The Hygiene Hypothesis Revisited: autoimmune diseases, intestinal microbiota and vitamin D's role

Trabajo Final de Máster Nutrición y Salud

Autora: Allison Clark
Profesora: Núria Mach Casellas

Julio de 2016

ÍNDICE

The Hygiene Hypothesis Revisited: autoimmune diseases, intestinal microbiota and vitamin D's role

Running Head: Hygiene hypothesis: immune system, microbiota, and vitamin D

Authors: Allison Clark[a] Núria Mach[a,b]

[a]Health Science Department, International Graduate Institute of the Open University of Catalonia (UOC), 08035, Barcelona, Spain.

[b]Animal Genetics and Integrative Biology unit (GABI), INRA, AgroParisTech, Université Paris-Saclay, 78350, Jouy-en-Josas, France.

*Corresponding author: allisonsclark@gmail.com

Manuscript submitted to *Frontiers in Immune Microbiology*

Word Count: 11,796

Number of figures: 4

Abstract
The hygiene hypothesis postulates that higher levels of hygiene and improper exposure to microorganisms early in childhood could disturb the intestinal microbiome functions resulting in abnormal immune responses that can later lead to allergies and autoimmune diseases. Additionally, vitamin D deficiency and vitamin D receptor (VDR) polymorphisms and function might also trigger abnormal immune responses that can lead to an autoimmune disease. Therefore, this review explores the role Western lifestyle factors that lead to intestinal dysbiosis and vitamin D deficiency, and the effect intestinal microbiota, vitamin D and VDR plays in the pathogenesis of autoimmune diseases in the context of the hygiene hypothesis.

The maintenance of a healthy core microbiome to prevent dysbiosis, infections or other disruptions that are associated with many inflammatory and autoimmune diseases is necessary for immune homeostasis. An excessively hygienic lifestyle such as antibiotic treatment, antibacterial soap and the Western diet, can deplete indigenous microbes, which is a growing concern since unhealthy microbiomes can then be inherited and vertically transferred to offspring. Literature shows that the one of main factors that lead to autoimmune diseases is VDR dysfunction and vitamin D_3 deficiency, which is the main VDR ligand. Vitamin D_3 has anti-inflammatory and antimicrobial properties that stimulate more tolerogenic immune responses and promotes immune homeostasis by maintaining intestinal barrier function, inducing the production of antimicrobial peptides and autophagy which produce anti-inflammatory effects in autoimmune patients. We found 13 studies that demonstrated that crosstalk occurs between vitamin D_3 and the gut microbiota and short chain fatty acids (SCFA) that promote gut homeostasis. On the other hand, VDR controls intestinal inflammation, susceptibility to pathogenic infection and lipopolysaccharides-induced endotoxemia.

Despite these findings, many population studies are inconclusive likely due to host genetic background, epigenetic marks and metagenomic differences, variations in lifestyle and heterogeneous study design. Furthermore there lacks a global consensus as to what defines vitamin D deficiency and sufficiency. Due to vitamin D's role in promoting immune homeostasis, maintaining adequate levels in at risk populations, especially pregnant and breastfeeding women and young children should be given more attention.

Key Words
Vitamin D_3, Vitamin D receptor, gut microbiota, hygiene hypothesis, autoimmune diseases, microbiota heritability, Western lifestyle

Abbreviations
antimicrobial peptide angiogerin-4 (*ANG4*), autophagy related 5(ATG5), autophagy related 16 like 1 (ATG16L1), cathelicidin antimicrobial peptide (CAMP), C-C motif chemokine ligand 22 (CCL22), 25-hydroxyvitamin D 24-hydroxylase (Cyp24), cytochrome P450 family 27 subfamily B member 1(Cyp27b1), dendritic cells (DC), dry matter (DM), dextran sulfate sodium (DSS), European Food Safety Authority (EFSA), guanine nucleotide binding protein 12 (GNA12*),* gut associated lymphoid tissue (GALT), genome-wide association study (GWAS), interleukin (IL), Institute of Medicine (IOM)**,** interleukin-1 receptor-associated kinase 4 (IRAK4*),* irritable bowel disease (IBD), knockout (KO), lipopolysaccharide (LPS), mitogen-activated protein kinase (MAPK), multiple sclerosis (MS), myeloid differentiation primary response protein 88 (MyD88), nucleotide-binding oligomerization domain 2 (NOD2), pathogen associated molecular pattern (PAMP), peroxisome proliferator activated receptor (PPAR), pattern recognition receptors (PRR), short chain fatty acids (SCFA), single nucleotide polymorphism (SNP), T helper 1 (Th1), T helper 2 (Th2), T helper 17 (Th17), toll-like receptor (TLR), tumor necrosis factor alpha (TNFα), regulatory T cell (Treg), vitamin D receptor (VDR), vitamin D responsive elements (VDRE), 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$), zonulin occluden-1

(ZO-1), zonulin occluden-2 (ZO-2)

1. Introduction

According to the hygiene hypothesis, the increased cleanliness, reduced family size and subsequent lack of proper microbial exposure in early childhood, a common occurrence in Western society, can disrupt the human microbiome causing a lack of indigenous microbes leading to aberrant immune responses such as allergies and autoimmune diseases (Cho and Blaser, 2012; Proal et al., 2013; Schnorr et al., 2014). The mammalian intestine harbors a diverse array of commensal and pathogenic organisms that are critical for shaping the development of the immune system (Owen and Mohamadzadeh, 2013; Proal et al., 2013). Studies analyzing the gut microbiota composition of indigenous cultures have shown that Westerners' gut microbiota lack diversity, which could affect host health and lead to a more inflammatory state (Clemente et al., 2012; Martínez et al., 2015; Schnorr et al., 2014; Yatsunenko et al., 2012). Additionally, Western lifestyle and its deleterious effects on microbial species could have detrimental effects for offspring as certain bacteria have been reported to be heritable such as *Actinobacteria* (Lim et al., 2016) perpetuating the prevalence of unbalanced gut microbiomes and an impaired immune system in subsequent generations (Proal et al., 2013; Rook, 2012; Sonnenburg et al., 2016).

Deehan and Walter (2016) declared that the Western diet is a key driver of diversity loss and the rises in non-communicable diseases, like autoimmune diseases. Furthermore, maternal lifestyle factors (Neu, 2015; Zijlmans et al., 2015) such as diet (Hashemi et al., 2016; Soderborg et al., 2016) probiotic consumption (Amarasekera et al., 2013; Hashemi et al., 2016) and antibiotic treatments during pregnancy (Forsythe, 2011; Rautava et al., 2012; Soderborg et al., 2016), as well as delivery mode (cesarean or vaginal) (Azad et al., 2011; Biasucci et al., 2008; Jašarević et al., 2015a; Neu, 2015) and breastfeeding (Amarasekera et al., 2013; Azad et al., 2011; Cacho and Neu, 2014; Funkhouser and Bordenstein, 2013) greatly impact the neonate's initial inoculation of beneficial bacteria and therefore immune development.

Autoimmune diseases are on the rise in Western countries, and are the third highest cause of deaths (Antico et al., 2012). Although autoimmune symptoms and severity differ greatly depending on the disease (Cooper and Stroehla, 2003), they are characterized by a loss of self-antigen tolerance (Antico et al., 2012) and increased auto-antibodies and/or auto-reactive lymphocytes (Ermann and Fathman, 2001). The key players in immune dysfunction and autoimmune disease development are believed to be the hygienic practices that disrupt the gut microbiota composition (Fasano, 2011), genetic background, loss of protective intestinal barrier functions and poor immunogenic lipopolysaccharide (LPS) levels (Hofer, 2016; Vatanen et al., 2016), as well as vitamin D_3 deficiency and vitamin D receptor (VDR) polymorphisms (Aranow, 2011; Cantorna and Mahon, 2005; Fujita et al., 2008; Jean-Claude Souberbielle, 2010; Kamen and Tangpricha, 2010; Kau et al., 2011; Kong et al., 2008; Proal et al., 2009).

Depending on the reference range and population, it is estimated that worldwide between 2-90% suffer from vitamin D_3 deficiency (Hilger et al., 2014). Vitamin D_3 status depends on synthesis from the skin's exposure to UVB rays or consumption of food or supplements (Spiro and Buttriss, 2014) that contain either vitamin D_2 (ergocalciferol from primarily mushrooms) or vitamin D_3 (cholecalciferol from oily fish) (Jean-Claude Souberbielle, 2010; Figure 1). Up to 90% of vitamin D_3 comes from synthesis from the sun's UVB rays (Lips, 2010; Spiro and Buttriss, 2014) and many autoimmune diseases such as multiple sclerosis (MS), are more common in countries that receive less sunshine (Pantazou et al., 2015; Jean-Claude Souberbielle, 2010). Vitamin D_3 activates the vitamin D receptor (VDR) (Adorini and Penna, 2008), which is located on chromosome 12 and has four polymorphisms (referred as *Bmsl*, *ApaI*,

TaqI and *FokI*). The functional significance of these four VDR polymorphisms remains unknown (Hossein-nezhad and Holick, 2013; Uitterlinden et al., 2004), but it is believed that strong linkage disequilibrium and one or more functional polymorphisms elsewhere in the VDR gene explain the observed associations between these SNPs and the prevalence of autoimmune diseases (Uitterlinden et al., 2004). In fact, $VDR^{-/-}$ knockout mice have shown to present low levels of VDR in the intestine, together with abnormal Paneth cells, impaired autophagy function, dysbiosis, reduced expression of the autophagy related 16 like 1 gene (*ATG16L1)* (Wu et al, 2015), which facilitates the removal of intracellular microorganisms, and is associated with a higher risk of infections, cancer, and inflammation (Jin et al, 2015). Similarly, $VDR^{-/-}$ knockout and germ free mice have presented increased bacterial burden and mortality upon enteric *Salmonella typhimurium* infection compared to the $VDR^{+/+}$ group (Wu et al, 2015) and greater susceptibility to LPS-induced endotoxemia and death (Froicu and Cantorna, 2007). In humans, serum vitamin D_3 levels above 40 ng/mL in untreated MS patients were associated with increased *Ruminococcaceae,* which might produce anti-inflammatory fermentation byproducts in the gut (Tankou et al., 2015), whereas in a cohort of 3,188 IBD patients, the higher plasma 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) was associated with significantly reduced risk of *Clostridium difficile* infection (Ananthakrishnan et al., 2014).

Given the ability of gut microbiota's to communicate with the immune system and its relationship with the vitamin D_3 deficiency and VDR function, the aim of this systematic review is to describe how the interaction between individual composition and function of the intestinal microbiota, serum vitamin D_3 levels, VDR function and Western lifestyle affect immune responses and could lead to the development of an autoimmune disease. More precisely, this review focuses on the rise in autoimmune diseases in Western countries in the context of the hygiene hypothesis, describing how the overly hygienic lifestyle and vitamin D_3 deficiency diminish microbial diversity and function and might cause aberrant immune responses in different ages.

2. Materials and Methods

We conducted a systematic review and synthesis of relevant qualitative research according to the requirements established in the preferred reporting items for systematic review and meta-analysis protocols (PRISMA) (Shamseer et al., 2015). The protocol was registered *a priori* with PROSPERO on April 11, 2016 (CRD42016037431).

Literature search strategy

A systematic and comprehensive search of electronic databases, including MEDLINE, Scopus, ClinicalTrials.gov, the PROSPERO International Prospective Register of Systematic Reviews, Science Direct, Springer Link, and EMBASE was done from March 2016 to June 2016.

The search process was completed using the keywords: "hygiene hypothesis", "gut microbiota", "microbiota heritability", "vitamin D_3", "VDR polymorphisms", "autoimmune disease", and "vertical transmission". The search was not restricted to the type of study (i.e. species, meta-analysis, case-control, prospective cohort studies, reviews), sample size, year of publication, publication status or follow-up. However, we only consulted articles published in English. Bibliographies of the identified reviews and original research publications were hand-selected for additional studies that may have been missed by the database searches. All articles were exported to the reference database Zotero. Due to the nature of this review, no request was performed for the ethics committee's approval.

Data extraction and synthesis

Full copies of citations coded as potentially relevant were obtained, and those meeting the inclusion criteria were read in detail and data extracted. One reviewer (AC) extracted information about the study

aim, population and sample size, experimental design and duration of follow-up, specie, individual characteristics, changes in the gut microbiota composition and immune response, association or not with an autoimmune disease. The primary outcome was the gut microbiota profile, aberrant changes in the immune response, vitamin D status or other clinically relevant outcomes related to autoimmune and immune related conditions. Details were then checked by a second reviewer (NM). If eligibility could be determined, the full article was retrieved.

The articles and extracted data were read and the findings organized into the following categories: (i) hygiene hypothesis, the gut microbiota and the immune system, including maternal lifestyle factors and transmission of the gut microbiota; (ii) vitamin D_3, VDR and the immune system; and (iii) experimental articles about the possible relationship between disturbances of the gut microbiota and/or vitamin D_3 deficiency, VDR dysfunction and autoimmune diseases.

3. Results

A search conducted in March 2016 resulted in the following list of key terms combinations (hygiene hypothesis, the gut microbiota and autoimmune disease =36; vitamin D_3 and autoimmune disease=54; vitamin D_3,VDR, intestinal microbiota and autoimmune diseases= 16). Finally, a total of 43 experimental studies and 71 reviews met the inclusion criteria and were included in the review. Most of the articles were reviews or randomized controlled trials. Periods of data collection spanned 1989 to 2016, proving data from humans and animals models (i.e. mice and rats).

While focusing on the context of the hygiene hypothesis in our modern world, 36 have demonstrated the crosstalk occurred between certain gut bacteria and the immune related pathways, which could play a role in the pathogenesis of various diseases. In particular, 31 studies reported that maternal lifestyle factors, maternal microbiota, vitamin D_3 status, birth mode and breastfeeding prove to be key factors in the microbiota and immune system development as the microbiome can be vertically transferred. Additionally, 10 studies demonstrated the intestinal microbiota genetic determinism. For instance, certain taxa among the Firmicutes and Proteobacteria phyla, including those related to immune health and disease, such as IBD, are consistently heritable among families. Discussion of these results is fully described in section 4.1. Moreover, 16 other studies showed crosstalk between the gut microbiota or short chain fatty acids (SCFA), vitamin D_2 ingestion, VDR function, and immune homeostasis in *in vitro* and animal model studies (Table 1).

Studies in Table 1 support the hypothesis that several factors cause autoimmune diseases, including aberrant adaptive and innate immune system responses and the cause/consequent dysbiosis, loss of protective intestinal barrier functions, vitamin D_3 deficiency and VDR genetic variants. Among them, 1 study in animals models described that vitamin D_3 can maintain intestinal barrier function, while 6 studies have demonstrated vitamin D_3's protective effects against pathogens through its antimicrobial activity. Most of the studies used VDR knockout mice to validate the functionality of VDR on immune homeostasis. Additionally, 9 demonstrated vitamin D's ability to induce autophagy and allow anti-inflammatory bacteria to proliferate in the gut, which could prevent or ameliorate autoimmune symptoms as seen in IBD and MS patients. One study demonstrated that vitamin D_3 supplementation has a positive impact on the gut microbiota in autoimmune patients (multiple sclerosis) (Table 2).

While most studies have focused of vitamin D's antimicrobial and anti-inflammatory effects and Th2 immune-promoting immune responses that could prevent autoimmune diseases, new evidence is emerging that it can modulate gut bacteria towards a more homeostatic composition by inducing antimicrobial peptides, decreasing inflammation and dysbiosis (15 studies). A total of 5 VDR knockout (KO) mice studies have demonstrated that VDR dysfunction increase the pro-inflammatory response,

the susceptible to LPS-induced endotoxemia, death and the prevalence of various autoimmune diseases such as IBD, Chron's disease, type I and II diabetes, and multiple sclerosis. VDR polymorphisms may be markers for functional variants that alter VDR expression rather than being the locus associated with disease or lower concentrations of $1,25(OH)_2D_3$.

Table 2 shows the relationship between vitamin D_3 deficiencies, VDR function, the gut microbiota and autoimmune diseases in human studies. In particular, 3 studies in humans have reported the relationship between hygienic lifestyle and an unhealthy gut microbiota composition which was found to be related to aberrant immune responses and the possible rise in autoimmune diseases such as MS, diabetes, IBD and autoimmune encephalomyelitis. Among them, 2 studies in humans showed that vitamin D_3 supplementation improved gut microbiota composition (e.g. increased *Faecalibacterium* and *Coprococcus*) and MS and IBD symptoms respectively, although there lacks well designed studies to make definite conclusions as to whether vitamin D deficiency or VDR polymorphisms play a role in autoimmune disease pathogenesis. The full description of the results is found in the section 4.2. There also lacks a consensus of what defines vitamin D_3 deficiency, sufficiency and optimal range and many studies are heterogeneous in their design and methods of analyzing vitamin D status in humans making it difficult to make definite conclusions.

