Aus dem Lehrstuhl
für Neurologie
Prof. Dr. med. Ralf Linker
der Fakultät für Medizin
der Universität Regensburg

Immunological effects of probiotic *Lactobacillus*-supplementation in people with multiple sclerosis

Inaugural – Dissertation
zur Erlangung des Doktorgrades
der Medizin

der
Fakultät für Medizin
der Universität Regensburg

vorgelegt von
Maria Kaiser

2025

Aus dem Lehrstuhl
für Neurologie
Prof. Dr. med. Ralf Linker
der Fakultät für Medizin
der Universität Regensburg

Immunological effects of probiotic *Lactobacillus*-supplementation in people with multiple sclerosis

Inaugural – Dissertation
zur Erlangung des Doktorgrades
der Medizin

der
Fakultät für Medizin
der Universität Regensburg

vorgelegt von
Maria Kaiser

2025

Dekan:	Prof. Dr. Dirk Hellwig
1. Berichterstatter:	Prof. Dr. med. Ralf Linker
2. Berichterstatter:	Prof. Dr. med. Romuald Brunner
Tag der mündlichen Prüfung:	19.08.2025

List of contents

1 Zusammenfassung

1.1 Hintergrund und Ziele

Die steigende Inzidenz der Multiplen Sklerose (MS) besonders in westlichen Ländern wird vermehrt mit dem Einfluss verschiedener Umweltfaktoren auf die Pathophysiologie der MS in Verbindung gebracht. Neben niedrigen Vitamin-D Spiegeln und einer frühen Infektion mit dem Epstein Barr Virus scheinen bestimmte Ernährungsfaktoren sowie der Darm und dessen Bakterien eine wichtige Rolle zu spielen (1). Ergebnisse aktueller Studien zeigten bereits einen Zusammenhang zwischen dem Darmmikrobiom, der „westlichen Ernährung“ und dem Immunsystem. Ein wesentlicher Bestandteil der „westlichen Ernährung“ ist Salz. Eine Studie im Tiermodell der MS, der experimentellen autoimmunen Enzephalomyelitis (EAE), ergab, dass eine salzreiche Ernährung zur Depletion von *Laktobazillen* im Darmmikrobiom führte, was mit einer Steigerung entzündungstreibender T Helfer (Th) 17 Zellen sowie einer klinischen Verschlechterung der EAE einherging (2). Eine Supplementierung von *Laktobazillen* zeitgleich zur salzreichen Ernährung konnte diesen Effekt verhindern (2). In einer darauffolgenden Pilotstudie mit gesunden Menschen führte eine salzreiche Ernährung ebenfalls zu Veränderungen im Darmmikrobiom, insbesondere *Laktobazillen* waren stark vermindert (2). Viele weitere Studien unterstreichen ebenfalls einen Zusammenhang zwischen dem Darmmikrobiom und dem Immunsystem bei verschiedenen (Autoimmun-)Erkrankungen, wie beispielsweise bei MS, rheumatoider Arthritis oder Hypertonie. Eine Modulation des Darmmikrobioms durch Einnahme eines Probiotikums könnte sich demnach positiv für immunvermittelte Erkrankungen wie die MS auswirken. Im Rahmen dieser Doktorarbeit wurde daher der Einfluss von Vivomixx®, einem probiotischen Nahrungsergänzungsmittel mit einem großen Anteil an *Laktobazillen*, auf die Immunzellen im Blut von MS Patienten und gesunden Kontrollen untersucht.

1.2 Methoden

Teilnehmende MS Betroffene und gesunde Kontrollen hatten je vier Termine (Ersttermin vor Beginn der Vivomixx® Einnahme, zwei Termine während der Einnahme von Vivomixx® für sechs Wochen und einen Kontrolltermin nach Beendigung der Einnahme), bei welchen Blut- und Stuhlproben entnommen wurden. Zusätzlich zu den ohnehin stattfindenden Routineuntersuchungen der MS Patienten wurden bei den Terminen eine Anamnese sowie klinisch-neurologische Untersuchung von MS Patienten sowie gesunden Kontrollen durchgeführt. Um die

Ernährungsgewohnheiten der Probanden zu erheben, wurde ein Ernährungsfragebogen einmal im gesamten Studienzeitraum ausgefüllt. Mit den Blutproben wurden eine Routineblutanalyse und eine Oberflächenfärbung von Immunzellen im Vollblut zur Analyse von B-Zellen, natürlichen Killer-Zellen (NK-Zellen), Monozyten, $CD4^+$, $CD8^+$ und $CD4^+CD8^+$ T-Zellen mittels Durchflusszytometrie (Fluoreszenz-aktivierte Zellsortierung, FACS) durchgeführt. Zudem wurde der Einfluss des Probiotikums auf T Helfer Zellen (Th1, Th17, Th2) sowie regulatorische T Zellen (Treg) untersucht.

1.3 Ergebnisse

Die Analyse der Routineblutwerte zeigte eine signifikante Verringerung von Cholesterin nach zweiwöchiger *Laktobazillen* Supplementation bei MS Patienten und bei gesunden Kontrollen. Diverse Unterschiede in der Menge der aufgenommenen Nährwerte zwischen MS Patienten und gesunden Kontrollen fielen in der Analyse der Ernährungsfragebögen auf, jedoch wurde dies im Rahmen dieser Arbeit nicht näher untersucht. Wichtig für diese Studie war die Aufnahme von Salz über die Nahrung, jedoch zeigte sich kein signifikanter Unterschied zwischen MS Patienten und gesunden Kontrollen.

Die Analyse von Immunzellen im Vollblut vor der Einnahme von Vivomixx® und nach zwei bzw. sechs Wochen zeigte keine signifikanten Effekte auf B-Zellen und NK-Zellen, sowohl bei MS Patienten als auch bei gesunden Kontrollen. Jedoch gab es einige signifikante Effekte auf Monozyten, $CD4^+$, $CD8^+$ und $CD4^+CD8^+$ T-Zellen, wobei weitere Studien benötigt werden, um diese Effekte mit der Einnahme von Vivomixx® in direkte Verbindung bringen zu können. Dagegen ergab die Analyse der $FoxP3^+$ Treg Zellen bei MS Patienten eine signifikante Steigerung nach zwei Wochen Vivomixx® Einnahme. Eine Zunahme der $FoxP3^+$ Treg Zellen war auch bei gesunden Kontrollpersonen zu erkennen, jedoch nicht signifikant. Eine Zunahme der Th17 Zellen war bei MS Patienten beim Vergleich der Zeitpunkte zum Kontrolltermin nach Beendigung der Einnahme von Vivomixx® signifikant. Bei gesunden Kontrollen war auch der Vergleich während des Einnahmezeitraumes signifikant. Th1 Zellen von MS Patienten und MS Patienten unter Therapie mit Ocrelizumab waren zwei Wochen nach Vivomixx® Einnahme signifikant vermindert. Dieser Effekt zeigte sich nicht bei gesunden Kontrollen. Die Einnahme von Vivomixx® hatte keinen Einfluss auf die Frequenz von Th2 Zellen, weder bei MS Patienten noch bei gesunden Kontrollen.

1.4 Diskussion und Schlussfolgerungen

Diese Ergebnisse sprechen für einen potentiellen Benefit des Probiotikums Vivomixx® auf das Immunsystem, besonders in Bezug auf die steigenden FoxP3^{+} Treg Zellen und die sinkenden Th1 Zellen. Andererseits zeigten die Th17 Zellen nicht die hypothetisch erwartete Abnahme. Diese Pilotstudie ist eine der Ersten, welche sich mit den Effekten von Probiotika auf Immunzellen von MS Patienten fokussiert und nicht nur auf gesunde Kontrollen oder Tierexperimente. Daher fungiert diese Studie als Basis für weitere Forschung in diesem Bereich.
Unter Berücksichtigung der bisherigen Forschung und den Ergebnissen dieser Studie kann von einem Zusammenhang zwischen der Ernährung, dem Darmmikrobiom und dem Immunsystem ausgegangen werden. Es bleibt jedoch spannend, wie ausgeprägt dieser Zusammenhang ist und wie wir auf diesem Weg die Behandlung von Autoimmunerkrankungen, wie der MS, verbessern können.

2 Abstract

2.1 Background and Objective

The rising incidence of multiple sclerosis (MS) especially in western countries, is increasingly associated with the influence of various environmental factors on the pathophysiology of MS. Besides low vitamin D levels and an early infection with the Epstein Barr virus, certain nutritional factors as well as the gut and its bacteria seem to play an important role (1). Results of recent studies have already shown a link between the gut microbiome, the so-called "Western diet" and the immune system. An essential component of the so-called "Western diet" is salt. A study in an animal model of MS, the Experimental Autoimmune Encephalomyelitis (EAE), was able to show that a high-salt diet (HSD) led to depletion of *lactobacilli* in the intestinal microbiome, which was accompanied by an increase in inflammatory T helper (Th) 17 cells as well as a clinical worsening of EAE (2). Supplementation of *lactobacilli* concurrent with a HSD prevented this effect (2). In a subsequent pilot study in healthy individuals, a HSD also led to changes in the intestinal microbiome, and *lactobacilli* in particular were greatly reduced (2). Many other studies also underline a connection between the gut microbiome and the immune system in various (autoimmune) diseases, such as MS, rheumatoid arthritis or high blood pressure. A modulation of the intestinal microbiome by the intake of a probiotic could therefore be positive for immune-mediated diseases such as MS. Within the framework of my doctoral thesis, the influence of Vivomixx®, a probiotic dietary supplement with a large proportion of *lactobacilli*, on immune cells in the blood of people with MS (pwMS) and healthy controls (HC) was investigated.

2.2 Methods

Participating pwMS and HC had four appointments each (one at baseline before starting the intake of Vivomixx®, two while taking Vivomixx® for six weeks and one as a control after ending the intake of Vivomixx®), at which blood and stool samples were taken. In addition to the routine examinations of pwMS, a medical history and clinical-neurological examination of pwMS and HC were performed during the appointments. For analysis of dietary habits of participants, a food questionnaire was filled in once during the study. The blood samples were analysed for routine blood parameters and characterised for the presence of different immune cells including B cells, natural killer cells (NK cells), monocytes, $CD4^+$-, $CD8^+$- and $CD4^+CD8^+$ T cells with flow cytometry (Fluorescence-activated Cell Sorting, FACS). Moreover, the effect

of the probiotic supplementation on T helper cells (Th1, Th17, Th2) as well as on regulatory T cells (Treg) was investigated.

2.3 Results

Analysis of routine blood levels showed a significant reduction in cholesterol after two weeks of *lactobacillus* supplementation in pwMS and healthy controls.

Evaluation of the food questionnaire revealed various differences in the amount of nutrients ingested by pwMS and HC, however, this was not investigated in more detail within the scope of this work. Important for this study was the intake of dietary salt, however, there were no significant differences between pwMS and HC.

Surface staining of immune cells in whole blood before starting the intake of Vivomixx® and after two or six weeks showed no significant effects on B cells or NK cells in pwMS and HC. There have been some significant fluctuations in monocytes, $CD4^+$, $CD8^+$ and $CD4^+CD8^+$ T cells, whereby further studies are required to link these effects to the intake of Vivomixx®. In contrast, analysis of $FoxP3^+$ Treg cells in pwMS showed a significant increase after Vivomixx® intake for two weeks. An increase of $FoxP3^+$ Treg cells was also observed for HC, although not statistically significant. Th17 cells were increased significantly in pwMS when comparing timepoints with the control timepoint after stopping the intake of Vivomixx®. In HC, the comparison to control points during the intake period also showed significant effects. In pwMS and pwMS under Ocrelizumab therapy, Th1 cells were significantly decreased after two weeks of Vivomixx® intake. This effect was not visible in HC. The intake of Vivomixx® had no effect on the frequencies of Th2 cells, neither in pwMS nor in HC.

