University of Applied Sciences Hamburg

Life Sciences Faculty

Study Programme Ecotrophology

The Role of Vitamin D Substitution in Autoimmune Diseases – Physiological Mechanisms and Evidence based Therapeutical Efficacy

Bachelor thesis

submitted by

Joann Kiebach

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Supervising examiner: Prof. Dr. Jürgen Lorenz (HAW Hamburg)

Second examiner: Prof. Dr. Sibylle Adam (HAW Hamburg)

Preface

The present bachelor thesis was drawn up as part of my Ecotrophology studies at the Hamburg University of Applied Sciences.

The idea for this topic already occurred during earlier stages of the degree course. Therefore, at this point I would like to particularly thank Prof. Dr. Lorenz for supporting my choice of topic and language as well as the whole writing process as my supervisor.

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Furthermore, I would like to thank my family for being a big support and encouragement throughout my whole bachelor's degree programme.

Hamburg, September 2nd, 2019

Joann Kiebach

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I. List of abbreviations

25(OH)D	Calcidiol
ACR	American College of Rheumatology
ANA	Anti-Nuclear-Antibodies
Anti-CCP	Anti-Cyclic Citrullinated Peptide Antibodies
anti-dsDNA	anti-double-stranded-DNA Antibodies
ARR	Annual Relapse Rate
BILAG Index	British Isles Lupus Assessment Group index
BW	Body Weight
CG	Control Group
CIA	Collagen-Induced Arthritis
CNS	Central Nervous System
CRP	C-reactive Protein
DAS28	Disease Activity Score 28
DGE	Deutsche Gesellschaft für Ernährung
EAE	Experimental Autoimmune Encephalomyelitis
ECLAM	European Consensus Lupus Activity Measurement
EDSS	Expanded Disability Status Scale
ESR	Erythrocyte Sedimentation Rate
EULAR	European League Against Rheumatism
FIS	Fatigue Impact Scale
FSS	Fatigue Severity Scale
GD	Graves’ disease
HAQ	Health Assessment Questionnaire
HLA	Human Leukocyte Antigen
HRUSG	High Resolution Ultrasonography

HT	Hashimoto Thyroiditis
IFN	Interferon
IG	Intervention Group
IL	Interleukin
IOM	Institute of Medicine
i-PTH	Intact Parathormone
IU	International Units
LT4	Levothyroxine
MS	Multiple Sclerosis
MSFC	Multiple Sclerosis Functional Composite
NOD-mice	Non-Obese Diabetic mice
PP	Per-Protocol Population
PTH	Parathormone
QoL	Quality of Life
RA	Rheumatoid Arthritis
RAID	RA Impact of Disease
SLE	Systemic Lupus Erythematosus
SLEDAI(-2K)	Systemic Lupus Erythematosus Disease Activity Index (-2000)
T3	Triiodothyronine
T4	Thyroxine
Tg-Ab	Thyroglobulin Antibodies
TGF	Transforming Growth Factor
TNF	Tumor Necrosis Factor
Th1 cells	T-Helper Cells Type 1
Th17 cells	T-Helper Cells Type 17

Th2 cells	T-Helper Cells Type 2
TPO-Ab	Thyroid Peroxidase Antibodies
Treg	Regulatory T Cells
TRH	Thyrotropin-releasing hormone
TSH	Thyroid-stimulating Hormone
VAS	Visual Analogue Scale
VitD	Vitamin D

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1. Introduction

Autoimmune diseases, including Multiple Sclerosis, Rheumatoid Arthritis and Systemic Lupus Erythematosus, are characterized by an abnormal immune response directed against harmless self-antigens. Consequential inflammations lead to tissue damage and loss of function of the affected organs or joints (Dankers, et al., 2017). The estimated worldwide prevalence is 7.6 - 9.4 % (Cooper, Bynum, & Somers, 2009) with an increasing tendency (Lerner, Jeremias, & Matthias, 2015), thus, the social burden is increasing as well. Although the etiology of autoimmune diseases remains widely unknown, it is assumed that in susceptible individuals, factors like genetic polymorphisms, environmental causes, epidemiological risks and hormonal conditions may trigger the disease pathogenesis (Antico, et al., 2012; Rosen, et al., 2016). More than 80 different autoimmune conditions have been identified, which cannot be cured, even if progression and symptoms may be managed. Indeed, the treatment has improved due to the development of so-called biologics targeting specific signaling pathways instead of non-specific immunosuppressants (Tavakolpour, 2017). However, many patients are still not responding adequately to these therapies (Dankers, et al., 2017), which highlights the necessity of new therapy options as well as improvement or completion of current strategies.

In this context, vitamin D might be a promising agent. The steroid hormone is contained in few foods, but mainly synthesized endogenously in the skin through sunlight (Bizzaro, et al., 2017). Several approaches suggest its beneficial effect on autoimmunity. A first hint is the connection between the prevalence of some autoimmune diseases like multiple sclerosis and increasing latitude, and hence, a decreasing sunlight exposure (Dankers, et al., 2017). In addition, epidemiological data show a high prevalence of vitamin D deficiency in different autoimmune disorders (Agmon-Levin, et al., 2013). Originally known for its role in calcium homeostasis and bone health, it has become clear during the last decades that the impact of vitamin D goes beyond this regulation. This importance is demonstrated by the finding of the vitamin D receptor (VDR) on almost all cell types including immune cells. Binding of the active form of vitamin D – $1,25(OH)_2D$ or calcitriol – to the intracellular VDR affects more than 900 genes participating in various physiological processes (Bizzaro, et al., 2017). Thereby, vitamin D can modulate both innate and adaptive immunity. For example, it decreases the proliferation of B cells and their antibody production, inhibits Th1 lymphocytes that are capable of producing proinflammatory cytokines, while promoting the (rather anti-inflammatory) Th2 cell response, and increases the quantity of regulatory T cells (Antico, et al., 2012). These pathways are also assumed to play a role in the development of autoimmune diseases (Dankers, et al., 2017). Additionally, gene polymorphisms of the VDR have been associated with pathological conditions, such as inflammation and

autoimmunity (Colotta, Jansson, & Bonelli, 2017). In summary, this evidence reveals that vitamin D supplementation might help to prevent or to decrease disease activity by balancing and regulating the aberrant immune response causing autoimmunity.

This bachelor thesis aims to present the accumulated data on the efficacy of a vitamin D supplementation in patients with autoimmune diseases. Firstly, the properties of vitamin D and, in that context, the current knowledge about the molecular mechanisms underlying its immunomodulatory effects, will be described. In addition to this biological plausibility for a possible role in autoimmunity, findings that have been made in epidemiological studies as well as experimental models will be depicted. Secondly, a systematic review of controlled clinical trials administering vitamin D for multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus, autoimmune thyroid diseases, type 1 diabetes and myasthenia gravis has been performed. These results are conclusively discussed with regard to potential benefits of vitamin D substitution in clinical practice and further research directions.

2. Properties and Immunomodulatory Effects of Vitamin D

2.1 Physiological Characteristics, Metabolism and Sufficiency

Vitamin D, or Calciferol, a steroid-hormone primarily known for its role in calcium homeostasis, has been gaining more attention regarding its possible immunomodulatory properties during the past years. Already in the 19^{h} century, first hints were found for extra skeletal effects, when vitamin D was used as treatment against tuberculosis, even before the discovery of antibiotics (Dankers, et al., 2017). Vitamin D exists in two forms: vitamin D_3 (cholecalciferol) and D_2 (ergocalciferol). Although its potency is still debated, cholecalciferol is considered the more effective form for raising serum vitamin D levels (Tripkovic, et al., 2012).

As only few foods (naturally) contain vitamin D, of which the most relevant probably is fatty sea fish, endogenous synthesis in the skin – especially during summer months – is the main source. Additionally, endogenously produced vitamin D, compared to ingested, may circulate at least twice as long in the blood (Holick, et al., 2011). This production is induced by UVB radiation, which causes the synthesis of cholecalciferol in the skin, from where it is transported to the liver bound to the vitamin D-binding protein. There it is converted to the inactive prohormone 25(OH)D_3 (calcidiol) and further transported to the kidneys, where it is hydroxylated to the active metabolite, 1,25$(OH)_2D_3$ or calcitriol (see figure 1). Signaling cascades of calcitriol are initiated by binding to the vitamin D receptor (VDR), which is expressed on almost all human cell types (Dankers, et al., 2017). Remarkably, the

hydroxylation for the conversion to the active calcitriol does not only take place in the kidneys (which are the main site, after all) but also in plenty other tissues. This implies the involvement in several physiological processes (Colotta, Jansson, & Bonelli, 2017).

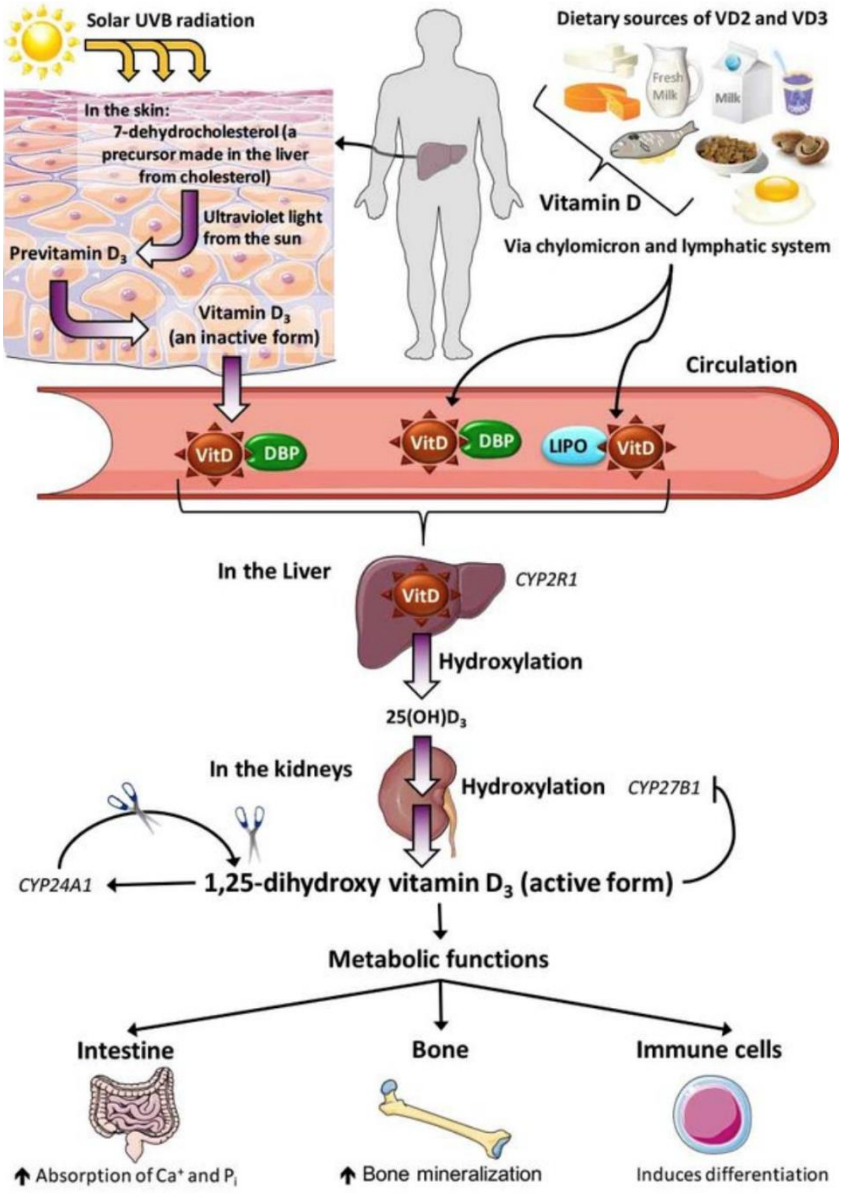

Figure 1: Human vitamin D-metabolism (Keane, et al., 2017)

Dependent on the question whether there are indeed effects apart from bone health, a discussion has occurred in the United States on adequate and sufficient vitamin D levels (Sintzel, Rametta, & Reder, 2018). Serum levels are usually measured using the inactive form, 25(OH)D, as it includes endogenously produced as well as exogenously ingested Vitamin D and has a longer plasma half-life (3 weeks) than the active metabolite, calcitriol (5-8 h) (Colotta, Jansson, & Bonelli, 2017). The recommendations given from the Institute of Medicine (IOM) and the Endocrine Society are based on different foundational models.

The IOM focuses on bone health using a population model with the aim to prevent deficiency in its majority (97.5%). Therefore, they concluded that a serum 25(OH)D level of >20ng/mL (50nmol/L) can be considered sufficient (IOM (Institute of Medicine), 2011). In accordance with the IOM, the German DGE (Deutsche Gesellschaft für Ernährung, German Society for Nutrition) also recommends a serum concentration of 25(OH)D of at least 50 nmol/L (Deutsche Gesellschaft für Ernährung, 2012). In contrast, the Endocrine Society recommendations, based on a medical model taking into account skeletal and also extra skeletal effects as well as the low toxicity, set >30ng/mL (>75nmol/L) as sufficient (Holick, et al., 2011), 40-60ng/mL as ideal and up to 100ng/mL as safe (Bischoff-Ferrari, et al., 2006).

As the serum vitamin D level is mainly dependent on subcutaneous synthesis, and hence, UVB radiation, it is affected by season and latitude, but also clothing and skin exposure (Colotta, Jansson, & Bonelli, 2017). Besides, synthesis is reduced by increasing age, skin pigmentation and BMI. For example, in an adult in bathing clothes that is exposed to UV radiation causing erythema (mild pinkness of the skin) for 24 hours, the produced vitamin D will be equivalent to 10,000 to 25.000IU (International Units; 40IU equivale 1µg of vitamin D (IOM (Institute of Medicine), 2011)). But this production is changed drastically, when the zenith angle of the sun is shifted by latitude, daytime or season. Effectively this means, in latitudes below and above approximately 33° the synthesis of vitamin D in winter is very low or even absent (Holick, et al., 2011). For comparison, 33° corresponds to Casablanca (Morocco) or Atlanta, Georgia (USA) in the northern, and Santiago (Chile) or Cape Town (South Africa), in the southern hemisphere. This results in an insufficient synthesis on the entire European continent, but also big parts of North America and Asia during winter months.

A vitamin D deficiency has been associated with various disease conditions like infectious diseases, cancers, cardiovascular diseases, but also mental disorders and autoimmune diseases. These are linked to a level that is sufficient to prevent rickets – a disease that is known as a result from vitamin D deficiency – but still seem to be suboptimal. Knowing about a possible relation between low vitamin D levels and autoimmune diseases, which have an increasing incidence, a possibly elevated threshold regarding these diseases must be taken into consideration (Sintzel, Rametta, & Reder, 2018).

2.2 Immunomodulatory mechanisms

Activation, proliferation and differentiation of immune and inflammatory cells of the innate, as well as the adaptive immune system are modulated by calcitriol through the vitamin D

receptor expressed in these cells, that are even able to convert 25(OH)D into calcitriol (Colotta, Jansson, & Bonelli, 2017).

The main effects of calcitriol on the different immune and inflammatory cells will be briefly summarized in this subchapter (See figure 2, general effects; table 1, effect mechanisms including IL-production).