4. Discussion

4.1. The context of the hygiene hypothesis in our modern world: Western lifestyle, microbiota's role and genetics

The hygiene hypothesis was officially created by Strachan in 1989 (Strachan, 1989) who believed that smaller families and hygienic conditions led to less microbial exposure early in life increases the possibility of suffering allergies and hay fever later in life (Versini et al., 2015). A recent study by Vatanen *et al* (2016) followed the gut microbiome development of 222 infants from Finland, Estonia and Russia from birth until age three. In line with the hygiene hypothesis, the authors discovered that the Russian babies' microbiota communities were more stable than the Finnish, which is a marker of a healthy microbiome, likely because they had a higher microbial exposure. The Finnish and Estonian infants presented higher levels of Bacteroides and LPS from *Bacteroides dorei,* which has been associated with type I diabetes and led to diminished endotoxin tolerance. Moreover, the Finnish babies presented more type I diabetes antibodies whereas the Russian children showed less incidences of egg and milk allergies and type I diabetes antibodies, illustrating that the gut microbiota composition and the type of LPS present affect immune tolerance and therefore the development of autoimmune diseases. Therefore, they concluded that early exposure to various microorganisms is critical for proper immune system development and balance so our bodies "learn" what is foreign and what is self, and what warrants an inflammatory immune response or not (Elston, 2006).

The human microbiome is the "forgotten organ" and is as unique as a fingerprint (Franzosa et al., 2015). Humans are home to trillions of microbes such as archea, small eukaryotes, fungi, parasites, viruses and yeast that outnumber our cells 10 to 1 (Lepage et al., 2013; Ursell et al., 2012), which are fundamental to host metabolism and immune function (Clemente et al., 2012; Lozupone et al., 2012; Neu, 2015). Gut microorganisms are essential for host nutrient digestion, SCFA production, synthesis of vitamins, gut hormone production, fat storage, gut permeability, and determining the host's susceptibility to gastrointestinal infections. The gut microbiota can also protect the host from pathogens (Brown et al., 2015). Although studies are still in their infancy, experts agree that characteristics of a healthy and diverse microbiome ecosystem include stability, resistance to change and the ability to return to equilibrium after biotic (e.g. virus, bacteria, small eukaryote, prion or metazoan) or abiotic stress (Morgan and Huttenhower, 2012). Along with overly hygienic lifestyle practices such as the overuse of antibacterial soap (Blaser and Falkow, 2009) and drinking chlorinated water (Rook, 2012), the well-

know abiotic stressors are the Western diet (Brown et al., 2012; Falony et al., 2016; Kau et al., 2011), toxins (Spanogiannopoulos et al., 2016), antiobiotics (Littman and Pamer, 2011), medication (Falony et al., 2016; Spanogiannopoulos et al., 2016; Wilson and Nicholson, 2009) such as proton pump inhibitors, metaformin, statins and laxatives, and smoking (Zhernakova et al., 2016) among others. Antibiotic treatment in healthy adults can cause considerable shifts in enrichment of the gut microbiota, including a reduction of SCFA production, for up to one year (Zaura et al., 2015). In infants, antibiotics can be detrimental to their developing gut microbiota (Palmer et al., 2007; Tanaka et al., 2009), and antibiotic resistant genes have been shown to peak after antibiotic treatment (Vatanen et al., 2016) making it a great public health concern is the rise in antibiotic resistant bacteria (Gombart, 2005).

4.1.1. Western lifestyle: urbanization and the microbiome

Urbanization around the world is expected to continue growing with 1.35 billion people inhabiting cities in the next 15 years, and the rate of noncommunicable diseases continue to rise in industrialized regions (Logan et al., 2015). The shift from Paleolithic times to industrialization has greatly affected the human microbiome (Cho and Blaser, 2012; Rook, 2012). With the modern conveniences of urban lifestyle has led to less exposure to indigenous viruses such as Hepatitis A, pathogenic bacteria such as *Heliobacter pylori, Salmonella spp., Mycobacterium tuberculosis,* parasites such as helminths and *Toxoplasma gondii* and commensal bacteria resulting in a less diverse and more dysbiotic gut microbiota compositions (Rook, 2012). Unlike most people who live in an industrialized society, indigenous populations do not lack microbial diversity in their microbiome. A study by Clemente *et al* (2015) in the Yanomamis of the Amazon and indigenous people of Papua New Guinea show that they have up to 50 times more microbial diversity than Americans probably because they do not take antibiotics, nor do they have a hyper-hygienic Westernized lifestyle (Martínez et al., 2015). Other studies comparing the fecal compositions of indigenous populations (Martínez et al., 2015; Yatsunenko et al., 2012) and mice (Turnbaugh et al., 2009) have shown how the Western lifestyle can reduce gut microbial diversity and SCFA production (Ríos-Covián et al., 2016) which are anti-inflammatory metabolites produced by commensal bacteria (Del Chierico et al., 2014; Figure 2).

4.1.2. Western lifestyle: diet and gut microbiota

New research has shown that diet can greatly influence the gut microbiota composition (Fasano, 2011) which can play a big role in the host's health. Dietary changes can account for up to 57% of gut microbiota changes, whereas genes account for no more than 12% (Brown et al., 2012). Short term consumption of a mostly animal or mostly plant diet can dramatically alter the microbiota composition and function (David et al., 2014) as fast as 24 hours (Aguirre et al., 2016).

Furthermore, the gut microbiota ferment and metabolize animal and vegetable proteins differently (Hughes et al., 2000). Bacteria, such as those from the Bacteroides phylum, ferment certain amino acids and proteins resulting in other potentially toxic byproducts such as ammonia, amines, volatile sulfur compounds, phelonic compounds and sulphides (Hughes et al., 2000), which have been associated with IBD, intestinal permeability and other inflammatory conditions (Marchesi et al., 2016). Moreover, diets high in animal protein and fat, such as the Western diet, can lead to higher colonic pH that facilitates the colonization of pathogenic bacteria such as *Escherichia coli* (Marchesi et al., 2016). However, eating resistant starch can ameliorate the negative effects of high animal protein consumption (Taciak et al., 2015; Toden et al., 2006). Artificial sweeteners such as aspartame can also negatively affect gut composition and have been shown to cause dysbiosis in rat models, which increases the risk for suffering an autoimmune disease (Suez et al., 2014). In summary, Deehan and Walter (2016) declared that the Western diet is a key driver of microbiota diversity loss and the rises in non-communicable diseases, like autoimmune diseases (Figure 2).

4.1.3. Maternal lifestyle in Western countries: vertical transmission of the microbiota

The composition of the gut microbiota is also influenced by the maternal microbiota via vertical transmission, which can prepare offspring for host-microbial mutualism facilitating the neonate's innate immune system development and the transmission of antibodies during pregnancy and those found in breast milk (Agüero et al., 2016; Figure 3). Evidence now shows that fetuses are not sterile and the earliest microbial inoculation comes from maternal intestinal, urogenital and oral bacteria (Rautava et al., 2012) that enter the uterus and amniotic fluid most likely via the bloodstream (Funkhouser and Bordenstein, 2013). Agaard *et al* (2014) discovered that the placenta has its own microbial niche that consists of commensal bacteria from the phyla Firmicutes, Tenericures, Proteobacteria, Bacteriodes and Fusobacteria. Furthermore, fetuses swallow about 400- 500 mL of amniotic fluid per day at term and the most common taxa found was *Escherichia coli,* which normally come from the mother's intestines (Neu, 2015). Bacteria genera such as *Lactobacillus* and *Enterococcus* from the mother's intestinal tract and urogenital region have been found in the umbilical cord, amniotic fluid, placenta membranes and meconium (Thum et al., 2012).

What is most worrisome is that several maternal lifestyle factors such as diet, health status, metabolism, type of birth, gestational weight gain, and antibiotic treatment affect the development of the offspring's microbial composition (Soderborg et al., 2016). For example, the delivery mode (caesarean or vaginal) affects the development of the human microbiome and immune system. Babies born caesarean are more susceptible to developing an autoimmune disease, which is alarming when nearly 50% of all births in the US and China are Caesarean and up to 80% in Brazil (Neu, 2015). Babies born cesarean are not exposed to commensal fecal and vaginal bacteria such as *Bifidobacterium, Protovella, Lactobacillus* and *Bacteroides* like babies born vaginally and have elevated levels of *Clostridia* (Salminen et al., 2004), pathogenic *Clostridium difficile* (Azad et al., 2011). Moreover, they have a higher exposure to microbes found on the mother's skin like *Staphylococcus spp.* making infants more susceptible to infections (Dominguez-Bello et al., 2010), allergies, asthma (Forsythe, 2011) and IBD (Bager et al., 2012). These differences in microbial composition can last between 6 months of age up to 7 years of age (Azad et al., 2011). Regardless of birth mode, many hospitals wipe off all of the new born's vertix, which is a thick coating on the skin that contains antimicrobial peptides such as defensins and catehelicidins as well as the innate immune proteins lysozyme and lactoferrin (Singh and Archana, 2008). Due to the vertix's antibacterial and wound healing properties, many experts including the OMS agree that the routine removal of the vertix immediately after birth is an unnecessary overly hygienic procedure (Singh and Archana, 2008).

Additionally, it has been observed that high maternal stress levels can negatively alter the gut microbiota composition and function, which is later transmitted to the infant leaving the offspring more prone to immune dysfunction. A study by Polloni *et al* (2015) showed that mothers who had more difficult pregnancies and experienced stressful events were much more likely to have children who developed food allergies, gastrointestinal symptoms and eczema compared to infants of mothers who experienced low stress levels during pregnancy (Figure 3). Theses results showed that the transmission of an unbalanced maternal microbiota could lead to aberrant immune responses in offspring.
Breast milk and the duration of breastfeeding is also critical aspect to proper colonization of the infant gut microbiota and immune system, for these reasons the World Health Organization (National Center for Chronic Disease Prevention and Health Protection, 2016) recommends exclusive breastfeeding from birth until 6 months of age (Hennet and Borsig, 2016). However, according to the Centers for Disease Control and Prevention's 2014 Breastfeeding Report Card, only 18.8% of babies in the U.S. are exclusively breastfed until 6 months of age, which is far below their 2020 goal of 60.6% (Centers for Disease Control and Prevention, 2015) and the global average of 39% (UNICEF, 2013).

Breast milk has its unique microbial composition (100-600 bacterial species; Funkhouser and Bordenstein, 2013) and is rich in approximately 200 different prebiotic oligosaccharides that are digested by beneficial bacteria such as *Bifidobacterium spp.* and *Bacteroides spp.,* and the unique composition of milk oligosaccharides therefore influences the colonization of the gut by certain bacteria (Hennet and Borsig, 2016) (Figure 3). The transfer of the anti-inflammatory cytoquines TGF-β and IL-10 from breast milk to neonate reduces bacterial translocation and promotes tolerogenic responses to the microbiota (Koch et al., 2016). On the other hand, babies who are formula-fed have higher levels of *Clostridium difficile,* which is found in the feces of babies who suffer allergies (Amarasekera et al., 2013). Importantly, breast milk passively transmits immune modulators to the infant such as IgA and IgG antibodies, neutrophils, macrophages and cytokines such as TNFα, and TGFβ (Azad et al., 2011). Furthermore, IgG2b and IgG3 antibodies regulate responses to translocated microbiota perhaps more than IgA, which promotes immune tolerance to the newly acquired microbes early in life (Koch et al., 2016). A study in 10 obese and 8 normal weight pregnant women showed that obesity and elective cesarean births caused less bacterial diversity in their breast milk compared to non-elective cesarean and vaginal births and mothers who were normal weight.

4.1.4. Microbiota genetic determinism

Besides hygienic practices, diet and the effect maternal lifestyle has on gut microbiota composition, genetic background may shape gut microbiota structure (Benson et al., 2010; Davenport, 2016; Goodrich et al., 2014, 2016; Lim et al., 2016; Org et al., 2015). One major concern with host genetic studies of microbioma is that vertical transmission of bacteria from parent to offspring can be confounded with measures of heritability. In a study by Lim *et al.* (2016) that evaluated the association of metabolic syndrome status with the gut microbiota and estimated the heritability of each taxon, the overall gut microbial community structures were more similar between monozygotic twins pairs than between dizygotic twin pairs, other family members or unrelated individuals. Among the taxa associated with metabolic syndrome, the phylum Actinobacteria had the highest heritability (45.7%), with the heritable estimate (h^2) values ranging between 13.1% and 45.7% for a total of 50 taxa. They also suggested that genetic variants in apolipoproteins (*APOA1/C3/A4/A5*) gene cluster are associated with specific gut microbial taxa, which might be important for gut microbiota related diseases. Similarly, Goodrich *et al.* (2014) examined the heritability of the gut microbiome using β-diversity metrics diversity of > 1,000 fecal samples from 416 monozygotic and dizygotic twin pairs. They observed that the relative abundance of the microbiota composition was significantly more similar between monozygotic than dizygotic twins, and that the most heritable taxon overall was from the *Christensenellaceae* family, followed by the *Ruminococcaceae* and *Lachnospiraceae* families. Interestingly the Bacteroidetes phylum was in general not heritable suggesting that environmental factors shape their colonization (Goodrich et al., 2014).

In a follow up study by Goodrich *et al.* (2016) in 416 monozygotic and dizygotic twin pairs analyzing 909 taxa shared similar findings from their previous study, yet their novel findings included an association between inheritable taxa and certain genotypes related with diseases involved in immune function and intestinal barrier function. For example, they found a significant association between the genus *SMB53* and genetic variants in the gene guanine nucleotide binding protein 12 (*GNA12)*, which is involved in barrier defense and ulcerative colitis. In the same line, Knights *et al*. (2014) analyzed 16S rRNA sequences of bacteria coming from intestinal mucosal biopsies from IBD patients and found that nucleotide-binding oligomerization domain 2 (*NOD2)* risk allele was associated with increased *Enterobacteriaceae* abundance. Davenport *et al*. (2016) identified between 10-14 common fecal bacterial taxa from Proteobacteria and Firmicutes phyla as heritable within each season using a 200,000 SNPs chip of the Hutterites population. Therefore, it is suggested that certain taxa, in particular within Firmicutes and Proteobacteria phyla, are consistently heritable regardless of environmental and cultural

differences between human populations.

Although not all human twin studies have clearly shown that the gut microbiome is heritable (Turnbaugh et al., 2009; Yatsunenko et al., 2012), animal studies have shown promising results that certain taxa are heritable and have confirmed that the crosstalk between the unique host's immune-related genes and microbiota could play a role in disease. For example, a genome-wide association study (GWAS) by Blekhman *et al.* (2015) revealed associations between the abundance of certain microbial taxa and 83 genetic variations, some of then related to immune-related pathway genes. A SNP-based approach was used to estimate the heritability of the gut microbiome in a controlled environment in mice. Heritability levels (from 0.3 to more than 0.5) were discovered for 350 major taxa in mice, including *Roseburia spp.* whose abundance could be correlated with obesity and interleukin-1 receptor-associated kinase 4 (*IRAK4)* expression which is involved in TLR signaling (Org et al., 2015). Benson *et al* (2010) examined possible genetic factors that affect the gut microbiota of 645 mice using 16S rRNA pyrosequencing and concluded that individual host genotype largely contributes to 64 taxonomic groups.

4.2. The context of the hygiene hypothesis in our modern world: autoimmune diseases in Western countries and possible causes

There are about 80 identified autoimmune diseases including MS, systemic lupus erythematosus, rheumatoid arthritis, type I diabetes and Sjögren's syndrome (National Institutes of Health Fiscal Years 2010 & 2011). In the U.S., approximately 8% of the population suffers from autoimmune diseases, 78% of whom are women (Fairweather and Rose, 2004) and the rates of several autoimmune diseases continue to increase such as inflammatory bowel disease (IBD), which include Crohn's disease and ulcerative colitis characterized by small intestine and colon inflammation, and MS which is damage of the myelin that insulate the brain, spinal cord and nerve cells (Versini et al., 2015). In an analysis of the global prevalence of 31 common autoimmune diseases, Cooper *et al* (2009) concluded that 5.3% of the global population have autoimmune diseases, the majority of whom are from Europe and the U.S. However, studies from Europe and the U.S. report that the prevalence of less severe diseases such as alopecia, vitiligo and psoriasis may be underestimated by 5–10 times (Cooper et al., 2009).
Although it is known that autoimmune diseases are classified by a lack of immune function and tissue damage due to an immune attack directed against a specific organ (Fasano, 2011) the exact causes remain elusive (Proal et al., 2013). One hypothesis is that several factors cause autoimmune diseases: (i) aberrant adaptive and innate immune system responses and the cause/consequent dysbiosis; (ii) loss of protective intestinal barrier functions; (iii) vitamin D_3 deficiency; and (iv) host genetic background.

4.2.1. Aberrant adaptive and innate immune system responses: role of gut microbiota and parasites in autoimmune diseases

The enteric immune system plays a critical role in innate and adaptive immunological functions (Jašarević et al., 2015; Owen and Mohamadzadeh, 2013), and about 70% of the human immune system resides in the GALT (Vighi et al., 2008). The innate immune system is primarily located within the GALT, which mediates innate and adaptive responses for specific host defense and oral tolerance induction (Mowat, 2003). The GALT is mainly composed of Peyer’s patches, lymphoid nodules embedded in the submucosa of the small intestine, and lymphocytes distributed throughout the lamina propria. Within the lamina propria of the intestines lies a variety of important immune components such as T helper, IgA-secreting B cells, dendritic cells and macrophages (Purchiaroni et al., 2013).

Gut commensal microorganisms, pathogens and nutrients that pass through the intestinal lumen are the first point of contact with the enteric immune system (Figure 4). There is a constant crosstalk between epithelial cells, gut microbiota and the GALT. Studies using germ-free animals have shown that

exposition to microorganisms and adequate commensal bacteria are crucial for proper host immune development and response (Wu and Wu, 2012). Germ-free animals, which are born in sterile conditions, have impaired GALT development and antibody production, and present less Peyer's patches, cellular lamina propria and plasma cells in germinal center of the mesenteric lymph node compared to animals raised in normal conditions (Wu and Wu, 2012).