2.4 Discussion and conclusion

These results suggest a potential benefit of probiotic Vivomixx® intake on the immune system, especially concerning the increase of $FoxP3^+$ Treg cells as well as the decrease of Th1 cells. On the other hand, Th17 cells did not show any decrease. Yet, this pilot study is one of the first that deals with the effects of probiotics on immune cells in pwMS and not only in HC or mice. Therefore, it may serve as a basis for future research in this field. In view of previous research and the results of the current study, there may be a connection between diet, the gut microbiome and the immune system. It remains interesting, how strong this connection actually is and how it may contribute to treat autoimmune diseases like MS through probiotic modulations.

3 Introduction

3.1 Multiple Sclerosis (MS)

3.1.1 Definition, subtypes and epidemiology of MS

MS is a chronic, neuroinflammatory disease of the central nervous system (CNS), which is characterized by multifocal demyelinated lesions in the brain and spinal cord (3).
There are different subtypes of MS, namely relapsing remitting MS (RRMS), secondary progressive MS (SPMS) and primary progressive MS (PPMS) (4). RRMS (initially diagnosed in about 85% of pwMS (5)) describes a subtype characterized by remitting relapses with good recovery afterwards, whereas patients with PPMS (about 10% of all patients initial diagnosis (5)) are characterized by progressive deterioration and permanent disability beginning from the onset of the disease (4). The majority of patients with RRMS shows a so called clinical isolated syndrome (CIS) before developing RRMS, which is an acute clinical attack without a lesion or with a silent lesion in magnetic resonance imaging (MRI) (4). If not adequately treated, SPMS is a frequent consequence of RRMS (approximately 50-60% of patients with RRMS (5)), where recovery is incomplete and disability remains permanent (4).
The highest prevalence of MS is detected in Northern America and Europe (4). So, prevalence is higher with increasing distance from the equator in industrialized countries and the incidence of MS is increasing (3). Mainly, MS is seen in women in early adult life with an onset of approximately 30 years of age (4). However, concerning PPMS the age of onset is later (on average 40 years of age) and the women-men-ratio is equal or even with a slight male dominance (4). Moreover, there is an increase in the onset of MS already in childhood (6).

3.1.2 Aetiology of MS

The aetiology of MS is still unknown, but it is well accepted that environmental and genetic factors play an important role (4).
Regarding environmental factors, discussed risk factors are low vitamin D levels, diet, obesity, infections and cigarette smoking (6). Studies have shown that vitamin D interacts with immune cells, especially with the T cell compartment as well as various immune cells have vitamin D receptors, which underlines the connection of low vitamin D levels and the risk of MS (7). Concerning infections, especially the Epstein Barr virus (EBV) is discussed as a risk factor for

developing MS (6). The comparison of pwMS and controls without MS showed significantly more EBV positive individuals in the MS group, whereas this effect could not be found for the cytomegalovirus (CMV) (8). Cigarette smoking as a risk factor for MS may be linked to a pro-inflammatory effect as well as the direct toxicity of cigarette smoking (9). Moreover, the progression of MS may be negatively influenced by continuing smoking after the diagnosis (10). Interestingly, environmental factors seem to play major roles in childhood or adolescence, which underlines the importance of early preventive actions (1).

The influence of the ethnic background as well as the family history of disease on developing MS links to an important role of genetics in the pathogenesis of MS (11). The major histocompatibility complex (MHC) region is highly linked with vulnerability for MS (12). MHC, also called HLA in human-beings, denotes surface proteins which are important for immune response associated with T-lymphocytes (13). HLA-class I proteins are recognized by $CD8^+$ T cells, whereas HLA-class II proteins are recognized by $CD4^+$ T cells (13). Because of the wide range of HLA subtypes, it is not easy to decipher strong connections between specific HLA alleles and diseases such as MS (13). In addition to the influence of MHC on the risk of MS, a study on single-nucleotide polymorphisms (SNPs) reveals that SNPs in the interleukin 2 (IL2RA) and the interleukin 7 receptor (IL7RA) are associated with strong effects on contribution to MS (14). Furthermore, genomic mapping of pwMS showed a susceptibility region for MS on the X chromosome, whereby no such regions were found on the Y chromosome (15). This could be one explaining factor for the majority of cases in female individuals (15). The fact that most risk alleles are linked to the immune system indicates the important role of the immune system in developing MS (16).

3.1.3 Immunology of MS

The common view of MS as a T cell mediated disease is currently changing to a view that MS immunopathology involves a complex interplay of diverse immune cells (17). Therefore, it is important to include different immune cells in the investigations on MS (17).

Regarding the innate immune system, phagocytes such as microglia and macrophages may play an important role in the pathogenesis of MS (16). Phagocytes communicate with the adaptive immune system, but are also able to destroy tissues by themselves, e.g. phagocytes damage myelin or are found in the area of damaged axons (16). Moreover, these cells lead to oxidative stress, which contributes to the formation of lesions (16). Although phagocytes may have an important part in demyelination, there are some phagocytes that may also support recovery (16).

Many data point to a well-accepted impact of T cells on MS pathology. $CD4^+$ T cells are activated by HLA class II expressing cells. In contrast, $CD8^+$ T cells interact with HLA class I molecules and it is presumed that such T cell responses influence progression of MS (16). However, the recognised epitopes by T cells are still unknown (16). $CD4^+$ T cells can, among others, differentiate towards pro-inflammatory Th1 and Th17 cells, producing IFNγ and IL17, which are known to be higher in pwMS with disease activity (16, 17). High numbers of Th1 and Th17 cells occur in the CNS and cerebrospinal fluid of pwMS, contributing to the damage of the blood-brain-barrier (BBB) as well as the activation of astrocytes and microglia, further enhancing the neuroinflammatory process (18).

$CD4^+$ T cells can also differentiate towards anti-inflammatory Th2 or Treg cells (17) . Especially Treg cells seem to be another important cell type in MS pathology, because they suppress $CD4^+$ effector cells and thereby suppress autoimmunity (5). However, most pwMS do not show lower numbers of Treg cells compared to HC, but Treg cells exhibit a defective suppressive functionality (19). This might be responsible for higher numbers of pro-inflammatory T cells and the respective cytokines as well as increased autoantibody producing B cells in MS (5). However, not only the decreased functionality of Treg cells in pwMS was found, but also errors in genes associated to Treg cells and a higher vulnerability to apoptosis of Treg cells were described in other studies (5). The main subtypes of Treg cells express the transcription factor Forkhead-Box-Protein P3 (FoxP3) (5) and these cells have been investigated in detail in this project. Besides T cells, B cells play an important role in MS immunopathology. B cells, plasmablasts and plasma cells, accompanied by B cell cytokines and survival factors, can be found in MS lesions (16). Moreover, isolated oligoclonal bands in the cerebrospinal fluid of pwMS speak for an antibody mediated damage as well, but the targeted antigens are still unknown (16).

3.1.4 Diagnosis and treatment of MS

The diagnosis of MS is mainly clinically based and MRI plays an important role as well (6). Clinical symptoms of MS are dependent on degenerated and inflamed areas in the CNS and are wide-ranged (18).

The treatment of MS comprises acute relapse management, disease-modifying therapies (DMTs) and symptomatic treatment approaches (4). Most of the used drugs are interfering with the immune system and aim at modulating excessive immune reactions, which underlines the importance of immune cells in the pathogenesis of MS (4).

3.2 Impact of the gut microbiome and the diet on autoimmune diseases such as MS

The gastrointestinal tract is home to about 10^{14} microorganisms, which form the complex and dynamic gut microbiome, that may have a great impact on homeostasis and disease (20). Since activation and differentiation of T cells may be associated with the human gut, microbiota may play an important role in pathology of autoimmune diseases (16). It is well accepted that a long-term diet and especially the "Western" diet, a high-fat, high-salt and high-sugar diet, is influencing the gut microbiome and thereby the pathology of autoimmune diseases (21). A study comparing a "plant-based diet" with an "animal-based diet" found that the gut microbiome changes already one day after the given diet reached the distal gut microbiota, although there were no significant differences in microbiota of participants before starting the "study-diet" (21). Additionally, the gut microbiota changed back to initial stage two days after ending the diet (21). Hence, changes in microbiome caused by diet occur quickly, but only seem to be relevant when ingesting a diet for a longer duration of time (22).

3.2.1 The microbiome-gut-brain axis

Studies found several correlations between the gut microbiome and neuro-immune as well as neuro-psychiatric disorders (23, 24).

The CNS affects the gut microbiome, for instance, via neural and endocrine pathways (23). Influence can be exerted directly, for example with the help of enterochromaffin cells or indirectly via changes in permeability or motility (24). The gut microbiome may influence the CNS as well, as shown in a study investigating the BBB in germ-free versus pathogen-free mice with a normal gut flora (25).

Germ-free mice showed a greater permeability of the BBB already in utero as well as in the further course of their life, which was associated with decreased levels of tight junction proteins essential for the BBB (25). Interestingly, when colonizing germ-free mice with the microbiome of pathogen-free mice, these proteins increased and the BBB became less permeable, which underlines a connection between the gut microbiome and the CNS (25).

The composition of the gut microbiome may influence exploratory behaviour and brain biochemistry, shown in a study whereby pathogen-free mice receiving oral antimicrobials showed increased exploratory behaviour and higher levels of brain-derived neurotropic factor as well as germ-free mice did when colonized by microbiota from other mice (26). Moreover, a study

with newborn rats, states, that rats without weaning from their mother and maternal milk showed a depressive-like behaviour, significantly lower *Clostridium histolyticum* and increased stress-associated species in gut microbiome (27).
Additionally, a study of specific pathogen-free and germ-free mice showed a strong influence of the microbiome on developing EAE in mice associated with myelin-specific $CD4^+$ T cells, which underlines the assumption that MS may be strongly triggered by the immune system in association with the gut microbiome (28).

3.2.2 The gut microbiome of pwMS and related animal models

Many studies found an altered gut microbiome in pwMS compared to healthy individuals (29). For instansce,16S rRNA sequencing found that the microbes *Methanobrevibacter*, a pro-inflammatory microbe, and *Akkermansia* are increased in pwMS, whereas *Butyricimonas* are decreased compared to HC (30). Interestingly, patients under DMTs showed higher numbers of *Prevotella* and *Sutterella* compared to untreated patients, which showed reduced numbers compared to HC (30). This speaks for an impact of DMTs on normalizing some of the changes in the gut microbiome of pwMS (30). When analysing gene expression in T cells and monocytes, positive correlations of *Methanobrevibacter* and *Akkermansia* for pro-inflammatory signal pathways were found while some negative correlations to anti-inflammatory processes were described (30).
A study tested the effect of treatment with an antibiotic-cocktail and thereby modifying the gut microbiome on SJL- and C57BL/6 mice with EAE (31). Oral antibiotic treatment resulted in a significant decrease of EAE, coherent with reduced pro-inflammatory and increased anti-inflammatory responses in lymph nodes compared to control mice and mice treated intraperitoneally with antibiotics (31).
Moreover, when transplanting microbiota of twins into mice, the incidence of autoimmunity increased in mice which received stool from one twin with MS compared to the mice that received the stool of the healthy twin (32). In this study, mainly the microbial strain *Sutterella*, which may be protective in autoimmune diseases, was reduced in mice with the microbiome of twins with MS (32).

