues and act much more quickly than adaptive lymphocytes. VAD was found to cause their response to shift from ILCs associated with the pro-inflammatory antibacterial activity of type-3 immunity (ILC3s) to the anti-worm, tissue-repair activity of type-2 immunity (ILC2s). This finding suggests that malnutrition redirects the immune system to protect against parasites that might compete for food. In addition, it also hints to an even more fundamental role for vitamin A in immunity, since ILC3s also induce the development of lymph nodes (Eberl, 2014).

The discovery that RA is critical for the generation of IgA-secreting B cells offers further evidence of a multifactorial role of RA in mucosal immunity (Hall et al., 2011). RA strongly interacts with IL-4 and IFN-γ to regulate the expression of polymeric immunoglobulin receptor in human colon adenocarcinoma grade 2 cells, suggesting that vitamin A may be required for proper regulation of IgA transport in response to mucosal infections (Sarkar et al., 1998).

To summarize, VAD does not only interfere with the first-line defense mechanisms. It increases susceptibility to infection, impairs the initial response to substances ingested orally and also adversely affects second-line defense, as it affects the development and regulation of proper lymphocytes.

2.5 INTERACTION WITH THE MICROBIOME

A considerable body of research exists detailing the numerous effects of vitamin A on host immune responses. However, little is known about how the bacteria in our bodies interact with this vitamin. Our microbiome coexists with our immune system. As we work to advance

our immunological insights into diseases, it has become clear that we also need to identify the mechanisms that allow specific members of the microbiota to modulate their host's immune health. New connections between the microbiome and the immune system are starting to emerge. Changes in the composition and behavior of the microbiome may even have therapeutic potential for the treatment of infections and inflammatory diseases.

Synergy between RA and microbial-driven signals promotes Th17 cell differentiation *in vivo* (Hall et al., 2011). Diet-induced VAD in mice elicited high levels of MUC2 by goblet-cell hyperplasia and subsequently reduced the gut microbiome, including segmented filamentous bacteria (SFA), a member of the *Clostridiaceae* family that drives the intestinal Th17 responses in mice. This suggests that RA deficiency can alter the gut microbiome, which, in turn, inhibits Th17 differentiation (Cha et al., 2010; Kau et al., 2011; Ross, 2012).

VAD reduces the concentrations of antimicrobial peptides and alters the intestinal microflora. It causes a decrease in the relative proportion of *Lactobacillus* spp. and is also associated with the appearance of *E. coli* strains (Amit-Romach et al., 2009). VAD also increases bacterial translocation, which influences the severity of disturbances of the intestinal epithelium (Kozakova et al., 2003). This can lead to dysbiosis, a condition which can disrupt the normal process of immune-cell development, intestinal tolerance and homeostasis (Sirisinha, 2015).

In addition, VAD can alter the structure of fucosylated glycoproteins secreted by goblet cells (Rojanapo et al., 1980), by decreasing the production of ILC3s and cytokines (Sirisinha, 2015). This may adversely affect the fucosylation process, which is essential for the support commensal microbiota. Fucose that is liberated from the fucosylated glycoproteins, metabolized

by commensal microbes and then used by them as an energy source may help protect the host from endogenous pathogens or increase its tolerance of infection (Pickard et al., 2014).

As mentioned earlier, VAD adversely affects sIgA, which can negatively affect the ability of sIgA to directly quench bacterial virulence factors. This hints to the manner in which VAD influences the composition of the intestinal microbiota (Mantis et al., 2011). Additionally, it was shown that RA signaling in B cells is essential for both microflora composition and oral immunization, by affecting IgA plasma cell differentiation (Pantazi et al., 2015).

The interaction between vitamin A and bacteria in the body goes both ways, as the microbiota plays a role in the biosynthesis and metabolism of vitamin A. The stimulation of DC through TLR2 increases the expression of host genes associated with the production of RA (Kau et al., 2011). Specialized DC subsets located in intestinal epithelial cells are responsible for producing RA from vitamin A, resulting in the conversion of naive T cells into $Foxp3^+$ Treg cells. The presence of certain bacterial strains and TLR2 signaling within the gastrointestinal tract may induce the functional activity of enzymes, such as retinaldehyde dehydrogenase type 2 (RALDH2), an enzyme which produces retinoic acid from retinal (Konieczna et al., 2012), which stimulates $Foxp3^+$ Treg cells to eventually suppress pro-inflammatory cytokines and their mediated autoimmune responses (Manicassamy et al., 2009). $CD103^+$ $RALDH^+$ DCs were shown to decrease in control animals with inflammations, while $CD103^+$ $RALDH^+$ DCs numbers were maintained in subjects fed *Bifidobacterium infantis*. This finding implies that appropriate DC processing of the microbiota promotes intestinal homeostasis and protects against aberrant inflammatory responses (Konieczna et al., 2013). Thus, vitamin A derivatives and their

metabolism in DCs are important for the effects of the microbiota on mucosal immuno-regulation.