Epithelial cells, Peyer's patch dendritic cells (DCs), and macrophages within the lamina propria present pattern recognition receptors (PRRs), which are responsible for different immune responses when facing dysbiosis or abiotic stress (Purchiaroni et al., 2013). TLRs are a class of PRR that are found on cell membranes or in endosomes, whereas NOD2 receptors, another type of PPR, are located in the cytosols (Belkaid and Hand, 2014). TLRs include: TRL2 which fortifies the intestinal barrier, TLR4 which recognizes LPS (Antico et al., 2012), TLR5 which recognize bacteria flagellin in intestinal epithelial cells and DCs (Fulde and Hornef, 2014) and TLR7 which recognizes viral RNA (Kabouridis and Pachnis, 2015). Upon biotic stress, TLRs recruit the signaling adaptors myeloid differentiation primary response protein 88 (MyD88) and the TIR-domain-containing adaptor protein inducing interferon-β which signals molecules by activating nuclear factor- kappa β (NF-κβ), p50 and p65 subunits and the mitogen-activated protein kinase (MAPK) pathway (Peterson and Artis, 2014). The NF-κβ signaling pathway regulates the expression of genes involved in inflammation, immune responses as well as cell proliferation, survival and apoptosis. Besides interferon-β, LPS and proinflammatory cytokines such as TNF-α and IL-Iβ are also able to activate the release of NF-κβ and subsequently the transcription of its inflammation genes (Pasparakis, 2008; Figure 4).

Like TRLs, NOD2 proteins signal through pathogen associated molecular patterns (PAMPs) (Biswas et al., 2012). The *NOD2* gene is expressed in intestinal epithelial cells, DCs, Paneth cells and in a low levels in T cells, and play a key role in regulating antimicrobial activity of crypts that are independent of the TLR-MyD88 pathway. Additionally, upon pathogen recognition, pro-inflammatory cytokines such as IL-2 stimulates the production of IFN-δ-secreting T-helper 1 (Th1) response, whereas IL-1, IL-6 and IL-23 production drives T helper 17 (Th17) response. In turn, Th1 and Th17 release pro-inflammatory cytokines that stimulate macrophages and neutrophils to attack pathogens (Clemente et al., 2012). On the other hand, when immature DCs interact with TGF-β or in the presence of IL-10, they can stimulate and anti-inflammatory Treg response (Belkaid and Hand, 2014; Mowat, 2003; Purchiaroni et al., 2013) resulting in local IgA production, systemic tolerance and enteric immune homeostasis (Mowat, 2003). IgA is the most abundantly produced antibody in the body and plays an important role in mucosal defense against viruses, toxins and have been shown to be bacteria-specific (Geuking et al., 2014). Treg cells functionality in the intestines and colon can be decreased by an antibiotic treatment (Tomkovich and Jobin, 2016), which could later affect immune tolerance to enteric microorganisms.

Currently many microbiologists and immunologists believe that infections (viral, bacterial or from parasites) can help the immune system develop properly reducing the possibility for developing allergies or autoimmune diseases, especially when this exposure occurs early in life (Elston, 2006; Maizels et al., 2014). Common missing microbes in Western countries are parasites that can increase immune tolerance and down regulate autoimmune reactions (Clemente et al., 2012; Martínez et al., 2015; Schnorr et al., 2014; Yatsunenko et al., 2012). More than 400 parasite species infect humans (Lukeš et al., 2014) and approximately one fourth of the population is infected with a helminth parasite (Maizels et al., 2014). Each parasite species adapts to the host and alters the immune system in a unique way for their own survival (Berrilli et al., 2012). For example, the *Nectar americanus* helminth and the *Trichuris suis* eggs can stimulate Treg cells and sometimes TGF-β cells that lower allergic symptoms (Campolina et al., 2013; Maizels et al., 2014)**.** In general, helminths can increase a Th2 response while reducing Th1/Th17 differentiation (Bancroft et al., 2012) thereby stimulating the production of Treg and B cells and type 2 macrophages. Therefore, a mild immune reaction from a parasitic infection stimulates

Treg cell production reducing inflammatory responses that permit the parasite to survive in the host. Interestingly, helminth proteins have a similar structure to about 40% of common allergens such as dust mites and cockroaches suggesting that helminth infections can "educate" the immune system and inhibit a possibly overactive response to a benign allergen (Helmby, 2015).

A recent study supporting the hygiene hypothesis in *NOD2* $^{-/-}$ knockout mice showed that intestinal helminth infection prevented the colonization of inflammatory *Bacteriodes vulgatus* and promoted the colonization of protective microbiota enriched in Clostridiales, which was caused by a Th2 immune response. On the other hand, de-worming treatment decreased Clostridiales and increased Bacteroidales. The authors concluded that genetically susceptible individuals, such as IBD patients, might benefit from altering the gut towards a more healthy composition (Ramanan et al., 2016). Additionally, helminths might have an anti-inflammatory effect as they contain glycoprotein ES-62 which has been used therapeutically for its ability to reduce histamine and pro-inflammatory cytokine production (Wammes et al., 2014). In fact, anecdotal evidence has shown that parasitic infection diminishes or eliminates allergic reactions (Clemente et al., 2012) probably because helminths for example can modulate the gut microbiota and modulate DCs towards a more tolerogenic phenotype (Versini et al., 2015).

Though highly controversial, some believe that helminth therapy is an effective way to "re-educate" the immune system and restore immunological homeostasis to reduce or eliminate allergies, food intolerances and even autoimmune diseases (Giacomin et al., 2016; Versini et al., 2015). There is evidence that parasite infection can reduce a typical IgE allergic response and symptoms, especially in adolescents who have food allergies (Bashir et al., 2002; Lukeš et al., 2014). Some patients who are infected with a low dose of helminths seem to tolerate them well without too many adverse reactions and a reduction in allergic symptoms (Elston, 2006). In a survey of 7,155 Ethiopian children, Dagoye *et al* (2003) concluded that *Ascaris* and probably other helminths protect against asthma in young children. Despite some positive results, that does not mean that this alternative treatment is recommended for those with allergies or autoimmune diseases. There is a lack of well-designed studies and many results are inconclusive (Campolina et al., 2013; Jouvin and Kinet, 2012; Wammes et al., 2014). Moreover, these experiments are also very risky and can cause tissue damage, inflammation (Rautava et al., 2012) anemia, nutrient deficiencies (Mpairwe et al., 2014) and other unpleasant side effects. For example, high doses of *Tricuris suis* and *N. americanus* can be pathogenic (Schnorr et al., 2014) losing their immunogenic regulatory effects. The inconsistent results could be due to host genetics, the species used, dose of infection, duration of the experiment, crosstalk between gut microbiota and parasites, and host lifestyle factors (Wammes et al., 2014).

4.2.2. Loss of protective intestinal barrier functions and autoimmune diseases

Aforesaid, another factor associated with the autoimmune diseases is the loss of protective intestinal barrier functions (Fasano, 2011; Figure 4). Toxins, pathogens and certain inflammatory proteins such as gluten can cause a loosening of the tight junction proteins, or intestinal permeability, that leads to the escape of macromolecules and bacterial components like LPS from the intestines into the blood stream triggering an inflammatory TNF-α and IFN-γ response (Fasano, 2011; Figure 4). The consumption of high amounts of fat may increase the levels of LPS, produce low levels of chronic inflammation, and increase intestinal permeability (de La Serre et al., 2010), while affecting TRL signaling (Myles, 2014) or MyD88 (Fulde and Hornef, 2014). However, early exposure to ligands such as LPS "train" the intestinal epithelial cells not to be hyper-responsive to TLR induction thus preventing excessive pro-inflammatory immune responses (Belkaid and Hand, 2014; Chen et al., 2015b).
Pendyala et al. (2012) reported that healthy humans who ate a high-fat Western diet for a month had up to 71% increase in plasma endotoxins such as LPS. Similarly, a diet high in sugar, salt and saturated fat is pro-inflammatory and can disrupt the immune system. Simple sugars can decrease white blood cell

phagocytosis and can increase inflammatory cytokines (Myles, 2014). The Academy of Nutrition and Dietetics has recently established that adequate fiber intake is approximately 25 g for adult women and 38 g for adult men (Dahl and Stewart, 2015), yet the Western diet is characteristically low in fiber and consequently SCFAs which are important for Treg cells in the gut. Furthermore, diets high in processed foods tend to be deficient in nutrients (Logan et al., 2015), such as vitamins A, B3, B9 and D, are necessary for proper immune function, further illustrating the diet-gut-autoimmune connection (Deehan and Walter, 2016). On the other hand, diets rich in fiber increase gut microbial diversity and immune health because the microbiota feed on foods such as legumes, cereals, vegetables and other polysaccharides produce SCFA such as acetate, butyrate and propionate (Sonnenburg and Sonnenburg, 2014) that are anti-inflammatory (Del Chierico et al., 2014; Schwab et al., 2007; Selmi, 2011; Vinolo et al., 2011). These beneficial metabolites improve intestinal barrier function, inhibit the noxious effects of pathogens (Puddu et al., 2014) serve as the main energy source for colonocytes and also prevent mucosal degradation (Canani et al., 2011), which could prevent hyper intestinal permeability (Ríos-Covián et al., 2016) and prevent LPS translocation and inflammatory immune responses. Butyrate and propionate help control intestinal inflammation, which may benefit IBD patients via inducing Treg differentiation (Ríos-Covián et al., 2016), and can modulate probiotics to stimulate Treg cells production (Dwivedi et al., 2016). High fiber and plant-based food intake can also diminish the harmful effects of byproducts from digested protein such as amines and phenolic compounds (Martínez et al., 2015).

4.2.3. Vitamin D_3 deficiency and immune system

Vitamin D_3 is known mostly for its maintenance of calcium, blood pressure and electrolyte regulation, but it is also an essential component of our immune system (Zhang et al., 2013). Although the hormone leptin is known to have a role in autoimmune diseases due to its Th1 promotion and proliferation of Treg cells (Matarese et al., 2007), the biologically active component $1,25(OH)_2D_3$ of vitamin D_3 plays a more important role in regulating inflammation, pathogen proliferation and intestinal barrier maintenance, as well as gut dysbiosis (Table 3).

Vitamin D_3 regulates immune homeostasis by inhibiting the synthesis of pro-inflammatory cytokines in a variety of cells types and promotes tolerogenic responses through the reduction of Th1 and Th17 cells (Kamen and Tangpricha, 2010, Adorini, 2002; Albert et al., 2009; Assa et al., 2014; Kamen and Tangpricha, 2010; Proal et al., 2013; Sun, 2010). D_3 also plays a role in promoting a healthy gut by maintaining intestinal barrier health, controlling the expression of the tight junction proteins (Zhang et al., 2013), preventing dysbiosis (Assa et al., 2014; Fujita et al., 2008; Kamen and Tangpricha, 2010, Wu et al., 2015) and pathogen colonization (e.g. *Salmonella*) when inflammation is mounted (Ooi et al., 2012; Ananthakrishnan et al., 2014; Liu et al., 2006; Weiss, 2011).

Additionally, $1,25(OH)_2D_3$ and vitamin D_3 analogs can up regulate the production of antimicrobial peptides such as cathelicidin and β-defensin making it a key factor in innate and adaptive immune responses in the gut and other barrier sites (Kamen and Tangpricha, 2010). For instance, BALB/c mice that were pretreated with $1,25(OH)D_3$ *in vitro* produced antimicrobial effects upon *Tricuris gondii* infection, possibly by stimulating a TLR2-induced cathelicidin expression in macrophages (Rajapakse et al., 2007). Yuk *et al* (2009) showed that $1,25(OH)_2D_3$ induces the antimicrobial peptide cathelicidin which activates the transcription of autophagy-related genes *Beclin-1* and autophagy related 5 (*ATG5)* in human monocytes (Yuk et al., 2009), which plays a role in regulating the adaptive immune system as well as the pathogenesis of Chron's disease (Sun, 2010).

Vitamin D_3 deficiency has also been associated with an increased risk of rheumatoid arthritis, systematic lupus erythematosus, MS, type I diabetes, IBD and other autoimmune diseases (Kamen and Tangpricha,

2010). Moreover, vitamin D_3 status has been inversely related to an increased risk for cesarean birth (Merewood et al., 2009; Scholl et al., 2012), preterm birth, small birth weight (Pérez-López et al., 2015), child allergies, eczema, asthma (Genuis, 2015) and type I diabetes (Sørensen et al., 2012). Of importance is the high rate of vitamin D_3 deficient pregnant women and babies, and there is a strong correlation between maternal and infant vitamin D_3 levels and infant health (Dawodu and Wagner, 2012). Disanto *et al.* (2012) performed a study in women from the UK and concluded that gestational UVB exposure could affect whether or not their offspring would suffer an immune related disease such as colitis and MS due to vitamin D_3 deficiency. Another study in mothers who supplemented with 6400IU/ day of vitamin D_3 were able to effectively and safely provide their infant with adequate vitamin D_3 through just breastfeeding (Hollis et al., 2015). Therefore, levels of maternal dietary vitamin D_3 intake and serum $1,25(OH)_2D_3$ can directly affect infant vitamin D_3 status, immune system development (Clancy et al., 2013; Pludowski et al., 2013; Spiro and Buttriss, 2014) as well as risk of developing autoimmune diseases (Disanto et al., 2012; Fronczak et al., 2003; Lucas et al., 2008; Mirzaei et al., 2011; Sørensen et al., 2012) and allergies (Nwaru et al., 2010)(Wagner et al., 2006; Figure 1)**.** Maternal vitamin D_3 status is an important health concern that needs more attention especially since supplementation in the mother and/or infant has proven to be effective at improving vitamin D_3 levels (Wagner et al., 2006). For this reason it is recommended that women who are pregnant or breastfeeding supplement daily with vitamin D_2 (Jean-Claude Souberbielle, 2010; O'Mahony et al., 2011; Wagner et al., 2006) to avoid adverse pregnancy, birth outcomes and deficiency (Mulligan et al., 2010; Palacios and Gonzalez, 2014; Figure 3; Table 4).

Though global population studies of vitamin D status have several limitations, approximately one billion people worldwide suffer from vitamin D deficiency (Gombart, 2009). An estimated 20-80% of the population in the Canadian and European population are vitamin D deficient while approximately one third of the U.S. population, 70% of the black non-Hispanic and 40% of the Mexican population are deficient (Hossein-nezhad and Holick, 2013). Deficiency is very common in people from Asia and the Middle East (Holick and Chen, 2008; Hossein-nezhad and Holick, 2013; Palacios et al., 2016), yet there is a surprising lack of research in African and South American populations (Palacios et al., 2016). Other populations at risk are infants and children > 5 years old, and the elderly 65 years and older (Hossein-nezhad and Holick, 2013; Jean-Claude Souberbielle, 2010; Spiro and Buttriss, 2014). People with dark-skin color and those who wear clothes that cover the whole body such as in the Middle East (Kamen and Tangpricha, 2010; Spiro and Buttriss, 2014).

The lack of consensus for what defines "optimal" serum $1,25(OH)_2D_3$ levels make it difficult to diagnose deficiency as well as implement fortification policies (Lips, 2010; Spiro and Buttriss, 2014). However, a review of 25 experts recommends that serum $1,25(OH)_2D_3$ levels be at least 30-40 ng/mL for optimal health benefit and that autoimmune patients could benefit from supplementation (Jean-Claude Souberbielle, 2010). Yet this amount may not be sufficient for populations at risk such as pregnant mothers, babies, elderly people (Spiro and Buttriss, 2014) or countries in latitudes north of 40º (Spiro and Buttriss, 2014; Figure 3).

Though not universally accepted (Proal et al., 2009; Waterhouse et al., 2009), the vitamin D_3 deficiency model arose due to many studies that have illustrated that autoimmune patients have low serum $1,25(OH)_2D$ levels and that vitamin D_3 supplementation helps alleviate symptoms (Albert et al., 2009). Supplementation of $1,25(OH)_2D_3$ attenuates the Th2 response (Coughlan et al., 2012). When supplementing daily, vitamin D_2 and vitamin D_3 have similar effects on $1,25(OH)_2D_3$ levels (Holick et al., 2008), however, vitamin D_3 appears to be more effective when supplementing intermittently (Jean-Claude Souberbielle, 2010). However, vitamin D's role in autoimmune diseases is not as simple as the presence or not of its deficiency.

$1,25(OH)_2D_3$ and its metabolites are the results of many integrated enzymatic and non-enzymatic transformations with numerous intermediaries that are regulated by the host genome, host epigenome, gut microbiota, food and drink consumption, drug use and exposure to pollutants. The need for a deeper understanding of how regulatory metabolic networks associated with vitamin D function in response to autoimmune diseases has led to increased efforts to model multiple "omic" dimensions simultaneously to gain a better understanding of how different biological systems are connected. For instance, $1,25(OH)_2D_3$ also epigenetically regulates various histone acetylases, deacetylases and demethylases (Falony et al., 2016) and is associated with at least 160 pathways including those of autoimmune diseases and cancer (Hossein-nezhad et al., 2013) representing a much wider array of genetic control by D_3 than previously thought. Moreover, severe vitamin D_3 deficiency can cause leukocyte DNA methylation changes which could affect leukocyte differentiation, cellular development and other metabolic processes (Fetahu et al., 2014), whereas genetic variants in the VDR can also modify the molecular control associated to immune response (see below). In fact, autoimmune diseases occur in ~50% of identical twins (Christen and Herrath, 2005; Ermann and Fathman, 2001) illustrating that genetic background and environmental factors and lifestyle play a big role in the pathogenesis of these diseases.