3.2.3 Role of microbiota metabolites on the immune system

Further investigations showed that not only the microbial composition but also microbiota metabolites may affect MS (32). These metabolites are produced by the metabolization of dietary components, vitamins or amino acids, among others (33). For instance, tryptophan, as an essential amino acid, is metabolized by microbes such as *lactobacilli* into immune-modulating factors like indoles. Indoles trigger a signalling cascade via the aryl hydrocarbon receptor (AhR), thus influencing various immune responses (33). Some of these indole compound interactions with the AhR start a signalling pathway which leads to the production of IL-22 and the release of antimicrobial peptides (34). The IL-22-dependent mucosal reaction results in mixed microbial communities and protects against fungal infections with Candida albicans and other inflammations (35). Other microbiota metabolites that were recently demonstrated to directly impact on the immune system during neuroinflammation are the short-chain fatty acid propionate (36, 37) or the tryptophan metabolite indole-3-lactic acid (2) .

3.2.4 Impacts of dietary fatty-acids and a high-salt diet

As mentioned above, the so called “Western diet”, which is characterized by high-fat, high-salt and high-sugar intake, is seen as an important environmental factor in the pathogenesis of autoimmune diseases as MS (38).

First studies demonstrated that obesity in childhood is a risk factor for developing MS (39). Later studies identified that mainly long-chain-fatty acids may contribute to disease pathogenesis, since they enhance the differentiation of $CD4^+$ T cells into Th1 or Th17 cells producing pro-inflammatory cytokines, whereas they inhibit the differentiation into Treg cells (40, 41). In contrast, short-chain-fatty acids led to the opposite effect (41). They increased the differentiation of Treg cells *in vitro* and *in vivo* and improved the clinical signs in EAE diseased mice (41). Interestingly, short-chain fatty acids are the main metabolites of dietary fiber digestion by the gut microbiota, indicating that gut microbes and microbial metabolites can impact on MS pathology (41).

Another dietary component potentially influencing the immune system during neuroinflammation is salt (42). Higher sodium-chloride (NaCl) concentrations *in vitro* led dose dependently to higher expressions of IL-17A by naïve $CD4^+$ T cells (42). This effect was maintained when reverting to normal salt concentrations (42). *In vivo*, HSD feeding in EAE diseased mice resulted in a more severe course of EAE, coinciding with higher numbers of Th17 cells (42). Additionally, another study focusing on the impact of HSD on the immune system, especially

on Th17 cells and the gut microbiome showed a decrease in *lactobacillus* in mice under HSD (2). Supplementation of *L. murinus* was accompanied by an amelioration of EAE with lower Th17 numbers and a reduction in blood pressure in mice (2). A following pilot study on healthy human individuals also demonstrated reduced *Lactobacillus spp.* colonization in the gut, increased Th17 cells as well as high blood pressure under HSD, even though *Lactobacillus* is not as dominant in humans as in mice (2). Another study found that the administration of potassium iodide (KI), sodium iodide (NaI) or sodium chloride (NaCl) stimulated pro-inflammatory IL-8 production via macrophages and osmotic stress (43). Moreover, there was a positive link between high sodium intake and relapse rate as well as MRI lesions in a clinical MS study (44). Yet, other studies observed no correlation between high-salt intake and MS severity (45, 46) . Nevertheless, a novel method to determine the salt load in humans, namely sodium MRI, revealed higher sodium contents in pwMS compared to healthy individuals independent from dietary salt intake (47). These observations indicate the potential importance of salt and other dietary components on developing MS or relapses.

3.2.5 First studies investigating the effect of probiotic supplementation on the immune system

Probiotics are compounds containing microorganisms, which show resemblance with beneficial human gut bacteria (48).

Supplementation of *Lactobacillus johnsonii* N6.2 (*L. johnsonii*) in healthy subjects versus placebo-group resulted in significant higher numbers of monocytes and natural killer (NK) cells, Th1 effector cells and $CD8^+$ T cell subsets as well as a trend towards increased Treg cells (49). Focusing on metabolomic effects of microbes, supplementation of *L. johnsonii* led to higher concentrations of tryptophan and therefore to a lower kynurenine:tryptophan ratio in healthy individuals (49). Interestingly, these findings associated with *L. johnsonii* were often only significant after the washout period (49).

Investigating the impact of the multispecies probiotics “Lactibiane iki” and Vivomixx® on mice with EAE showed a dose-dependent improvement of EAE under “Lactibiane iki” with reduced demyelination and inflammation as well as decreased pro-inflammatory and increased immunoregulatory mechanisms (50). Vivomixx® showed lower demyelination and T cell infiltration compared to vehicle-treated mice as well but did not significantly increase immunoregulatory or decrease pro-inflammatory mechanisms (50). Both probiotics improved motor coordination skills in mice and led to a higher percentage of myeloid dendritic cells (DCs) (50).

Effects of Vivomixx® have also been investigated in a murine model of progressive MS (51). Microbial changes in the gut, clinical improvement of motor impairment, decreased CNS leukocyte infiltration, higher numbers of regulatory B cells in the CNS as well as higher IL-10 gene expression besides lower IL-1β and IL6 expression in spinal cord, changes in metabolomic aspects and a decreased IL-17 production in the periphery could be observed (51).
In the present study, the probiotic Vivomixx® is used for investigating its impact on pwMS compared to HC. Several studies have declared *Lactobacilli*, which is the main bacterium strain in this probiotic, as safe (52). Moreover, *Bifidobacterium*, another bacterial strain in Vivomixx®, belongs to bacteria that are "generally regarded as safe" (52).

3.3 Lactobacilli containing probiotics in other diseases

First studies on other diseases as rheumatoid arthritis (RA), high blood pressure and diabetes mellitus showed positive impacts of probiotic supplementations on disease courses.
In RA, it is well-accepted that the gut and also the oral microbiomes play important roles (53, 54). For instance, *L. casei* supplementation in female patients with RA led to lower serum levels of C-reactive protein (CRP), beneficial changes in immune cells, less tender and swollen joint counts as well as better global health score and DAS28, a score for clinical relevance of RA (53).
HSD is triggering a high blood pressure in mice as well as in human beings coinciding with higher Th17 numbers and a reduction of *Lactobacillus spp.* in the gut microbiome (2). Additionally, a review of nine randomized controlled trials (RCTs) with 543 patients in total showed significant reductions in numbers of systolic and diastolic blood pressure under probiotic supplementation dependent on duration and concentration of probiotic intake as well as on the number of species used (55). An exploratory study already showed that Vivomixx® prevented a HSD-induced hypertension in healthy subjects, a current study is investigating the effect of Vivomixx® on hypertension (56).
A meta-analysis examining 15 RCTs, using probiotics containing *lactobacilli* and *Bifidobacterium* in patients with diabetes mellitus type II showed a significant decrease in glycated haemoglobin, homeostasis model assessment of insulin resistance and fasting blood glucose in the probiotic groups versus placebo (57).

3.4 Hypotheses and starting point of the current study

We hypothesize that pwMS may have increased pro-inflammatory immune cells compared to HC coinciding with reduced *lactobacilli* in the gut. In addition, an intake of the *Lactobacillus*-containing probiotic Vivomixx® may increase *lactobacilli* in the gut, thereby decreasing circulating Th17 cells and increasing Treg cells.

To investigate the potential benefit of dietary probiotic supplementation on the immune system in pwMS, we analysed different blood immune cells by flow cytometry. Furthermore, stool samples from the participants were harvested to investigate the frequency of *lactobacilli* in the gut microbiota and to analyse metabolomic products. Parts of the samples were processed immediately, whereas other samples were frozen and preserved for later analyses. All analyses were performed in pwMS and HC prior to the probiotic intake (baseline), after two and six weeks of daily Vivomixx® intake and four to six weeks after the last Vivomixx® intake. Salt-intake and the consumption of other dietary-products were analysed by a food questionnaire.

4 Material

Table 1: List of antibodies and isotype controls

Antibody	**Clone**	**Source**
CD3 eFluor	UCHT1	Invitrogen (Darmstadt, Germany)
CD4 PerCP Cy 5,5	L200	BD Biosciences (Heidelberg, Germany)
CD4 PerCP	SK3	BioLegend (Koblenz, Germany)
CD8 APC	SK1	BD Biosciences (Heidelberg, Germany)
CD45 RO PE-Cy7	UCHL1	Invitrogen (Darmstadt, Germany)
CCR7 PE	3D12	eBioscience (Frankfurt, Germany)
CD14 APC	MφP3	BD Biosciences (Heidelberg, Germany)
CD16 FITC	eBioCB16	eBioscience (Frankfurt, Germany)
CD19 PE Cy7	HIB19	BioLegend (Koblenz, Germany)
CD56 PE	HCD56	BioLegend (Koblenz, Germany)
CD4 Pacific Blue	SK3	BioLegend (Koblenz, Germany)
IL-17A PE	eBio64DEC17	eBioscience (Frankfurt, Germany)
IFNγ APC	B27	BioLegend (Koblenz, Germany)
IL-4 FITC	MP4-25D2	BioLegend (Koblenz, Germany)
FoxP3 PE	206D	BioLegend (Koblenz, Germany)
PE Mouse IgG1 K	MOPC-21	BD Biosciences (Heidelberg, Germany)
APC Mouse IgG1 K	MOPC-21	BD Biosciences (Heidelberg, Germany)
FITC Rat IgG1 K	RTK2071	BioLegend (Koblenz, Germany)

Table 2: List of buffers, media and solutions

Buffers, Media, Solutions	**Composition (Source)**
FACS-Buffer	MACS BSA Stock Solution (Miltenyi Biotec, Bergisch Gladbach, Germany) Auto MACS Rinsing Solution containing EDTA and PBS (Miltenyi Biotec, Bergisch Gladbach, Germany)
DPBS (1x):	Dulbecco´s Phosphate Buffered Saline without $CaCl_2$ and $MgCl_2$ (Gibco via Life Technologies, Darmstadt, Germany)
Erythrocyte Lysis Buffer	Ammoniumchlorid (NH_4Cl): 99,5+%NH_4Cl A.C.S Reagenz (SIGMA-ALDRICH, Steinheim, Germany) Aqua destilliert
RLT Lysis Buffer	RNeasy Plus lysis buffer (QIAGEN, Hilden, Germany)
R10	RPMI Medium 1640 with GlutaMAX™ (Gibco via Life Technologies, Darmstadt, Germany) 1% L-Glutamine 200mM sterile filtered (PAN™ BIOTECH, Aidenbach, Germany) 1% Penicillin-Streptomycin P4458, 100ml (SIGMA-ALDRICH, Steinheim, Germany) 1% Sodium pyruvate solution 100mM (SIGMA-ALDRICH, Steinheim, Germany) 1% Non-essential Amino-Acids (NEM-NEAA) 100x (Gibco via Life Technologies, Darmstadt, Germany) 10% FBS (Fetal Bovine Serum) (Gibco via Life Technologies, Darmstadt, Germany)
FcR Blocking Reagent, human	MiltenyiBiotec (Bergisch Gladbach, Germany)
Fixable viability dye eFluor780	eBioscience (Frankfurt, Germany)
Golgi-Stop, Protein Transport Inhibitor	BD Biosciences (Heidelberg, Germany)
Ionomycin, c=0,75 mg/ml, dissolved in DMSO	SIGMA-ALDRICH (Steinheim, Germany)