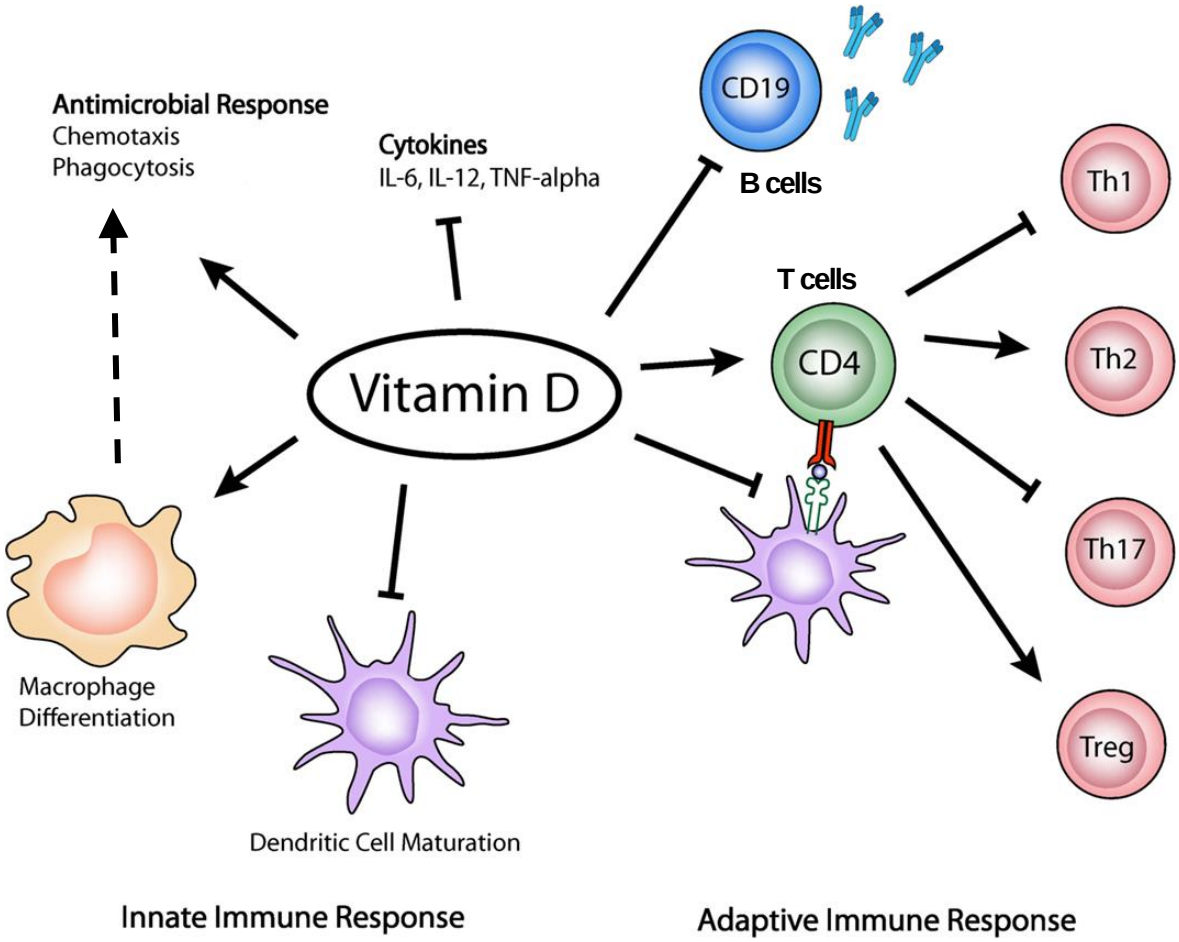

Figure 2: General effects of calcitriol on the innate and adaptive immune system, adapted from: Iruretagoyena et al., 2015

Monocytes and macrophages are an important part of the innate immune response, serving as first and quick defense against outside agents due to their anti-microbial activity, chemotactic and phagocytic capacity. They are subdivided into M1 and M2 macrophages; and while M1 produce proinflammatory mediators and promote a T helper 1 (Th1) and Th17 immune response (that is also rather proinflammatory), M2 produce interleukin 10 (IL-10), which has anti-inflammatory properties (Murray & Wynn, 2011). Calcitriol can stimulate the differentiation of these macrophages and monocytes. But yet it might be able to decrease their production of proinflammatory cytokines such as IL-1, IL-6 and tumor necrosis factor α (TNFα) while increasing IL-10-production, and thus, acts anti-inflammatorily (Zhang, et al., 2012).

Dendritic cells are an important connection between the innate and adaptive immune system, as they are the most important antigen-presenting cells. They process antigens and present them as peptides on HLA molecules (human leukocyte antigen). They are presented to T cells and antigen-specific T cells subsequently differentiate to T effector cells with either pro- or anti-inflammatory properties. This depends on the cytokines secreted by the dendritic cell (Dankers, et al., 2017). Calcitriol can cause a decreased production of pro-inflammatory cytokines like IL-6 and IL-12 and increase the production of IL-10 (Ferreira, et al., 2011). Besides, it promotes the differentiation to so-called tolerogenic dendritic cells. These immature-like cells are less capable of inducing proliferation and cytokine production of proinflammatory T cells. Instead, the differentiation of regulatory T cells (Tregs) is promoted (Unger, et al., 2009). Tregs are a subgroup of T cells suppressing the immune answer and thus, regulating self-tolerance and preventing the development of autoimmune diseases. Additionally, tolerogenic dendritic cells are able to induce apoptosis in autoreactive T cells while not affecting other T cells (van Halteren, et al., 2004).

B cells have several activities in immune responses. They differentiate to plasma cells and produce antibodies, but they also act as antigen-presenting cells and modulate other immune and inflammatory cells by secreting cytokines. In antibody-mediated autoimmune diseases they play a crucial role by producing auto-reactive antibodies, for example anti-citrullinated peptide autoantibodies in rheumatoid arthritis and anti-nuclear antibodies in systemic lupus erythematosus (Dankers, et al., 2017). Calcitriol inhibits the differentiation of B cells to plasma cells. It also may induce apoptosis in B cells and suppresses immunoglobulin class switching (Chen, et al., 2007; Lemire, et al., 1984). The vitamin D receptor is able to directly bind to the promoter region of IL-10 in B cells, upregulating IL-10 production (Heine, et al., 2008).

Originally, dendritic cells were thought to be the main target of calcitriol and effects observed on T cells were mediated by those. But the vitamin D receptor was found on different T cell populations, so T cells are also directly targeted by calcitriol. (Dankers, et al., 2017). T cells are subdivided into CD4+ T cells and CD8+ (cytotoxic) T cells. Th1, Th2, Th17 and Treg cells belong to the CD4+ group. For a long time Th1 cells have been thought to be the main mediator in disease pathogenesis of autoimmune diseases. But with the finding of Th17, this subpopulation is now considered to be one of the most important drivers of autoimmunity. However, Th1 cells may also still have a role in pathogenesis (Dankers, et al., 2017). Calcitriol inhibits the production of the proinflammatory interferon-γ (IFN-γ) and IL-2 in Th1 cells (Pichler, et al., 2002).

Th2 cells are pathogenic in the development of asthma and allergies, but in the context of autoimmunity, they might have a protective role in Th17 driven diseases, in contrast to Th1 (Dankers, et al., 2017). Calcitriol may promote the production of the Th2-cytokine IL-4, but only when its levels are diminished (Colin, et al., 2010). In the presence of IL-4, the cellular IL-4 production is unaffected or even inhibited (Pichler, et al., 2002). So calcitriol might promote a Th2 differentiation along with IL-4 production and help suppress autoimmunity, but only when available IL-4 is insufficient (Dankers, et al., 2017).

In Th17, calcitriol might decrease activity by inhibiting IL-17A, IL-17F and IL-22-expression, and also suppresses Th17 differentiation (Colin, et al., 2010).

Tregs are able to downregulate the activity of macrophages, dendritic cells, CD4+ and CD8+ T cells, and they produce anti-inflammatory cytokines like IL-10 and transforming growth factor β (TGFβ). Tregs also express the transcriptional factor FoxP3 and are programmed by this. Calcitriol upregulates FoxP3 expression by binding to its promoter and induces Tregs differentiation (Kang, et al., 2012). In CD8+ T cells, calcitriol inhibits IFNγ and TNFα-expression (Lysandropoulos, et al., 2011).

As all these immune cells are also involved in the pathogenesis of autoimmune disease, a possibly modulating agent like vitamin D is of course of particular interest.

Table 1: Effect of calcitriol on immune cells including interleukin-inhibition/expression (references, see text)

	Immune Cell	***Induction / Effect***
Innate Immune System	Monocytes / Macrophages	Differentiation ↑ IL-1, IL6, TNFα ↓ IL-10 ↑
	Dendritic Cells (DC)	IL-12, TNFα ↓ IL-10 ↑ Tolerogenic DCs ↑ → proliferation, cytokine prod. of proinflammatory T cells ↓ → Tregs ↑ → apoptosis in autoreactive T cells ↑
Adaptive Immune System	B Cells	Differentiation to plasma cells ↓ Apoptosis ↑ Immunoglobulin class switching ↓ IL-10 ↑
	T Cells: Th1 Th2 Th17 Treg Cytotoxic T cells	 IFN-γ ↓ (IL-4 ↑) IL-17A, IL-17F, IL-22 ↓ Differentiation ↓ Differentiation ↑ FoxP3 expression ↑ IFN-γ, TNF-α ↓

3. Vitamin D and Autoimmunity

3.1 Principles of Autoimmunity

Autoimmune diseases result from immune reactions directed towards self-antigens with a consequential tissue damage. This destruction is caused by the formation of either autoreactive antibodies or T cells, but as in every immune response, various immune and inflammatory cells are involved, and it is not solely one part of the immune system acting. Etiology as well as the exact pathophysiology are still widely unknown. Most likely, one of the main mechanisms is the disruption of self-tolerance. Immune tolerance can be defined as the immune systems' capability to prevent itself from targeting body's own structures, thus molecules, cells or tissues. This immune homeostasis is regulated by several key

concepts. Central tolerance in the thymus and bone marrow is one of them. In the thymus, immature lymphocytes go through positive selection before maturation and release to the circulation, meaning possibly self-reactive lymphocytes are deleted in the thymic medulla. Similarly, if immature B cells express surface antigens recognizing general self-antigens, they are eliminated as well. Peripheral tolerance is a second selection process outside the thymus or bone marrow, where self-reactive T and B cells are either deleted or they become anergic, thus inactive. But even in healthy individuals, small numbers of possibly self-directed lymphocytes can escape these mechanisms and circulate in the periphery. This does not necessarily lead to autoimmunity, because even if they are detected and targeted, the immune response normally is limited by modulators like Tregs. Physiological autoimmunity, as this state is also called, is usually transient and has no further consequences. This also explains the existence of autoantibodies like the rheumatic factor and antinuclear antibodies in healthy persons. However, in these susceptible individuals triggers like genetic predisposition or environmental factors may lead to an inflammation and positive feedback loops contribute to the onset of an autoimmune disease. This process is summarized in figure 3. Genome-wide association studies identified that several possible genetic variants are associated with autoimmunity, especially HLA, which are involved in antigen-presentation (Wang, Wang, & Gershwin, 2015). Environmental factors particularly include infections, but also nutrition, tobacco smoke and hormones (Murphy & Weaver, 2018).

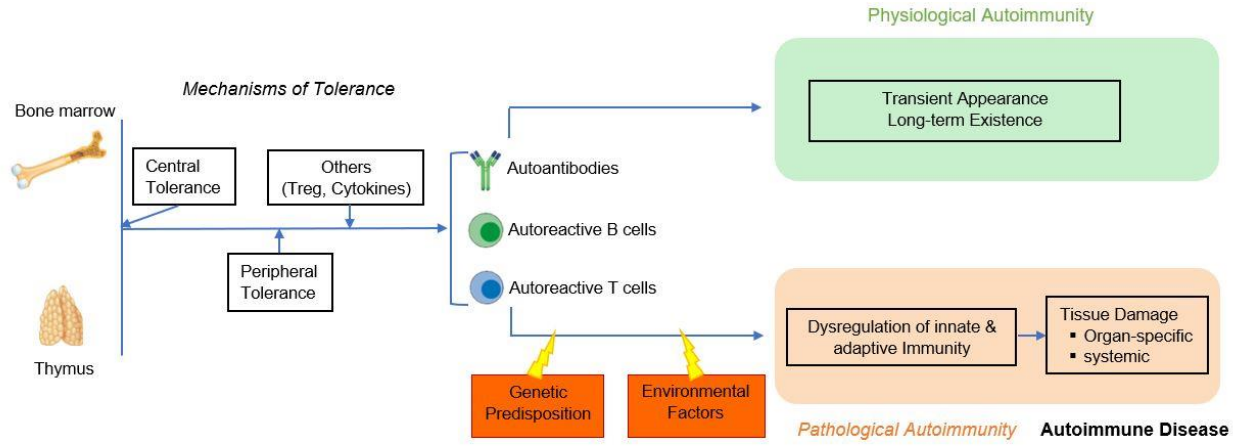

Figure 3: Schematic development of autoimmune diseases, adapted from: Wang, Wang, & Gershwin, 2015

In manifested autoimmune diseases, two types of autoimmune diseases are distinguished (table 2): Organ-specific autoimmune diseases and systemic autoimmune diseases. While in organ-specific diseases the reaction is directed towards autoantigens existing in only one or few organs, hence, limiting the disease to these areas, systemic diseases cause damage in several tissues and organs, because the attacked autoantigens occur on different cell types (Murphy & Weaver, 2018).

Table 2: Classification of autoimmune diseases (Murphy & Weaver, 2018)

Organ specific autoimmune diseases	*Systemic autoimmune diseases*
Multiple sclerosis (CNS)	**Rheumatoid arthritis**
Graves' disease Hashimoto thyroiditis (Thyroid gland)	**Systemic lupus erythematosus**
Type 1 diabetes mellitus (Pancreas)	**Systemic sclerosis**
Myasthenia gravis (acetylcholine receptors on motor end plates)	

The complex and not fully understood pathogenesis including and combining so many individual factors, is probably a main reason for the absence of a therapy that is able to cure an autoimmune disease. It is hoped to find a possibility to modify the immune system towards a restoration of tolerance. This succeeded in murine models of autoimmunity but has not been effective yet in humans (Wang, Wang, & Gershwin, 2015). Current therapy usually consists of two parts: the symptomatic or replacement therapy and the immunosuppressive or immunomodulating therapy. These approaches are used individually or combined. The symptomatic therapy primarily aims do decrease symptoms and maintain function. An example are autoimmune thyroid diseases, where it is tried to reduce the thyroxin production in case of hyperfunction or substitute the hormone at hypofunction (Chandrashekara, 2012). The immunomodulating therapy is a more aggressive therapy form aiming to modulate the inflammatory and/or effector pathways with the use of biologic agents. Substances that block TNFα were approved first and used for various autoimmune diseases like rheumatoid arthritis, multiple sclerosis, psoriasis and systemic lupus erythematosus. Other drugs include mycophenolate-mofetil, an immunosuppressive agent with antiproliferative and pro-apoptotic effects especially on activated T cells, but also B cells, or methotrexate with similar effects. Also, IL6- and IL-12 inhibitors are used (Wang, Wang, & Gershwin, 2015). Alongside these biologic agents, also unspecific immunosuppressive drugs like corticosteroids are used, which may rapidly induce remission in many patients, but have problematic long-term effects with an impact on patients' quality of life. Monotherapy with other nonspecific agents causing less side effects remains problematic, as they all influence many cells in the body and block several

signaling pathways. This augments the risk of secondary conditions like cancer (Tavakolpour, 2017).

Comparing the pathways of the biologic drugs developed in the past years, e.g. targeting TNFα, with the possible effects of vitamin D described in chapter 2.2, various similarities are noticeable. This supports the interest in vitamin D as treatment for autoimmune disease, as there is an urgent need for further specific therapies that do not affect the normal immune response.