4.2.4. Vitamin D receptor polymorphisms and immune system

Vitamin D_3 activates the vitamin D receptor (VDR) which is a nuclear receptor located in immune such as macrophages, DCs and activated T cells and other types of cells in about 30 different tissues (Gombart, 2009). The activated VDR binds to vitamin D responsive elements (VDRE) to form a heterodimer (Zhang et al., 2012), which can interact with histone acetylases to induce transcription and regulate the expression of more than 2,000 genes (Hossein-nezhad and Holick, 2013; Proal et al., 2013; Figure 1). For instance, VDREs are found in the promoter region of HLA DRB1 genes (Antico et al., 2012) and predominantly in introns and intergenic intervals, as well as in the promoters of the antimicrobial *CAMP* and beta defensin 2 (*defB2*) (Zhang et al., 2012). VDRs are also known to stabilize tight junction structure of the intestinal epithelial cells in a vitamin D_3 -dependent manner (Sun, 2010; Zhang et al., 2013). Froicu and Cantorna (2007) discovered that VDR expression was critical for a proper innate immune response in mice with colitis, and VDR knockout mice were more susceptible to LPS-induced endotoxemia and death (Froicu and Cantorna, 2007; Table 3). Remarkably, VDR agonist olmesartan and subinhibitory dosages of certain bacteriostatic antibiotics can reverse systemic lupus erythematosis, rheumatoid arthritis, autoimmune thyroid disease, type I and II diabetes mellitus, among other autoimmune diseases (Sun, 2010; Figure 4).

Recently, the genetic variants of VDR gene has been associated with anti-inflammatory immune response (Sun, 2010), lower serum vitamin D_3 and with various autoimmune diseases such as IBD, Chron's disease, type I and II diabetes, multiple sclerosis (Laing and Ferguson, 2015). VDR gene which is located on chromosome 12q13.1 spans over 1~00kb and has an extensive promoter region (Uitterlinden et al., 2004) and four described polymorphisms. Three polymorphisms, *Bmsl* (rs1544410) and *ApaI* (rs7975232) in intron 8 and *TaqI* (rs731236) in exon 9 have been identified at the 3'-end of the gene, whereas *FokI (*rs10735810) is located in the start codon (Kamel et al., 2014). Uitterlinden *et al* (2004) believe that >100 polymorphisms could lie in the VDR gene suggesting that the mechanisms for how this gene and its SNPs function remains largely unknown. Kamel *et al* (2014) hypothesize that the VDR polymorphisms may be a marker for functional variants that alter VDR expression rather than being the locus associated with disease or lower concentrations of $1,25(OH)_2D_3$. In fact, a GWAS study in 5,575 people showed that VDR genetic variants accounted for only 5.2% of the variance in $1,25(OH)_2D_3$ levels (Hossein-nezhad and Holick, 2013). To make matters more complex, several genomic studies associating VDR genetic variants with autoimmune diseases among diverse populations have heterogeneous outcomes possibly due to genotype variation and its effect on the immune system (Laing and Ferguson, 2015) illustrating that the mechanisms in which the VDR

promoter region interacts with the rest of the human genome remains largely unknown. The inconclusive results could be due to varying lifestyles, epigenetic changes and microbiota subproducts that might affect the promoters of genes associated with immune responses.

In summary, it is important to note that the host genome is not the only factor in autoimmune disease development because epigenetic alterations, metagenome profiles and lifestyle factors, such as vitamin D_3 supplementation also play a role in the transcriptome expression that affect immune health and autoimmune disease development (Hossein-nezhad and Holick, 2013).

4.3. The context of the hygiene hypothesis in our modern world: the connection between microbiota, vitamin D_3, VDR function and autoimmune diseases

Aforesaid, proper vitamin D_3 levels, VDR function and healthy microbiota are playing a significant role on restoring gut homeostasis and thus attenuate autoimmune symptoms. In mice, vitamin D_3 has been shown to interact with the symbiont *Bacteroides fragilis* which produces polysaccharide A that induces Treg cells in the gut and protect against experimental autoimmune encephalomyelitis in mice (Ochoa-Repáraz and Kasper, 2014). Another study showed that mice raised on a vitamin D_3 sufficient diet had 50 times more colonic bacteria than mice raised on a vitamin D_3 poor diet (Lagishetty et al., 2010). The vitamin D_3 deficient mice also presented deregulated colonic containment of enteric bacteria which the authors believe could be a possible mechanism behind colitis susceptibility. Furthermore, they hypothesized that colonic metabolism of $25(OH)D_3$ to $1,25(OH)_2D_3$ promoted antimicrobial peptide angiogerin-4 (*ANG4*) expression in the intestines promoting enteric bacteria regulation, though it is unclear as to whether vitamin D_3 directly regulates transcription or not (Lagishetty et al., 2010). Another experiment illustrated in C57BL/6 mice that $1,25(OH)_2D$ and VDR attenuated DSS-induced colitis by decreasing *Helicobacteraceae* (Proteobacteria phyla) which lead to less colitis symptoms (Ooi et al., 2012). In humans, vitamin D deficiency plays a potential role in MS patients by altering the gut microbiota composition by increasing the abundance of microbes that produce immune tolerance (*Akkermansia*) and anti-inflammatory butyrate such as *Faecalibacterium* and *Coprococcus* for example (Cantarel et al., 2015). Interestingly, butyrate can induce mRNA expression of the VDRs, which can be further increased by the binding of $1,25(OH)_2D_3$ to its receptor (Schröder et al., 2011), and decrease inflammation in a colitis model (Sun, 2015). Furthermore, serum vitamin D_3 levels above 40ng/mL in untreated MS patients have been associated with increased *Ruminococcaceae* which produces anti-inflammatory SCFA, suggesting intestinal inflammation and vitamin D_3 deficiency may be underlying causes of MS (Tankou et al., 2015).

Perhaps the most well studied effects vitamin D_3 has on microbiota and autoimmune diseases is in IBD. Vitamin D_3 supplementation has also shown to decrease the risk of *Clostridium difficile* infection in IBD patients by inducing cathelicidin (Ananthakrishnan et al., 2014) and promoting microbiota homeostasis. Furthermore, $1,25(OH)_2D_3$ has also been shown to induce autophagy upon intracellular *Mycobacterium tuberculosis* infection further demonstrating vitamin D_3 's role in altering the gut microbiota towards homeostasis (Yuk et al., 2009).

When focusing on the VDR functions, Wu *et al* (2010) discovered that enteric *Salmonella typhimurium* infection increased colonic VDR concentrations in specific pathogen free VDR-/- knockout mice which positively correlated with regulation of NF-kB, and increased levels of *Salmonella* infection in the cells of the upper and lower epithelial crypts. Furthermore, VDR-/- knockout mice had increased bacterial burden and mortality upon infection compared to the VDR+/+ group. *Cyp27b1* and VDR-/- knockout mice have also shown higher prevalence of dysbiosis because they had less antimicrobial peptide E-cadherin expression in the gut and less tolerogenic DCs than wild type mice (Ooi et al., 2012). Another experiment illustrated in C57BL/6 mice that $1,25(OH)_2D_3$ and VDR attenuated dextran sulfate sodium

(DSS)-induced colitis by decreasing *Helicobacteraceae* (Proteobacteria phyla) which lead to less colitis symptoms (Ooi et al., 2012). Wu *et al* (2015) conducted a study in VDR-/- knockout mice with induced colitis to see the role of VDR and autophagy play in IBD pathogenesis. They induced colitis using DSS. They discovered that $VDR^{-/-}$ knockout mice led to a down regulation at the transcription and translation level of *ATG16L1*, and caused impaired Paneth cell function, dysbiosis and inflammation. Interestingly, without any DSS treatment, $VDR^{-/-}$ knockout mice had less bacterial abundance and butyrate producing bacteria and higher levels of *Escherichia coli* and *Bacteroides* than the control group. Butyrate administration up regulated VDR expression in the intestines which attenuated inflammation in a colitis model (Wu et al., 2015) as seen in a similar experiment (Sun, 2015; Table 2). These are probably the results of mRNA expression activation of the VDRs, which can be further increased by the binding of 1,25-dihydroxyvitamin D_3 to its receptor (Schröder et al., 2011).

Similarly, it has been observed that some pathogenic microorganisms (*Borrelia burgdorferi, Cytomegalovirus, Mycobacterium leprae, Aspergillus fumigatus* and *Mycobacterium tuberculosis* and the Epstein-Barr virus) can produce substances that block VDRs thus causing immune impairment and allowing pathogens to accumulate within the microbiota ecology (Proal et al., 2013; Waterhouse et al., 2009). Subsequently pathogens can flourish in the gut further inhibiting the innate immune system causing a down regulation of thyroid-α-1, adrenal, and glucocorticoid nuclear receptors among others which also produce antimicrobial peptides (Proal et al., 2009; Waterhouse et al., 2009). For these reasons Waterhouse et al (2009) believe that restoring VDR function, not vitamin D_3 levels, is key to preventing or improving autoimmune symptoms. Improving VDR function is a new therapeutic target for autoimmune diseases (Proal et al., 2013), yet more studies are needed to fully understand the role VDR function plays in preventing autoimmune diseases.

5. Conclusion

Western society's overly hygienic lifestyle, such as antibacterial soaps, excessive antibiotic use and de-worming, which leads to reduced exposure to beneficial microbes that help humans develop proper immune responses. Research also suggests that western lifestyle and maternal lifestyle factors, such as diet, stress and vitamin D_3 status, greatly affect the offspring's gut microbiota, which can subsequently alter the development of the immune system. Furthermore, recent evidence shows that the microbiota is heritable suggesting that the deleterious effects of modern society could perpetuate throughout generations and lead to an increase in unhealthy gut microbiota and aberrant immune responses and thus, a rise in autoimmune diseases. Along with this, one of the main factors that leads to autoimmune diseases is vitamin D_3 deficiency and vitamin D receptor (VDR) dysfunction. Vitamin D_3 has a significant immunological role in the presence of an infection, pathogen and dysbiosis in the attenuation and prevention of an autoimmune disease. Therefore, more emphasis needs to be put on maintaining sufficient vitamin D_3 levels for at risk populations such as pregnant women.

Studies in humans and mice have demonstrated that vitamin D_3 also promotes gut homeostasis by inducing antimicrobial peptides angiogenin-4 and E-cadherin in colitis models as well as autophagy. Additionally, in humans, vitamin D_3 supplementation have modified gut microbiota composition towards homeostasis and helped to prevent autoimmune disease. Therefore, healthy gut microbiota promoting lifestyle habits and maintaining adequate vitamin D_3 levels should be encouraged to ensure a healthy immune system. Vitamin D_3 supplementation has proven to increase the colonization of beneficial bacteria in the intestines such as *Ruminococcaceae, Akkermansia, Faecalibacterium, and Coprococcus.* Vitamin D_3 recommendations varies from 5-20 μg depending on the age, population and country. Interestingly, VDR knockout studies in mice have demonstrated the role of VDR and its genetic variants have on controlling intestinal inflammation, susceptibility to pathogenic infection and LPS-induced endotoxemia. More well designed studies of vitamin D status, VDR function and metagenomics in human cohorts are needed to better understand how the development of the gut

microbiota sets the stage for autoimmune disease development. It is therefore important for future research to tease out how vitamin D_3, VDR and the intestinal microbiota influence and regulate immune responses in order to better understand how Western lifestyle is impacting the intestinal microbiota. In the future, it may be possible to use the gut microbiome as a tool to predict potential autoimmune disorders before conventional diagnostic tools.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

Not applicable

Competing interests

The authors declare no competing interests

Funding

Not applicable

Authors' contributions

AC wrote and designed the main text and designed all figures. NM provided feedback and revision of manuscript. Both authors have edited and approved the final version of the manuscript.

Literature Cited

Aagaard, K., Ma, J., Antony, K.M., Ganu, R., Petrosino, J., and Versalovic, J. (2014). The placenta harbors a unique microbiome. Sci. Transl. Med. *6*, 237ra65.

Adorini, L. (2002). Immunomodulatory effects of vitamin D receptor ligands in autoimmune diseases. Int. Immunopharmacol. *2*, 1017–1028.

Adorini, L., and Penna, G. (2008). Control of autoimmune diseases by the vitamin D endocrine system. Nat. Rev. Rheumatol. *4*, 404–412.

Agüero, M.G. de, Ganal-Vonarburg, S.C., Fuhrer, T., Rupp, S., Uchimura, Y., Li, H., Steinert, A., Heikenwalder, M., Hapfelmeier, S., Sauer, U., et al. (2016). The maternal microbiota drives early postnatal innate immune development. Science *351*, 1296–1302.

Aguirre, M., Eck, A., Koenen, M.E., Savelkoul, P.H.M., Budding, A.E., and Venema, K. (2016). Diet drives quick changes in the metabolic activity and composition of human gut microbiota in a validated in vitro gut model. Res. Microbiol. *167*, 114–125.

Albert, P.J., Proal, A.D., and Marshall, T.G. (2009). Vitamin D: the alternative hypothesis. Autoimmun. Rev. *8*, 639–644.

Amarasekera, M., Prescott, S.L., and Palmer, D.J. (2013). Nutrition in early life, immune-programming and allergies: the role of epigenetics. Asian Pac. J. Allergy Immunol. Launched Allergy Immunol. Soc. Thail. *31*, 175–182.

Antico, A., Tampoia, M., Tozzoli, R., and Bizzaro, N. (2012). Can supplementation with vitamin D reduce the risk or modify the course of autoimmune diseases? A systematic review of the literature. Autoimmun. Rev. *12*, 127–136.

Aranow, C. (2011). Vitamin D and the Immune System. J. Investig. Med. Off. Publ. Am. Fed. Clin. Res. *59*, 881–886.

Assa, A., Vong, L., Pinnell, L.J., Avitzur, N., Johnson-Henry, K.C., and Sherman, P.M. (2014). Vitamin D Deficiency Promotes Epithelial Barrier Dysfunction and Intestinal Inflammation. J. Infect. Dis. *210*, 1296–1305.

Azad, M.B., Kozyrskyj, A.L., Azad, M.B., and Kozyrskyj, A.L. (2011). Perinatal Programming of Asthma: The Role of Gut Microbiota, Perinatal Programming of Asthma: The Role of Gut Microbiota. J. Immunol. Res. J. Immunol. Res. *2012*, *2012*, e932072.

Bashir, M.E.H., Andersen, P., Fuss, I.J., Shi, H.N., and Nagler-Anderson, C. (2002). An Enteric Helminth Infection Protects Against an Allergic Response to Dietary Antigen. J. Immunol. *169*, 3284–3292.

Belkaid, Y., and Hand, T.W. (2014). Role of the Microbiota in Immunity and Inflammation. Cell *157*, 121–141.

Benson, A.K., Kelly, S.A., Legge, R., Ma, F., Low, S.J., Kim, J., Zhang, M., Oh, P.L., Nehrenberg, D., Hua, K., et al. (2010). Individuality in gut microbiota composition is a complex polygenic trait shaped by multiple environmental and host genetic factors. Proc. Natl. Acad. Sci. U. S. A. *107*, 18933–18938.

Biswas, A., Petnicki-Ocwieja, T., and Kobayashi, K.S. (2012). Nod2: a key regulator linking microbiota to intestinal mucosal immunity. J. Mol. Med. Berl. Ger. *90*, 15–24.

Blekhman, R., Goodrich, J.K., Huang, K., Sun, Q., Bukowski, R., Bell, J.T., Spector, T.D., Keinan, A., Ley, R.E., Gevers, D., et al. (2015). Host genetic variation impacts microbiome composition across human body sites. Genome Biol. *16*, 191.

Brown, K., DeCoffe, D., Molcan, E., and Gibson, D.L. (2012). Diet-Induced Dysbiosis of the Intestinal Microbiota and the Effects on Immunity and Disease. Nutrients *4*, 1095–1119.

Brown, W.M.C., Davison, G.W., McClean, C.M., and Murphy, M.H. (2015). A Systematic Review of the Acute Effects of Exercise on Immune and Inflammatory Indices in Untrained Adults. Sports Med. - Open *1*.

Cacho, N., and Neu, J. (2014). Manipulation of the Intestinal Microbiome in Newborn Infants. Adv. Nutr. Int. Rev. J. *5*, 114–118.

Campolina, S.S., Araujo, M.S.S., Rezende, T.M.R.L., Matoso, L., Quites, H.F.O., Teixeira-Carvalho, A., Martins-Filho, O.A., Gazzinelli, A., and Correa-Oliveira, R. (2013). Effective anthelmintic therapy of residents living in endemic area of high prevalence for Hookworm and Schistosoma mansoni infections enhances the levels of allergy risk factor anti-Der p1 IgE. Results Immunol. *5*, 6–12.

Cantarel, B.L., Waubant, E., Chehoud, C., Kuczynski, J., DeSantis, T.Z., Warrington, J., Venkatesan, A., Fraser, C.M., and Mowry, E.M. (2015). Gut microbiota in MS: possible influence of immunomodulators. J. Investig. Med. Off. Publ. Am. Fed. Clin. Res. *63*, 729–734.

Cantorna, M.T., and Mahon, B.D. (2005). D-hormone and the immune system. J. Rheumatol. Suppl. *76*, 11–20.

Cantorna, M.T., McDaniel, K., Bora, S., Chen, J., and James, J. (2014). Vitamin D, immune regulation, the microbiota, and inflammatory bowel disease. Exp. Biol. Med. Maywood NJ *239*, 1524–1530.