PMA, c=1mg/ml, dissolved in DMSO	SIGMA-ALDRICH (Steinheim, Germany)
Acridine Orange Propiodium Iodide	Logos Biosystems (Bremen, Germany)
Lymphoprep	Lymphoprep™ via STEMCELL™ TECHNOLOGIES (Köln, Germany)
Pancoll human	PAN™ BIOTECH (Aidenbach, Germany)

Table 3: List of kits

Kits	**Composition (Source)**
FoxP3/Transcription Factor Staining Buffer Set	Fixation/Permeabilization Concentrate Fixation/Permeabilization Diluent Permeabilization Buffer 10x (eBioscience, Frankfurt, Germany)

Table 4: List of consumables

Consumables	**Source**
Pipettes Research Plus (2,5µl, 10µl, 20µl, 200µl, 1000µl)	Eppendorf (Hamburg, Germany)
Pipett-tips (5ml, 10ml, 25ml) (10µl, 200µl, 1000µl)	Sarstedt (Nümbrecht, Germany)
Integra Pipetboy 2	Integra (Biebertal, Germany)
Glass Pasteur Pipettes 150mm	VWR™ International (Ismaning, Germany)
FACS-tubes (5ml, 15x12mm)	Sarstedt (Nümbrecht, Germany)
1.5ml Eppendorf-Cups	Sarstedt (Nümbrecht, Germany)
0.5ml Eppendorf-Cups	Sarstedt (Nümbrecht, Germany)
Falcon-Tubes 50ml	Sarstedt (Nümbrecht, Germany)
Falcon-Tubes 15ml	Sarstedt (Nümbrecht, Germany)
Cryo-Babies	DiversifiedBiotech via Carl Roth GmBH + Co. KG (Karlsruhe, Germany)
Parafilm “M”	Bemis via Carl Roth GmbH + Co. KG, (Karlsruhe, Germany)
TC-Platte 6 Well Cell+	Sarstedt (Nümbrecht, Germany)
Tissue Culture Testplate 6	TPP (Klettgau, Germany)
Corrugated Panel, Nuclon™, Delta Surface	Thermo Scientific™ (Schwerte, Germany)

Table 5: List of devices and software

Devices and Software	**Source**
Centrifuge 5702R	Eppendorf (Hamburg, Germany)
Centrifuge 5810R	Eppendorf (Hamburg, Germany)
Centrifuge 5430R	Eppendorf (Hamburg, Germany)
Centrifuge 5418R	Eppendorf (Hamburg, Germany)
Centrifuge Hettich EBA 8S	SIGMA-ALDRICH (Steinheim, Germany)
Vortex Mix	NeoLab (Heidelberg, Germany)
Roll Mixer	Phoenix Instrument (Garbsen, Germany)
Incubator with mixing function	VWR Incubating Mini Shaker (avantor™ deliverend by vwr™ Darmstadt, Germany)
Incubator 37°C, 5% CO_2	HERAcell 150 (Thermo Scientific™, Schwerte, Germany)
Suction device	BVC control, VACUUBRAND GMBH + CO KG, Wertheim, Germany)
Luna™ Dual Fluorescence Cell Counter	Logos Biosystems (Bremen, Germany)
Luna™ Cell Counting Slides	Logos Biosystems (Bremen, Germany)
BD FACS Canto II	BD Biosciences (Heidelberg, Germany)
FlowJo v10.6.2	FlowJo Software by Dongle-Lizenz
GraphPad Prism 9	GraphPad Software (San Diego, CA, USA)

Table 6: List of consumables used at the neurological outpatient clinic

Consumables neurological outpatient clinic	**Source**
Safety-Multifly-Kanüle	Sarstedt (Nümbrecht, Germany)
S-Monovette 7.5ml Z-Gel (for Serum)	Sarstedt (Nümbrecht, Germany)
S-Monovette 2.7ml K3E: 1.6 mg EDTA/ml	Sarstedt (Nümbrecht, Germany)
S-Monovette 9ml K3E: 1.6mg EDTA/ml	Sarstedt (Nümbrecht, Germany)
Plaster strips Leukosilk	BSN medical GmbH (Hamburg, Germany)
Hand disinfectant Octeniderm	Schülke & Mayr GmbH (Norstedt, Germany)
Tupfer Pur-Zellin	Hartmann (Aachen, Germany)
Stuhlauffanghilfe	Labortechnik Süsse (Hessen, Germany)
Faeces Tube 79x16mm	Sarstedt (Nümbrecht, Germany)
DNA/RNA Shield Fecal Collection Tube 20x76mm (9ml Fl./1ml sample)	PANGEA Laboratory/Licensed from Zymo Research Corp. (CA, USA)

5 Methods

5.1 Study design and study participants

The current unblinded, parallel-grouped, pre-post designed study investigated the effect of a *lactobacillus*-supplementation by the intake of Vivomixx® in pwMS (n=16) compared to HC (n=18). The study was permitted by the Ethics Committee of the Friedrich-Alexander University Erlangen-Nuremberg.

Participants were recruited from August 2020 until January 2021 (figure 1). Exclusion criteria were lactose intolerance, probiotic intake, vaccinations four to six weeks before the study, acute infections, drug- or alcohol abuse or insufficient mental or physical capacity to adhere to the study protocol. One HC (female, aged 24) ended the study after the second appointment because of side effects (diarrhoea and abdominal pain).

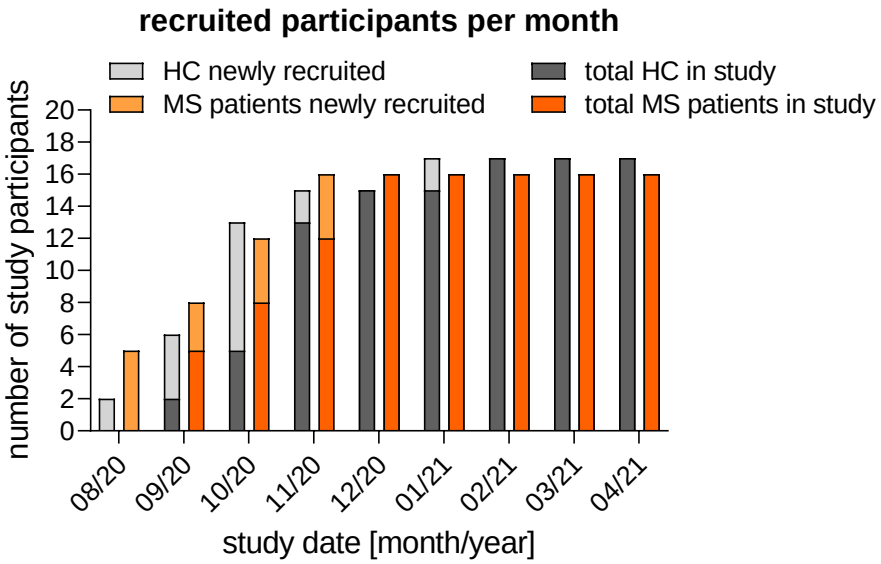

Figure 1: This figure shows the recruited participants per month from August 2020 (start of the study) until April 2021 (end of the study). The newly recruited participants are shown in a light colour on top of the total numbers of study participants (dark colours) in the respective months.

Every participant had four appointments at the neurological outpatient clinic of the University Hospital Regensburg. After the first appointment (L0: before *lactobacilli* intake), participants were controlled again after two (L2: *lactobacilli* intake for 2 weeks) and six weeks (L6: *lactobacilli* intake for 6 weeks) of Vivomixx® intake and four to six weeks after the last Vivomixx® intake (WO: washout).

After obtaining written informed consent at the first appointment, the following parameters

were recorded: height and weight to determine the BMI (afterwards calculated by "bmirechner.net"), current MS immunotherapy and former medical treatment of MS if proceeded, EDSS (Expanded Disability Status Scale), form of MS, initial manifestation and diagnosis dates, smoking status, previous diseases and surgeries, family history concerning autoimmune diseases, food supplements, medication besides MS therapy and allergies. Some of those aspects have been copied from up-to-date medical reports from the clinic or have been evaluated after the first appointment. At every appointment, study participants were asked for current health problems, infections or vaccinations in the last four to six weeks, changes concerning MS and tolerance and regularity of Vivomixx® intake. Furthermore, pwMS and HC were neurologically examined at every appointment.

Moreover, participants filled in a food questionnaire ("Ernährungsfragebogen of DEGS of Robert Koch Institut, RKI" (58)) one time during the study. Questionnaires were digitalized via Adobe Acrobat and were evaluated with the software DGExpert by collaboration partners at the Halle University, Nutritional Sciences headed by Prof. Dr. Gabriele Stangl**.**

Blood samples were taken at every appointment (three 9ml EDTA-tubes, one 2.7ml EDTA-tube and two 7.5ml serum-tubes) for analysing routine blood parameters (blood cell count, liver values, kidney values, HbA1c, CRP and blood lipids), serum asservation and flow cytometry analysis of blood lymphocytes.

Moreover, two stool samples were provided at every appointment. One sample was stored in a DNA-stabilizing solution for later gut microbiome analysis and one sample was stored without any solution for later microbial metabolite analysis.

After the first appointment, all participants took the probiotic Vivomixx® (Mendes SA, Lugano, Switzerland), containing eight different strains of bacteria: *Streptococcus thermophilus* NCIMB 30438, *Bifidobacterium breve* NCIMB 30441, *Bifidobacterium longum* NCIMB 30435 (Re-classified as *B. lactis*), *Bifidobacterium infantis* NCIMB 30436 (Re-classified as *B. lactis*), *Lactobacillus acidophilus* NCIMB 30442, *Lactobacillus plantarum* NCIMB 30437, *Lactobacillus paracasei* NCIMB 30439, *Lactobacillus delbrueckii subsp. bulgaricus* NCIMB 30440 (Re-classified as *L. helveticus*). The participants ingested six sachets of Vivomixx® every day for six weeks (two in the morning, two at lunch and two in the evening). Participants dissolved the powder in water, yoghurt, milk or juice. The total amount of bacteria intake per day was 2.700 billion.

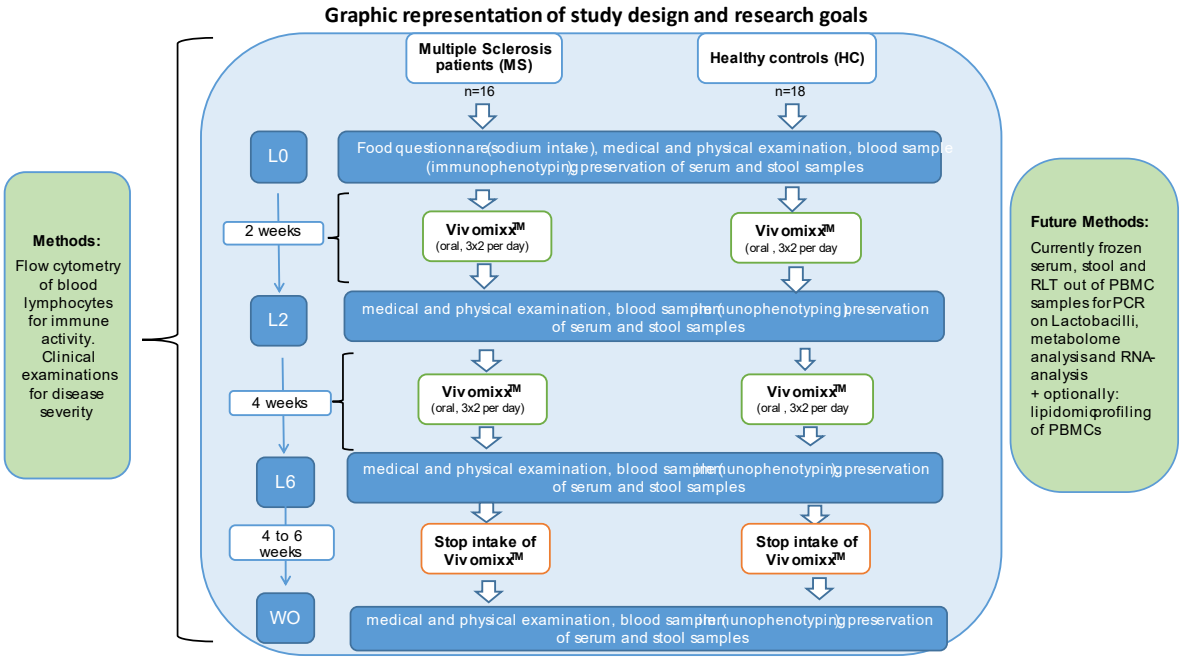

Figure 2: Graphic sketch of study design, research methods and planned methods with currently preserved samples.