3.2 Epidemiological Studies

Various studies investigated a possible relation between vitamin D and the incidence and disease activity of autoimmune diseases. One first hint for a correlation was found for multiple sclerosis, because the disease prevalence appeared to increase with increasing latitude and accordingly decreasing UVB radiation (Simpson, et al., 2011). This link was also found for other autoimmune diseases like type I diabetes mellitus (Mohr, et al., 2008). Further evidence for the impact of sun exposure provides the finding of a relation between the risk of multiple sclerosis development and birth month. In the northern hemisphere, the risk was higher in April, thus, with a pregnancy during winter months, whereas the risk was decreased in October and November. Crucial in this context is also the fact that this correlation was only found in regions with a change in UV radiation throughout the year (Dobson, Giovannoni, & Ramagopalan, 2013). But also the vitamin D intake through food or supplementation might be relevant. In terms of diabetes mellitus type I, it could be demonstrated that a supplementation of vitamin D during early childhood can, depending on the frequency, reduce the disease risk up 30%. Additionally, a correlation between maternal vitamin D intake and diabetes risk in the offspring was recorded but could not be confirmed yet (Dong, et al., 2013). Rheumatoid arthritis is another autoimmune disease for which an inverse correlation between incidence and vitamin D intake could be found (Song, Bae, & Lee, 2012). Because measuring vitamin D intake and UV exposure is mostly based on estimations, studies analyzing correlations with 25(OH)D levels might be more accurate. Also here, evidence shows that patients suffering from rheumatoid arthritis often have lower serum 25(OH)D levels compared to healthy controls and lower vitamin D levels are also associated with disease activity (Lin, et al., 2016). Importantly, these epidemiological studies can only show correlations, so it must be clarified whether low vitamin D levels are the cause or rather a result of the autoimmune disease. For example, this is particularly questionable in systemic lupus erythematosus, because UV radiation may trigger relapses and patients are therefore advised to avoid direct sun exposure and apply sunscreen (Agmon-Levin, et al., 2013).

3.3 Experimental Models

In addition to the evidence provided by epidemiological studies, many animal studies have been performed to investigate potential effects of vitamin D using experimental models of different autoimmune diseases.

Two of these models, the autoimmune encephalomyelitis (EAE) and the collagen-induced arthritis (CIA), are used to represent multiple sclerosis and rheumatoid arthritis, respectively (Dankers, et al., 2017). A treatment with calcitriol could prevent the initiation as well as the progression in both models (Cantorna, Hayes, & DeLuca, 1996; 1998). In EAE, calcitriol was able to prevent and reverse paralysis (Cantorna, Hayes, & DeLuca, 1996). Beneficial effects were also observed after administering high doses of dietary vitamin D. From a therapeutic point of view, it is very interesting that the combination of vitamin D with IFN-β, which is commonly used in multiple sclerosis, seems to be more effective than the two agents alone (van Etten, et al., 2007). However, the suppressive effects seem to require dietary calcium. Positive effects of calcitriol in the EAE-model are associated with the inhibition of IL-12 and IL-17. Besides, IL-10-signaling seems to be necessary (Mattner, et al., 2000; Spach, et al., 2006; Joshi, et al., 2011). Consequently, the effect seems to be connected to Th17 cells, which are thought to be one of the major cell types involved in the disease with the expression of IFN-γ, among others (Hirota, et al., 2011). Additionally, there is a possible effect of calcitriol on CD4+ T cells, as it may inhibit the migration of those cells into the CNS, hence, suppressing the immune reaction (Grishkan, et al., 2013). In CIA, vitamin D was also able to reduce swellings in hind paws (Tsuji, et al., 1994).

A protective effect of vitamin D could also be observed in nonobese diabetic (NOD) mice, supposedly mediated by decreasing T effector cells while inducing Tregs (Takiishi, et al., 2014) In models of systemic lupus erythematosus, calcitriol could reduce disease severity (Lemire, Ince, & Takashima, 1992). If vitamin D was administered before disease induction, the expression of IL-17, IL-23 and IFN-γ as well as titers of IL-17 and importantly, anti-double-stranded DNA antibodies, as often found in SLE, could be reduced significantly. However, the treatment with vitamin D after disease onset showed no effects (Faraji, et al., 2016). This especially supports a preventive role of vitamin D in this condition.

Taken together, these results indicate an involvement of vitamin D in autoimmunity and highlight its possible efficacy in disease treatment. Results of human studies investigating the direct impact of vitamin D supplementation in different autoimmune disease will be described and evaluated in the following chapters.

4. Methods

A systematic research has been performed from April 23 to May 2, 2019, using PubMed and ScienceDirect databases.

Six autoimmune diseases were considered in the review, namely multiple sclerosis, systemic lupus erythematosus, rheumatoid arthritis, autoimmune thyroid diseases, diabetes mellitus type I and myasthenia gravis. Used search terms are shown in Table 2.

Table 3: Search term combinations used in PubMed and ScienceDirect

Keywords	**PubMed**	**ScienceDirect**
((Multiple Sclerosis) OR MS) AND Vitamin D	101	343
(((Systemic Lupus erythematosus) OR SLE) OR Lupus) AND Vitamin D	27	47
((Rheumatoid Arthritis) OR RA) AND Vitamin D	30	48
((((Hashimoto thyroiditis) OR Basedow's disease) OR Graves disease) OR autoimmune thyroid disease) AND Vitamin D	9	55
(((type 1 Diabetes mellitus) OR juvenile Diabetes mellitus) OR type I Diabetes mellitus) AND vitamin D	7	26
(Myasthenia gravis) AND Vitamin D	1	2

When searching PubMed, the following filters were used:

- Article types: Clinical Study, Clinical Trial, Meta-Analysis
- Publication dates: 2012-2019
- Languages: English, German
- Species: Human
- Search in: Title/abstract

Filters used in Science Direct were:

- years: 2012-2019;
- search in: Title, Abstract, keywords;
- article Type: research articles + review articles

For every search term combination, titles and abstracts of results were screened and full texts of studies that might be relevant to the review were obtained or requested, if not available.

Additionally, a manual search has been performed screening bibliographic references of identified studies for other possibly eligible articles.

Clinical trials investigating the effects of Vitamin D supplementation (Vitamin D_3, D_2, Calcitriol or Vitamin D analogues) compared to either placebo, second treatment (other form of Vitamin D/different dose) or no treatment (open label) were considered eligible.

The following criteria led to study exclusion:

(1) non-controlled trial (no comparison group)
(2) no clinical outcomes, only surrogate markers in MS, RA, SLE, Myasthenia gravis (in type I Diabetes and autoimmune thyroid diseases there are not primarily clinical symptoms)
(3) Vitamin D as add-on therapy only (as clearly stated in study aims/outcomes/endpoints), except for insulin therapy in type I diabetes and levothyroxine therapy in autoimmune thyroid diseases (hormone substitution rather than immune-modulating or -suppressing therapy)
(4) Treatment naïve patients beginning conventional therapy as well
(5) meta-analyses (partly) reviewing studies that met abovementioned criteria.

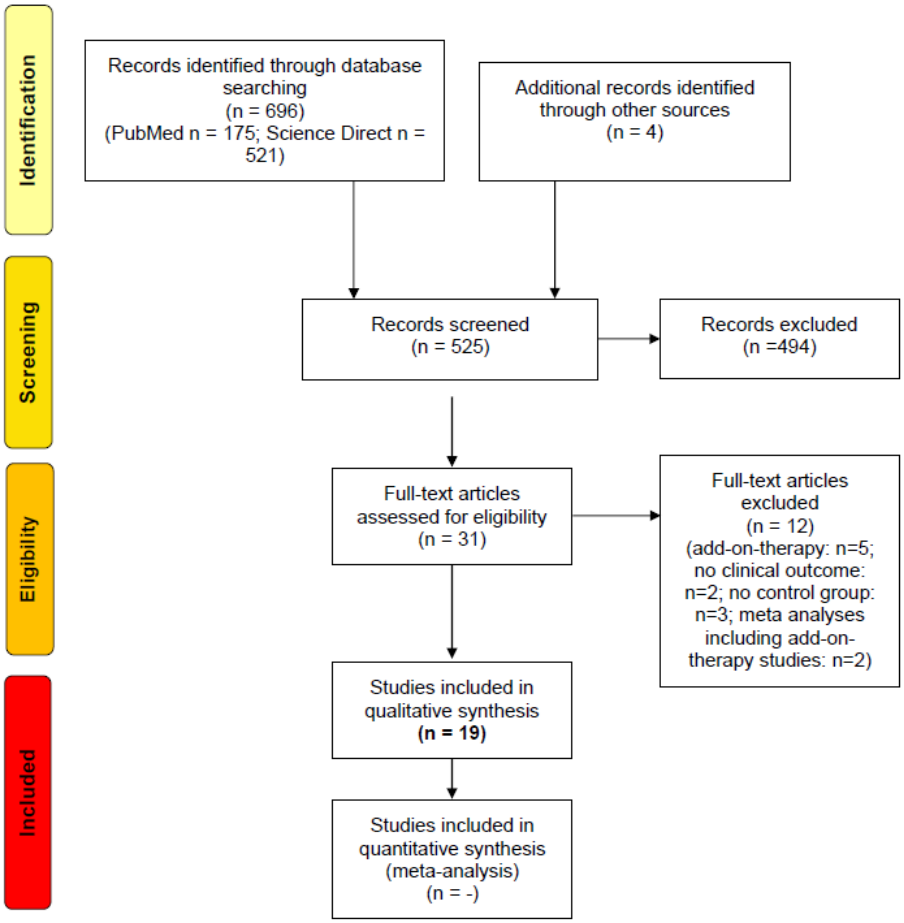

Figure 4: Flow chart of study selection according to PRISMA-guidelines (Moher, et al., 2009)

The PRISMA flow chart for study selection processes and included studies is presented in Figure 1. Overall, 19 Studies were deemed eligible for inclusion (MS: 3, RA: 5; Autoimmune thyroid diseases: 5; Diabetes mellitus type I: 3; Myasthenia gravis: 0)

5. Reviewed Studies

Hereinafter, results of the surveyed studies will be described and summarized. Methodological quality has been considered and will be involved, but due to the heterogeneity of study types included (randomized, double-blind controlled trials as well as nonrandomized and open-label studies), the use of a tool for methodological quality or risk of bias assessment has been waived. As these tools are usually developed to assess either RCTs or other study types, their applicability for both is arguable (Higgins & Green, 2011)

5.1 Multiple Sclerosis

Multiple sclerosis, a chronic inflammatory demyelinating disorder located in the CNS, is characterized by a focal inflammatory infiltration (lesions) by immune cells causing demyelination and neurodegeneration (Compston & Coles, 2008). Disease etiology remains widely unknown, it is believed to be triggered by genetic and environmental factors, as cases are clustered within families and several gene loci have been revealed as risk factors. Ebstein-Barr virus infection has been strongly linked to MS (Ascherio & Munger, 2007) and also vitamin D has been associated with the disease, as described above.

Pathogenesis involves an immune reaction against CNS antigens mediated through T cells of the CD4+ type, but with additional involvement of monocytes, macrophages and B cells. The evidence from animal models suggests that Th1 cells releasing IFN-γ, IL-2 and TNF-α along with Th17 play a key role in the mediation of inflammation in MS, whereas Th2 cells (secreting IL-4, 5 and 10) and Tregs have an inhibiting function (Garg & Smith, 2015).

Multiple sclerosis often has a relapsing-remitting course and clinical symptoms differ between patients. They may include neurological (sensory, visual) as well as motor disabilities often accumulating throughout the disease course (Garg & Smith, 2015).

Based on the epidemiological link between sun exposure and the incidence of MS, the connection has been further investigated in interventional studies.

Three studies on multiple sclerosis met the selection criteria. Their characteristics are shown in table 4.

Achiron et al. aimed to evaluate the effect of Alphacalcidol, a vitamin D analogue, on fatigue in MS. In a randomized and double-blind study, they have treated 158 multiple sclerosis patients with either alphacalcidol or placebo for six months and followed them for two more months. They observed a significantly greater fatigue reduction (measured with the Fatigue Impact scale, a validated 40 items questionnaire) in the treatment arm, compared to the control arm (-41,6% vs. -27.4%; p=0.007). Also, the number of relapses was significantly lower in the treatment group than in the placebo group, and the quality of life was significantly improved in both social and psychological subscales of the used instrument. However, no improvement in the Expanded Disability Status Scale (EDSS) (a common and verified method to quantify disability in MS-patients, based on examination of different functional systems, like brain stem, sensory, visual and other (Kurtzke, 1983)) was observed (Achiron, et al., 2015).

The randomized, placebo-controlled trial by Kampman and colleagues was initially designed to assess the effect of vitamin D on bone mineral density in MS-patients, with no effects found. This current paper reported the impact on exploratory outcomes of the study. 71 participants have been treated with cholecalciferol or placebo for 96 weeks but were allowed to continue vitamin D supplementation they used at baseline. Although the 25(OH)D level was successfully raised to at least 75nmol/L in 80% of patients in the intervention group, no differences in EDSS score, fatigue, grip strength or annual relapse rate have been observed. Median time until the first relapse tended to be longer in the intervention group, but without statistical significance (Kampman, et al., 2012).

Shaygannejad et al. investigated the effect of vitamin D treatment on the progression of relapsing-remitting MS in a double-blind study. Patients continued their ongoing disease-modifying treatment, but intake was not limited to certain drugs, which is why this trial was not considered an add-on-design in the first place. 50 participants were randomized to receive either calcitriol or placebo for 12 months. A significant increase in EDSS was observed in the placebo group, compared to no change in the intervention group, but the between-group difference was insignificant. Relapse rate decreased significantly in both groups, with no difference between groups (Shaygannejad, et al., 2012).

Taken together, no conclusive benefit of vitamin D supplementation can be reasoned for patients suffering from multiple sclerosis, as only one of three studies observed clear effects. However, it is remarkable that they all use different forms and doses of vitamin D, namely Alphacalcidol 1µg daily (Achiron, et al., 2015), cholecalciferol 20,000IU (500µg) weekly (Kampman, et al., 2012) and calcitriol 0.5µg (0.25µg for first two weeks) daily (administered twice daily) (Shaygannejad, et al., 2012). This makes it hard to draw

comparisons between the trials. As described above, studies also differed regarding sample size. The three single-center studies were carried out in Israel (Achiron, et al., 2015), Iran (Shaygannejad, et al., 2012) and Norway (Kampman, et al., 2012); all of them having a similar, but wide age range (total range: 15-60 years) of included patients and using placebo in the control arm. In Achiron as well as Shaygannejad et al. patients continued their standard treatment, whereas there is no report on ongoing medication in Kampman et al. except for vitamin D supplementation, which was allowed to be continued in both treatment arms. All studies paid attention to safety and adverse events, but no severe adverse events or side effects were observed (Achiron, et al., 2015; Kampman, et al., 2012; Shaygannejad, et al., 2012).

The three studies reviewed on multiple sclerosis were considered similar in terms of study quality. Positively, they all performed intention to treat-analyses, recorded dropouts and described blinding methods. Kampman et al. also described randomization procedure and Achiron and colleagues used adequate allocation concealment. Noteworthy, the trial by Achiron was the only one receiving grants from a pharmaceutical company. However, the authors assured the company had no influence on study design, data collection and analysis or publication (Achiron, et al., 2015).