Cho, I., and Blaser, M.J. (2012). The human microbiome: at the interface of health and disease. Nat. Rev. Genet. *13*, 260–270.

Clemente, J.C., Ursell, L.K., Parfrey, L.W., and Knight, R. (2012). The Impact of the Gut Microbiota on Human Health: An Integrative View. Cell *148*, 1258–1270.

Clemente, J.C., Pehrsson, E.C., Blaser, M.J., Sandhu, K., Gao, Z., Wang, B., Magris, M., Hidalgo, G., Contreras, M., Noya-Alarcón, Ó., et al. (2015). The microbiome of uncontacted Amerindians. Science Advances *1*, e1500183.

Cooper, G.S., and Stroehla, B.C. (2003). The epidemiology of autoimmune diseases. Autoimmun. Rev. *2*, 119–125.

Cooper, G.S., Bynum, M.L.K., and Somers, E.C. (2009). Recent insights in the epidemiology of autoimmune diseases: Improved prevalence estimates and understanding of clustering of diseases. J. Autoimmun. *33*, 197–207.

Coughlan, C.A., Chotirmall, S.H., Renwick, J., Hassan, T., Low, T.B., Bergsson, G., Eshwika, A., Bennett, K., Dunne, K., Greene, C.M., et al. (2012). The Effect of Aspergillus fumigatus Infection on Vitamin D Receptor Expression in Cystic Fibrosis. Am. J. Respir. Crit. Care Med. *186*, 999–1007.

Dagoye, D., Bekele, Z., Woldemichael, K., Nida, H., Yimam, M., Hall, A., Venn, A.J., Britton, J.R., Hubbard, R., and Lewis, S.A. (2003). Wheezing, allergy, and parasite infection in children in urban and rural Ethiopia. Am. J. Respir. Crit. Care Med. *167*, 1369–1373.

Davenport, E.R. (2016). Elucidating the role of the host genome in shaping microbiome composition. Gut Microbes *7*, 178–184.

David, L.A., Maurice, C.F., Carmody, R.N., Gootenberg, D.B., Button, J.E., Wolfe, B.E., Ling, A.V., Devlin, A.S., Varma, Y., Fischbach, M.A., et al. (2014). Diet rapidly and reproducibly alters the human gut microbiome. Nature *505*, 559–563.

Deehan, E.C., and Walter, J. (2016). The Fiber Gap and the Disappearing Gut Microbiome: Implications for Human Nutrition. Trends in Endocrinology & Metabolism *27*, 239–242.

Elston, D.M. (2006). The hygiene hypothesis and atopy: bring back the parasites? J. Am. Acad. Dermatol. *54*, 172–179.

Ermann, J., and Fathman, C.G. (2001). Autoimmune diseases: genes, bugs and failed regulation. Nat. Immunol. *2*, 759–761.

European Food Safety Authority. "Public Consultation on the Draft Scientific Opinion on Dietary Reference Values for Vitamin D." Home. May 16, 2016. Accessed June 20, 2016. https://www.efsa.europa.eu/en/consultations/call/160321.

Fairweather, D., and Rose, N.R. (2004). Women and Autoimmune Diseases1. Emerg. Infect. Dis. *10*, 2005–2011.

Falony, G., Joossens, M., Vieira-Silva, S., Wang, J., Darzi, Y., Faust, K., Kurilshikov, A., Bonder, M.J., Valles-Colomer, M., Vandeputte, D., et al. (2016). Population-level analysis of gut microbiome variation. Science *352*, 560–564.

Fasano, A. (2011). Leaky Gut and Autoimmune Diseases. Clin. Rev. Allergy Immunol. *42*, 71–78.

Fetahu, I.S., Höbaus, J., and Kállay, E. (2014). Vitamin D and the epigenome. Integr. Physiol. *5*, 164.

Franzosa, E.A., Huang, K., Meadow, J.F., Gevers, D., Lemon, K.P., Bohannan, B.J.M., and Huttenhower, C. (2015). Identifying personal microbiomes using metagenomic codes. Proc. Natl. Acad. Sci. U. S. A. *112*, E2930-2938.

Fujita, H., Sugimoto, K., Inatomi, S., Maeda, T., Osanai, M., Uchiyama, Y., Yamamoto, Y., Wada, T., Kojima, T., Yokozaki, H., et al. (2008). Tight Junction Proteins Claudin-2 and -12 Are Critical for Vitamin D-dependent Ca2+ Absorption between Enterocytes. Mol. Biol. Cell *19*, 1912–1921.

Fulde, M., and Hornef, M.W. (2014). Maturation of the enteric mucosal innate immune system during the postnatal period. Immunol. Rev. *260*, 21–34.

Funkhouser, L.J., and Bordenstein, S.R. (2013). Mom Knows Best: The Universality of Maternal Microbial Transmission. PLOS Biol *11*, e1001631.

Genuis, S.J. (2015). Maternal and Pediatric Health Outcomes in relation to Gestational Vitamin D Sufficiency. Obstet. Gynecol. Int. *2015*.

Giacomin, P., Agha, Z., and Loukas, A. (2016). Helminths and Intestinal Flora Team Up to Improve Gut Health. Trends Parasitol. *0*.

Gombart, A.F. (2005). Human cathelicidin antimicrobial peptide (CAMP) gene is a direct target of the vitamin D receptor and is strongly up-regulated in myeloid cells by 1,25-dihydroxyvitamin D3. FASEB J. *19*, 1067–1077.

Gombart, A.F. (2009). The vitamin D–antimicrobial peptide pathway and its role in protection against infection. Future Microbiol. *4*, 1151.

Goodrich, J.K., Waters, J.L., Poole, A.C., Sutter, J.L., Koren, O., Blekhman, R., Beaumont, M., Van Treuren, W., Knight, R., Bell, J.T., et al. (2014). Human Genetics Shape the Gut Microbiome. Cell *159*, 789–799.

Goodrich, J.K., Davenport, E.R., Beaumont, M., Jackson, M.A., Knight, R., Ober, C., Spector, T.D., Bell, J.T., Clark, A.G., and Ley, R.E. (2016). Genetic Determinants of the Gut Microbiome in UK Twins. Cell Host Microbe *19*, 731–743.

Hennet, T., and Borsig, L. (2016). Breastfed at Tiffany's. Trends Biochem. Sci. *41*, 508–518.

Hilger, J., Friedel, A., Herr, R., Rausch, T., Roos, F., Wahl, D.A., Pierroz, D.D., Weber, P., and Hoffmann, K. (2014). A systematic review of vitamin D status in populations worldwide. Br. J. Nutr. *111*, 23–45.

Hofer, U. (2016). Microbiome: Is LPS the key to the hygiene hypothesis? Nat. Rev. Microbiol. *14*, 334–335.

Holick, M.F., and Chen, T.C. (2008). Vitamin D deficiency: a worldwide problem with health consequences. Am. J. Clin. Nutr. *87*, 1080S–1086S.

Hossein-nezhad, A., and Holick, M.F. (2013). Vitamin D for Health: A Global Perspective. Mayo Clin. Proc. Mayo Clin. *88*, 720–755.

Hossein-nezhad, A., Spira, A., and Holick, M.F. (2013). Influence of Vitamin D Status and Vitamin D 3 Supplementation on Genome Wide Expression of White Blood Cells: A Randomized Double-Blind Clinical Trial. PLOS ONE *8*, e58725.

Hughes, R., Magee, E.A., and Bingham, S. (2000). Protein degradation in the large intestine: relevance to colorectal cancer. Curr. Issues Intest. Microbiol. *1*, 51–58.

Jašarević, E., Howerton, C.L., Howard, C.D., and Bale, T.L. (2015). Alterations in the Vaginal Microbiome by Maternal Stress Are Associated With Metabolic Reprogramming of the Offspring Gut and Brain. Endocrinology *156*, 3265–3276.

Jean-Claude Souberbielle, J.-J.B. (2010). Souberbielle JC, Body JJ, Lappe JM, Plebani M, Shoenfeld Y, Wang TJ et al.. Vitamin D and musculoskeletal health, cardiovascular disease, autoimmunity and cancer: Recommendations for clinical practice. Autoimmun Rev 9, 709-715. Autoimmun. Rev. *9*, 709–715.

Jouvin, M.-H., and Kinet, J.-P. (2012). Trichuris suis ova: testing a helminth-based therapy as an extension of the hygiene hypothesis. J. Allergy Clin. Immunol. *130*, 3-10-12.

Kabouridis, P.S., and Pachnis, V. (2015). Emerging roles of gut microbiota and the immune system in the development of the enteric nervous system. J. Clin. Invest. *125*, 956–964.

Kamel, M.M., Fouad, S.A., Salaheldin, O., El-Razek, A.E.-R.A.A., and El-Fatah, A.I.A. (2014). Impact of vitamin D receptor gene polymorphisms in pathogenesis of Type-1 diabetes mellitus. Int. J. Clin. Exp. Med. *7*, 5505–5510.

Kamen, D.L., and Tangpricha, V. (2010). Vitamin D and molecular actions on the immune system: modulation of innate and autoimmunity. J. Mol. Med. Berl. Ger. *88*, 441–450.

Kau, A.L., Ahern, P.P., Griffin, N.W., Goodman, A.L., and Gordon, J.I. (2011). Human nutrition, the gut microbiome and the immune system. Nature *474*, 327–336.

Koch, M.A., Reiner, G.L., Lugo, K.A., Kreuk, L.S.M., Stanbery, A.G., Ansaldo, E., Seher, T.D., Ludington, W.B., and Barton, G.M. (2016). Maternal IgG and IgA Antibodies Dampen Mucosal T Helper Cell Responses in Early Life. Cell *165*, 827–841.

Kong, J., Zhang, Z., Musch, M.W., Ning, G., Sun, J., Hart, J., Bissonnette, M., and Li, Y.C. (2008). Novel role of the vitamin D receptor in maintaining the integrity of the intestinal mucosal barrier. Am. J. Physiol. - Gastrointest. Liver Physiol. *294*, G208–G216.

Laing, B.B., and Ferguson, L.R. (2015). Genetic variations in Vitamin D metabolism genes and the microbiome, in the presence of adverse environmental changes, increase immune dysregulation.

Lepage, P., Leclerc, M.C., Joossens, M., Mondot, S., Blottière, H.M., Raes, J., Ehrlich, D., and Doré, J. (2013). A metagenomic insight into our gut's microbiome. Gut *62*, 146–158.

Lim, M.Y., You, H.J., Yoon, H.S., Kwon, B., Lee, J.Y., Lee, S., Song, Y.-M., Lee, K., Sung, J., and Ko, G. (2016). The effect of heritability and host genetics on the gut microbiota and metabolic syndrome. Gut gutjnl-2015-311326.

Lips, P. (2010). Worldwide status of vitamin D nutrition. J. Steroid Biochem. Mol. Biol. *121*, 297–300.

Littman, D.R., and Pamer, E.G. (2011). Role of the Commensal Microbiota in Normal and Pathogenic Host Immune Responses. Cell Host Microbe *10*, 311–323.

Lozupone, C.A., Stombaugh, J.I., Gordon, J.I., Jansson, J.K., and Knight, R. (2012). Diversity, stability and resilience of the human gut microbiota. Nature *489*, 220–230.

Lukeš, J., Kuchta, R., Scholz, T., and Pomajbíková, K. (2014). (Self-) infections with parasites: re-interpretations for the present. Trends Parasitol. *30*, 377–385.

Ly, N.P., Litonjua, A., Gold, D.R., and Celedón, J.C. (2011). Gut Microbiota, Probiotics, and Vitamin D: Interrelated Exposures Influencing Allergy, Asthma, and Obesity? J. Allergy Clin. Immunol. *127*, 1087–1094.

Maizels, R.M., McSorley, H.J., and Smyth, D.J. (2014). Helminths in the hygiene hypothesis: sooner or later? Clin. Exp. Immunol. *177*, 38–46.

Marchesi, J.R., Adams, D.H., Fava, F., Hermes, G.D.A., Hirschfield, G.M., Hold, G., Quraishi, M.N., Kinross, J., Smidt, H., Tuohy, K.M., et al. (2016). The gut microbiota and host health: a new clinical frontier. Gut *65*, 330–339.

Martínez, I., Stegen, J.C., Maldonado-Gómez, M.X., Eren, A.M., Siba, P.M., Greenhill, A.R., and Walter, J. (2015). The gut microbiota of rural papua new guineans: composition, diversity patterns, and ecological processes. Cell Rep. *11*, 527–538.

Matarese, G., Leiter, E.H., and La Cava, A. (2007). Leptin in autoimmunity: many questions, some answers. Tissue Antigens *70*, 87–95.

Merewood, A., Mehta, S.D., Chen, T.C., Bauchner, H., and Holick, M.F. (2009). Association between Vitamin D Deficiency and Primary Cesarean Section. J. Clin. Endocrinol. Metab. *94*, 940–945.

Morgan, X.C., and Huttenhower, C. (2012). Chapter 12: Human Microbiome Analysis. PLOS Comput Biol *8*, e1002808.

Mowat, A.M. (2003). Anatomical basis of tolerance and immunity to intestinal antigens. Nat. Rev. Immunol. *3*, 331–341.

Mpairwe, H., Tweyongyere, R., and Elliott, A. (2014). Pregnancy and helminth infections. Parasite Immunol. *36*, 328–337.

Mulligan, M.L., Felton, S.K., Riek, A.E., and Bernal-Mizrachi, C. (2010). Implications of vitamin D deficiency in pregnancy and lactation. Am. J. Obstet. Gynecol. *202*, 429.e1-9.

National Center for Chronic Disease Prevention and Health Promotion. Breastfeeding Report Cards. Centers for Disease Control and Prevention. 2014. Accessed June 20, 2016. http://www.cdc.gov/breastfeeding/data/reportcard.htm.

National Institutes for Health. Biennial Report of the Director, National Institutes of Health Fiscal Years 2010 & 2011. Accessed June 20, 2016. https://report.nih.gov/biennialreport/.

National Institutes of Health Office of Dietary Supplements. Office of Dietary Supplements - Vitamin D. Vitamin D — Health Professional Fact Sheet. Accessed June 20, 2016. https://ods.od.nih.gov/factsheets/VitaminD-HealthProfessional/#h2.

Neu, J. (2015). Developmental aspects of maternal-fetal, and infant gut microbiota and implications for long-term health. Matern. Health Neonatol. Perinatol. *1*, 6.

Nordic Council of Ministers, N.C. of M. (2008). Nordic Nutrition Recommendations 2012. Nord. Nutr. Recomm. 2012 *5*, 1–3.

Ochoa-Repáraz, J., and Kasper, L.H. (2014). Gut microbiome and the risk factors in central nervous system autoimmunity. FEBS Lett. *588*, 4214–4222.

O'Mahony, L., Stepien, M., Gibney, M.J., Nugent, A.P., and Brennan, L. (2011). The Potential Role of Vitamin D Enhanced Foods in Improving Vitamin D Status. Nutrients *3*, 1023–1041.

Ooi, J.H., Chen, J., and Cantorna, M.T. (2012). Vitamin D regulation of immune function in the gut: Why do T cells have vitamin D receptors? Mol. Aspects Med. *33*, 77–82.

Org, E., Parks, B.W., Joo, J.W.J., Emert, B., Schwartzman, W., Kang, E.Y., Mehrabian, M., Pan, C., Knight, R., Gunsalus, R., et al. (2015). Genetic and environmental control of host-gut microbiota interactions. Genome Res. *25*, 1558–1569.

Owen, J.L., and Mohamadzadeh, M. (2013). Microbial Activation of Gut Dendritic Cells and the Control of Mucosal Immunity. J. Interferon Cytokine Res. *33*, 619–631.

Palacios, C., and Gonzalez, L. (2014). Is vitamin D deficiency a major global public health problem? J. Steroid Biochem. Mol. Biol. *144PA*, 138–145.

Palacios, C., De-Regil, L.M., Lombardo, L.K., and Peña-Rosas, J.P. (2016). Vitamin D supplementation during pregnancy: Updated meta-analysis on maternal outcomes. J. Steroid Biochem. Mol. Biol.

Palmer, C., Bik, E.M., DiGiulio, D.B., Relman, D.A., and Brown, P.O. (2007). Development of the Human Infant Intestinal Microbiota. PLOS Biol *5*, e177.

Pantazou, V., Schluep, M., and Du Pasquier, R. (2015). Environmental factors in multiple sclerosis. Presse Médicale Paris Fr. 1983 *44*, e113-120.

Pasparakis, M. (2008). IKK/NF-κB signaling in intestinal epithelial cells controls immune homeostasis in the gut. Mucosal Immunol. *1*, S54–S57.

Peterson, L.W., and Artis, D. (2014). Intestinal epithelial cells: regulators of barrier function and immune homeostasis. Nat. Rev. Immunol. *14*, 141–153.

Polloni, L., Ferruzza, E., Ronconi, L., Lazzarotto, F., Toniolo, A., Bonaguro, R., and Muraro, A. (2015). Perinatal stress and food allergy: a preliminary study on maternal reports. Psychol. Health Med. *20*, 732–741.

Proal, A.D., Albert, P.J., and Marshall, T.G. (2009). Dysregulation of the vitamin D nuclear receptor may contribute to the higher prevalence of some autoimmune diseases in women. Ann. N. Y. Acad. Sci. *1173*, 252–259.

Proal, A.D., Albert, P.J., and Marshall, T.G. (2013). The human microbiome and autoimmunity. Curr. Opin. Rheumatol. *25*, 234–240.