5.2 Experimental Methods

5.2.1 Asservation of serum samples

Serum tubes were kept at room temperature, transported to the laboratory and centrifuged for 10 minutes at 4000 rpm. The serum was aliquoted and is currently preserved at -80°C for future examinations of cytokine concentrations or metabolite measurements.

5.2.2 Asservation of stool samples

Stool samples were transferred in a cooling bag from the clinics to the laboratory. Samples with DNA stabilizing solution are currently stored at 4°C for later microbiome analysis. Samples without DNA stabilizing solution are preserved at -80°C for later microbial metabolite analysis.

5.2.3 Immunophenotyping in whole-blood samples

100µl blood were filled into two separate FACS-tubes (A and B) and incubated with 10µl Fc-receptor blocking reagent for 10 minutes at 4°C. To identify $CD4^+$ T cells, $CD8^+$ T cells, $CD4^+CD8^+$ T cells, memory ($CD45RO^-CCR7^+$) and effector ($CD45RO^+CCR7^-$) $CD4^+/CD8^+$ T

cells, B-cells ($CD19^{+}$), monocytes (classical ($CD14^{++}CD16^{-}$), intermediate ($CD14^{++}CD16^{+}$) and non-classical ($CD14^{+}CD16^{++}$) monocytes) and NK-cells ($CD56^{bright}CD16^{dim}$, $CD56^{bright}CD16^{-}$ and $CD56^{dim}CD16^{bright}$), the following antibodies were added to the blood samples:

Tube A: CD3 eFluor 1µl / CD4 PerCP (Cy5.5) 2µl / CD8 APC 1µl /
CD45 RO PE Cy-7 2.5µl / CCR7 PE 2.5µl

Tube B: CD3 eFluor 1µl / CD14APC 1µl / CD16 FITC 2.5µl / CD19 PE Cy7 2.5µl /
CD56 PE 2.5µl

After vortexing and incubating for 30 minutes at 4°C, 2ml of erythrocyte-lysis-buffer (0.14M NH_4Cl) were added per tube followed by an incubation for 10 minutes at room temperature in the dark on a shaker with a speed of 270 rpm. Cells were washed two to three times with FACS-Buffer and centrifuged for 5 minutes at 4°C and 0.3 x 1000 rcf, respectively. 200µl cell solution were measured with the FACS Canto II.

5.2.4 Immunophenotyping of Th17($IL17^{+}$)-, Treg ($FoxP3^{+}$)-, Th1($IFN\gamma^{+}$)- and Th2($IL4^{+}$)- cells

5.2.4.1 Isolation of PBMCs

In a first step, EDTA-blood was transferred into a sterile 50ml Falcon-tube together with 5ml of sterile DPBS. In order to obtain leukocytes (PBMC = Peripheral Blood Mononuclear Cells) a density gradient was performed by using the fractionation-solution Lymphoprep. Therefore, the blood samples were undercoated with 15ml Lymphoprep.

The density gradient was centrifuged for 20 minutes at room temperature and 0.8 x 1000 rcf without brake. From top to bottom the following layers were visible: Serum – PBMC – "Lymphoprep" – Erythrocytes/cell debris. PBMCs were transferred with a 10ml pipette tip into a new 50ml Falcon tube and washed with sterile DPBS (centrifugation for 10 minutes at 4°C and 0.3 x1000rcf).

Cells were resuspended in 10ml sterile DPBS and counted with the LUNA Fluorescence cell counter.

5.2.4.2 Cell counting

1.5µl of Acridine Orange Propiodium Iodide were added to 13.5µl of PBMC solution and gently mixed. 13.5µl of this solution were then transferred onto a cell counting slide and cells were counted by LUNA Fluorescence cell counter in the Fluorescence cell counting mode. The total number of cells per ml, live cells per ml, dead cells per ml, viability [%], diameter [µm] and the total number of cells in the counted field were documented. Cells were washed with DPBS and adjusted to 10 x 10^6 cells/ml in R10 culture medium for intracellular flow cytometry analysis (5.2.4.3) or in DPBS for asservation and later RNA isolation (5.2.5).

5.2.4.3 Intracellular staining for flow cytometry analysis

Prior to intracellular cytokine staining, 5 x 10^6 PBMCs were incubated with 4µl Ionomycin (stock concentration) plus 150µl of 1:1000 PMA (stock concentration) and 6µl Golgi-Stop solution (containing Monensin) in a cell culture plate for three hours at 37°C and 5% CO_2. Additionally, 5 x 10^6 unstimulated cells only treated with Golgi-Stop were used as controls.
After this incubation, the cells were transferred to FACS-tubes labelled with "A-D stimulated" and "A-D non-stimulated".
Dead cells were stained by adding 0.5µl of the Fixable Viability Dye eFluor 780. Cells were mixed and incubated for 30 minutes at 4°C. Cells were washed with 2ml FACS-Buffer per tube and centrifuged for 5 minutes at 4°C and 0.3 x 1000 rcf. After discarding the supernatant, cells were mixed and 10µl of Fc-receptor-blocking-reagent were added for 10 minutes at 4°C. The cells were then incubated with 1µl of anti-CD4 pacific blue for 30 minutes at 4°C.
Cells were washed with FACS-Buffer and 150µl of Fixation-Permeabilization-Buffer (eBioscience™ FoxP3/Transcription Factor Staining Buffer Set) were added to every tube. Cells were incubated for at least 30 minutes at 4°C, but in most cases were stored in the fridge overnight for a maximum of 18 hours.
After fixation, the cells were washed with 2ml Permeabilization-Buffer (1:10 solution of Permeabilization-Buffer10x and Aqua distilled), centrifuged for 5 minutes at 4°C and 0.3 x 1000 rcf. The supernatant was discarded and cells were vortexed for two times. For intracellular staining the following antibodies were used:
Tube A: IL-17 PE 2.5µl / IFNγ APC 2.5µl / IL-4 FITC 2.5µl
Tube B: IgG1 PE 2.5µl / IgG 1 APC 2.5µl / rat IgG1 FITC 0.2µl
Tube C: FoxP3 PE 2.5µl

Tube D: IgG1 PE 3µl

Antibodies were mixed in 50µl Permeabilization-Buffer per tube and cells were incubated for 45 minutes at 4°C.

Cells were washed one time with Permeabilization-Buffer and one time with FACS-Buffer.

200µl cell solution were measured with the FACS Canto II.

5.2.5 PBMC asservation for later RNA expression analysis

5-10 x 10^6 PBMCs were washed with DPBS and centrifuged for 5 minutes at room temperature and 0.3 x 1000 rcf. The supernatant was discarded and 350µl of RLT Lysis-Buffer were added and mixed for 30 seconds. This solution is currently frozen at -80°C for later RNA analysis.

5.3 Analysing Methods

5.3.1 Fluorescence-activated cell sorting (FACS-) analysis

Immune cells as stained in sections 5.2.3 and 5.2.4 were measured by flow cytometry with a BD FACS Canto II. All FACS-tubes were measured for three minutes at a medium flow rate. Raw data were analyzed by FlowJo v10.6.2. The gating strategy and isotype control are representatively shown for Treg cells (figure 3).

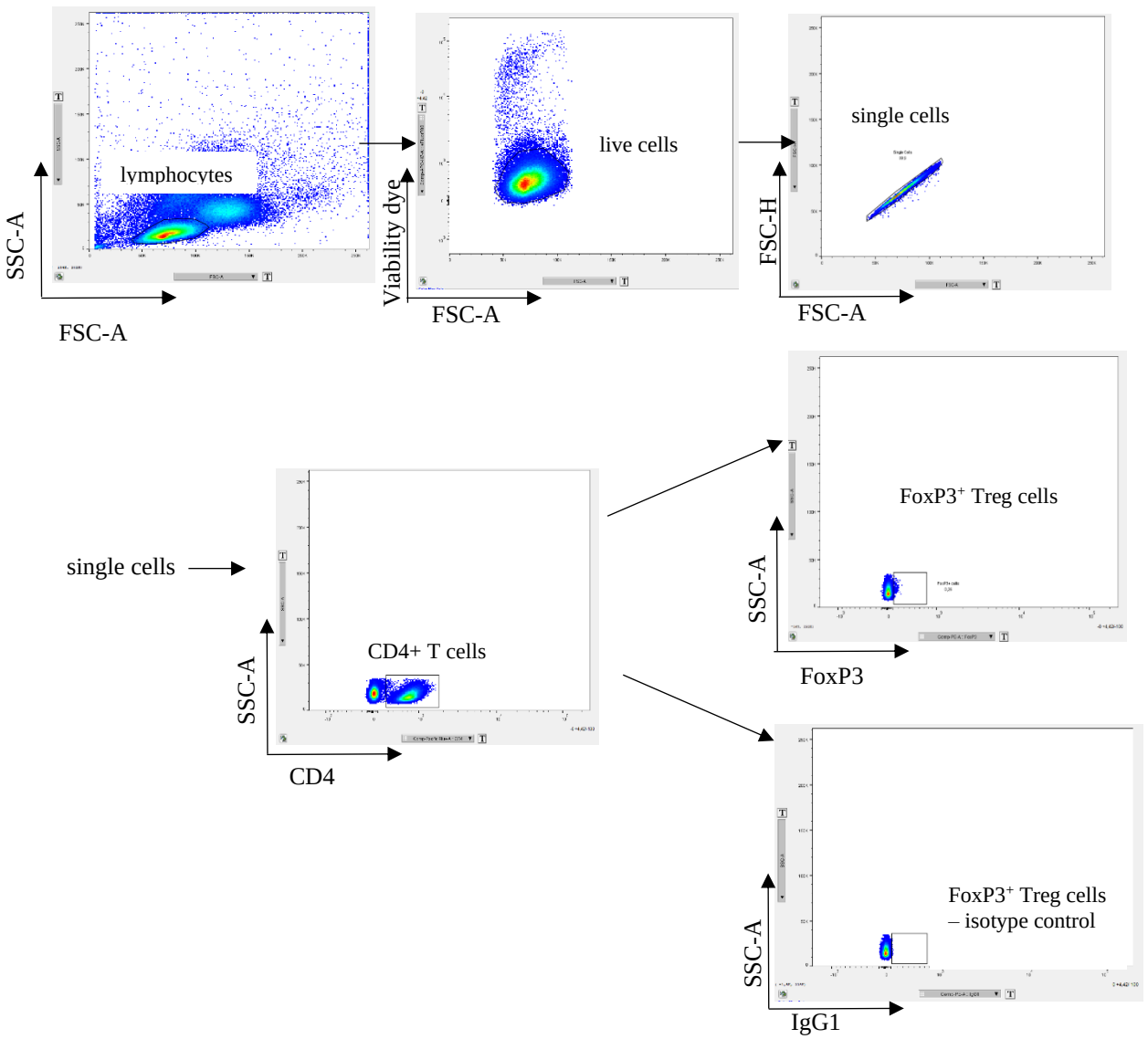

Figure 3: Gating strategy is shown for FoxP3$^+$ Treg cells.