Table 4: Characteristics of included studies on multiple sclerosis

Achiron et al. (2015) – Effect of Alfacalcidol on multiple sclerosis-related fatigue: A randomized, double-blind placebo-controlled study				
Methods	**Participants**	**Interventions**	**Outcomes**	**Results**
- prospective, randomized, double-blind, placebo-controlled trial - country: Israel - study years: not reported	- randomized: n=158 - inclusion criteria: clinically definite MS by McDonald criteria, fatigue interfering with work/family/social life, FIS score of ≥40, age 18-55, neurological disability in EDSS score ≤5.5 - exclusion criteria: relapse within 30 days prior to study, blood calcium >10.5mg/dl, history of hypersensitivity/intolerance to Alphacalcidol, life threatening/unstable clinical condition, alcohol/drug abuse	- Intervention group (n=80): VitD analogue (Alphacalcidol; 1µg) orally daily - Control group (n=78): placebo with arachis oil identical in appearance - administration for 6 months - follow-up at month 8 - Co-Intervention: continuation of ongoing treatment, relapses treated with intravenous methylprednisolone (1000mg/day for 5 days)	- primary outcome: FIS score, modified FIS (with impact of fatigue on patient's life) - secondary outcome: RAYS QoL score, neurological disability in EDSS, No. of acute relapses	- FIS and modified FIS reduction from baseline to month 6 sign. greater in IG compared to CG (p=0.007 and p=0.005) - no sign. differences in EDSS - No. of relapses sign. lower in IG vs. CG, also sign. bigger proportion of relapse-free patients in IG vs. CG - relapse reduction became sign. at month 4, was sustained at month 6 and decayed at follow-up - sign. greater improvement in social and psychological QoL-scale in Alphacalcidol (vs. placebo) - no differences in adverse events between groups, no serious events
Kampman et al. (2012) – Effect of vitamin D_3 supplementation on relapses, disease progression, and measures of function in persons with multiple sclerosis: exploratory outcomes from a double-blind randomized controlled trial				
Methods	**Participants**	**Interventions**	**Outcomes**	**Results**
- prospective, randomized, double-blind, placebo-controlled trial - country: Norway - study years: Apr 2008 to Jan 2010	- randomized: n=71 - inclusion criteria: age 18-50 years, MS according to McDonald criteria, EDSS ≤4.5 - exclusion criteria: inability to walk ≥500m, diseases affecting bone, pregnancy/lactation during past 6 months, use of bone-active medications other than intravenous methylprednisolone for relapse treatment; history of nephrolithiasis, menopause	- Intervention group (n=35): $VitD_3$ (cholecalciferol; 20,000IU) capsules 1x weekly - Control group (n=36): identical placebo - administration for 96 weeks - Co-Intervention: 500mg calcium daily (both groups); permission to continue VitD supplements if used	- serum 25(OH)D, ARR, EDSS, MSFC compo-nents, grip strength, FSS	- no side effects - serum 25(OH)D levels above 75nmol/L were reached in 80% of patients in IG (24% in CG) - proportion of relapse-free subjects did not differ between groups - median time to first relapse was 29 weeks vs. 39 weeks (CG vs. IG; p=0.48) - no sign. differences in ARR, EDSS, MSFC, grip strength, FSS

Shaygannejad et al. (2012) – Effects of Adjunct Low-Dose Vitamin D on Relapsing-Remitting Multiple Sclerosis Progression: Preliminary Findings of a Randomized Placebo-Controlled Trial				
Methods	**Participants**	**Interventions**	**Outcomes**	**Results**
- prospective, randomized, double-blind, placebo-controlled trial - country: Iran - study years: Oct 2007 to Mar 2009	- randomized: n=50 - inclusion criteria: age 15 to 60 years, MRI, clinical or laboratory supported diagnosis of definite RRMS, stable neurological functioning for ≥1 month prior to study, EDSS ≤6, serum 25(OH)D >40ng/ml, willingness to continue current medication - exclusion criteria: abnormalities in neurological, psychiatric, cardiac, endocrinological, hematologic, hepatic, renal, metabolic functions, use of digitalis, VitD suppl., pregnancy	- Intervention group (n=25): Calcitriol ($1,25(OH)_2D_3$; 0.25µg/day, after 2 weeks increased to 0.5µg/day) capsules twice daily - Control group (n=25): placebo capsules twice daily - administration for 12 months - Co-Intervention: continuation of ongoing treatment	- primary outcome: EDSS - secondary outcome: relapse rate, side effects	- sign. increase in EDSS in placebo group (baseline vs. 12 months), no change in treatment group; yet no sign. difference between groups - relapse rate decreased sign. in both groups, with no statistical difference between groups - no difference in adverse events between groups

5.2 Systemic Lupus Erythematosus

Systemic lupus erythematosus is a complex, chronic autoimmune disease with a changing clinical symptomology. Without treatment it is possibly life-threatening. Similar to multiple sclerosis, genetic, hormonal and environmental factors are probably contributing to the disease risk. B cells are main mediators of this disease, but there is also an involvement of autoreactive T cells. Typically, autoantibodies are found, which are directed against parts of the nucleus (antinuclear antibodies, ANAs) and RNA-binding proteins. Additionally, in approximately 70 - 98% of the patients, antibodies against double-stranded DNA (anti-dsDNA-antibodies) are detectable and their serum concentrations correlate with disease activity (Tsokos, 2011). The disease is characterized by immune complexes that are formed by autoantigens and autoantibodies in an antigen-antibody-reactions. These complexes deposit in several tissues like skin and joints, but also in connective tissues of blood vessels and inner organs, where they cause many SLE-symptoms like thrombosis, lupus nephritis, vasculitis and arthritis (Toong, et al., 2011).

SLE-patients are sensitive to sunlight and UV radiation, as it might cause relapses and an exacerbation of the disease. Therefore, patients are advised to avoid the sun and to use sunscreen, which might be one of the reasons for vitamin D deficiency among patients with systemic lupus erythematosus (Agmon-Levin, et al., 2013). However, vitamin D might also have positive effects of the disease, as described above. For this reason, studies aim to determine whether low vitamin D levels are rather reason or cause of the disease and whether a repletion of low levels might result in a decreased activity.

According to the beforementioned criteria, three studies regarding systemic lupus erythematosus were selected and reviewed (table 5).

Andreoli and colleagues conducted a crossover study investigating the impact of two different regimens of vitamin D on pre-menopausal women with SLE. In a randomized open-label (assumed, as no report on blinding) study, 34 participants have been treated with either low or high dose of vitamin D for 12 months, and then a further 12 months with the other dose. The intense regimen increased serum 25(OH)D significantly compared to standard regimen with the biggest effect after 3 months due to a high bolus dose at baseline. However, there was no effect in SLEDAI-score (standard disease activity assessment using clinical history, physical examination, organ specific function tests and serologic parameters (Bombardier, et al., 1992)), and no changes in anti-dsDNA and complement levels (Andreoli, et al., 2015).

A double-blind, randomized and placebo-controlled multicenter-study by Aranow et al. explored whether there is an effect of vitamin D on the interferon signature in SLE patients, but also examined the changes in disease activity and other clinical outcome parameters. 57 participants with stable inactive disease were randomized to receive either placebo, low or high dose of oral vitamin D for 12 weeks. Vitamin D treatment significantly increased serum 25(OH)D in the intervention groups compared to placebo, with slightly higher levels in high dose group. There was no difference in IFN-signature between placebo and intervention groups at study end. Disease activity remained stable with no differences among treatment arms. Also, anti-dsDNA antibody status in subjects did not change, except for two participants in the high-dose group, who turned negative (Aranow, et al., 2015).

Lima et al. aimed to evaluate the effect of vitamin D on disease activity and fatigue in patients with juvenile-onset SLE in a randomized, double-blind and placebo-controlled trial. 45 female patients were randomly assigned to receive vitamin D or identical placebo for 6 months. There was a significant increase in serum 25(OH)D in the treatment group compared to placebo, with 70% of patients achieving 30ng/ml vs. 0% in the control group. SLEDAI and ECLAM (another index measuring disease activity, similar to SLEDAI, derived from a large European cohort of SLE patients) scores significantly improved in the treatment group compared to placebo ($p=0.011$ and $p=0.006$). In the vitamin D group, 15% of the patients became anti-dsDNA negative, compared to 0% in the control group. Besides, vitamin D-treated patients had a significantly better global fatigue score than the placebo group at the end of the study ($p=0.012$) (Lima, et al., 2016).

Overall, there is no clear evidence for a beneficial effect of vitamin D for SLE-patients resulting from these three studies with the trial by Lima et al. being the only one showing significant improvements. Again, the studies were noticeably divers in their methods and characteristics. Trials were carried out on three different continents, the two single-center studies in Italy (Andreoli, et al., 2015) and Brazil (Lima, et al., 2016), and a multi-center study in eight centers of the United States (Aranow, et al., 2015). Age range differs between studies, as Aranow and Andreoli recruited adults, whereas Lima studied juvenile onset patients up to 25 years. All considered studies use the same form of vitamin D, namely cholecalciferol (vitamin D_3), but doses and administration differ. While Aranow and colleagues used daily doses of 4.000IU or 2.000IU daily vs placebo, Lima et al. administered weekly doses of 50.000IU or placebo and the trial by Andreoli used 300.000IU as baseline bolus plus 50.000IU in a monthly administration versus no bolus and 25.000IU monthly (no placebo). Comparing these, the highest dose is used by Lima, followed by Aranow (high dose). The lowest dose was administered by Andreoli and colleagues. Nonetheless, the different frequency complicates common conclusions. In all trials, patients continued their

ongoing standard treatment on stable doses. Safety and adverse events were documented in every study, but except for few cases of slight transitory hypercalcemia or hypercalciuria, no events were observed (Andreoli, et al., 2015; Aranow, et al., 2015; Lima, et al., 2016).

Of the three studies evaluated on systemic lupus erythematosus, every study reported dropouts, but only one used the intention to treat-analysis (Aranow, et al., 2015). Within the two randomized double-blind trials, no information on adequacy of blinding or randomization procedures were given (Aranow, et al., 2015; Lima, et al., 2016).

Table 5: Characteristics of included studies on systemic lupus erythematosus

Andreoli et al. (2015) – A 24-month prospective study on the efficacy and safety of two different monthly regimens of vitamin D supplementation in pre-menopausal women with systemic lupus erythematosus				
Methods	**Participants**	**Interventions**	**Outcomes**	**Results**
- prospective, randomized crossover trial (no information on blinding) - country: Italy - Study years: enrolment from May 2011 to January 2012	- randomized: n=34 (only females) - inclusion criteria: stable disease inactivity, SLEDAI-2K score <6 in year prior to enrolment, discontinuation of VitD supplementation 1 month prior to study - exclusion criteria not stated	- Intensive regimen (IR) (n=16 in first year): $VitD_3$ (cholecalciferol; 300,000 IU at baseline, then 50,000 IU) monthly - Standard regimen (SR) (n=18 in first year): Cholecalciferol (25,000 IU) monthly → cross-over after 12 months - Co-Intervention: ongoing immunosuppressive treatment was not changed	Serum 25(OH)D, (i-PTH), anti-dsDNA, C3, C4, CH50 serum levels (complement proteins), serum calcium and phosphorus, urinary calcium and phosphorus, SLEDAI-2K	- IR sign. raised 25(OH)D compared to SR, greatest difference at 3 months (bolus effect); sign. more participants in sufficiency range after 1 year (IR vs. SR; p=0.001) - no sign. changes in safety parameters observed; only 3 transitory slight hypercalciuria (2 in IR, 1 in SR) - no sign. change in SLEDAI score - no changes in anti-dsDNA and complement levels - no dose change in immunosuppressants was needed throughout study period, except for 3 patients with disease flare
Aranow et al. (2015) – Double-Blind Randomized Placebo-Controlled Trial of the Effect of Vitamin D3 on the Interferon Signature in Patients with Systemic Lupus Erythematosus				
Methods	**Participants**	**Interventions**	**Outcomes**	**Results**
- prospective multicenter, randomized, double-blind, placebo-controlled trial - country: USA (8 centers)	- randomized: n=57 - inclusion criteria: meeting 1997 ACR Classification criteria for SLE, age ≥18 years, serum 25(OH)D ≤20 ng/ml, presence of IFN signature (either presence of 1 of 3 IFNα inducible genes at least 4 SD above mean, or expression of 2 of 3 genes ≥2 SD), stable inactive disease	- high dose group (n=18): $VitD_3$ (4,000IU) orally daily - low dose group (n=17): $VitD_3$ (2,000IU) orally daily - placebo group (n=19): placebo orally daily - administration for 12 weeks	- primary outcome: levels of 3 IFNα inducible genes (→"IFN signature response") and serum 25(OH)D	- sign. increase in serum 25(OH)D in both IGs compared to placebo, higher levels in high dose group than low dose, but not sign. - IFN signature was not sign. different in CG compared to IG - serum 25(OH)D had no sign. effect on IFN signature -patients taking anti-malarials as ongoing treatment were 3.2 times more

- study years: Dec 3, 2008 to Apr 19, 2011 (screening)	(SELENA-SLEDAI ≤4 and no BILAG A or B score in any organ system except mucocutaneous), anti-dsDNA positivity, stable immune-suppressive medications, prednisone dose ≤20 mg/d - exclusion criteria: VitD supplementation >800 IU/day within previous 3 months, hypercalcemia, hypercalciuria, kidney, liver, thyroid diseases, treatment with biologic agents and certain other agents	- Co-Intervention: ongoing stable immunosuppressive treatment	- secondary outcome: - SELENA-SLEDAI score, BILAG index, complement levels, anti-dsDNA, PTH, urinary calcium/creatinine, assessment of adverse events	likely to have IFN signature response than subjects not taking it (p=0.057) - no correlation between individual IFN inducible gene expression changes and 25(OH)D - Disease activity remained stable, no sign. differences among treatment groups - anti-dsDNA antibody status did not change, except for 2 subjects in high dose group (positive to negative) - no sign. changes in BILAG index - no severe adverse events, 2 cases of grade 1 hypercalcemia
Lima et al. (2016) – Vitamin D Supplementation in Adolescents and Young Adults With Juvenile Systemic Lupus Erythematosus for Improvement in Disease Activity and Fatigue Scores: A Randomized, Double-Blind, Placebo-Controlled Trial				
Methods	**Participants**	**Interventions**	**Outcomes**	**Results**
- prospective, randomized, double-blind, placebo-controlled trial - country: Brazil - study years: Jul 2012 to Aug 2013 (enrollment), study completion Feb 2014	- randomized: n=45 - inclusion criteria: female patients with juvenile-onset SLE up to 25 years, fulfilling ACR-classification criteria for SLE, symptoms before age 16 - exclusion criteria: SLEDAI score >12, other autoimmune diseases or infectious conditions, renal impairment, renal stones, hypercalcemia, liver disease, history of drugs for osteoporosis treatment, pregnancy, treatment with high doses of immunosuppressants within previous 3 months	- Intervention group (n=22): $VitD_3$ (cholecalciferol, 50,000IU) orally once weekly - Control group (n=23): identical placebo tablets - administration for 6 months - Co-Intervention: patients allowed to continue ongoing standard therapy, with stable doses	- SEDAI score, ECLAM score - Kids Fatigue Severity Scale questionnaire - serum 25(OH)D, - anti-dsDNA-Ab, serum complement (C3 and C4), calcium and creatinine, complete blood count, 24-hr urinary protein - adverse events	- sign. higher serum 25(OH)D in IG than PG after 6 months - 70% of patients in VitD-group reached >30ng/ml (0% in placebo group) - sign. improvement in SLEDAI (p=0.011) and ECLAM (p=0.006) scores in IG compared to CG - 15% of subjects in IG became anti-dsDNA negative (0% in CG) - sign. better global fatigue score after 6 months (IG vs. CG, p=0.012); sign. improvement also for social life - no safety difference between groups; no serious adverse events

5.3 Rheumatoid Arthritis

Rheumatoid Arthritis is the most common autoimmune disease, affecting around 1% of the world population. It is characterized by a chronic synovial inflammation leading to destruction of the joints and, hence, to impairment of the mobility in severe cases. Patients suffer from swollen and tender joints with a stiffness occurring particularly in the morning. The etiology of rheumatoid arthritis involves genetic as well as environmental factors, similar to other autoimmune diseases. There is an involvement of both innate and adaptive immunity in the disease pathogenesis. A main aspect is the formation of pannus, a typical inflammatory tissue in rheumatoid arthritis leading to synovial inflammation (synovitis) in the joints. Monocytes and macrophages are the major cells found in the synovial fluid, although many parts of the innate immunity involved in the inflammation. Altogether with T cells (Th1), they produce inflammatory cytokines like IL-1, IL-6 and TNFα leading to synovitis while inhibiting Treg differentiation. Also, Th17 cells are involved in the inflammatory process whereas Th2 cells are thought to have a regulatory role mediated by IL-4, which inhibits the abovementioned inflammatory cytokines. Furthermore, B cells play an important role in the pathogenetic processes due to their antibody-production and formation of immune complexes. The so-called rheumatoid factor that binds to a specific part of IgG-antibodies, was considered the most important autoantibody in rheumatoid arthritis for a long time. It is still used for diagnoses, but the link to disease severity seems arguable. Besides, rheumatoid factors are also found in healthy subjects. And therefore, antibodies against anti-cyclic citrullinated peptides (anti-CCP) are currently assumed as second criterion for diagnoses, also because they could even be detected before the beginning of the disease (Ishikawa, et al., 2017).