Purchiaroni, F., Tortora, A., Gabrielli, M., Bertucci, F., Gigante, G., Ianiro, G., Ojetti, V., Scarpellini, E., and Gasbarrini, A. (2013). The role of intestinal microbiota and the immune system. Eur. Rev. Med. Pharmacol. Sci. *17*, 323–333.

Ramanan, D., Bowcutt, R., Lee, S.C., Tang, M.S., Kurtz, Z.D., Ding, Y., Honda, K., Gause, W.C., Blaser, M.J., Bonneau, R.A., et al. (2016). Helminth infection promotes colonization resistance via type 2 immunity. Science *352*, 608–612.

Rautava, S., Luoto, R., Salminen, S., and Isolauri, E. (2012). Microbial contact during pregnancy, intestinal colonization and human disease. Nat. Rev. Gastroenterol. Hepatol. *9*, 565–576.

Rook, G.A.W. (2012). Hygiene hypothesis and autoimmune diseases. Clin. Rev. Allergy Immunol. *42*, 5–15.

Ross, A.C., Manson, J.E., Abrams, S.A., Aloia, J.F., Brannon, P.M., Clinton, S.K., Durazo-Arvizu, R.A., Gallagher, J.C., Gallo, R.L., Jones, G., et al. (2011). The 2011 Report on Dietary Reference Intakes for Calcium and Vitamin D from the Institute of Medicine: What Clinicians Need to Know. J. Clin. Endocrinol. Metab. *96*, 53–58.

Schnorr, S.L., Candela, M., Rampelli, S., Centanni, M., Consolandi, C., Basaglia, G., Turroni, S., Biagi,

E., Peano, C., Severgnini, M., et al. (2014). Gut microbiome of the Hadza hunter-gatherers. Nat. Commun. *5*, 3654.

Scholl, T.O., Chen, X., and Stein, P. (2012). Maternal Vitamin D Status and Delivery by Cesarean. Nutrients *4*, 319–330.

Shamseer, L., Moher, D., Clarke, M., Ghersi, D., Liberati, A., Petticrew, M., Shekelle, P., Stewart, L.A., and PRISMA-P Group (2015). Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015: elaboration and explanation. BMJ *349*, g7647.

Soderborg, T.K., Borengasser, S.J., Barbour, L.A., and Friedman, J.E. (2016). Microbial transmission from mothers with obesity or diabetes to infants: an innovative opportunity to interrupt a vicious cycle. Diabetologia 1–12.

Sonnenburg, E.D., Smits, S.A., Tikhonov, M., Higginbottom, S.K., Wingreen, N.S., and Sonnenburg, J.L. (2016). Diet-induced extinctions in the gut microbiota compound over generations. Nature *529*, 212–215.

Spanogiannopoulos, P., Bess, E.N., Carmody, R.N., and Turnbaugh, P.J. (2016). The microbial pharmacists within us: a metagenomic view of xenobiotic metabolism. Nat. Rev. Microbiol. *14*, 273–287.

Spiro, A., and Buttriss, J.L. (2014). Vitamin D: An overview of vitamin D status and intake in Europe. Nutr. Bull. Bnf *39*, 322–350.

Strachan, D.P. (1989). Hay fever, hygiene, and household size. BMJ *299*, 1259–1260.

Suez, J., Korem, T., Zeevi, D., Zilberman-Schapira, G., Thaiss, C.A., Maza, O., Israeli, D., Zmora, N., Gilad, S., Weinberger, A., et al. (2014). Artificial sweeteners induce glucose intolerance by altering the gut microbiota. Nature *514*, 181–186.

Sun, J. (2010). Vitamin D and mucosal immune function. Curr. Opin. Gastroenterol. *26*, 591–595.

Taciak, M., Barszcz, M., Tuśnio, A., and Pastuszewska, B. (2015). Interactive Effects of Indigestible Carbohydrates, Protein Type, and Protein Level on Biomarkers of Large Intestine Health in Rats. PLOS ONE *10*, e0142176.

Tanaka, S., Kobayashi, T., Songjinda, P., Tateyama, A., Tsubouchi, M., Kiyohara, C., Shirakawa, T., Sonomoto, K., and Nakayama, J. (2009). Influence of antibiotic exposure in the early postnatal period on the development of intestinal microbiota. FEMS Immunol. Med. Microbiol. *56*, 80–87.

Tankou, S., Bry, L., Gerber, G., Lin, N., Glanz, B., Cook, S., Stuart, F., Weiner, M., Melo, K., Ghandi, R., et al. (2015). Effect of Vitamin D level in the gut microbiome of MS patients (P2.206). Neurology *84*, P2.206.

Thum, C., Cookson, A.L., Otter, D.E., McNabb, W.C., Hodgkinson, A.J., Dyer, J., and Roy, N.C. (2012). Can Nutritional Modulation of Maternal Intestinal Microbiota Influence the Development of the Infant Gastrointestinal Tract? J. Nutr. *142*, 1921–1928.

Toden, S., Bird, A.R., Topping, D.L., and Conlon, M.A. (2006). Resistant starch prevents colonic DNA damage induced by high dietary cooked red meat or casein in rats. Cancer Biol. Ther. *5*, 267–272.

Tomkovich, S., and Jobin, C. (2016). Microbiota and host immune responses: a love-hate relationship. Immunology *147*, 1–10.

Turnbaugh, P.J., Ridaura, V.K., Faith, J.J., Rey, F.E., Knight, R., and Gordon, J.I. (2009). The effect of diet on the human gut microbiome: a metagenomic analysis in humanized gnotobiotic mice. Sci. Transl. Med. *1*, 6ra14.

Uitterlinden, A.G., Fang, Y., van Meurs, J.B.J., Pols, H.A.P., and van Leeuwen, J.P.T.M. (2004). Genetics and biology of vitamin D receptor polymorphisms. Gene *338*, 143–156.

Ursell, L.K., Metcalf, J.L., Parfrey, L.W., and Knight, R. (2012). Defining the Human Microbiome. Nutr. Rev. *70*, S38–S44.

Vatanen, T., Kostic, A.D., d'Hennezel, E., Siljander, H., Franzosa, E.A., Yassour, M., Kolde, R., Vlamakis, H., Arthur, T.D., Hämäläinen, A.-M., et al. (2016). Variation in Microbiome LPS Immunogenicity Contributes to Autoimmunity in Humans. Cell *165*, 842–853.

Versini, M., Jeandel, P.-Y., Bashi, T., Bizzaro, G., Blank, M., and Shoenfeld, Y. (2015). Unraveling the Hygiene Hypothesis of helminthes and autoimmunity: origins, pathophysiology, and clinical applications. BMC Med. *13*, 81.

Vighi, G., Marcucci, F., Sensi, L., Di Cara, G., and Frati, F. (2008). Allergy and the gastrointestinal system. Clin. Exp. Immunol. *153*, 3–6.

Wagner, C.L., Hulsey, T.C., Fanning, D., Ebeling, M., and Hollis, B.W. (2006). High-Dose Vitamin D3 Supplementation in a Cohort of Breastfeeding Mothers and Their Infants: A 6-Month Follow-Up Pilot Study. Breastfeed. Med. *1*, 59–70.

Wammes, L.J., Mpairwe, H., Elliott, A.M., and Yazdanbakhsh, M. (2014). Helminth therapy or elimination: epidemiological, immunological, and clinical considerations. Lancet Infect. Dis. *14*, 1150–1162.

Wang, T.J., Zhang, F., Richards, J.B., Kestenbaum, B., van Meurs, J.B., Berry, D., Kiel, D., Streeten, E.A., Ohlsson, C., Koller, D.L., et al. (2010). Common genetic determinants of vitamin D insufficiency: a genome-wide association study. Lancet *376*, 180–188.

Wang, T.-T., Nestel, F.P., Bourdeau, V., Nagai, Y., Wang, Q., Liao, J., Tavera-Mendoza, L., Lin, R., Hanrahan, J.H., Mader, S., et al. (2004). Cutting Edge: 1,25-Dihydroxyvitamin D3 Is a Direct Inducer of Antimicrobial Peptide Gene Expression. J Immunol *173*, 2909–2912.

Waterhouse, J.C., Perez, T.H., and Albert, P.J. (2009). Reversing Bacteria-induced Vitamin D Receptor Dysfunction Is Key to Autoimmune Disease. Ann. N. Y. Acad. Sci. *1173*, 757–765.

Wilson, I.D., and Nicholson, J.K. (2009). The Role of Gut Microbiota in Drug Response. Curr. Pharm. Des. *15*, 1519–1523.

Wu, S., Liao, A.P., Xia, Y., Li, Y.C., Li, J.-D., Sartor, R.B., and Sun, J. (2010). Vitamin D Receptor Negatively Regulates Bacterial-Stimulated NF-κB Activity in Intestine. Am J Pathol *177*, 686–697.

Wu, S., Zhang, Y., Lu, R., Xia, Y., Zhou, D., Petrof, E.O., Claud, E.C., Chen, D., Chang, E.B., Carmeliet, G., et al. (2015). Intestinal epithelial vitamin D receptor deletion leads to defective autophagy in colitis. Gut *64*, 1082–1094.

Yatsunenko, T., Rey, F.E., Manary, M.J., Trehan, I., Dominguez-Bello, M.G., Contreras, M., Magris, M., Hidalgo, G., Baldassano, R.N., Anokhin, A.P., et al. (2012). Human gut microbiome viewed across age and geography. Nature *486*, 222–227.

Zaura, E., Brandt, B.W., Mattos, M.J.T. de, Buijs, M.J., Caspers, M.P.M., Rashid, M.-U., Weintraub, A., Nord, C.E., Savell, A., Hu, Y., et al. (2015). Same Exposure but Two Radically Different Responses to Antibiotics: Resilience of the Salivary Microbiome versus Long-Term Microbial Shifts in Feces. mBio *6*, e01693-15.

Yassour, M., Vatanen, T., Siljander, H., Hämäläinen, A.-M., Härkönen, T., Ryhänen, S.J., Franzosa, E.A., Vlamakis, H., Huttenhower, C., Gevers, D., et al. (2016). Natural history of the infant gut microbiome and impact of antibiotic treatment on bacterial strain diversity and stability. Science Translational Medicine *8*, 343ra81-343ra81.

Yuk, J.-M., Shin, D.-M., Lee, H.-M., Yang, C.-S., Jin, H.S., Kim, K.-K., Lee, Z.-W., Lee, S.-H., Kim, J.-M., and Jo, E.-K. (2009). Vitamin D3 Induces Autophagy in Human Monocytes/Macrophages via Cathelicidin. Cell Host Microbe *6*, 231–243.

Zhang, Y., Leung, D.Y.M., Richers, B.N., Liu, Y., Remigio, L.K., Riches, D.W., and Goleva, E. (2012). Vitamin D Inhibits Monocyte/macrophage Pro-inflammatory Cytokine Production by Targeting Mitogen-Activated Protein Kinase Phosphatase 1. J. Immunol. Baltim. Md 1950 *188*, 2127–2135.

Zhang, Y., Wu, S., and Sun, J. (2013). Vitamin D, vitamin D receptor and tissue barriers. Tissue Barriers *1*, e23118.

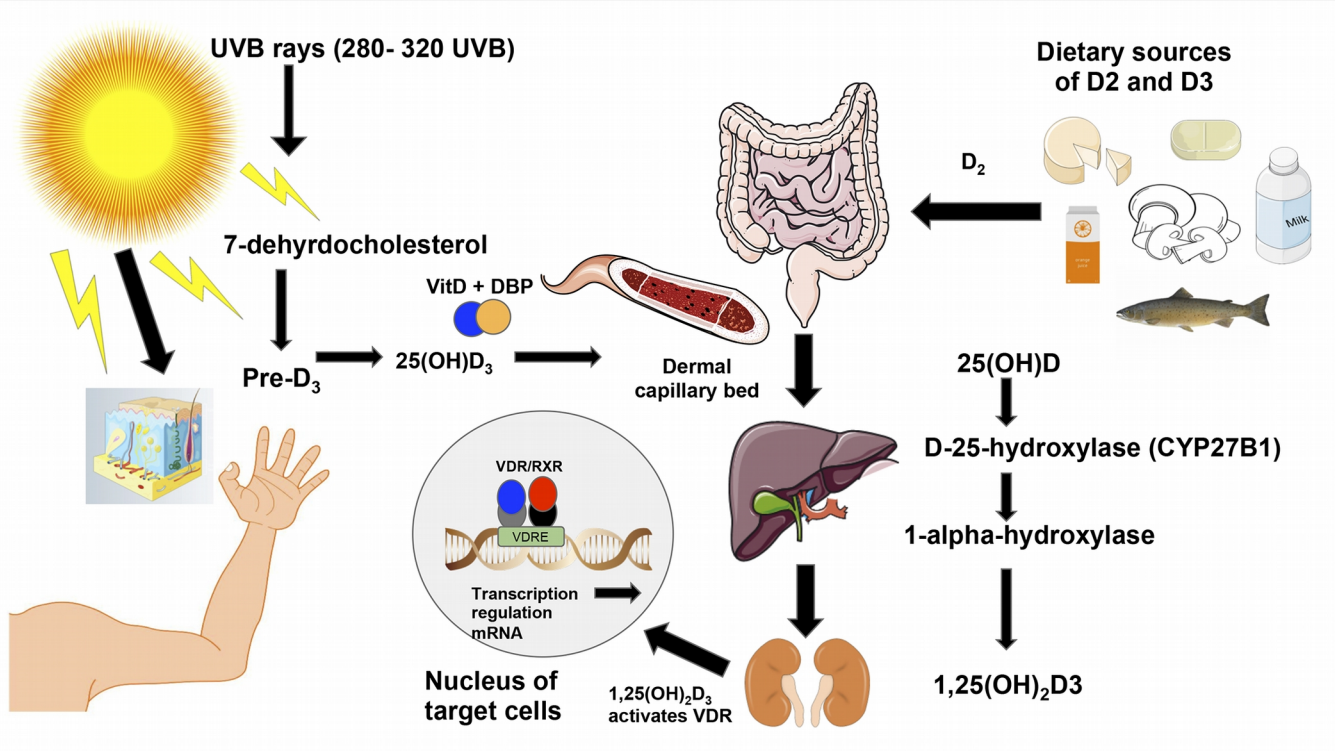
Zhao, H., Zhang, H., Wu, H., Li, H., Liu, L., Guo, J., Li, C., Shih, D.Q., and Zhang, X. (2012). Protective role of 1,25(OH)2vitamin D3 in the mucosal injury and epithelial barrier disruption in DSS-induced acute colitis in mice. BMC Gastroenterol. *12*, 57.

Zhernakova, A., Kurilshikov, A., Bonder, M.J., Tigchelaar, E.F., Schirmer, M., Vatanen, T., Mujagic, Z., Vila, A.V., Falony, G., Vieira-Silva, S., et al. (2016). Population-based metagenomics analysis reveals markers for gut microbiome composition and diversity. Science *352*, 565–569.

World Health Organization, and Food and Agricultural Organization of the United Nations. "Vitamin and Mineral Requirements in Human Nutrition." WHO. 2004. Accessed June 20, 2016. http://www.who.int/nutrition/publications/micronutrients/9241546123/en/.