To conclude, vitamin A can modulate immune responses directly, by communicating with immune cells, or indirectly, by interacting with the microbiome, which in turn, can affect vitamin A metabolism. Recognition of the complex interaction of VAD with ILCs and the microbiome adds a new dimension to our understanding of the role of vitamin A in regulating immunity and bacterial homeostasis.

2.6 REPRODUCTION AND GROWTH

As stated earlier, nutrient imbalance, such as VAD, can result in malnutrition and inflammation, which may affect growth, development and functional outcomes. Vitamin A is necessary for the development and growth of all types of cells. Vitamin A is not only essential for both male and female reproduction processes (Clagett-Dame and Knutson, 2011), it is also crucial for the prevention of malformations, the maintenance of vertebral identity and for the development of skeletal elements during embryogenesis, (Baume et al., 1972) as well as for proper growth and maintenance throughout the life cycle.

2.6.1 Reproduction

The requirement of vitamin A for reproduction was first recognized in the early 1900's. Large numbers of developmental processes that require vitamin A were first identified in nutritional deficiency studies of rat embryos, and later in genetic studies in mice (Clagett-Dame and Knutson, 2011).

Vitamin A plays an essential role in both male and female reproduction. The functional form of vitamin A in these activities is all-trans retinoic acid (atRA), which has the ability to reverse most reproductive blocks associated with VAD. Moreover, the importance of atRA is exemplified by the appearance of VAD defects when retinoic acid receptors (RAR) are treated with competitive antagonists or mutated. In female rats with severe VAD, reproduction fails prior to implantation; whereas when pregnant rats with VAD are given small amounts of vitamin A early in organogenesis, embryos form, but exhibit a collection of defects (Clagett-Dame and Knutson, 2011). The manipulation of atRA levels in the diet of VAD female rats undergoing a reproductive cycle has provided a means of generating large numbers of embryos at later stages of development with the VAD syndrome (Clagett-Dame and DeLuca, 2002).

Vitamin A is also essential for the maintenance of the male genital tract and spermatogenesis. It was recently suggested that RA is needed for both adult male spermatogonial differentiation and the entrance into meiosis (Clagett-Dame and Knutson, 2011; Hogarth and Griswold, 2010; Matson et al., 2010). In cases of VAD, the epithelia of the epididymis, prostate and seminal vesicle are replaced with stratified squamous keratinizing epithelium and spermatogenesis ceases (Mason, 1933; Wolbach and Howe, 1925). Upon the addition of vitamin A, spermatogenesis can resume (van Pelt and de Rooij, 1990).

2.6.2 Prenatal and neonatal growth

Vitamin A is of utmost importance in many developing embryonic organ systems such as the nervous system, heart, kidney, lung and axial skeleton. In the 1930's, a teratogenic effect of maternal VAD was first reported in pigs. The defects found in the progeny included

anophthalmia or microphthalmia, palatal fissuring, subcutaneous cyst formation and renal displacement (Hale, 1935; Hale, 1977). Furthermore, prenatal VAD impairs adaptive immune responses to some vaccines in neonatal pigs (Kandasamy et al., 2014).

Other studies have reported ocular, facial, urogenital, renal, diaphragmatic and cardiac malformations along with severe enamel and dentin dysplasia in the progeny of rats with VAD (Baume et al., 1972). During the third trimester of pregnancy, VAD is associated with an increased risk of preterm delivery and maternal anemia in humans (Radhika et al., 2002). Subclinical VAD is also a problem during the third trimester of pregnancy. A serum retinol concentration of <20 Ag/dL appears to indicate a problematic lack of vitamin A and is associated with increased risk of pre-term delivery and maternal anemia.