As vitamin D might influence almost all of the involved parts of the immune system, and due to the direct influence on bone metabolism a vitamin D supplementation might be positive especially in rheumatoid arthritis. For instance, a correlation between bone erosion and high parathyroid hormone (PTH, a hormone involved in calcium homeostasis inducing bone loss in hypo calcemic conditions) has been found in patients with rheumatoid arthritis. Consequently, vitamin D may be beneficial due to the regulation of PTH-levels (Rossini, et al., 2011).

Five studies on rheumatoid arthritis were deemed eligible according to the selection criteria and their characteristics are shown in table 6.

Dehghan et al. conducted a randomized, double-blind and placebo-controlled trial investigating the effect of vitamin D supplementation on disease activity and dosages of

disease modifying antirheumatic drugs. 80 participants in remission phase were randomized to receive either vitamin D or similar placebo pearls once weekly for 6 months. Disease flares were observed in 17.5% of the treatment group, but 27.5% of the placebo group, which is, however, nonsignificant (p=0.42). Vitamin D supplementation did not lead to reduction of standard medication dosages. Patients with flare-ups did not differ regarding vitamin D level or DAS28 score (an examination of 28 defined joints for measuring rheumatoid arthritis disease activity) (Dehghan, et al., 2014).

Another randomized, double-blind, placebo-controlled study by Hansen and colleagues evaluated the effect of high dose vitamin D on patients with low serum 25(OH)D levels. 22 patients were randomly assigned to receive oral vitamin D or placebo for 12 months. No significant effects on bone mineral density or health outcomes could be demonstrated. TNFα even increased and the physical function domain of the SF-36 questionnaire for quality of life declined in treatment group (Hansen, et al., 2014).

The phase II trial carried out by Li and colleagues was also randomized, double-blind and placebo-controlled, aiming to compare the efficacy and safety of 22-oxa-calcitriol with calcitriol. 369 participants were randomly assigned to the three study arms: 22-oxa-calcitriol, calcitriol and placebo were administered weekly for 6 weeks. Both treatments significantly decreased swollen joints ($p<0.001$ for each 22-oxa-calcitriol and calcitriol), reduced c-reactive protein ($p=0.023$ and $p=0.03$) and pain ($p=0.005$ and $P=0.018$), and improved HAQ-DI (an instrument measuring functional status and disability; $p=0.011$ and $P=0.0483$) compared to placebo. Improvements in all outcomes were similar in 22-oxa-calcitriol and calcitriol group, except for morning stiffness duration, where the strongest effect was found in the calcitriol group (however, both treatments reduced it significantly). Calcitriol raised serum calcium, whereas 22-oxa-calcitriol intake had no significant impact on calcium levels. Subgroup analysis showed significantly better improvements in females and younger patients compared to male/older participants (Li, et al., 2018).

The fourth reviewed study by Soubrier et al. (also randomized, placebo-controlled and double-blind) was conducted to investigate whether a high-dose vitamin D supplementation improves functional handicap in vitamin D-deficient rheumatoid arthritis patients in non-remission. Therefor, 59 participants received vitamin D or placebo for 24 weeks. Although HAQ score tended to decrease in treatment group and increased in placebo group, the difference was not significant ($p=0.11$). But, after adjusting for gender, age, season and initial vitamin D level, the difference became significant ($p=0.046$). After this adjustment, also erythrocyte sedimentation rate and c-reactive protein levels in the intervention group improved significantly. There was no difference in EULAR response, global assessment,

pain, activity or fatigue, RAID or quality of life (Soubrier, et al., 2018). (EULAR response measures whether patients are non-, moderate or good responders to a therapy depending on changes in disease activity (Fransen & van Riel, 2009); RAID is a validated questionnaire measuring the impact of disease on patients' life. (EULAR, n.d.))

Yang et al. explored the effect of a vitamin D supplementation on the recurrence rate of rheumatoid arthritis in a randomized open label study. 377 patients were included and divided into vitamin D sufficient or deficient at a cut-off value of 30ng/ml. 192 of these participants were considered deficient and randomly assigned to vitamin D-analogue treatment or control (no treatment). The group with normal vitamin D levels had no treatment either. Study duration was 24 months. The highest recurrence rate was 29.5% in the deficient control group, recurrence rates in the normal vitamin D group and the deficient treatment group were 16.5% and 19.0%. This difference is significant between the normal vitamin D and the control group, but not for the two deficient subgroups ($p=0.02$ and $p=0.11$) (Yang et al., 2015).

Summarizing the study results, no conclusive beneficial effects have been reported. Nonetheless, there seemed to be an improvement in inflammatory markers like erythrocyte sedimentation rate and c-reactive protein rather than in clinical outcomes, although positive effects on HAQ, thus disease severity have been observed. Noteworthy, all the considered studies were performed in single centers, two of them in China (Li, et al., 2018; Yang, et al., 2015), one in Iran (Dehghan, et al., 2014), France (Soubrier, et al., 2018) and the USA (Hansen, et al., 2014) each. Age of the participants was not reported at all in one study (Dehghan, et al., 2014), the others stated means, which ranged from 41 to 63 years. All studies used placebo as control group, only Yang et al. performed an open-label study. The forms and dosages used in the trials differed. Hansen and colleagues used Ergocalciferol 50.000IU three times weekly for the first 4 weeks, then 50.000IU twice a month, whereas Li et al. used 22-oxa-calcitriol and calcitriol at a dosage of 50.000IU weekly each (equivalent to 1.25mg, Li, et al., 2018). In the treatment group of deficient patients in the trial by Yang et al. the vitamin D analogue alphacalcidol was administered at a dosage of 0.25µg twice daily. Neither Dehghan nor Soubrier specified the type of used vitamin D, the former gave 50.000IU weekly and the latter administered an initial vitamin D dose depending on the baseline 25(OH)D levels. Patients with a level below 10ng/ml received 200.000IU every two weeks for 2 months, patients with 10-20ng/ml received the same dose for 1.5 months and those with a 25(OH)D level of 20-30ng/ml received this dose for 1 month. After this loading period, patients were administered 100.000IU vitamin D every 4 weeks. Two studies allowed patients to continue their standard/corticosteroid treatment (Soubrier, et al., 2018; Dehghan, et al., 2014). Li and colleagues gave no information on ongoing treatment and

Hansen et al. only reported an additional calcium intake of 500mg thrice daily and the request to apply sunscreen in summer months. Yang et al. did not clearly state a prohibition of standard medication but reported that participants not receiving vitamin D were allowed to continue the ongoing treatment. Studies varied not only in terms of form and dosage of vitamin D, but also in duration and sample size. Only three of the five studies made statements about safety of the treatment (Hansen, et al., 2014; Li, et al., 2018; Yang, et al., 2015), but none of those reported any significant side effects.

Studies were relatively similar in quality, for example, blinding was only described by two studies (Dehghan, et al., 2014; Li, et al., 2018) as well as randomization procedure (Yang, et al. 2015; Li, et al., 2018). Not every study recorded dropouts nor performed an intention to treat analysis. The only study fulfilling all these quality criteria was performed by Li et al.

Table 6: Characteristics of included studies on rheumatoid arthritis

Dehghan et al. (2014) – Role of vitamin D in flare ups of rheumatoid arthritis				
Methods	**Participants**	**Interventions**	**Outcomes**	**Results**
- prospective, randomized, double-blind, placebo-controlled trial - country: Iran - study years: Oct 2012 to Feb 2013	- randomized: n=80 - inclusion criteria: RA diagnose based on 2010 ACR-EULAR criteria, remission during past 2 months (determined by number of tender joints, number of swollen joints, C-reactive protein, global assessment), VitD level <30 ng/dl - exclusion criteria: RA symptoms overlapped by other rheumatologic diseases, abnormal calcium and phosphorus levels	- Intervention group (n=40): VitD (50,000IU) orally once weekly - Control group (n=40): similar placebo pearls - administration for 6 months - Co-Intervention: continuation of standard therapy (prednisolone, methotrexate, hydroxychloroquine)	Numbers of involved, swollen and painful joints, DAS28	- flare observed in 17.5% and 27.5% of subjects (IG vs. CG, p=0.42) - not using VitD causes 17% increased risk of recurrence (non-sign.) - VitD intake did not lead to lower necessary doses of standard medication - no sign. differences in flared patients regarding VitD level or DAS28
Hansen et al. (2014) – An Evaluation of High-Dose Vitamin D for Rheumatoid Arthritis				
Methods	**Participants**	**Interventions**	**Outcomes**	**Results**
- prospective, randomized, double-blind, placebo-controlled trial - country: USA - study years: 2004 to 2009 (enrollment)	- randomized: n=22 - inclusion criteria: RA according to ARA 1987 revised criteria for classification of rheumatoid arthritis, serum 25(OH)D of 6.1-24.9 ng/mL - exclusion criteria: hypercalcemia/-calciuria, calcium intake 2 g/day, kidney diseases, Paget's disease, hyperthyroidism, pregnancy, women 45-55 years old/ menopause, osteoporosis medication, estrogen, spine to hip T-score ≤3	- Intervention group (n=11): $VitD_2$ (Ergocalciferol; 50,000IU 3x weekly for 4 weeks, then 50,000IU orally twice monthly) - Control group (n=11): matching placebo - administration for 12 months - Co-Intervention: 500mg calcium 3x daily for all subjects, asked to apply sunscreen between May and Sep	- primary outcome: PTH - secondary outcomes: bone mineral density, DAS28, HAQ and SF-36-scores, cytokines	- no sign. effect on PTH, N-telopeptide or bone mineral density - increase of bone formation (reflected by bone specific alkaline phosphatase) - TNFα increased in treatment group (however, already higher at baseline) - DAS28 scores remained unaffected, but patient's global health and RA assessments worsened - physical function domain of SF-36 declined in IG - no adverse events

Li et al. (2018) – Efficacy and Safety of 22-Oxa-Calcitriol in Patients with Rheumatoid Arthritis: A Phase II Trial				
Methods	**Participants**	**Interventions**	**Outcomes**	**Results**
- prospective, randomized, double-blind, placebo-controlled trial - country: China - study years: Feb 3, 2017 to Jan 1, 2018 (enrollment)	- randomized: n=369 - inclusion criteria: age ≥18 years, active RA (defined as ≥4 swollen joints), erosions in radiographic images, morning stiffness - exclusion criteria: negative rheumatoid factor, intake of methotrexate, anti-TNFα, anakinra, alefacept, infliximab, etanercept, efalizumab, painkillers or systemic corticosteroids	- Intervention group (n=123): VitD analogue (22-oxa-Calcitriol; 50,000IU) once weekly - Control group (n=123): VitD (calcitriol; 50,000IU) once weekly - Placebo group (n=123): lactose powder 5g/week - administration for 6 weeks	- primary: urinary protein, albumin and creatinine, serum VitD and calcium, CRP, ESR - secondary: number of swollen joints, duration of morning stiffness, VAS for pain, HAQ-DI (disease activity)	- 22-oxa-calcitriol and calcitriol sign. decreased swollen joints compared to placebo - both treatment increased serum vitamin D levels, but placebo did not - besides, calcitriol raised serum calcium and caused hypercalcemia ($p<0.001$); 22-oxa-calcitriol did not sign. change calcium levels - both treatments reduced C-reactive protein and pain in patients (baseline vs. week 6), placebo did not - morning stiffness was sign. reduced in all groups, strongest effect was found in calcitriol group - both treatment groups improved erythrocyte sedimentation rate, urine albumin and creatinine as well as HAQ-DI
Soubrier et al. (2018) – A randomised, double blind, placebo-controlled study assessing the efficacy of high doses of vitamin D on functional disability in patients with rheumatoid arthritis				
Methods	**Participants**	**Interventions**	**Outcomes**	**Results**
- prospective, randomized, double-blind, placebo-controlled trial - country: France - study years: Nov 2011 to Aug 2016	- randomized: n=59 - inclusion criteria: age ≥18 years, RA according to 1987 ACR criteria; non remission (DAS28 >2.6), serum 25(OH)D <30 ng/mL, no expected treatment modification, no change in disease-modifying treatment within last 3 months, no anti-articular infiltrations over last 2 months, corticosteroid	- Intervention group (n=29): VitD (100,000IU) vial every 4 weeks (initial dose based on 25(OH)D levels: <10ng/ml → 2 vials/2 weeks for 2 months, 10-20ng/ml → 2 vials/2 weeks for 1.5 months, 20-30ng/ml → 2 vials/2 weeks for 1 month	- primary: HAQ (functional handicap) - secondary: DAS28-ESR, DAS28-CRP, number of swollen and tender joints, VAS for pain, VAS for activity, ESR	- no sign. difference in HAQ between groups, although score tended to increase in CG while decreasing in IG ($p=0.11$); after adjusting for age, gender, season, initial vitamin D sign. difference ($p=0.046$) - in patients with vitamin D <20ng/mL sign. decrease in HAQ in IG compared to CG

	intake only on stable daily dose <10 mg prednisone equivalent - exclusion criteria: ACR functional class IV, hypercalcemia, hypercalciuria, history of kidney stone colic, thiazide intake, pregnancy/lactation	- Control group (n=30): placebo - administration for 24 weeks - Co-Intervention: patients allowed to continue ongoing treatment	and CRP, EULAR response, decrease in asthenia (VAS and FACIT-fatigue), RAID score, QoL (SF-36)	- after adjustment sign. improvement in ESR and CRP levels in vitamin D group - no differences in EULAR response, global assessments, VAS for pain, activity or fatigue, RAID and quality of life
Yang et al. (2015) – Effect of vitamin D on the recurrence rate of rheumatoid arthritis				
Methods	**Participants**	**Interventions**	**Outcomes**	**Results**
- prospective, randomized, open-label, controlled trial - country: China - study years: Oct 2010 to Feb 2014 (enrollment)	- included: n=377 - randomized: n=192 - included patients were divided into VitD sufficient or deficient (cut off value 30ng/ml); deficient patients were randomly assigned to treatment or control - inclusion criteria: RA according to 2010 ACR-EULAR classification criteria, in remission phase in previous 2-3 months (according to ACR-EULAR criteria; <1 swollen joint, 1 tender joint, CRP <1 mg/dl, global assessment ≤1), no glucocorticoids in past 3 months - exclusion criteria: other comorbid rheumatic diseases, abnormal serum calcium or phosphorus	- normal VitD group (n=168): continuation of previously used treatment method - Intervention (VitD deficient sub-) group (n=84): VitD analogue (alphacalcidol; 0.25µg) twice a day - Control (VitD deficient sub-) group (n=84): continuation of previously used treatment method - duration: 24 months	- VAS, number of swollen and tender joints, CRP, ESR, activity level (DAS28; score ≥3.2 deemed as recurrence), serum calcium and phosphorus (only in intervention group), $VitD_3$	- recurrence rates were 16.7%, 19.0% and 29.5% (normal VitD group, VitD treatment subgroup, control subgroup) - Sign. difference between normal VitD group and control subgroup in recurrence rate (p=0.02) but not between deficient subgroups (p=0.11)

5.4 Diabetes Mellitus Type I

Type I diabetes mellitus is characterized by an absolute insulin deficiency due to the destruction of β-cells in the pancreas, which are responsible for insulin-production. Most likely, the disease is likewise triggered by genetical predisposition and environmental factors. Different subpopulations of T cells play a key role in the disease pathogenesis, but also B cells, dendritic cells and other parts of the immune system are involved (Li, Song, & Qin, 2014).