Cells are shown in different graphs where the x- and y-axis shows either FSC/SSC or the respective antibody for the shown cell-type. The different graphs result after gating and using the last gate as the basis for the next graph.

This example shows from the first to the last graph: lymphocytes, live cells, single cells, CD4+ T cells, FoxP3$^+$ Treg cells, isotype control.

5.3.2 Data representation and statistical analysis

The percentages of investigated immune cell populations were transferred to GraphPad Prism 9 for generating graphs and statistics.

The Shapiro-Wilk-Test was employed to analyze the data for normal distribution.

Data with normal distribution were analyzed by parametric paired T-test. Data without normal distribution were analyzed by non-parametric Wilcoxon matched-pairs signed rank test.

For unpaired data the parametric unpaired T-test or the non-parametric Mann-Whitney-test were performed.

Flow cytometry data are presented as mean±SD. *$p<0.05$, **$p<0.01$, ***$p<0.001$ were considered as statistically significant. Clinical data and some experimental results are presented as median (interquartile range), respectively.

6 Results

6.1 Clinical parameters

This pilot study included 16 pwMS and 18 HC. One HC (female, aged 24) opted to end the study after the second appointment, wherefore discussion of results includes 17 HC. The mean age of the MS-group was 48.3 ± 11.8 years and in the HC-group was 27.6 ± 10.2 years. The MS-group consisted of four females (25%) and twelve males. Ten female (59%) and seven male HC participated in the study (table 7).

Table 7: Overview of baseline parameters of participating pwMS and HC.

Parameter	**Multiple Sclerosis (MS)**	**Healthy Controls (HC)**
Individuals, n	16	17
Female	4	10
Male	12	7
Age [years] [mean±SD]	48.3±11.8	27.6±10.2
BMI [kg/m^2][mean±SD]	26.6±3.7	23.0±3.0
Smokers	4	0
Previous smokers	3	0

Clinical parameters of pwMS are shown in table 8. The recruited pwMS had a median EDSS of 3.0 and a median disease duration of 10.5 years. Twelve of the sixteen pwMS were diagnosed with RRMS, three patients suffered from SPMS and one patient from PPMS. Except of two therapy naïve pwMS, the MS cohort received immune modulatory medications such as Ocrelizumab (n=11), Fingolimod (n=2) and Natalizumab (n=1). Moreover, one patient received a transient corticosteroid pulse therapy in addition to the DMT during the study.

Table 8: Clinical parameters of participating pwMS. Disease Duration is calculated from initial manifestation, except for four patients for whom only the date of initial diagnosis has been elicitable.

Parameter	**pwMS**
Individuals, n	16
EDSS [median (interquartile range)]	3.0 (1.6-4.0)
Disease Duration [mean±SD]	10.6±7.6
Ocrelizumab treated	11
Fingolimod treated	2
Natalizumab treated	1
Therapy naive	2
Relapsing-remitting MS	12
Secondary-progressive MS	3
Primary-progressive MS	1

Regarding allergies, 17.6% of HC reported allergies, 11.8% mentioned intolerances and one HC was suspected of actually having an allergy. 31.3% of pwMS mentioned allergies. This comprises allergies or intolerances against different medicines, house dust, insect venom, plasters, nuts and hay fever.

Acute infections and vaccinations constituted criteria for exclusion. However, 29.4% of HC and 18.8% of pwMS suffered from a mild cold without fever and other symptoms of illness over the course of the study. Two pwMS mentioned gastrointestinal infections, one HC got sick with Covid-19 and one HC had a bladder infection while participating in the study. Concerning vaccinations, participants have been asked to keep a gap of at least four weeks between study appointments. However, 47% of HC and 37.5% of pwMS in total reported any vaccinations less than four weeks before an appointment.

In total, there were only 10 delays in 132 appointments with pwMS and HC. Six of these delays concerned the washout timepoint. Reasons for delays were personal and sometimes because of quarantine due to the Covid-19-pandemic.

According to the probands, Vivomixx® was taken regularly in up to 90% of individuals.

Two HC showed side effects of Vivomixx®, which is why one HC opted out of the study and one HC reduced the dose by one third. All other participants did not show any side effects, except for slight changes in bowel movements.

As mentioned above, pwMS and HC were examined neurologically at every appointment. Regarding the HC group no changes in the clinical examinations were found. At timepoint L6, a slight recovery could be determined in the neurological examination of two pwMS, mainly related to the balance.

Furthermore, existing data on relapse rate and MRI activity of pwMS was recorded independent of Vivomixx® intake. There were no differences in relapse rate and MRI-activity between patients under Ocrelizumab therapy and patients under another therapy than Ocrelizumab visible.

6.2 Routine blood parameter analysis

The routine blood parameter analysis comprised the analysis of CRP, a detailed blood cell count, sodium, potassium, calcium, liver values, kidney values, blood lipids and HbA1c. These parameters were analysed by the laboratory of Bezirksklinikum Regensburg. The results are shown in the supplement (section 9.1) as [mean ± SD] for all timepoints for pwMS and HC (tables 27 and 28). Statistical analysis between timepoints L0 and L2 in both groups and comparation of baseline parameters between pwMS and HC are shown in tables 9, 10 and 11.

When comparing baseline and timepoint L2 of pwMS, most of the routine blood parameter values were not significantly changed (table 9). Significant decreases were found in erythrocytes, sodium and γ-GT, whereas these changes could not be confirmed for HC (table 10). Interestingly, cholesterol was significantly decreased in pwMS and HC two weeks after *lactobacilli* intake (table 9 and 10).

Table 9: Routine blood parameters of pwMS at baseline and timepoint L2 in [mean ± SD].
At baseline there are missing some values of the first two patients, because of initial ambiguities what should be analysed (deviating "n" are listed in the table).

Parameters	**MS (L0)**	**MS (L2)**	**p (MS_L0 vs. MS_L2)**
Individuals, n	16	16	16
CRP [mg/l]	1.4±1.7	1.0±1.2	0.5313 - np
Leukocytes/BB [x10^3/µl]	6.8±3.2	6.3±2.1	0.6230 - np
Lymphocytes [%]	21.7±8.9 (n=15)	21.3±8.9	0.8916 - p
Neutrophiles [%]	65.1±8.7 (n=15)	65.9±8.4	0.6611 - p
Monocytes [%]	10.8±2.5 (n=15)	10.0±3.1	0.2539 - np
Basophiles [%]	0.7±0.7 (n=15)	0.9±0.6	0.5000 - np
Eosinophiles [%]	2.0±1.3 (n=15)	2.3±1.5	0.6534 - p
Erythrocytes/BB [/pl]	4.9±0.5	4.8±0.5	*** 0.0295 - p**
Haemoglobin [g/dl]	14.8±1.5	14.6±1.3	0.1353 - p
Haematocrite [%]	42.5±3.7	42.3±3.5	0.4831 - p
medium cell volume [fl]	88.4±5.9	88.6±5.4	0.5302 - p
medium cell-HB [pg]	30.6±2.1	30.7±2.2	0.3761 - p
medium HB-conc. [g Hb/dl Ery]	34.7±1.1	34.7±0.9	0.9649 - p
Thrombocytes [x10^3/µl]	260.6±52.9	258.5±48.9	0.7916 - np
Sodium [mmol/l]	140.8±1.7	139.7±1.7	**** 0.0097 - p**
Potassium [mmol/l]	4.3±0.3	4.3±0.2	0.5226 - p
Calcium [mmol/l]	2.4±0.1 (n=14)	2.4±0.1	>0.9999 - np
GOT [U/l]	23.6±5.8	22.6±5.8	0.8198 - np
GPT [U/l]	30.9±33.2	25.1±13.6	0.5325 - np
γ-GT [U/l]	55.4±50.2 (n=14)	43.9±32.5	**** 0.0020 - np**
Creatinine [mg/dl]	0.9±0.2	0.9±0.2	0.8482 - p
Urea [mg/dl]	14.3±5.9	14.5±6.3	0.7190 - np
GFR [ml/min]	89.2±22.7	87.4±20.6	0.2994 - p
Cholesterol [mg/dl]	213.9±44.0 (n=14)	193.6±39.2	*** 0.0232 - p**
Triglycerides [mg/dl]	145.0±84.8 (n=14)	132.3±97.3	0.9032 - np
HbA1c [%]	5.6±0.3 (n=14)	5.6±0.4	0.2733 - p

List of abbreviations: CRP=C-reactive protein, HB=haemoglobin, conc.=concentration, GOT=Glutamate-Oxalacetate-Transaminase, GPT=Glutamate-Pyruvate-Transaminase, GFR=glomerular filtration rate, np=non-parametric statistical test (Wilcoxon matched-pairs signed rank test), p=parametric statistical test (paired t-test).

Comparing the routine blood parameters before (L0) and after two weeks of *lactobacilli* intake (L2) in HC revealed significant changes in haematocrite, calcium, urea and cholesterol values (table 10).

Table 10: Routine blood parameters of HC at baseline and timepoint L2 in [mean ± SD].

Parameters	**HC (L0)**	**HC (L2)**	**p (HC_L0 vs. HC_L2)**
Individuals, n	17	17	17
CRP [mg/l]	1.4±2.0	1.0±1.1	0.8066 - np
Leukocytes/BB [x10^3/µl]	5.8±1.2	5.8±0.9	0.9329 - p
Lymphocytes [%]	33.2±7.1	32.7±6.1	0.6290 - p
Neutrophiles [%]	55.8±7.8	56.2±7.2	0.6751 - p
Monocytes [%]	7.9±2.2	8.0±2.3	0.8717 - p
Basophiles [%]	0.9±0.2	0.9±0.3	> 0.9999 - np
Eosinophiles [%]	2.6±2.0	2.6±1.7	> 0.9999 - np
Erythrocytes/BB [/pl]	4.8±0.5	4.7±0.4	0.1495 - p
Haemoglobin [g/dl]	14.4±1.3	14.2±1.3	0.0727 - p
Haematocrite [%]	41.3±3.7	40.5±3.5	*** 0.0183 - p**
medium cell volume [fl]	86.7±3.2	86.4±3.8	0.4219 - np
medium cell-HB [pg]	30.3±1.2	30.2±1.3	0.2292 - p
medium HB-conc. [g Hb/dl Ery]	34.9±0.7	35.0±0.8	0.5924 - p
Thrombocytes [x10^3/µl]	245.7±48.4	246.1±55.3	0.9386 - p
Sodium [mmol/l]	139.4±2.5	139.0±1.8	0.5684 - p
Potassium [mmol/l]	4.2±0.3	4.2±0.3	0.8566 - np
Calcium [mmol/l]	2.4±0.1	2.4±0.1	*** 0.0449 - np**
GOT [U/l]	23.9±11.9	22.8±7.0	0.6289 - np
GPT [U/l]	17.7±6.2	20.9±11.1	0.1281 - np
γ-GT [U/l]	16.1±5.2	16.3±7.2	0.9688 - np
Creatinine [mg/dl]	0.8±0.2	0.8±0.2	0.7337 - p
Urea [mg/dl]	11.6±3.3	9.5±2.2	**** 0.0011 - p**
GFR [ml/min]	98.4±20.3	97.9±15.6	0.8462 - p
Cholesterol [mg/dl]	178.1±39.7	170.5±35.5	**** 0.0091 - p**
Triglycerides [mg/dl]	95.1±40.6	99.7±38.1	0.5794 - p
HbA1c [%]	5.2±0.2	5.2±0.3	0.3828 - np

List of abbreviations: CRP=C-reactive protein, HB=haemoglobin, conc.=concentration, GOT=Glutamate-Oxalacetate-Transaminase, GPT=Glutamate-Pyruvate-Transaminase, GFR=glomerular filtration rate, np=non-parametric statistical test (Wilcoxon matched-pairs signed rank test), p=parametric statistical test (paired t-test).