In the embryonic axis, fibroblast growth factor (FGF) signaling is important for somitogenesis. RA from the pre-somitic mesoderm and somites is required for neural differentiation, as it inhibits the expression of FGF8 (Diez del Corral et al., 2003). The combination of FGF and RA signaling is also important for the regulation of somite size. Vitamin A-deficient quail embryos have smaller somites than their vitamin A-sufficient counterparts and also have an expanded FGF8 domain in the pre-somitic mesoderm (Diez del Corral et al., 2003), which prevents the initiation of differentiation (Aulehla and Pourquie, 2010). RA may also be involved in the termination of the process of segmentation (Tenin et al., 2010). Furthermore, atRA can reverse developmental embryonic blocks including the block that occurs in RALDH2-mutant mice (Clagett-Dame and DeLuca, 2002).

RA is involved in the specification of the axial identity of future somites. Rat embryos with VAD show anterior vertebral transformations throughout the axial skeleton. These problems can be repaired if vitamin A is provided during specific periods of embryogenesis (Kaiser et al., 2003). In addition, genetic deletion of several RARs, similar to the situation seen in VAD embryos, produces defects in axial development with anterior cervical transformations (Ghyselinck et al., 1997; Lohnes et al., 1993; Lohnes et al., 1994).

Rat embryos that became vitamin A-deficient later in embryogenesis exhibited hypoplastic cranial bones and defects of the thyroid, cricoid and tracheal cartilage, as well as failure of development of the neural arch of cervical vertebrae 1. Late-VAD rat embryos also exhibited grossly malformed sternal and pelvic regions (See et al., 2008).

Some RARs in neural crest cells act to direct morphogenesis of skeletal elements. Failure of frontonasal segment development has also been reported in RAR-null mutant mice and RBP-null mutant mice on a VAD diet (Clagett-Dame and Knutson, 2011). Malformation of the skull and the cervical vertebrae has also been described in lions kept in captivity worldwide and this problem has been related to VAD (Gross-Tsubery et al., 2010; Shamir et al., 2008).

Treatment with vitamin A during pregnancy and lactation has been shown to increase newborn femur length; whereas direct vitamin A supplementation of newborns increased the length of their hypertrophic zones, altering neonatal bone formation and chondrocyte gene expression (Zhang et al., 2012).

2.6.3 Growth aspects of children and adults

The effects of micronutrient deficiencies on growth and body composition are most important in the pediatric age group (Mehta et al., 2013). Vitamin A levels are correlated with nocturnal growth hormone (GH) secretion and total dietary vitamin A intake has been found to be significantly lower in short children with abnormal nocturnal GH secretion than in normal children and endocrinologically normal, short children. Moreover, vitamin A supplementation for three months increased nocturnal GH secretion in these children (Evain-Brion et al., 1994).

A recent study assessed the effect of vitamin A supplementation and protein on anthropometric indices of primary and middle school students of a low socioeconomic status. That study found that supplementation increased mean height for age and the mean weight for age z-scores, implying that vitamin A supplementation may prevent growth problems (Cao et al., 2013).

Subnormal vitamin A intake is one of the etiological factors in delayed puberty. Giving supplemental vitamin A and iron to normal constitutionally delayed children with subnormal vitamin A intake has been shown to be as effective as hormonal therapy for the induction of growth and puberty (Zadik et al., 2004). Similarly, six months of vitamin A, iron and zinc supplementation has been shown to accelerate the growth of short children who were born small for their gestational age and exhibit subnormal nutrient intake, similar to growth hormone therapy (Zadik et al., 2010). In addition, a Gambian study showed that during seasons in which foods containing vitamin A precursors were plentiful, children's' growth was least impaired, infection was at its lowest and gut integrity was at its best (Thurnham et al., 2000).

Vitamin A also interacts with docosahexaenoic acid (DHA), which is involved in a myriad of vital physiological functions pertaining to growth and development. The synthesis of DHA is partially regulated by RXR and it has been demonstrated that dietary VAD profoundly reduces DHA, regardless of the level of dietary alpha linolenic acid, by impairing peroxisomal proliferation and inhibiting the activity of acyl-CoA oxidase (Zhou et al., 2006).