A possible link between vitamin D and type I diabetes has been found in several epidemiological and experimental studies, but yet studies on supplementation remain inconclusive. As the destruction of beta cells cannot be reversed, it seems plausible to administer vitamin D in the earliest disease stages when there are still functioning beta cells.

According to the selection criteria, three studies on Diabetes mellitus type I were included in the review (table 7).

A randomized, single-blinded and placebo-controlled trial by Ataie-Jafari et al. aimed to investigate whether vitamin D supplementation may preserve beta cell function in newly diagnosed children and adolescents. 61 patients were included and randomized to receive either a vitamin D analogue (alphacalcidol) or placebo capsules daily for 6 months. Within-subject analysis showed the biggest difference between groups from month 3 to 6 for fasting C-peptide (a laboratory marker reflecting insulin secretion) ($p=0.049$) and from baseline to month 3 for the needed daily insulin dosage ($p=0.052$). This dosage difference became nonsignificant again at month 6. Between-subject effects show sign. differences in the daily insulin dose between groups, and also between genders, where males had a stronger response. For fasting C-peptide, values increased in males at month 6 but not in females (Ataie-Jafari, et al., 2013).

Gabbay and colleagues carried out a randomized, double-blind and placebo-controlled trial with 38 participants to evaluate the effect of a vitamin D supplementation on peripheral cytokines, regulatory T cells as well as residual beta cell function in patients with new-onset autoimmune diabetes. Vitamin D or placebo were administered daily for a period of 18 months. There was a significant increase in serum 25(OH)D in the intervention group, but no change in the insulin dosage throughout the study time and between groups. No significant differences could be detected in cytokines except for IL-12 which decreased within the first 6 months in both groups ($p=0.05$). there was a significant increase in the number of Tregs in the vitamin D group at month 12 ($p=0.04$), however, the amount decreased again until month 18. Stimulated C-peptide values were significantly enhanced

in the intervention group compared to placebo, although the effect was stronger in the first 12 months (p=0.01) and attenuated until month 18. Besides, the cumulative incidence of undetectable fasting C-peptide levels was significantly higher in the placebo group compared to vitamin D (Gabbay, et al., 2012).

Another randomized, double-blind and placebo-controlled trial was conducted by Treiber et al. and had a similar aim, namely the effect of vitamin D on Treg number and function, peripheral immune cells and the residual beta cell function. 30 patients were recruited and received either weekly oral doses of vitamin D or placebo for 12 months. Although there was no difference in Treg percentages or its changes between groups, the suppressive capacity of these regulatory T cells was significantly higher in the intervention group compared to placebo at the study end (p=0.017). Apoptosis measurements, immune cell percentages and HbA1c did not differ significantly between groups. The fasting C-peptide tended to decrease slower in the vitamin D group throughout the study time, but there were no significant differences in fasting or stimulated C-peptide between groups. In the control group the average insulin requirements significantly increased (p<0.001), whereas there was no change in the intervention group (Treiber, et al., 2015).

In summary, some positive effects have been observed regarding C-peptide, thus insulin secretion. Notwithstanding, conclusive benefits on HbA1c or the required insulin doses could not be detected in every study. All of the studies were carried out in single centers in different geographical areas, more precisely in Iran (Ataie-Jafari, et al., 2013), Brazil (Gabbay, et al., 2012) and Austria (Treiber, et al., 2015). As every study focused on new-onset diabetes patients, the participants' age was similar with children and adolescents being recruited. Albeit Gabbay et al. included patients up to the age of 30 years. Placebo was used as control in all studies, but the used forms and dosages of vitamin D varied. While Ataie-Jafari used the calcitriol-analogue alphacalcidol at a dose of 0.25µg daily for the first two weeks and then increased to 0.5µg, Gabbay and Treiber used cholecalciferol. Participants in the former received 2000IU orally daily, and the latter administered it once weekly according to body weight of the patient (140IU per kg per day within the first month, then 70IU/kg/d). So theoretically, considering the average weight of 52.2 kg in the treatment group, the mean cholecalciferol dose was 3645IU daily or 7308IU within the first month, respectively. No study reported additional or ongoing treatments, except for insulin administration. Additionally, studies were different in sample sizes, which were generally small, after all, and duration. Safety parameters were investigated in all studies, but these measurements stayed within normal ranges and no adverse events were reported (Ataie-Jafari, et al., 2013; Gabbay, et al., 2012; Treiber, et al., 2015).

The quality of the studies performed can be considered similar. Although they reported dropouts, none of them used the intention to treat-principle for analyzing the data. Only Treiber and colleagues described blinding procedure and randomization method, which was appropriate, whereas Gabbay et al. made no such descriptions. In the trial by Ataie-Jafari, randomization procedure was described and adequate, but only subjects and laboratory personnel, not care providers and investigators were blinded (Ataie-Jafari, et al., 2013; Gabbay, et al., 2012; Treiber, et al., 2015).

Table 7: Characteristics of included studies on diabetes mellitus type I

Ataie-Jafari et al. (2013) – A randomized placebo-controlled trial of alphacalcidol on the preservation of beta cell function in children with recent onset type 1 diabetes				
Methods	**Participants**	**Interventions**	**Outcomes**	**Results**
- prospective, participant-blinded, parallel group, randomized placebo-controlled trial - Country: Iran - Study years: recruitment Sep to Dec 2010)	- Randomized: n=61 - Inclusion criteria: age between 8 and 15 years at time of recruitment, satisfied diagnosis criteria of type 1 diabetes, clinical disease duration <8 weeks, no medical co-morbidities or chronic diseases, stable diabetic diet for previous week - Exclusion criteria: intake of cholecalciferol, calcium, multivitamin or mineral supplements during past 3 months, regular consumption of VitD-fortified foods, hypercalcemia (serum calcium >2.7mmol/L)	- Intervention group (n=29): VitD analogue (alphacalcidol; 0.25µg for first 2 weeks, if normal serum calcium → increased to 0.5µg) daily in capsules - Control group (n=25): equal number of capsules daily - duration: 6 months - Co-Intervention: standard insulin treatment in both groups	- serum calcium, albumin, phosphate, HbA1c, serum 25(OH)D, fasting serum C-peptide (FCP), residual islet beta cell function (measured by FCP and daily insulin dosage (DID) to maintain euglycemia, sun exposure (questionnaire)	- HbA1c tended to be lower in IG at months 3, 6 (non-sign.) - within-subject contrasts: differences between groups most marked between month 3 and 6 for FCP, between month 0 and 3 for DID - DID difference between groups again became non-sign. at month 6 - between-subject effects: sign. differences in DID between treatment groups and also genders - stronger response in males - between-subject contrasts not sign. for FCP, but males improved at month 6 while females did not - serum calcium and phosphate normal in all subjects
Gabbay et al. (2012) – Effect of Cholecalciferol as Adjunctive Therapy With Insulin on Protective Immunologic Profile and Decline of Residual ß-Cell Function in New-Onset Type 1 Diabetes Mellitus				
Methods	**Participants**	**Interventions**	**Outcomes**	**Results**
- prospective, randomized, double-blinded, placebo-controlled trial - country: Brazil	- randomized: n=38 - Inclusion criteria: age between 7 and 30 years, disease duration <6 months (first insulin injection), positive test results for islet cell autoantibodies, fasting or 2-hour postmeal	- Intervention group (n=17): $VitD_3$ (Cholecalciferol; 2000IU) orally daily - Control group (n=18): identical placebo - administration for 18-months period	- complete blood cell count, aminotransferase levels, phosphate and alkaline phosphatase levels, HbA1c, Serum C-peptide	- sign. increase in serum 25(OH)D in IG, non-sign. variation in CG - no sign. change in insulin dose throughout the study and between groups -HbA1c in IG sign. higher at baseline, fell sign. after 6 months, but no sign.

- study years: Mar 10, 2006 to Oct 28, 2008	stimulated serum C-peptide ≥0.6ng/mL during mixed meal tolerance test - Exclusion criteria: severe systemic disease, disorders in calcium metabolism	- Co-Intervention: intensive insulin regimen in both trial arms	(fasting and stimulated (SCP)), antiglutamic acid decarboxylase 65 and antiprotein tyrosine phosphatase (islet cell autoantibodies), serum ionized calcium, 25(OH)D, cytokines, Tregs	difference between groups at other time points - no sign. differences in cytokines, only IL-12 decreased sign. from baseline to month 6 in both groups - Tregs increased sign. at 12 months of follow-up study in IG - SCP enhanced in first 12 months, less decay until 18 months IG vs. CG) (both time periods sign.) - sign. higher cumulative incidence of undetectable FCP in CG vs. IG - safety determinants within normal ranges in both groups - no clinical adverse events
Treiber et al. (2015) – Cholecalciferol supplementation improves suppressive capacity of regulatory T-cells in young patients with new-onset type 1 diabetes mellitus – A randomized controlled trial				
Methods	**Participants**	**Interventions**	**Outcomes**	**Results**
- prospective, randomized, double-blind, parallel group, placebo-controlled trial - Country: Austria - Study years: Nov 2011 to Nov 2012	- Randomized: n=30 - Inclusion criteria: >6 years, disease duration <3 months (diabetes onset defined according to ADA criteria), normal Ca and Creatinine plasma levels - Exclusion criteria: pre-existing hypercalcemia, inflammatory systemic diseases, disorders in Ca metabolism, kidney diseases, pregnancy	- Intervention group (n=14): $VitD_3$ (Cholecalciferol; 70IU/kg BW/d) orally once weekly (in first month 140/IU/kg BW/d) - Control group (n=15): equal amount of vegetable oil once weekly - administration for 12 months	- primary: Treg number, function - secondary: frequencies of immune cells (innate and adaptive), apoptosis of Tregs and Teffs, FCP, SCP, HbA1c, safety assessments (serum 25(OH)D, serum Calcium and PTH, urinary calcium/creatinine ratio)	- mean Tregs percentage or its changes did not differ between groups at any time - suppressive capacity sign. higher at month 12 in IG (37.2 ± 25) than in CG (0.7 ± 28.9) (p=0.017) (increase in IG, decrease in CG compared to baseline) - apoptosis measurement values similar in both groups - no sign. difference in FCP or SCP at any time between groups (FCP tended to decrease slower from baseline to 12 months in IG than in CG (p=0.078) - average insulin requirements sign. increased in CG (p<0.001), but not IG - HbA1c was similar in both groups - safety measurements did not differ between groups

5.5 Autoimmune Thyroid Diseases

Autoimmune thyroid diseases include Hashimoto thyroiditis and Graves' disease. They are characterized by an infiltration of lymphocytes into the thyroid gland. Like other autoimmune diseases, they occur due to a combination of genetic predisposition and environmental factors like infections or smoking (Kim, 2017).

Thyroid hormones are regulated by TRH (thyrotropin-releasing hormone) and TSH (thyroid-stimulating hormone or thyrotropin). TRH stimulates TSH, which again stimulates protein transcription and epithelial growth in the thyroid gland and is therefore responsible for the synthesis of thyroid hormones, namely thyroxine (T4) and triiodothyronine (T3). In healthy individuals, the synthesis of TRH and TSH is suppressed by high concentrations of T4 and T3 in a negative feedback loop.

Hashimoto thyroiditis, or chronic autoimmune thyroiditis is characterized by an intrathyroidal infiltration of B and T cells, mainly Th1 cells. The detection of anti-thyroid-peroxidase antibodies (anti-TPO) and/or anti thyroglobulin (anti-Tg) is common, although they are not found in all patients. Hashimoto thyroiditis involves a hypothyroid state of varying degrees. However, Graves' disease leads to a hyperthyroidism. In contrast to Hashimoto, the infiltration of lymphocytes is low in Graves' disease and there is a predominance of Th2 cells. This causes secretion of antibodies which bind to the TSH receptor and stimulation of thyroid cell growth (Kim, 2017).

Despite the significant role of vitamin D in immunomodulation, evidence of correlations between vitamin D and autoimmune thyroid diseases remains contradictory. Therefore, studies assessing the effect of a vitamin D supplementation on thyroid autoimmunity have been performed.

Regarding autoimmune thyroid diseases, two studies have been found by database search. Wang et al. performed a meta-analysis of RCTs and the second result, a trial by Krysiak and colleagues, was one of the six there included studies. A further three studies have then been found by manual search, but the remaining two studies in the meta-analysis were in Chinese and therefore not reviewed. All reviewed studies are depicted in table 8.

The meta-analysis by Wang was performed in China, and PubMed, Embase, Cochrane as well as three Chinese databases were systematically searched. According to the authors, randomized controlled studies with autoimmune thyroiditis patients (either Hashimoto thyroiditis or Graves' disease), comparing vitamin D interventions to either placebo or no treatment were included. 6 Studies were analyzed, with a total of 344 participants. The two

Chinese studies used 0.25µg calcitriol daily, dosages and further information on the other studies will be described below (Wang et al., 2018). The results of the meta-analysis will be presented at the end of this sub-chapter, altogether with the combined consideration of the studies.

Krysiak at al. conducted a non-randomized and open-label controlled trial investigating whether vitamin D can improve thyroid antibody titers and hence, enhance the effects of levothyroxine in woman with Hashimoto thyroiditis. 34 participants were allocated to either receive vitamin D treatment or not, according to patients' preferences. Treatment duration was 6 months. At the end of the study, there was a significant increase in 25(OH)D and reduction of TPO-antibodies in treated patients, as all as slightly reduced Tg-Antibodies. No changes were observed in the control group. Subgroup analysis showed a greater decrease in both antibody titers in patients with subclinical hypothyroidism compared to participants with normal thyroid function, and the reduction was only significant in the former group. The changes in serum 25(OH)D correlated with the changes in both antibody titers, and also with baseline thyrotropin (Krysiak, Szkróbka, & Okopien, 2017).

The randomized and open-label controlled trial by Simsek and colleagues aimed to examine the effect of vitamin D supplementation on TPO and Tg-antibodies and therefore the effect on thyroid autoimmunity. The 82 included patients had either Hashimoto thyroiditis or Graves' disease and were all vitamin D-deficient (25(OH)D below 20ng/ml) and were assigned to receive either vitamin D or no treatment for one month. In the treatment group, serum vitamin D levels significantly increased, while TPO and Tg-antibody titers significantly decreased. There was no difference in the control group after one month. However, between-group analysis showed no significant difference between intervention and control for any thyroid antibody titer, but only for serum 25(OH)D (Simsek, et al., 2016).