When comparing routine blood parameters of pwMS and HC at baseline, lymphocytes were significantly lower, while neutrophils and monocytes were significantly higher in pwMS (table 11). Cholesterol, triglycerides, γ-GT and HbA1c were significantly higher in pwMS compared to HC (table 11).

Table 11: Routine blood parameters of HC and pwMS at baseline in [mean ± SD].

Parameters	**MS (L0)**	**HC (L0)**	**p (MS_L0 vs. HC_L0)**
Individuals, n	16	17	MS: 16, HC: 17
CRP [mg/l]	1.4±1.7	1.4±2.0	0.2687 – np
Leukocytes/BB [x10^3/µl]	6.8±3.2	5.8±1.2	0.2136 – np
Lymphocytes [%]	21.7±8.9 (n=15)	33.2±7.1	***** 0.0003 -p**
Neutrophiles [%]	65.1±8.7 (n=15)	55.8±7.8	**** 0.0032 -p**
Monocytes [%]	10.8±2.5 (n=15)	7.9±2.2	**** 0.0016 -p**
Basophiles [%]	0.7±0.7 (n=15)	0.9±0.2	0.1707 – np
Eosinophiles [%]	2.0±1.3 (n=15)	2.6±2.0	0.4099 – np
Erythrocytes/BB [/pl]	4.9±0.5	4.8±0.5	0.5149 – p
Haemoglobin [g/dl]	14.8±1.5	14.4±1.3	0.4307 – p
Haematocrite [%]	42.5±3.7	41.3±3.7	0.3505 – p
medium cell volume [fl]	88.4±5.9	86.7±3.2	0.3376 – np
medium cell-HB [pg]	30.6±2.1	30.3±1.2	0.5768 – p
medium HB-conc. [g Hb/dl Ery]	34.7±1.1	34.9±0.7	0.4185 – p
Thrombocytes [x10^3/µl]	260.6±52.9	245.7±48.4	0.5275 – np
Sodium [mmol/l]	140.8±1.7	139.4±2.5	0.0750 – p
Potassium [mmol/l]	4.3±0.3	4.2±0.3	0.1092 – np
Calcium [mmol/l]	2.4±0.1 (n=14)	2.4±0.1	0.4607 – np
GOT [U/l]	23.6±5.8	23.9±11.9	0.3760 – np
GPT [U/l]	30.9±33.2	17.7±6.2	0.1641 – np
γ-GT [U/l]	55.4±50.2 (n=14)	16.1±5.2	***** 0.0002 – np**
Creatinine [mg/dl]	0.9±0.2	0.8±0.2	0.2737 – p
Urea [mg/dl]	14.3±5.9	11.6±3.3	0.0593 – np
GFR [ml/min]	89.2±22.7	98.4±20.3	0.2299 – p
Cholesterol [mg/dl]	213.9±44.0 (n=14)	178.1±39.7	*** 0.0244 – p**
Triglycerides [mg/dl]	145.0±84.8 (n=14)	95.1±40.6	*** 0.0471 – np**
HbA1c [%]	5.6±0.3 (n=14)	5.2±0.2	**** 0.0019 – np**

List of abbreviations: CRP=C-reactive protein, HB=haemoglobin, conc.=concentration, GOT=Glutamate-Oxalacetate-Transaminase, GPT=Glutamate-Pyruvate-Transaminase, GFR=glomerular filtration rate, np=non-parametric statistical test (Mann-Whitney-test), p=parametric statistical test (unpaired t-test).

6.3 Dietary salt intake and analysis of food questionnaires

All study participants filled in a food questionnaire that allowed to analyse the total energy intake, the intake of carbohydrates, fat, sugar, salt, vitamins, amino acids and related factors. These 158 factors were summarized as median values per study group (data on file). The analysis showed that pwMS had an average higher energy intake than HC (total energy intake of pwMS 2183.0 kcal versus total energy intake of HC 1691.8 kcal).

Statistical analysis of the individual nutrients was not performed in this thesis. However, because of the special interest on dietary salt intake, this value was statistically analysed and presented in figure 4. All study participants consumed an average of 4.28g ± 0.718g salt per day (figure 4C) and there was no statistical difference between pwMS and HC. Moreover, statistical analysis of some fatty acids showed no significant results (data on file).

Study participants have been asked about their intake of food supplements. 35,3% of HC and 50% of pwMS ingested food supplements regularly, whereby vitamin D, vitamin B_{12}, magnesium and calcium were the most frequent ones.

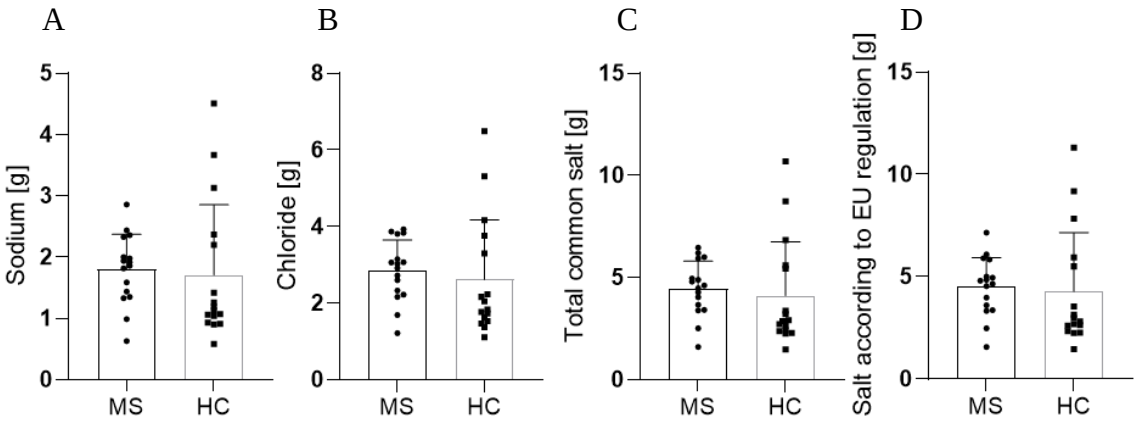

Figure 4: Dietary salt intake in MS patients and HC. All study participants filled in a food frequency questionnaire. The sodium intake (A), chloride intake (B), total salt intake (C) and the salt intake according to EU regulation (D) were determined by DGExpert. Dots represent individual study participants. Statistical analysis by unpaired t-test did not show any statistical significance.
p(sodium) = 0.1964, p(chloride) = 0.1625, p (total common salt) = 0.1381,
p (salt according to EU regulation) = 0.1964

6.4 Blood immunophenotyping in pwMS and HC

We performed an in-depth analysis of blood immune cells by multi-colour flow cytometry in pwMS and HC before, after two and six weeks of Vivomixx® intake and four to six weeks after ending the Vivomixx® intake. We analysed the frequencies of B cells ($CD19^{+}$), NK cells ($CD56^{bright}CD16^{dim}$, $CD56^{bright}CD16^{-}$ and $CD56^{dim}CD16^{bright}$), monocytes (classical $CD14^{++}CD16^{-}$, non-classical $CD14^{+}CD16^{++}$ and intermediate $CD14^{++}CD16^{+}$ monocytes), $CD4^{+}$, $CD8^{+}$ and $CD4^{+}CD8^{+}$ T cells as well as memory ($CD45RO^{-}CCR7^{+}$) and effector ($CD45RO^{+}CCR7^{-}$) subsets of these cells, T helper cells (pro-inflammatory Th1 and Th17 cells, anti-inflammatory Th2 cells) and regulatory T cells ($FoxP3^{+}$ Treg).

6.4.1 Baseline blood cell frequencies in pwMS and HC

At baseline, we detected significantly lower B cell frequencies in the blood of pwMS compared to HC (figure 5A). Moreover, pwMS showed increased frequencies of total monocytes (figure 5B), and more in depth characterization revealed higher frequencies of intermediate monocytes compared to HC (figure 5C). In contrast, we observed no differences in classical and non-classical monocytes (figure 5D and E). Analysis of NK cells revealed an increased frequency of $CD56^{bright}CD16^{dim}$ and $CD56^{dim}CD16^{bright}$ in pwMS compared to HC (figure 5F and G), whereas $CD56^{bright}CD16^{-}$ frequencies were comparable in both groups (figure 5H). Moreover, baseline $CD4^{+}$ (figure 5I), $CD8^{+}$ (figure 5J) and $CD4^{+}CD8^{+}$ (figure 5K) T cell frequencies showed no significant difference between pwMS and HC. Analysis of T helper cell subsets and Treg cells revealed no significant differences between pwMS and HC before the start of Vivomixx® intake (figure 6)

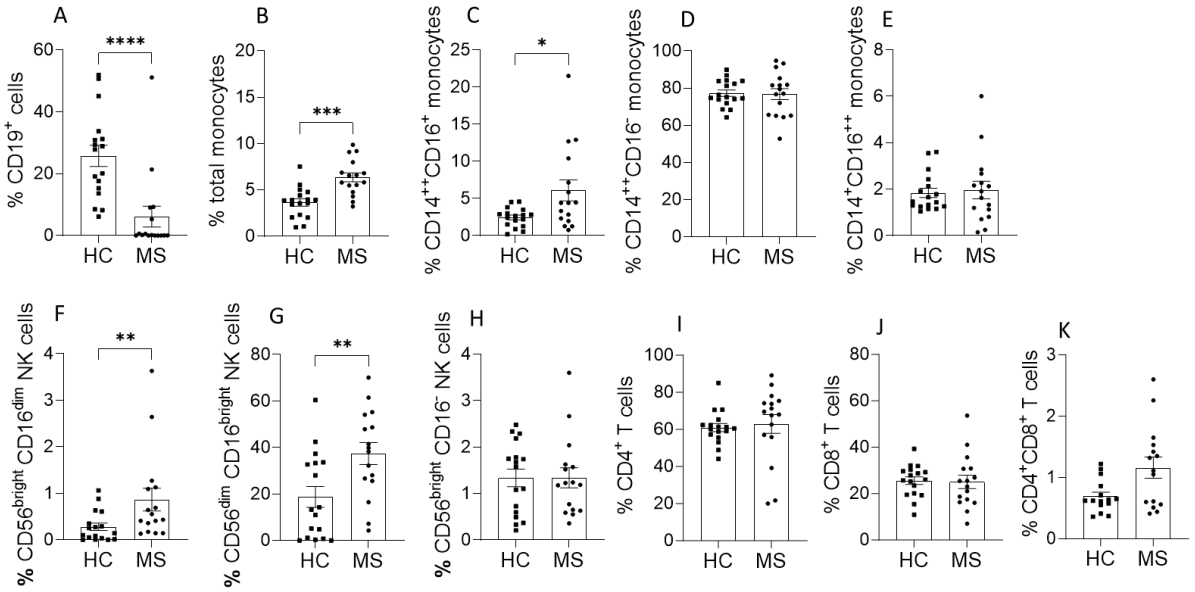

Figure 5: Baseline blood cell frequencies of pwMS and HC before the start of probiotic Vivomixx® intake.
Flow cytometry analysis of B cells (A), monocytes (B-E), NK cells (F-H), $CD4^+$ T cells (I), $CD8^+$ T cells (J) and $CD4^+CD8^+$ T cells (K) in the blood of pwMS and HC before probiotic Vivomixx® intake.
MS n=16, HC n=17.
**p<0.05, **p<0.01, ***p<0.001 by Mann-Whitney-test (non-parametric) or unpaired t-test (parametric).*
*****p ($CD19^+$B cells)<0.0001; ***p(total monocytes)<0.0001; *p($CD14^{++}CD16^+$ monocytes)=0.0455; **p($CD56^{bright}CD16^{dim}$ NK cells)=0.0040; **p($CD56^{dim}CD16^{bright}$ NK cells)=0.0097*