Vitamin A forms are also involved in the skeletal system of adults. Carotenoids, some of which are vitamin A precursors from plants, are associated with improved bone health (Tanumihardjo, 2013). There is evidence that carotenoids may protect bone health through their effect on oxidative stress, a state which may increase bone resorption through the activation of NF-κB, a mediator of TNFα, and osteoclastogenesis (Iotsova et al., 1997; Schreck et al., 1992; Sen and Packer, 1996). Carotenoids are capable of reducing oxidative stress by scavenging singlet oxygen and peroxyl radicals. Other ways in which carotenoids protect the bone may include retinoid-dependent signaling, stimulation of gap junction communications, effects on the regulation of cell growth, induction of detoxifying enzymes and up-regulation of the expression of genes like *connexin43* (Mehta et al., 1991; Stahl et al., 2002). The Framingham Osteoporosis Study showed a protective trend of beta-carotene for hip fracture (Sahni et al., 2009) and also reported a protective effect of total carotenoids against four-year loss of trochanter bone mineral density among men (Sahni et al., 2009). Therefore, it is suggested that carotenoids may help prevent osteoporosis. On the other hand, there has been some evidence that linked excess consumption of vitamin A from animal-source foods and fortified foods to greater risk of osteoporosis and hip fracture (Tanumihardjo, 2013).

2.7 VISION

The importance of vitamin A in eye development was first recognized in experiments conducted in the first half of the previous century, in which piglets with vitamin A-deficient mothers were born blind (Hale, 1935; Hale, 1977). The consequences of VAD in rat embryos include dysmorphogenesis of the anterior eye segment, retina and optic disc (Warkany and Schraffenberger, 1944; Warkany and Schraffenberger, 1946; Wilson et al., 1953).
Since then, numerous studies have focused on different forms of vitamin A as essential contributors to the visual system. The unique metabolism of vitamin A in the retinoid cycle in the eye greatly affects the structure and functioning of retinal pigment epithelial (RPE) cells. RPE cells produce a variety of extracellular matrix (ECM) proteins, thrombospondin-1 (TSP-1) and angiogenic factors. Among other things, these proteins are responsible for cellular structure and for the prevention of endothelial adhesion. Their expression is modulated by vitamin A (Uchida et al., 2005).

To sustain vision, humans require the continued cyclic regeneration of the vitamin A derivative, 11-cis-retinal (Perusek and Maeda, 2013), in order to continue the formation of rhodopsin, the visual pigment essential to the retinal receptors responsible for dark adaptation, inside the photoreceptor cell (Sommer, 2008). Vitamin A is required for regeneration of the visual pigments of rods and cones in the retina (Reddy and Vijayalaxmi, 1977). VAD can cause a significant interruption in the sequence of reactions needed for vision, damaging the overall health of the retina and the quality of color vision and dark adaptation (Perusek and Maeda, 2013; Reddy and Vijayalaxmi, 1977).

Xerophthalmia, the leading cause of preventable childhood blindness, is the classical expression and most specific disorder of VAD. It encompasses the clinical spectrum of ocular

manifestations of VAD. Under conditions of gradually worsening vitamin A status, the eye undergoes a series of conjunctival and corneal changes, beginning with night blindness, the inability to see under low levels of illumination and Bitot's spots, followed by potentially blinding stages of corneal xerosis, ulceration and necrosis, namely, keratomalacia (Akhtar et al., 2013; Sommer, 1995). Although night blindness and Bitot's spots are considered mild stages of eye disease, both represent moderate to severe systemic VAD, as evidenced by low serum concentrations of retinol (Sommer et al., 1980; WHO, 2009).

In the case of VAD mice, the expression of the factors involved in visual processing was found to be down-regulated relative to normal mice and to have an adverse effect on vision (Uchida et al., 2005).

It has also been shown that after vitamin A deprivation of approximately 10 months, the sensitivity of the fully dark-adapted eye of men with VAD was decreased relative to the starting point (Pirie, 1956).

Substantial study of structural change in the eye due to VAD has concentrated on the young. Early studies found that severe maternal VAD in rats induces retinal and other ocular malformation in their offspring (Warkany, 1954). Similarly, calves and rabbits with VAD may go blind due to distorted growth of the bone surrounding the optic foramen, with consequent constriction of the optic nerve (Mellanby, 1941; Moore, 1941; Pirie, 1956).

Supplementation with vitamin A derivatives has been shown to bypass defective steps in the visual cycle and regenerate pigments necessary for vision, in animal models of retinal degenerative diseases (Perusek and Maeda, 2013; Perusek et al., 2015). In Southeast Asia, there

is an excellent documentation indicating that vitamin A supplementation programs have ongoing positive effects on both ocular health and mortality (Bailey et al., 2015).

In summary, vitamin A is a central element in the system of vision and its absence reduces the ability to see. Xerophthalmia is certainly the most characteristic clinical sign of VAD, and it can be reversible. Continued emphasis should be placed on vitamin A supplementation for the treatment and prevention of eye disease.