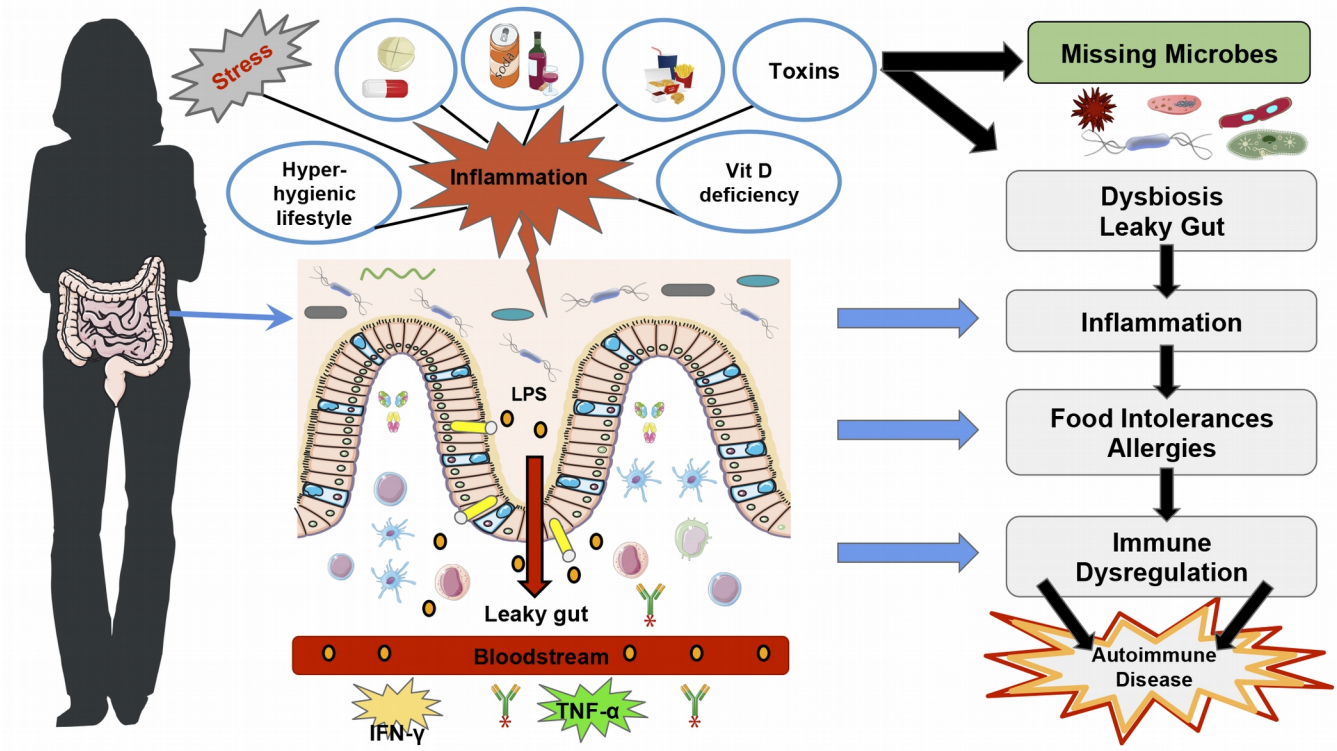
Figures

Figure 1. Vitamin D_3 synthesis from sun exposure and food



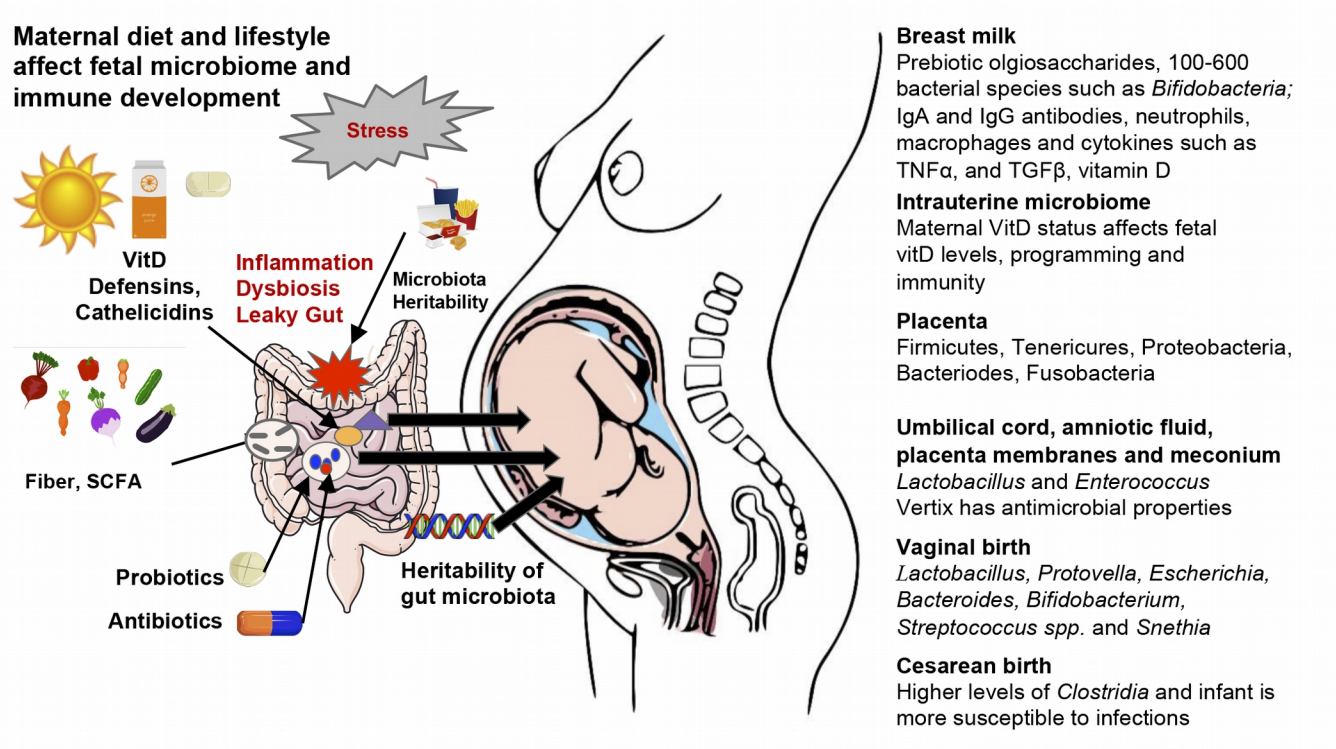
Vitamin D is synthesized from sun exposure or consumption of foods rich in vitamin D. On the left, UVB rays of frequencies between 280 - 320 from the sun hit the skin where 7-dehydrocholesterol is converted to pre-vitamin D_3 and then isomerized into vitamin D_3, or cholecalciferol (Bikle, 2014). Vitamin D binding protein (DBP) then facilitates vitamin D_3's entrance into the dermal capillary bed (Hossein-nezhad and Holick, 2013). On the right of the figure, ingested vitamin D_2 (ergocalciferol) from food or supplements is incorporated into chylomicrons which enter the lymphatic system and blood. Once in the bloodstream, both vitamin D_2 and vitamin D_3 pass to the liver where the enzyme cytochrome P450 hydroxylates both forms of the vitamin. Then the 1-α-hydroxylase enzyme (CYP27B1) further hydroxylates 25(OH)D (calcidiol) into the active form of the vitamin to $1,25(OH)_2D_3$, in the kidneys (Hossein-nezhad and Holick, 2013). Unlike D_2 or other vitamin D metabolites, vitamin D_3 is the active form that binds to the vitamin D receptor (VDR), which is located in about 30 different tissues (Gombart, 2009). The activated VDR binds to vitamin D responsive elements (VDRE) to form a heterodimer (Zhang et al., 2012) which can interact with histone acetylases to induce transcription and regulate the expression of more than 2,000 genes (Hossein-nezhad and Holick, 2013; Proal et al., 2013). Generally, 1,000 IU vitamin D_3/day increases $1,25(OH)_2D_3$ levels approximately 10 ng/mL, though individual results may vary (Jean-Claude Souberbielle, 2010), whereas an adult exposed to 1 minimal erythemal dose (slight pinkness to the skin 24 hours after exposure) is equivalent to oral intake of 20,000 IU (500 µg) of vitamin D_2 (Jean-Claude Souberbielle, 2010). Arm and leg exposure of 0.5 erythemal dose is equivalent of oral intake of 3,000 IU of vitamin D_3 (Hossein-nezhad and Holick, 2013). Lastly, about 30 minutes of sun exposure on the arms and face between latitudes 42°N and 42°S is equivalent to 200-400 IU of vitamin D_3 (WHO, 2004).

Figure 2: Western lifestyle factors that lead to autoimmunity diseases



In this representation of the hygiene hypothesis, some of the main factors that contribute to "missing microbes" and disruption of gut homeostasis are the Western diet (Brown et al., 2012), toxins (Spanogiannopoulos et al., 2016), drugs (Falony et al., 2016; Spanogiannopoulos et al., 2016; Wilson and Nicholson, 2009) antibiotics (Zhernakova et al., 2016), de-worming at an early age and chlorinated water (Rook, 2012). Vitamin D_3 deficiency and vitamin D receptor (VDR) polymorphisms have also been shown to disrupt gut homeostasis thus immune function (Aranow, 2011; Proal et al., 2009). All of these factors lead to intestinal dysbiosis, more susceptibility to pathogenic infections and intestinal permeability, which predispose to lipopolysaccharides (LPS) translocation and trigger inflammatory immune responses such as TNF-α and IFN-γ (Fasano, 2011). The loss of immune homeostasis can lead to food intolerances and allergies, which can subsequently lead to autoimmune disease development (Rook, 2012).

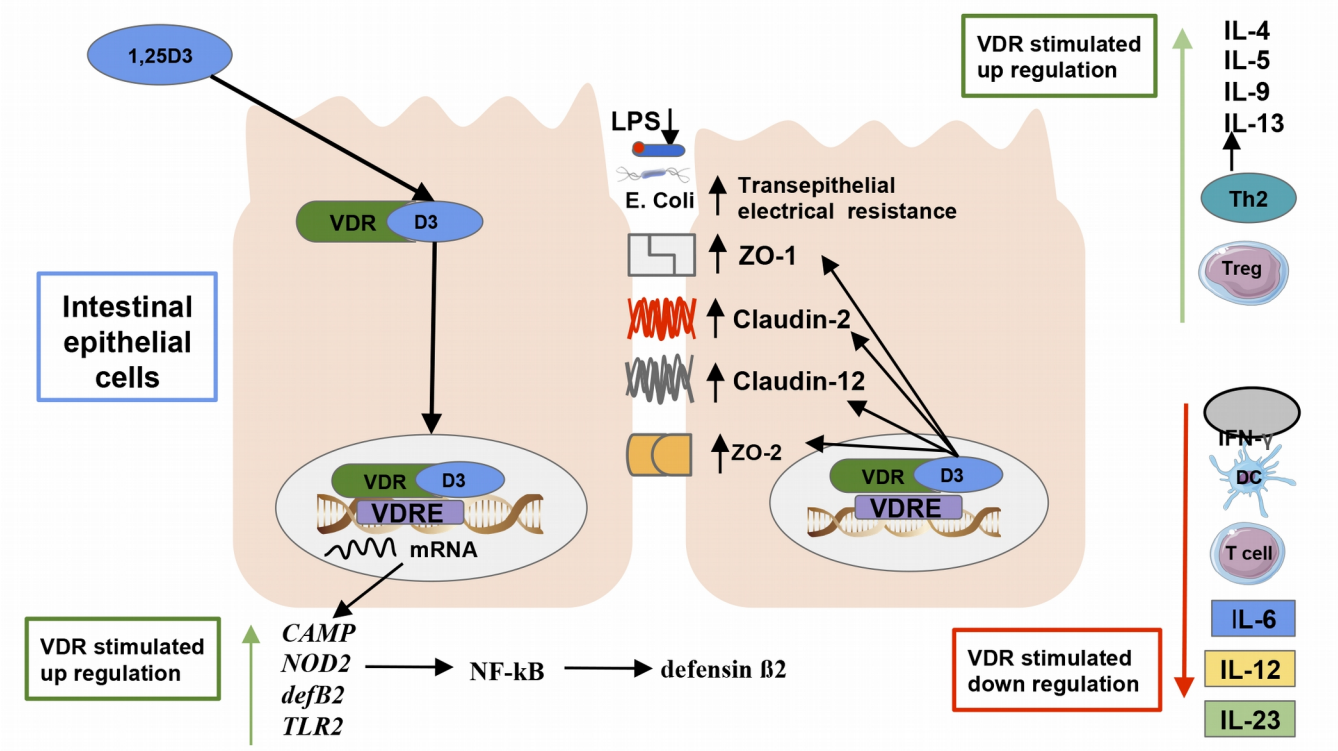
Figure 3: Transmission of maternal microbiota to offspring



(A) (Starting from the top left going counterclockwise): Maternal stress during pregnancy can lead to difficult pregnancies, food allergies, gastrointestinal symptoms and eczema in offspring compared to infants of mothers who experienced little stress during pregnancy (Polloni et al., 2015). Maternal diet can influence the offspring's microbiota composition, and mothers who eat a Western diet that is typically low in fiber means less short chain fatty acids (SCFA) are produced which can cause dysbiosis, intestinal permeability and inflammation in the mother's gut (Brown et al., 2012). Maternal vitamin D status from sun exposure or consumption of vitamin D_2 rich foods directly affects the infant's level and immune system development (Clancy et al., 2013; Pludowski et al., 2013; Spiro and Buttriss, 2014) and risk of developing autoimmune diseases (Disanto et al., 2012; Fronczak et al., 2003; Lucas et al., 2008; Mirzaei et al., 2011; Sørensen et al., 2012). Antibiotic treatment negatively affects the development of the offspring's microbial composition (Soderborg et al., 2016) whereas maternal probiotic treatment can improve infant health outcomes (Amarasekera et al., 2013; Hashemi et al., 2016). Genetic background may shape gut microbiota structure (Benson et al., 2010; Davenport, 2016; Goodrich et al., 2014, 2016; Lim et al., 2016; Org et al., 2015). **(B)** (Right of figure, top to bottom): Breast milk has its unique microbial composition (Cacho and Neu, 2014; Cabrera-Rubio et al., 2012) that is rich in prebiotic oligosaccharides and beneficial bacteria such as *Bifidobacteria* that promote gut homeostasis (Funkhouser and Bordenstein, 2013) as well as various immune modulators that are also transferred to the infant (Azad et al., 2011). It is believed that microbes from maternal intestinal, urogenital and oral bacteria (Rautava et al., 2012) enter the uterus and amniotic fluid most likely via the bloodstream (Funkhouser and Bordenstein, 2013). The placenta contains commensal bacteria from the phyla Firmicutes, Tenericures, Proteobacteria, Bacteriodes and Fusobacteria (Aagaard et al., 2014). *Lactobacillus* and *Enterococcus* from the mother's intestinal tract and urogenital region have been found in the umbilical cord, amniotic fluid, placenta membranes and meconium (Thum et al., 2012). The vertix contains immuno-protective and antimicrobial components such as defensins, catehelicidins, lysozyme and lactoferrin (Singh and Archana, 2008). Vaginal births expose neonates to commensal fecal and vaginal bacteria such as *Lactobacillus, Provotella, Bacteroides, Bifidobacterium, Strephococcus spp.* and *Snethia* (Biasucci et al., 2008; Jašarević et al., 2015b), whereas babies born

cesarean tend to have elevated levels of *Clostridia* (Salminen et al., 2004).

Figure 4: Vitamin D's effect on the gut microbiota and immunity



The active form of vitamin D_3 ($1,25(OH)_2D_3$) binds to vitamin D receptor (VDR) inside epithelial cells. The activated VDR then binds to vitamin D responsive elements (VDRE) to form a heterodimer in the nucleus (Zhang et al., 2012). In intestinal epithelial cells, the activated VDR up regulates the production of antimicrobial peptides such as cathelicidin, β-defensin (Proal et al., 2013), cathelicidin antimicrobial peptide (CAMP) and defensin β2 (Froicu and Cantorna, 2007; Wang et al., 2010). Activated VDR also regulates toll-like receptor 2 (TLR2) (Proal et al., 2013). Vitamin D_3 also induces the expression of the nucleotide-binding oligomerization domain 2 (*NOD2),* a pattern recognition receptor in monocytic and epithelial cells which then stimulates NF-kB transcription factor function which can also activate the expression of gene for defensin β2 (Wang et al., 2010). Vitamin D_3 plays a role in maintaining intestinal barrier function by controling the expression of the tight junction proteins zonulin occluden-1 (ZO-1), zonulin occluden-2 (ZO-2), up regulating claudin 2 and 12 and down regulating cadherin-17 in the intestine (Zhang et al., 2013). In the presence of pathogenic *Escherichia coli* O157:H7, vitamin D_3 reduces transepithelial barrier resistance and decreases intestinal permeability in epithelial cells (Assa et al., 2014) and reduces lipopolysaccharides (LPS) levels in Caco-2 cells (Zhao et al., 2012), which promotes gut and immune homeostasis. Vitamin D_3 also reduces dendritic cell (DC) maturation and pro-inflammatory IL-6, IL-12 and IL-23 production (Veldhoen and Brucklacher-Waldert, 2012). Furthermore, vitamin D_3 promotes tolerogenic immune responses by inhibiting IL-12 and increasing DC mediated IL-10 production, which increases Treg production promoting a Th2 response (Gombart, 2009) such as IL-3, IL-5, IL-9 and IL-13 production (Suez et al., 2014). Lastly, vitamin D_3 induces VDR expression and inhibits T cell proliferation, IL-2 and IFN-γ production and Th1 development (Adorini, 2002).