A third open-labeled trial (randomized, controlled), conducted by Chaudhary et al., also evaluated the effect of vitamin D treatment on thyroid autoimmunity, marked by TPO-antibody titers. 102 newly diagnosed autoimmune thyroid disease (or rather Hashimoto thyroiditis) patients were randomized to receive oral vitamin D or no treatment for 8 weeks. A significant decrease in TPO-antibodies was observed in the treatment group compared to control. 68% of the patients in the treatment group were considered responders (defined by >25% reduction of TPO-Ab-titers), compared to 44% in the control group ($p=0.015$). Subgroup analysis showed that in patients with TSH below 10mIU/L, supplementation with vitamin D lead to a significantly greater reduction compared to control, whereas no significant effect was observed for patients with a TSH level above 10mIU/L (Chaudhary, et al., 2016).

The only randomized, double-blind and placebo-controlled study on autoimmune thyroid diseases in this review, was carried out by Vahabi Anaraki and colleagues. The trial was designed to investigate the effect of vitamin D supplementation in deficient Hashimoto thyroiditis patients on thyroid function and TPO-antibodies. Either oral vitamin D or placebo was administered to 65 participants with a 25(OH)D level ≤20ng/ml for 12 weeks. The within-group comparison showed a significant decrease for PTH in the intervention group at the study end, but not for other outcome measures. No significant changes were observed in the placebo group. Comparing both groups, a significant difference was only found for PTH after adjusting for baseline values, which was lower in the intervention group than in the placebo group (Vahabi Anaraki, et al., 2017).

In sum, a positive effect of vitamin D on TPO-antibodies in patients with Hashimoto thyroiditis (and Graves' disease) has been observed in three of four studies, whereas the effect on Tg-antibodies was less conclusive. Additionally, studies measuring intact PTH found a decrease in vitamin D-treated patients. The four studies were carried out in single centers in Asia (India (Chaudhary, et al., 2016), Iran; (Vahabi Anaraki, et al., 2017), Turkey; (Simsek, et al., 2016)) and Europe (Poland (Krysiak, Szkróbka, & Okopien, 2017)). All of them included adults, mean ages ranged from around 28 (Chaudhary, et al., 2016) to 44 years (Vahabi Anaraki, et al., 2017). Simsek and Vahabi et al. included deficient patients with 25(OH)D levels below 20ng/mL only, while Krysiak only included those with levels above 30ng/mL and Chaudhary made no such restrictions. Three studies were open-labeled and therefore had no treatment in the control group, although Simsek et al. instructed participants without treatment to expose themselves to sunlight; Vahabi and colleagues used placebo as control. Furthermore, only the latter specified the type of vitamin used as cholecalciferol, while the others used the general term vitamin D. Dosages used were, sorted in ascending order, 1,000IU per day in the trial by Simsek, 2,000IU per day (Krysiak, Szkróbka, & Okopien, 2017), 50,000IU weekly administered by Vahabi et al. and 60,000IU given by Chaudhary. Patients in the trials by Krysiak and Vahabi continued their stable doses of levothyroxine treatment. Simsek and colleagues only included patients without prior medications affecting thyroid function, but initiated levothyroxine or else, methimazole therapy if patients had hyper- or hypothyroidic symptoms and TSH levels above or below the normal range. Similarly, Chaudhary et al. started levothyroxine in patients with TSH>10mIU/L, if not already initiated before the study. The trials were also heterogenous regarding sample size and study duration, as described above. Two studies did not explicitly report safety measurements (Simsek, et al., 2016; Vahabi Anaraki, et al., 2017), while the other two observed no significant adverse events or complications (Chaudhary, et al., 2016; Krysiak, Szkróbka, & Okopien, 2017).

The comprehensive analysis by Wang et al. showed a significant decrease of TPO antibodies at 6 months in the intervention group with data of three studies ($p<0.01$), but no significant effect at 1-3 months (data from three other studies; Krysiak et al. and two Chinese trials). Analysis of the four studies investigating Tg-antibody titers revealed a significant decrease in the intervention groups compared to the controls ($p=0.033$) Limitations the authors mentioned were mainly the small number of studies included and their small sample sizes, a short study duration and high heterogeneity of the studies, a lack of double-blind and placebo-controlled studies as well as a detected publication bias of the studies regarding Tg-antibodies. (Wang, et al., 2018).

The quality of the studies was similar. None of them performed an intention to treat-analysis, but while according to the study data no patients dropped out in the trials by Krysiak and Simsek, Chaudhary and Vahabi did record dropouts among the participants. An adequate randomization procedure was described by Chaudhary and Vahabi, whereas the latter was the only double-blind study and blinding procedure was stated (Chaudhary, et al., 2016; Krysiak, Szkróbka, & Okopien, 2017; Simsek, et al., 2016; Vahabi Anaraki, et al., 2017).

Table 8: Characteristics of included studies on autoimmune thyroid diseases

Wang et al. (2018) – The effect of Vitamin D supplementation on thyroid autoantibody levels in the treatment of autoimmune thyroiditis: a systematic review and a meta-analysis				
Methods	**Participants**	**Interventions**	**Outcomes**	**Results**
- Meta-Analysis Year: 2017 - Country: China (research) - Systematic Search of PubMed, Embase, Cochrane, Chinese Databases	- Inclusion Criteria: (1) RCT (2) Patients with autoimmune thyroiditis; either GD or HT (3) intervention with VitD vs. control with placebo/no treatment (4) TPO-Ab and Tg-Ab titers described - 6 studies included; n=344 (of which 330 with Hashimoto) - intervention groups n=178 - control groups n=166 - diagnostic criteria either based on high thyroid autoantibodies, ultrasound of the thyroid, or typical clinical signs and symptoms; no clear disease definition in 2 studies; 5 studies enrolled patients with deficient/insufficient 25(OH)D, 1 included normal levels	- 1 placebo-controlled study, 5 without treatment in control group - interventions: VitD 1000IU daily, 2000IU daily, 50,000IU weekly, cholecalciferol 60,000IU weekly, calcitriol 0.25µg daily (2 studies; chinese) - duration: 1-6 months - LT4 administration in 5 studies to maintain TSH, elemental calcium 500mg daily in 1 study	- TSH (all studies) - FT4 (4 studies) - FT3 (3 studies) - TPO-Ab as primary or secondary outcome (all studies) - Tg-Ab (4 studies)	Meta-Analysis: - sign. decrease of TPO-Ab in IGs at 6 months (3 studies; p<0.01; 0.0% heterogeneity), but not at 1-3 months (3 studies; p=0.67; 78.8% heterogeneity) - sign. decrease in Tg-Ab titers in VitD-groups compared to CGs (4 studies; p=0.033; 63.0% heterogeneity) (no subgroup analysis on duration due to limited data - no adverse events observed (2 studies)
Krysiak et al. (2016) – The Effect of Vitamin D on Thyroid Autoimmunity in Levothyroxine-Treated Women with Hashimoto's Thyroiditis and Normal Vitamin D Status				
Methods	**Participants**	**Interventions**	**Outcomes**	**Results**
- prospective, non-randomized, open-label, controlled trial - country: Poland - study years: not stated	- included: n=34 - inclusion criteria: women, age 20-50, LT4 treatment for ≥6 months before study, TPO-Ab titers >100IU/ml, reduced echogenicity of thyroid parenchyma on ultrasonography, serum 25(OH)D >30ng/mL, normal thyroid function or mild subclinical hypothyroidism	- Intervention group (n=16): VitD (2000IU) orally daily - Control group (n=18): no intervention - administration for 6 months	Thyrotropin, free T4, free T3, serum 25(OH)D, titers of TPO-Ab and Tg-Ab	- no changes in thyrotropin, free thyroid hormones 25(OH)D or thyroid antibodies in control group - sign. increase in 25(OH)D and reduction of TPO-Ab, slightly reduced Tg-Ab - decrease in TPO-Ab and Tg-Ab was greater in subgroup with

	- exclusion criteria: positive serum antibodies against thyrotropin receptor, other autoimmune diseases, diseases and conditions affecting heart, kidney, liver, BMI<40kg/m^2, Turner or Down syndrome, pregnancy or lactation	- Co-Intervention: continuation of stable LT4 treatment		subclinical hypothyroidism than patients with normal thyroid function, and only sign. in the former group - changes in 25(OH)D correlated with changes in both antibody titers and baseline thyrotropin - no sign. adverse events, complications
Simsek et al. (2016) – Effects of Vitamin D treatment on thyroid autoimmunity				
Methods	**Participants**	**Interventions**	**Outcomes**	**Results**
- prospective, randomized, open-label, controlled trial - country: Turkey - study years: Apr 2015 to Jun 2015 (enrollment)	- randomized: n=82 - inclusion criteria: diagnosis of autoimmune thyroid disease and VitD deficiency; HT diagnosed by high serum TSH and low/normal free T4 levels with positive TPO-Ab or Tg-Ab titers; GD diagnosed by low TSH and normal/high free T3 and/or T4 levels with positive antibody titers - exclusion criteria: VitD replacement therapy in past year, medications affecting thyroid function, e.g. steroid and LT4	- Intervention group (n=46): VitD (1000IU) daily - Control group (n=36): no intervention, but instructed to expose themselves to sunshine and monitor their diet - administration for 1 month - Co-Intervention: initiation of LT4/methimazole therapy if patients had hyper/hypothyroid symptoms and TSH levels above/below normal range	Serum TSH, free T3 and T4, TPO-Ab, Tg-Ab, VitD	- 25(OH)D sign. increased in IG, whereas TPO-Ab and Tg-Ab sign. decreased (baseline vs. month 1) - no sign. difference in control group after 1 month - between group comparison showed no sign. differences after 1 month of treatment (IG vs. CG) except for VitD level, which was sign. higher in IG
Chaudhary et al. (2016) – Vitamin D supplementation reduces thyroid peroxidase antibody levels in patients with autoimmune thyroid disease: An open-labeled randomized controlled trial				
Methods	**Participants**	**Interventions**	**Outcomes**	**Results**
- prospective, randomized, open-label, controlled trial - country: India	- randomized: n=102 - inclusion criteria: age >18 years, newly diagnosed hypothyroidism within last 3 months, thyroiditis diagnosed by ultrasonography, autoimmune thyroid disease	- Intervention group (n=51): $VitD_3$ (cholecalciferol, 60,000 IU) once weekly - Control group (n=51): no treatment	Free T4, intact PTH, TSH, TPO-Ab,	- sign. decrease in TPO-Ab titers in IG compared to CG - responder rate (>25% reduction in TPO-Ab) achieved in 68% vs. 44% (tIG vs. CG, p=0.015)

- study years: Feb 2013 to May 2015	defined as serum TPO-Ab titer >34kIU/L and/or HRUSG evidence of thyroiditis - exclusion criteria: chronic liver or cardiac disease, malignancy, epilepsy, tuberculosis, immunodeficiency, chronic medications possibly interfering with thyroid hormone or VitD metabolism	- administration for 8 weeks - Co-Intervention: 1250mg calcium carbonate/day (equivalent to 500mg elemental calcium); in patients with TSH>10mIU/L LT4 treatment, if not already initiated before study	calcium, phosphate, 25(OH)D	- sign. decrease in iPTH in IG vs. CG ($p<0.001$) - subgroup analyses showed that in patients with TSH<10mIU/L vitamin D supplementation was associated with sign. greater quantum of reduction in TPO-Ab titers compared to control; but supplementation did not result in sign. greater TPO-Ab reduction in patients with TSH>10mIU/L - no adverse events observed
Vahabi Anaraki et al. (2017) – Effect of Vitamin D deficiency treatment on thyroid function and autoimmunity markers in Hashimoto's thyroiditis: A double-blind randomized placebo-controlled clinical trial				
Methods	**Participants**	**Interventions**	**Outcomes**	**Results**
- prospective, randomized, double-blind, placebo-controlled trial - country: Iran - study years: Feb 2015 to Jul 2015	- randomized: n=65 - inclusion criteria: Hypothyroid patients, euthyroid, stable LT4 for at least 6 months or mild hypothyroidism at enrollment (TSH <15mU/L), 25(OH)D ≤20ng/ml - exclusion criteria: renal or liver disease, cancer, pregnancy, severe weight loss, immunosuppressive medication, insulin, sulfonamides	- Intervention group (n=33): VitD (50,000 IU) orally once weekly - Control group (n=32): similar placebo once weekly - administration for 12 weeks - Co-Intervention: continuation of treatment with stable doses of LT4, metformin or statin	25(OH)D, TSH, TPO-Ab, CRP, PTH, calcium, phosphorus, albumin blood urea nitrogen, creatinine	- within-group comparison showed sign. decrease for PTH, but not TSH, TPO-Ab or other variable after 12 weeks compared to baseline; no sign. changes within CG - between-group analyses showed sign. difference in PTH after adjustment for baseline values, but not in other variables (IG vs. CG)

6. Discussion

This current review aimed to evaluate the efficacy of a vitamin D supplementation in the treatment of autoimmune diseases. Therefore, a systematic search was performed to identify controlled clinical trials and meta-analyses that have been carried out since 2012 addressing this research question. Six autoimmune diseases were considered, i.e. multiple sclerosis, systemic lupus erythematosus, rheumatoid arthritis, autoimmune thyroid diseases, diabetes mellitus type I and myasthenia gravis; a total of 19 studies were included. Notably, no studies regarding myasthenia gravis (an autoimmune disease affecting various muscles due to autoantibodies directed towards acetylcholine receptors in the motoric end plates) could be reviewed, as the few results found did not meet the inclusion criteria.

This systematic review includes studies carried out in different countries and with different study characteristics. Besides, the duration of supplementation ranged from 1 to 24 months and the doses and forms of administered vitamin D differed. Although all studies found vitamin D to be safe, there were considerable differences in the effectiveness. Another aspect that may have led to discrepancies in study results is the different study quality (see chapter 5).

In multiple sclerosis, overall, no effects could be detected. This finding is consistent with the conclusion of a systematic review of the Cochrane collaboration on MS-studies, which all here considered studies are part of (Jagannath, et al., 2018). Main outcomes of the here included studies were clinical outcomes like EDSS, relapse rate and fatigue, whereas biomarkers were not investigated. The disease severity score EDSS has been measured in all trials, but only Shaygannejad et al. found a nonsignificant positive trend, whereas even Achiron and colleagues, who showed significant improvements in other domains like fatigue, failed to find differences in EDSS (Achiron, et al., 2015; Shaygannejad, et al., 2012). This might be due to the selection of participants with low EDSS already at baseline, consequently, possible effects in more severe disease stages cannot be ruled out. Evidence for an inverse correlation between 25(OH)D and EDSS score were also found in a Tasmanian study (van der Mei, et al., 2007), although it is not clear whether low vitamin D levels in more severe disease stages are a cause or rather a consequence. In patients with similarly low EDSS, a dose escalation trial by Burton and colleagues also only found a trend for lower scores but observed significantly less relapses. Additionally, a suppression of the T cell-proliferation was detected (Burton, et al., 2010). Studies in this review did not measure immune and inflammatory markers, but a possible effect on these is supported by a trial performed by Mosayebi et al. IFN-β-treated patients received monthly cholecalciferol injections (300,000 IU) for 6 months, whereupon peripheral blood mononuclear cell-

proliferation decreased and TGF-β as well as IL-10 levels increased (Mosayebi, et al., 2011). Furthermore, the combination of IFN-β and vitamin D treatment might be more effective, as shown in animal models (van Etten, et al., 2007) and confirmed by a prospective cohort study. There, vitamin D levels were only associated with a reduced relapse risk in patients on IFN-β and, vice versa, IFN-β only was only protective against relapses in patients with higher 25(OH)D-levels (Stewart, et al., 2012). Lastly, there was a trend for reduced relapse risk also in the reviewed trial by Kampman (Kampman, et al., 2012) which might reach significance in larger samples.