2.8 INCREASED MORBIDITY AND MORTALITY

Improving the vitamin A status of vitamin A-deficient children could, by itself, dramatically reduce morbidity and mortality (Sommer and West, 1996). Worldwide, VAD is an important public health problem and it is the second most prevalent nutritional disease after protein energy malnutrition (PEM), with which it is often associated (Mejia, 1986; Sherwin et al., 2012). Since VAD is related to malnutrition, inflammation and altered immunity, it is only logical that it may contribute to increased mortality from infectious diseases (De Sole et al., 1987; Goetghebuer et al., 1996; Sommer et al., 1984).

The high prevalence of VAD in South Asian developing countries leads to increased morbidity and mortality among infants, children and pregnant women (Akhtar et al., 2013). After observing that Indonesian children with mild xerophthalmia died at far higher rates than their non-xerophthalmic peers, it was hypothesized that preventing xerophthalmia and its associated VAD might reduce the mortality of young children, among whom even subclinical deficiency was associated with increased mortality (Sommer, 2008).

In various countries, vitamin A supplementation has reduced global and infection-related mortality and measles-associated morbidity among populations of children with a high prevalence of VAD (Goetghebuer et al., 1996). Thus, in 1987, WHO recommended vitamin A supplementation for children suffering from measles in developing countries (WHO, 1987).

Neonatal vitamin A supplementation may reduce infant mortality rates, as well as the prevalence of severe respiratory infection among young infants (Humphrey et al., 1996). Providing a high-dose capsule of vitamin A to children aged 6 to 60 months, including those who eat vitamin A-rich foods, may decrease the incidence of malnutrition, diarrhea and acute respiratory infections (Grubesic, 2004).

Treating children with a large dose of vitamin A at periodic intervals is a direct, versatile and relatively rapid means of improving vitamin A status and preventing associated blindness, morbidity and mortality (Sommer and West, 1996). It is extremely crucial to remain vigilant for VAD in nutritionally vulnerable population, as it plays a profound role in their survival.

2.9 EPIGENETICS - ONE KNOWN MECHANISM OF ACTION OF VITAMIN A

Nutrition is one of the primary environmental factors that determine health. Throughout the life cycle, nutrients can modify physiologic and pathologic processes through epigenetic mechanisms that are independent of primary DNA sequences and critical for gene expression. Vitamin A, especially in it RA form, is a potent agent capable of inducing epigenetic alterations and modifications yielding various phenotypic effects (Dadon Bar-El and Reifen, 2015).

According to the current model of retinoid signaling, in the absence of RA, RAR/RXR receptors interact directly with corresponsive elements (SMRT, NCoR and Sin3A) that recruit histone deacetylase complexes I/II, which deacetylate the lysine residues of histone tails. This

enables a tight association with the nucleosome DNA and establishes a "closed" chromatin state that is inaccessible to transcription. The presence of RA releases these co-repressors and induces histone acetylation, opening the compact chromatin structure that facilitates gene transcription.

As we increase our understanding of the relationship between diet and the epigenome, opportunities will arise for the clinical application of new knowledge. The medical benefits of vitamin A as an epigenetic modulator, especially with respect to its chronic use as a nutraceutical agent, will depend on our understanding of its epigenetic effects during health and disease, as well as through different generations.

3. CONCLUSION

3.1 THE VICIOUS CYCLE OF VITAMIN A DEFICIENCY

Vitamin A is critical throughout the life cycle. Together with its derivatives, it regulates a variety of processes including vision, reproduction, embryogenesis, growth, cellular differentiation and proliferation, normal metabolism and immune function. This ability of vitamin A to target and affect multiple pathways, as well as its tight homeostatic regulation (WHO, 2011), makes it a hormone-like compound active in a large number of tissues and organs.

We propose a scheme describing the vicious cycle of VAD (Figure 1). Entering the cycle and moving from one stage to another may lead to further deterioration. As the deficiency becomes more severe, its clinical and biochemical manifestations, only some of which are reversible, contribute to the vicious, multidirectional cycle of VAD. Although this may be true, we should be slightly careful when we extrapolate animal studies into people. Future studies should substantiate all these preclinical findings in the context of humans.