Tables

Table 1. *In vitro* and animal model studies based on the interaction between vitamin D ingestion, VDR function, intestinal microbiota and immune homeostasis

Species	Experimental Design	Effect on microbiota and/ or immune system	Reference
Vitamin D_3 and antimicrobial activity			
Human keratinocytes, monocytes and neutrophils	All tissue lines were cultured under recommended conditions. Assays were performed 24h later.	Vitamin D_3 induced antimicrobial peptide gene expression in isolated human keratinocytes, monocytes and neutrophils, and human cell lines. Together, vitamin D_3 and lipopolysaccharides (LPS) induced cathelicidin antimicrobial peptide (CAMP) expression in neutrophils. D_3 also induced antimicrobial secretion of antimicrobial activity against pathogens including *Pseudomonas aeruginosa.*	(Wang et al., 2004)
Human macrophages	Macrophages from serum of healthy donors with sufficient vitamin D_3 levels were infected with *Mycobacterium tuberculosis.*	Toll like receptors (TLR) activation of VDRs induced antimicrobial cathelicidin and killing of *M. tuberculosis* in African Americans who were more susceptible to infection possibly due to vitamin D_3 deficiency.	(Liu et al., 2006)
Human monocytes	Monocytes were incubated with vitamin D_3 at different concentrations and times.	Vitamin D_3 induces the antimicrobial peptide cathelicidin which activates the transcription of autophagy-related genes beclin-1 and autophagy related 5(*Atg5)* in human monocytes via regulatory function of human cathelicidin.	(Yuk et al., 2009)
Human monocytes	Cultures of human monocytes were treated with vitamin D_3 (100 nM) for 24h before RNA isolation for quantitative PCR analysis. 25-hydroxyvitamin D 24-hydroxylase (*CYP24*) mRNA levels were also monitored as a positive control for vitamin D_3 action.	Vitamin D_3 and its analogs up regulated antimicrobial peptide production such as cathelicidin and β-defensin as well as toll-like receptor 2 (TLR-2) gene expression, and induced the expression of the nucleotide-binding oligomerization domain 2 (*NOD2)* in monocytic and epithelial cells which then stimulated NF-kB transcription factor function thereby activating defensin β2 expression. This study links *NOD2* genetic predisposition with Crohn's and vitamin D_3 deficiency.	(Wang et al., 2010)

BALB/c mice	Mice were infected with *Toxoplasma gondii* and then administered 0.5µg/kg/ day for 2 days. Systemic pathological analysis of the small and large intestine was done 7 days after infection.	Mice treated with vitamin D_3 had less infections and lesions in the organs analyzed (lungs, brain, liver, spleen, small and large intestine) than mice not treated with vitamin D_3. Intestinal epithelial cells treated with vitamin D_3 *in vitro* produced antimicrobial effects upon *T. gondii* infection possibly by stimulating TLR2-induced cathelicidin expression in macrophages.	(Rajapakse et al., 2007)
C57BL/6 mice	Mice received a single oral dose (6, 12, 24, or 48 pmol) of $1,25(OH)_2D_3$ or β-gluc-$1,25(OH)_2D_3$ suspended in 50 µl of peanut oil. Blood was collected and duodenum, and colon mRNA transcriptome analysis was conducted. Another study consisted of dextran sulfate sodium (DSS)-induced colitis and administration of 24 or 120 pmol/day $1,25(OH)_2D_3$ and 24 or 120 pmol/day of β-gluc-$1,25(OH)_2D_3$.	The authors concluded that the analog β-gluc-25(OH)D was converted to 25(OH)D in the colon probably by bacteria such as *Bacteroides sp*. and competitively inhibited colon 24-hydroxylase, allowing $1,25(OH)_2D_3$ to persist longer exhibiting anti-inflammatory action by the liberated from the β-gluc-$1,25(OH)_2D_3$ in the colon.	(Goff et al., 2012)
C57BL/6 mice	Mice were raised from weaning on vitamin D_3-deficient or sufficient diets and then treated with DSS to induce colitis.	Vitamin D_3 deficiency might lead to homeostatic deregulation of enteric bacteria in the colon. Antimicrobial protein angiogenin-4, which is involved in enteric bacteria containment, is associated with Crohn's and has bactericidal activity, was down regulated in vitamin D_3 deficient mice even in the absence of colitis, and they had 50-fold amount of bacterial infiltration in colonic tissue.	(Lagishetty et al., 2010)
VDR, gut composition and immune homeostasis			
C57BL/6, C57BL/6 $VDR^{-/-}$ knockout (KO), germ-free and specific pathogen free mice	Mice were treated with 7.5 mg of the antibiotic streptomycin, and 24h later were infected with 1x 10^7 colony forming units (CFU) of *Salmonella typhimurium.* Cecum tissues were dissected for histology and PCR analyses for infection burden	Enteric *Salmonella typhimurium* infection increased VDR concentrations which correlated with VDRs' binding to and regulation of NF-kB p65. Commensal bacteria facilitated the distribution of VDRs in the colon where bacterial colonization is much richer than in the small intestine, which was not	(Wu et al, 2010)

	and RNA expression in epithelial cells.	observed as much in germ-free mice. $VDR^{-/-}$ KO mice had increased bacterial burden and mortality upon infection compared to the $VDR^{+/+}$ group.	
$VDR^{-/-}$ KO mice	Fecal and cecal stool samples were cultured from $VDR^{-/-}$ KO and wild type mice for bacterial DNA and later sequenced with pyrosequencing of 16S rRNA gene.	$VDR^{-/-}$ KO mice had diminished levels of *Lactobacillus* and increased *Clostridium* and *Bacteroides* levels. Compared to wild type mice, $VDR^{-/-}$ KO mice had a gut microbiota composition that is associated with higher risk for infections, cancer, inflammation and other conditions.	(Jin et al, 2015)
$VDR^{-/-}$ KO mice and $IL10^{-/-}$ KO mice	Immune-based $IL10^{-/-}$ colitis model	Low VDR level in the intestine was associated with abnormal Paneth cells, impaired autophagy function, dysbiosis and a reduction of autophagy gene called autophagy related 16 like 1 (*ATG16L1)*, which was regulated transcriptionally by VDR. Butyrate administration increased VDR expression in experimental colitis models.	(Sun, 2015)
C57BL/6 $VDR^{-/-}$ KO and germ-free wild type mice *C. rodentium* 48h later. Fecal DNA was then sequenced.	Mice were infected by oral gavage with 200 mL of *Clostridium rodentium* suspension that contained $5x10^9$ CFU. Cecal transplant from one wild type or VDR knockout donor was collected the cecal contents were orally administered to wild type germ-free mice, and they were then infected with *C. rodentium.*	$VDR^{-/-}$ KO mice were more susceptible to the pathogen *C. rodentium.* VDR regulated dysbiosis and *C. rodentium* infection susceptibility. Antibiotic administration in $VDR^{-/-}$ KO mice reversed colonization resistance to *C. rodentium* infection.	(Chen et al,, 2015a)
C57BL/6 and cytochrome P450 family 27 subfamily B member 1$(Cyp27b1)^{-/-}$ KO mice	Mice were treated with DSS to induce colitis. Some were treated with antibiotics and Cyp27b1 KO mice were given 1.25µg/100g vitamin D_3 daily. RNA was isolated and analyzed from colon tissues. Intestinal epithelial cells and lamina propia lymphocytes were isolated and analyzed.	Cyp27b1 knockout and VDR knockout mice had more bacteria from the Bacteroidetes and Proteobacteria phyla and fewer bacteria from the Firmicutes and Deferribacteres phyla in the feces compared to wild-type. Vitamin D_3 and VDR attenuated DSS-induced colitis by decreasing *Helicobacteraceae* (Proteobacteria phyla) which lead to less colitis symptoms. *Cyp27b1* and VDR knockout mice had more dysbiosis	(Ooi et al., 2012)

		due to less antimicrobial peptide E-cadherin expression in the gut.	
ApoE$^{-/-}$ KO mice	Mice were randomized to receive saline or 10×10^9 CFU/kg/day of VSL#3 probiotics for 3 months alone or in combination with 0.2% of DSS in drinking water.	VSL#3 reversed insulin resistance, a risk factor for type II diabetes mellitus. The probiotics modulated VDR expression.	(Mencarelli et al., 2012)
Naked mole rats (*Heterocephalus gluber*)	Control rats were fed a carrot diet, and the vitamin D_3 groups was administered 25 ng vitamin D_3/g food every 3 d during the experiment. Cecal samples were analyzed for short chain fatty acids (SCFAs).	Vitamin D_3 enhanced microbial-controlled fermentation processes in the cecum compared with animals not administered vitamin D_3. SCFA production increased more than l.4-fold per g DM cecal substrate.	(Yahav and Buffenstein, 1993)
VDR and LPS			
VDR$^{-/-}$ KO mice	Mice were treated with 0.5-3.5% DSS solution to develop colitis. Body weight, hematological and histological analysis were conducted for intestinal damage and inflammation.	VDR knockout mice were more susceptible to LPS-induced endotoxemia and death.	(Froicu and Cantorna, 2007)
Vitamin D_3 and intestinal barrier function			
C57BL/6 mice	DSS-induced acute colitis model. Mice groups other than the control group drank 2% DSS water *ad libitum* for 7 days. Mice in the vitamin D- treated group were injected with 0.2µg/25g of $1,25(OH)_2D_3$ for 14 days. Mice were executed at the end of the experiment and serum vitamin D_3, LPS and colons were analyzed for inflammation and intestinal permeability.	Mice treated with vitamin D_3 had rapid weight recover from day 9 to 21, lower mRNA levels of inflammation related genes, less LPS translocation and intestinal permeability and a lower histologic colitis score (5.00 ± 0.00 vs 8.60 ± 2.00) than the DSS. D_3 controlled the expression of the tight junction proteins zonulin occluden (ZO)-1, ZO-2, up regulated claudin 2 and 12 and down regulated cadherin-17 in Caco-2 cells thus reducing intestinal permeability.	(Zhao et al., 2012)

Table 2: The relationship between vitamin D_3, VDR function, the gut microbiota and autoimmune diseases in human studies

Species	Experimental design	Results	Reference
Human cohort of 3,188 inflammatory bowel disease (IBD) patients	Plasma 25(OH)D was measured at least once in patients. Multivariate logistic regression models adjusting for potential confounders were used to identify independent effect of plasma 25(OH)D on risk of *Clostridium difficile* infection.	Higher plasma 25(OH)D was associated with reduced risk of *C. difficile* infection in IBD patients. The mean plasma 25(OH)D level was much lower in patients with *C. difficile* infection (20.4ng/ml) compared to non-IBD patients (27.1ng/mL) (p=0.002).	(Ananthakrishnan et al., 2014)
Multiple Sclerosis (MS) patients	Serum 25(OH)D level was measured in all patients. Stool samples were collected and fecal DNA was extracted and sequenced.	Serum vitamin D_3 levels above 40ng/mL in untreated MS patients was associated with increased *Ruminococcaceae,* which produces anti-inflammatory SCFAs.	(Tankou et al., 2015)
Healthy white women and MS patients with insufficient vitamin D_3 levels	MS patients were either treated with glatiramer acetate or untreated. Stool was collected stool at baseline and after 90 days of vitamin D_3 (5,000 IU/day) supplementation. The abundance of OTUs was evaluated by hybridization of 16S rRNA to a DNA microarray.	*Akkermansia, Faecalibacterium*, and *Coprococcus* genera were increased in untreated MS patients after vitamin D_3 supplementation compared to the other groups.	(Cantarel et al., 2015)
Humans with or without IBD and C57BL/6 transgenic h vitamin D_3 receptor (hVDR) mice	Mice were treated with a 2-3% dextran sulfate sodium (DSS) solution. Colon biopsies underwent histological, anti-VDR immunohistochemical and RNA analysis.	VDR levels were greatly reduced in Crohn's patients. VDR deletion in mice led to severe colitis. The authors suggested that VDR signaling can reduce colonic inflammation by maintaining mucosal barrier function preventing luminal bacteria and antigens from interacting with the lamina propia.	(Liu et al., 2013)

Table 3. Effects vitamin D_3 and its biologically active component $1,25(OH)_2D$ has on the immune system

Immunological effect	References
Anti-inflammatory effects	
Promotes the maturation, survival and apoptosis of dendritic cells (DC), which is a key mechanism of the adaptive immune system	(Adorini, 2002)
Decreases synthesis of pro-inflammatory interleukin (IL)-6, IL-12, IL-23, IL-1, IL-8, IL-17, IL-21, TNF-α and IFN-γ in an a variety of cells types	(Adorini, 2002; Gombart, 2009; Kamen and Tangpricha, 2010; Li et al., 2013; Zhang et al., 2013)
Inhibits IL-12 and increases DC mediated IL-10 production which increases regulatory T cells (Treg) production promoting a Th2 response.	(Gombart, 2009)
Induces vitamin D receptor (VDR) expression and inhibits T cell proliferation, IL-2 and IFN-γ production and T helper 1(Th1) cells development.	(Adorini, 2002)
Decreases B cells and antibody production	(Veldhoen and Brucklacher-Waldert, 2012)
Decreases IgG and IgM production	(Veldhoen and Brucklacher-Waldert, 2012)
Decreases Th1 and T helper 17 (Th17) cell differentiation while enhancing tolerogenic responses	(Veldhoen and Brucklacher-Waldert, 2012)
Increases T helper 2 (Th2) cell differentiation	(Veldhoen and Brucklacher-Waldert, 2012)
Promotes Treg proliferation by increasing C-C motif chemokine ligand 22 (CCL22) expression	(Gombart, 2009)
Down regulation of Th1/Th17 and CD4+ T	(Kamen and Tangpricha, 2010)
Toll-like receptor (TLR) -2 expression regulation	(Proal et al., 2013)
Macrophages, DCs and activated T cells respond to vitamin D_3 activated VDRs	(Zhang et al., 2013)
Promotes autoimmune homeostasis by stimulating Treg and inhibiting TLR8 activity	(Adorini and Penna, 2008)
Activate macrophages, DCs and activated T cells through the activated VDRs	(Zhang et al., 2013)
Antimicrobial properties	
Up regulates the production of antimicrobial peptides such as cathelicidin and β-defensin and TLR2	(Proal et al., 2013)

Increases cathelicidin antimicrobial peptide (*CAMP*) and defensin β2 expression	(Froicu and Cantorna, 2007; Wang et al., 2010)
Regulates cathelicidin which activates the transcription of autophagy-related genes *Beclin-1* and autophagy related 5(*ATG5)* in human monocytes	(Yuk et al., 2009)
Induces nucleotide-binding oligomerization domain 2 (NOD2) in monocytic and epithelial cells which then stimulates NF-kB and defensin β2	(Wang et al., 2010)
Activates peroxisome proliferator activated receptor (PPAR) -gamma and alpha, glucocorticoids and androgens	(Albert et al., 2009)
Intestinal barrier maintenance	
D controls the expression of the tight junction proteins ZO-1, ZO-2, and can up regulate claudin 2 and 12 and down regulate cadherin-17 in the intestine.	(Zhang et al., 2013)
Inhibits pathogenic *Escherichia coli* O157:H7–induced reductions in transepithelial barrier resistance and decreasing intestinal permeability in epithelial cells.	(Assa et al., 2014)
Increases transepithelial electrical resistance and decreasing LPS levels in Caco-2 cells that were either incubated or not with DSS.	(Zhao et al., 2012)
Dysbiosis prevention	
Attenuates irritable bowel disease (IBD) through its anti-inflammatory properties and the prevention of dysbiosis.	(Cantorna et al., 2014)

Table 4. Definition of adequate and deficient level of serum $1,25(OH)_2D_3$ for the general population

Organization		References
National Institutes for Health 2016		
Deficiency	< 12 ng/mL (< 30 nmol/L)	(National Institutes for Health, 2011)
Insufficiency	12- < 20 ng/mL (30-< 50 nmol/L)	(National Institutes for Health, 2011)
Sufficiency	≥ 20 ng/mL (≥ 50 nmol/L)	(National Institutes for Health, 2011)
Safer upper limit	> 50 ng/mL (> 125 nmol/L)	(National Institutes for Health, 2011)
Institute of Medicine (IOM) 2011		
Deficiency	20 ng/mL or less (50 nml/L or less)	(Ross et al., 2011)
Insufficiency	> 50 ng/mL (> 125 nmol/L)	(Spiro and Buttriss, 2014)
Sufficiency	30 ng/mL for more than 97.5% of the population	(Hilger et al., 2014; Ross et al., 2011)
Optimal	No guidelines have been established for autoimmune diseases.	(Spiro and Buttriss, 2014)
Safe upper limit	100 ng/mL	(Jean-Claude Souberbielle, 2010)
World Health Organization (WHO)/ FAO 2004		
Deficiency	< 20 ng/mL (50 nmol/L)	(WHO, 2004)
Insufficiency	< 30 ng/mL (75 nmol/L)	(WHO, 2004)
Optimal	No guidelines have been established.	(WHO, 2004)
The Endocrine Society's Clinical Guidelines Subcommittee Task Force 2011 (U.S.)		
Deficiency	< 20 ng/mL (50 nmol/L)	(Holick et al., 2011)
Insufficiency	21–29 ng/mL (525–725 nmol/L)	(Holick et al., 2011)
Optimal	Few randomized controlled trials have raised serum levels above 30 ng/mL and there lacks evidence that levels about 50 ng/mL provide health benefits.	(Holick et al., 2011)

Nordic Council of Ministers 2013		
Deficiency	< 25 nmol/L	(Nordic Council of Ministers, 2008)
Insufficiency	30-50 nmol/L	(Nordic Council of Ministers, 2008)
Slight hypovitaminosis	< 40 nmol/L	(Nordic Council of Ministers, 2008)
Optimal	No established guidelines, but levels > 50 nmol/L were considered to be desirable.	(Nordic Council of Ministers, 2008)
European Food Safety Authority (EFSA) 2016		
Insufficiency	< 50 nmol/L	(European Food Safety Authority, 2016)
Optimal	50 nmol/L for all populations and age groups	(European Food Safety Authority, 2016)

Table 5. Daily vitamin D_3 intake recommendations (men and women) according to different organizations

Organization		**References**
Institute of Medicine (IOM) 2011		(Ross et al., 2011)
0-12 months	400 IU (10 μg)	
1- 70 years old	600 IU (15 μg)	
> 70 years old	800 IU (20 μg)	
Pregnant and breastfeeding women	600 IU (15 μg)	
World Health Organization (WHO)/ Food and Agriculture Organization of the United Nations (FAO) 2004		(WHO, 2004)
0-6 months- 50 years	200 IU (5 μg)	
Pregnant and breastfeeding women	200 IU (5 μg)	
51-65 years old	400 IU (10 μg)	
> 66 years old	600 IU (15 μg)	
European Food Safety Authority (EFSA) 2016		(European Food Safety Authority, 2016)
7-11 months	400 IU (10 μg)	
1-17 years	600 IU (15 μg)	
Pregnant and lactating women	600 IU (15 μg)	
Nordic Council of Ministers 2013		(Nordic Council of Ministers, 2008; WHO, 2004)
< 6 months- 74 years	400 IU (10 μg)	
≥ 75 years	800 IU (20 μg)	
Pregnant and breastfeeding women	400 IU (10 μg)	
DACH (Germany, Austria, Switzerland) 2013		(Spiro and Buttriss, 2014)
< 1 year	400 IU (10 μg)	
1- all adults	800 IU (20 μg)	
Pregnant and lactating women	800 IU (20 μg)	