Results regarding the rheumatic autoimmune diseases, systemic lupus erythematosus and rheumatoid arthritis were not quite conclusive either. In SLE-studies, biomarkers like anti-dsDNA-antibodies as well as clinical scores like SLEDAI have been measured. But, effects in SLEDAI score, anti-dsDNA antibodies and fatigue were only found in juvenile patients (Lima, et al., 2016), leading to the hypothesis that there might be a better disease response in early disease stages. Supporting this notion, a cross-sectional study reported a negative correlation between 25(OH)D-levels and SLEDAI-scores, thus disease activity in juvenile-onset SLE-patients (Casella, et al., 2012). So, vitamin D deficiency might contribute to an increased B cell activation and thereby increased auto-antibody production. Nevertheless, this correlation has also been found in adult SLE patients (Amital, et al., 2010), so there might indeed be an effect also in adults. As a matter of fact, the results of the studies in this review are not in accordance with some previously performed uncontrolled studies. A cross-sectional study with weekly, later daily vitamin D supplementation found a significant decrease in SLEDAI scores as well as increased Treg levels and decreased IL-17-levels (Marinho, et al., 2017). Also, a second prospective study reported significant improvements in immune cell markers after 6 months of weekly vitamin D administration in deficient SLE-patients (Terrier, et al., 2012). While Treg levels increased, there was a decrease of Th1 and Th17 cells as well as memory B cells and anti-dsDNA antibodies. Accordingly, vitamin D seems to enhance the immune response in SLE patients. Notably, both studies observed a successful raise of 25(OH)D-levels (Marinho, et al., 2017; Terrier, et al., 2012), while the reviewed studies by Aranow and Andreoli reported infrequent and ineffective vitamin D repletion in patients (Andreoli, et al., 2015;Aranow, et al., 2015). Andreoli and colleagues attributed this to the monthly supplementation scheme, consequently, a daily or weekly supplementation might be preferable. Another reason for a lack of effects in both trials might be, similar to the MS-studies, due to the inclusion of patients with stable inactive disease or minimal disease activity only. This makes it harder to detect differences especially in SLEDAI scores for disease activity.

In rheumatoid arthritis, beneficial effects were rather seen in inflammatory markers like erythrocyte sedimentation rate and C-reactive protein than in clinical scores like the HAQ, although an effect on the latter also has been partly observed. A nonsignificant decrease in flare-ups was reported by Dehghan et al., but also in this case, the lack of significance might be due to the selection of patients in remission. Indeed, in patients with moderate to high disease activity, vitamin D deficiency correlated with pain and disability as well as higher DAS scores (Haque & Bartlett, 2010). This finding is similar to multiple sclerosis. So, vitamin D deficiency is likely to have a negative effect at least in more advanced disease stages. Interestingly, although the reviewed study by Yang failed to show an effect of vitamin D supplementation, it reported a significant difference in recurrence rate in deficient patients compared to a normal status, suggesting that hypovitaminosis D might be a risk factor also for recurrence (Yang, et al., 2015). Of note, treatment in this study did not successfully raise 25(OH)D in participants. Other studies with RA-patients are similarly inconclusive. While some found positive effects by vitamin D, others could not confirm these results. Andjelkovic and colleagues treated patients receiving disease modifying anti-rheumatic drugs (DMARDs) with 2µg alphacalcidol for three months and reported a complete remission in 45% as well as significant improvements in 44% of the patients (Andjelkovic, et al., 1999). In a study by Gopinath, triple DMARD therapy was initiated in treatment naïve RA-patients together with vitamin D_3 (500IU/day) or placebo. Treatment with additional vitamin D lead to a significantly better pain relief (Gopinath & Danda, 2011). On the contrary, in a study with 117 patients on methotrexate therapy no significant differences in DAS could be detected after treatment with 50,000IU 25(OH)D weekly for 12 weeks (Salesi & Farajzadegan, 2012). In patients with early rheumatoid arthritis, a single dose of 300,000IU vitamin D significantly improved global health scores, although no effect on T helper subsets was found (Buondonno, et al., 2017). Although also B cell-, thus autoantibody-driven, the importance of anti CCP-antibodies or rheumatoid factor in RA declined, as they are only found in a part of the patients. This is probably why intervention-studies addressing them as outcomes have not been found. Yet, a possible effect of vitamin D on autoantibodies also in RA might be interesting to understand possible modes of action. Additionally, as described earlier, a direct effect on PTH levels might also contribute to effects of vitamin D in rheumatoid arthritis. But except for Hansen et al., who observed no effects on this outcome (Hansen, et al., 2014), PTH was unfortunately not investigated in the reviewed studies. For that reason, this link cannot be further discussed.

The three studies on autoimmune diabetes mellitus reported some significant results in terms of fasting or stimulated C-peptide and required insulin, but there was no consistency in findings. Notably, in many outcomes, like again insulin dosages, C-peptide and HbA1c,

effects were found but did not reach statistical significance. As sample sizes were small in all trials, one might assume a shift towards significance in larger samples. Gabbay et al. also measured immune response markers and found a significant increase in Tregs in the intervention group together with increased levels of the peripheral chemokine ligand 2 (CCL2), a factor having an important role in polarization of naïve T cells towards a Th2 phenotype (Gabbay, et al., 2012). This might lead to a better regulation of the Th1/Th2 disbalance that was found to contribute to β-cell loss in type 1 diabetes in combination with deficiency of Tregs (Lindley, et al., 2005). Other studies addressing vitamin D supplementation in type 1 diabetes were likewise contradictory. An uncontrolled before-after study with 70 subjects receiving 50,000IU cholecalciferol biweekly for 3 months showed a significant reduction in HbA1c levels (Ordooei, et al., 2017). However, Bizzari et al. found no protective effect of calcitriol on β-cell function in recent-onset diabetes patients (Bizzarri, et al., 2010) and neither did Walter and colleagues (Walter, et al., 2010). These findings make a long-term β-cell preservation seem unlikely. Albeit, study durations were too short to draw this final conclusion and the dosages used by Bizzari and Walter were relatively low. Interestingly, vitamin D might even have a direct effect on β-cells by rendering them more resistant to cellular stress occurring in diabetes (Wolden-Kirk, et al., 2011). Especially with this finding in mind, most effective forms of vitamin D administration need to be evaluated.

The considered studies on autoimmune thyroid diseases mainly included patients with Hashimoto thyroiditis. Here, relatively clear results were found regarding TPO-antibodies, but also some effects on Tg-antibodies were observed. These are supported by an association between 25(OH)D-levels and TPO-antibodies already found by Shin et al. (Shin, et al., 2014). Additionally, there seems to be a negative association between 25(OH)D and TSH as well as Hashimoto and especially overt hypothyroidism (Kim, 2016). The finding by Krysiak slightly supports this link, as their subgroup analysis revealed a stronger effect of vitamin D in patients with subclinical hypothyroidism than those with euthyroidism (Krysiak, Szkróbka, & Okopien, 2017). Chaudhary and colleagues performed a subgroup analysis as well and found that TPO-Ab-reduction was only effective in patients with low TSH-levels (Chaudhary, et al., 2016). Lower TSH-levels indicate a less advanced disease state, consequently, a vitamin D supplementation might be most beneficial in patients with newly diagnosed autoimmune thyroid disease. The reviewed trials included vitamin D deficient patients and also patients with normal levels. Yet, improvements have been observed in every study. In another study, Krysiak reported beneficial effects on autoantibody titers also in women with postpartum thyroiditis and deficient or insufficient vitamin D levels (Krysiak, Kowalcze, & Okopien, 2016). Altogether, this leads to the assumption that vitamin D might

be beneficial in deficient patients but also in those within the sufficiency range. In all studies, patients continued or started levothyroxine treatment, that is a hormone substitution therapy but may also decrease antibody titers (Schmidt, et al., 2008). Therefore, vitamin D might have a potentiating effect on this treatment, similar to IFN-β-therapy in MS.

Overall, the clearest positive effects seem to be achieved in autoimmune thyroid diseases and type 1 diabetes. This might be due to the use of biomarkers instead of disease scores, as the diseases are marked by loss of physiological function instead of typical and disease specific symptoms. Biomarkers are usually more sensitive than clinical scores in detecting changes, because scores consist of several parameters and an amelioration in one might be concealed by the worsening of another. For example, in type 1 diabetes mellitus, there seems to be an additional direct effect on β-cells, that might be responsible for positive effects rather than immunomodulation. In autoimmune thyroid diseases as well as diabetes and rheumatoid arthritis, beneficial effects sometimes seem to be more pronounced in early disease stages, hence, it might be an important factor to administer vitamin D as early as possible to achieve the best results. However, in multiple sclerosis, vitamin D supplementation had no effect on the conversion rate of optic neuritis to definite multiple sclerosis (Pihl-Jensen & Frederiksen, 2015). Epidemiological data indeed supports a protective role for vitamin D in all of the reviewed diseases. Consequently, it must be considered that vitamin D might be more effective in preventing the onset of autoimmune diseases than in their treatment. As many patients are vitamin D deficient or insufficient and this was partly associated with disease activity, a monitoring and repletion of 25(OH)D-levels seems reasonable in any case.

Drawing conclusions for modes of action of vitamin D in autoimmune diseases is hard due to their still poorly understood etiology and pathophysiology. Besides, they are heterogenous in symptoms and characteristics, these differences are distinct between diseases, but also within. Therefore, patients do not respond equally even to conservative treatments. The slightly better response in autoimmune thyroid diseases and diabetes might be due to the disease localization in glands, different from systemic diseases like SLE and rheumatoid arthritis involving many tissues and organs. The local production of the active calcitriol might also play a key role in efficacy. Possibly it is enhanced in glands due to hormonal activity, whereas it is diminished in the CNS as it is immunologically privileged and thus, more isolated. Importantly, these statements lack evidence and therefore need to be further investigated.

7. Conclusions

During the past decades, a lot of evidence on the efficacy of vitamin D in autoimmune diseases has been derived from epidemiological and experimental studies. This thesis exemplarily reviewed effects of vitamin D on multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus, autoimmune thyroid diseases and type I diabetes mellitus as some of the most prevalent autoimmune diseases. Yet, the results of the here presented 19 studies remain inconclusive. Nonetheless, there have been some positive and promising results, for instance in terms of autoantibody titer-reduction in autoimmune thyroiditis. The lack of consistent findings might be due to the differing qualities of the studies. Major limitations of almost all studies were small sample sizes and/or short study durations. Furthermore, many different types and dosages of vitamin D have been used, which makes it hard to draw overall conclusions. Another aspect limiting the power of this review is the inclusion of open-labeled and nonrandomized studies, which was necessary due to the lack of studies with a higher evidence level.

Notably, the divergence between epidemiological evidence and therapeutic efficacy might be partly explained by the fact that epidemiological studies are usually based on estimations, while in interventional studies, the actual blood parameters are measured.

Besides, also the use of 25(OH)D, the inactive prohormone, for the determination of vitamin D levels may possibly be biased. Due to a potential local hydroxylation of 25(OH)D to calcitriol the true level of calcitriol in the target organs and tissues might not be in accordance with the estimated level derived from 25(OH)D.

Ultimately, it is of high importance that none of the studies reported severe side effects caused by vitamin D supplementation, even at higher dosages. The Endocrine society proposed daily intakes of up to 10,000IU to be safe in adults (Sintzel, Rametta, & Reder, 2018). The dosages administered in the reviewed studies stayed below this upper limit (although it is difficult to draw direct comparisons in equivalents of vitamin D and calcitriol or its analogues). Remarkably, by means of the qualitative analysis in this review, studies using higher doses tended to have more positive results. This leads to the hypothesis that higher dosages of vitamin D might be necessary and acceptable to obtain more significant effects resulting in definite clinical advantages.

Therefore, it is crucial that well-designed randomized controlled trials with larger sample sizes, longer study durations and recruitment in multiple centers are performed to extend the current knowledge and clarify the efficacy of vitamin D in autoimmune conditions.

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Abstract

Vitamin D is a steroid hormone best known for its role in bone metabolism. But over the past decades it has become clear that its effects go beyond this regulation. An important extra skeletal effect is the immunomodulation. Upon activation of vitamin D receptors, that are expressed on almost all immune cells, it modulates activation, differentiation, proliferation and apoptosis of these cells. In this context, epidemiological data have demonstrated a strong correlation between low vitamin D levels and autoimmune diseases that has been confirmed by genetic and experimental studies, indicating a potential therapeutic benefit for these diseases. Thus, this bachelor thesis aims to present accumulated data on the efficacy of a vitamin D supplementation in patients with different autoimmune diseases. 19 controlled trials were reviewed regarding multiple sclerosis, systemic lupus erythematosus, rheumatoid arthritis, diabetes mellitus type 1 and autoimmune thyroid diseases. The results of these studies remain inconclusive. Nonetheless, some significant effects have been observed on important biomarkers, but also clinical outcomes in different autoimmune conditions. Yet, further studies are needed to understand possible modes of action and determine the proper vitamin D supplementation that is required for beneficial effects.

Zusammenfassung

Vitamin D ist ein Steroidhormon, welches am besten für seine Rolle im Knochenstoffwechsel bekannt ist. Doch in den letzten Jahrzehnten ist klar geworden, dass seine Wirkungen über diese Regulierung hinausgehen. Ein wichtiger extra-skelettaler Effekt ist die Modulation des Immunsystems. Durch die Aktivierung von Vitamin D-Rezeptoren, die auf fast allen Immunzellen exprimiert werden, hat es Einfluss auf Aktivierung, Differenzierung, Proliferation und Apoptose dieser Zellen. In diesem Zusammenhang haben epidemiologische Daten eine starke Korrelation zwischen niedrigen Vitamin-D-Spiegeln und Autoimmunerkrankungen gezeigt, die durch genetische und experimentelle Studien bestätigt wurde, was auf einen potenziellen therapeutischen Nutzen für diese Krankheiten hindeutet. Aus diesem Grund zielt diese Bachelorarbeit darauf ab, gesammelte Daten über die Wirksamkeit einer Vitamin-D-Supplementierung bei Patienten mit verschiedenen Autoimmunerkrankungen zu präsentieren. 19 kontrollierte Studien wurden in Bezug auf Multiple Sklerose, Systemischen Lupus Erythematodes, rheumatoide Arthritis, Diabetes mellitus Typ 1 und autoimmune Schilddrüsenerkrankungen betrachtet. Die

Ergebnisse dieser Studien bleiben uneindeutig. Dennoch wurden einige signifikante Effekte auf wichtige Biomarker, aber auch klinische Ergebnisse in verschiedenen Autoimmunerkrankungen beobachtet. Trotz alledem sind weitere Studien notwendig, um mögliche Wirkungsweisen zu verstehen und die richtige Vitamin-D-Supplementierung zu bestimmen, die für positive Effekte erforderlich ist.

Eidesstattliche Erklärung

Ich versichere hiermit, dass ich die vorliegende Bachelorarbeit ohne fremde Hilfe selbstständig verfasst und nur die angegebenen Quellen und Hilfsmittel benutzt habe.

Wörtlich oder dem Sinn nach aus anderen Werken entnommene Stellen sind unter Angabe der Quelle kenntlich gemacht.

Hamburg, 02.09.2019

Joann Kiebach