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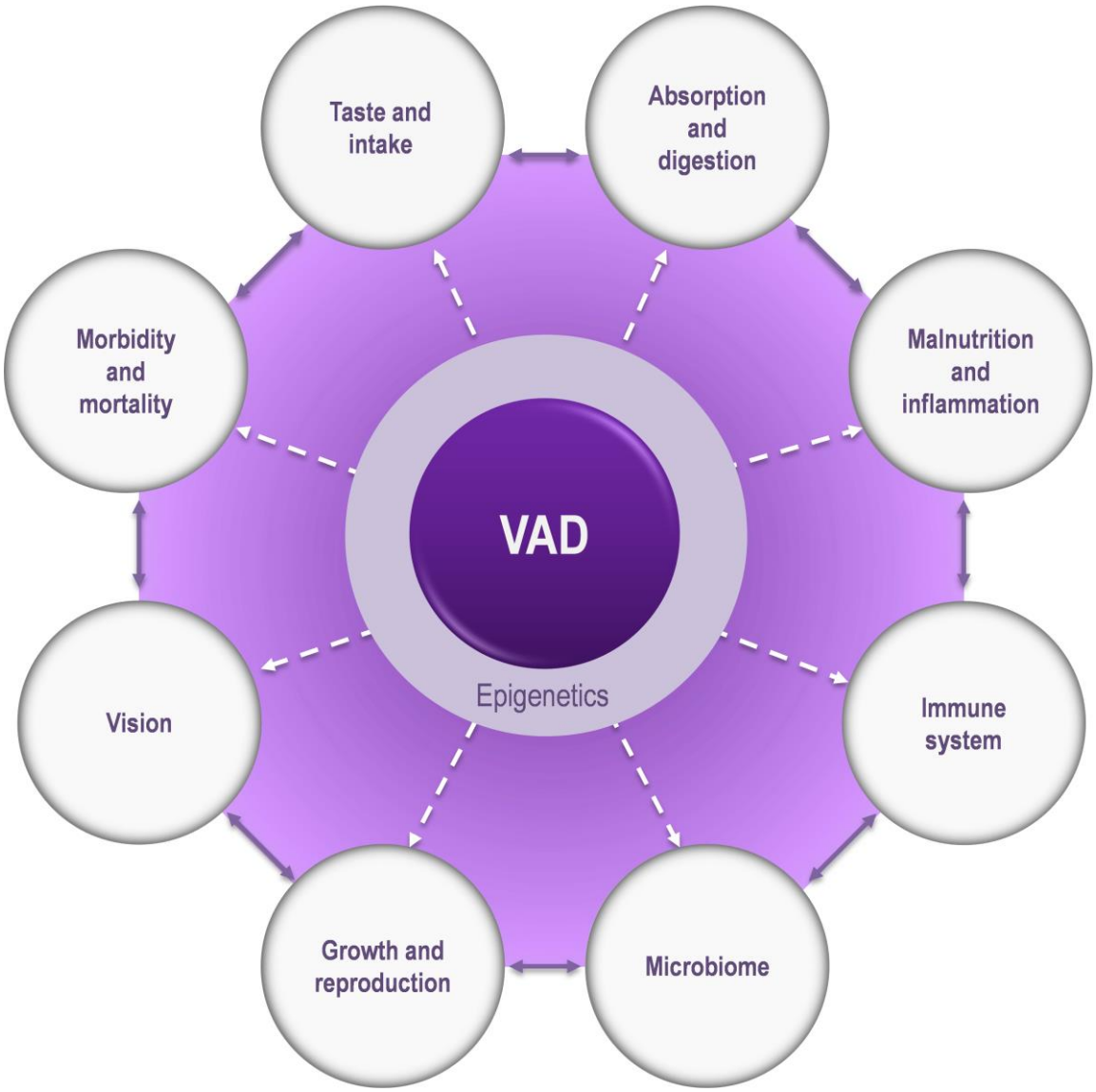

Figure 1: The vicious cycle of vitamin A deficiency (VAD). Typically, the cycle of VAD begins with reduced taste ability which leads to decreased food intake. This, along with impairment in absorption and digestion, may lead to malnutrition and may increase the risk for inflammation, which is exacerbated during VAD state. At the same time, VAD can reduce immune system's ability to protect the body through direct interaction with immune components/gene expression, or through the microbiome, that consequently damaged growth, bone development and

reproduction. As the VAD deepens, disturbed vision is witnessed. All mentioned above sets the stage for increased morbidity and mortality due to VAD, with the involvement of epigenetic mechanisms, among others.