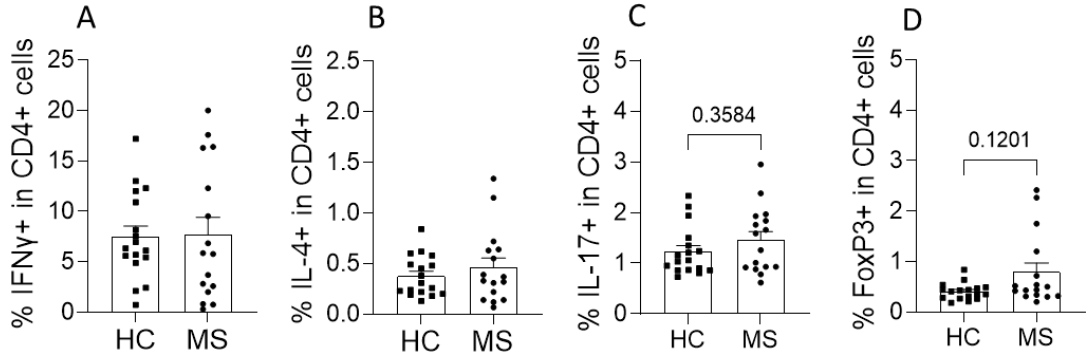

Figure 6: Baseline frequencies of Th cell subsets and Treg cells in pwMS and HC before the start of probiotic Vivomixx® intake.
Flow cytometry analysis of Th1 (A), Th2 (B), Th17 (C) and Treg (D) cells in pwMS and HC before probiotic Vivomixx® intake. pwMS n=16, HC n=17.
Mann-Whitney-test (non-parametric) or unpaired t-test (parametric) was used for statistical analysis.

6.4.2 No significant effect on B cells and NK cells under Vivomixx® intake

Flow cytometry analysis of B cells ($CD19^+$) revealed no effect of Vivomixx® intake in pwMS and HC (figure 7A and C). We additionally investigated effects of Vivomixx® intake on B cells in the subgroup of pwMS under Ocrelizumab therapy and observed no effects either (figure 7B). Statistical analysis when comparing all timepoints with all p-values can be found in the supplement (section 9.2.1, tables 29 and 30).

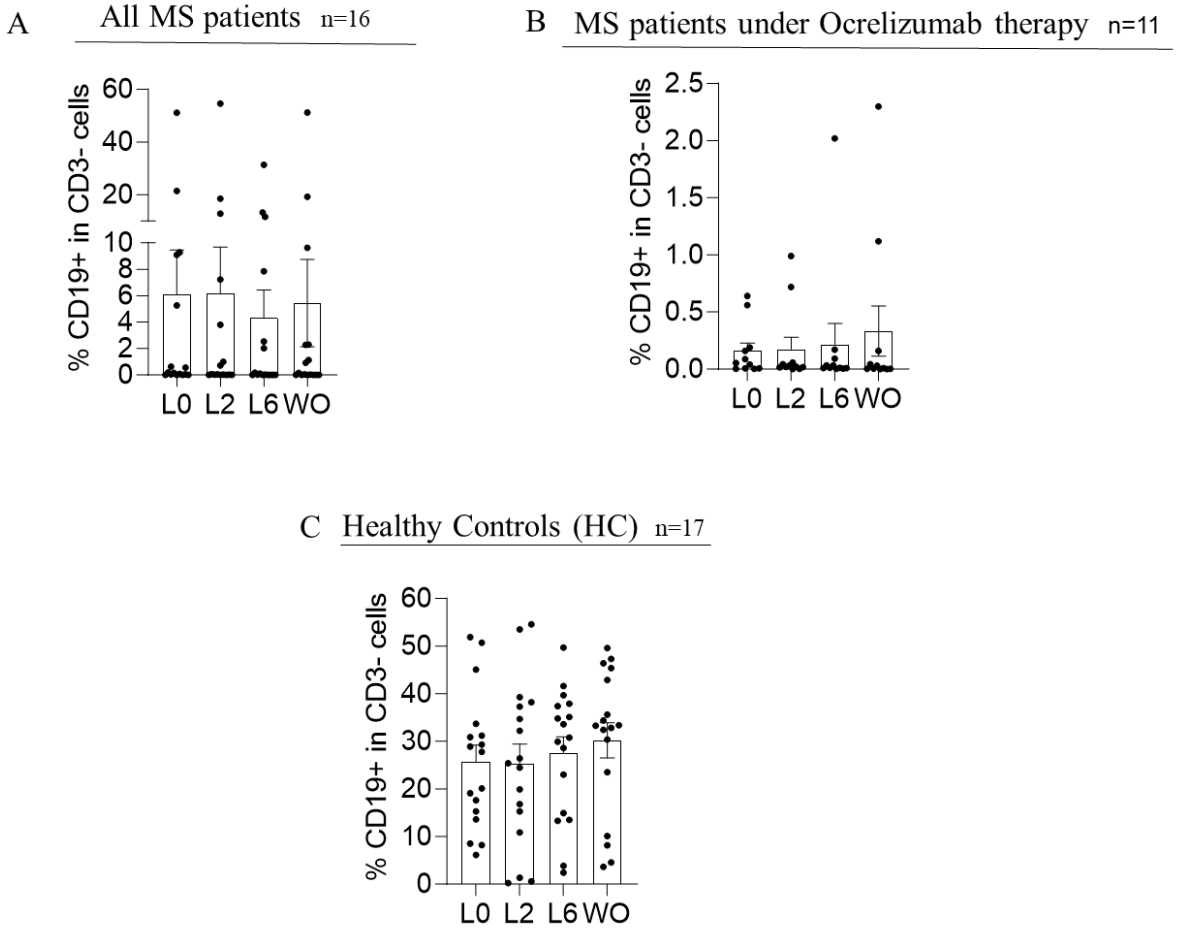

Figure 7: Vivomixx® intake does not affect B cell frequencies in pwMS, pwMS under Ocrelizumab therapy or HC.

Flow cytometry analysis of $CD19^+$ B cells in pwMS (A), pwMS under Ocrelizumab (B) and HC (C) showed no significant effects when comparing all timepoints (baseline, timepoints L2, L6 and WO).

pwMS n=16, pwMS under Ocrelizumab therapy n=11, HC n=17.

Statistical analysis with Wilcoxon matched-pairs signed rank test (non-parametric statistical analysis) or paired t-test (parametric statistical analysis).

Flow cytometry analysis of NK cells ($CD56^{bright}CD16^{-}$, $CD56^{bright}CD16^{dim}$ and $CD56^{dim}CD16^{bright}$) did not show any significant effects of a probiotic Vivomixx® intake in pwMS, the subgroup of pwMS under Ocrelizumab therapy and HC. Tables 12-14 show the numbers of NK cells in [mean±SD] at every timepoint analysed for pwMS, pwMS under Ocrelizumab therapy and HC. Statistical analysis when comparing all timepoints with all p-values can be found in the supplement (section 9.2.1, tables 29 and 30).

Table 12: Numbers of $CD56^{bright}CD16^{-}$, $CD56^{bright}CD16^{dim}$ and $CD56^{dim}CD16^{bright}$ NK cells in [mean±SD] at baseline (L0), timepoint L2, timepoint L6 and the washout timepoint (WO) for pwMS.

	pwMS			
cell population	**L0**	**L2**	**L6**	**WO**
$CD56^{bright}CD16^{-}$ NK cells	1.34 ± 0.86	1.28 ± 0.97	1.30 ± 0.74	1.17 ± 0.81
$CD56^{bright}CD16^{dim}$ NK cells	0.86 ± 0.97	0.92 ± 1.03	1.08 ± 0.98	0.76 ± 0.74
$CD56^{dim}CD16^{bright}$ NK cells	37.36 ± 18.83	35.88 ± 17.10	40.87 ± 16.58	34.34 ± 23.13

Table 13: Numbers of $CD56^{bright}CD16^{-}$, $CD56^{bright}CD16^{dim}$ and $CD56^{dim}CD16^{bright}$ NK cells in [mean±SD] at baseline (L0), timepoint L2, timepoint L6 and the washout timepoint (WO) for pwMS under Ocrelizumab therapy.

	pwMS under Ocrelizumab therapy			
cell population	**L0**	**L2**	**L6**	**WO**
$CD56^{bright}CD16^{-}$ NK cells	1.47 ± 0.99	1.31 ± 1.05	1.51 ± 0.75	1.30 ± 0.85
$CD56^{bright}CD16^{dim}$ NK cells	0.95 ± 1.17	1.04 ± 1.23	1.17 ± 1.08	0.83 ± 0.85
$CD56^{dim}CD16^{bright}$ NK cells	33.33 ± 20.02	32.63 ± 17.60	39.12 ± 15.62	32.03 ± 23.84

Table 14: Numbers of $CD56^{bright}CD16^{-}$, $CD56^{bright}CD16^{dim}$ and $CD56^{dim}CD16^{bright}$ NK cells in [mean±SD] at baseline (L0), timepoint L2, timepoint L6 and the washout timepoint (WO) for HC.

	HC			
cell population	**L0**	**L2**	**L6**	**WO**
$CD56^{bright}CD16^{-}$ NK cells	1.34 ± 0.78	1.28 ± 0.78	1.6 ± 0.91	1.48 ± 0.71
$CD56^{bright}CD16^{dim}$ NK cells	0.28 ± 0.33	0.29 ± 0.28	0.52 ± 0.66	0.42 ± 0.45
$CD56^{dim}CD16^{bright}$ NK cells	18.74 ± 18.56	16.44 ± 15.69	17.23 ± 16.79	17.23 ± 15.31

6.4.3 Decreased monocytes in pwMS under Vivomixx®

Total frequencies of monocytes were numerically decreased in the blood of pwMS after two weeks of probiotic Vivomixx® intake and were significantly decreased when comparing baseline and the washout timepoint in pwMS and pwMS under Ocrelizumab therapy. However, more in depth analysis on different monocyte subpopulations did not confirm this observation. Vivomixx® intake did not affect the frequencies of classical ($CD14^{++}CD16^{-}$), intermediate ($CD14^{++}CD16^{+}$) or non-classical ($CD14^{+}CD16^{++}$) monocytes in pwMS, pwMS under Ocrelizumab therapy or HC at any timepoint.

Tables 15 to 17 show the numbers of monocytes in [mean±SD] at every timepoint analysed for pwMS, pwMS under Ocrelizumab therapy and HC. Significant p-values can be found in the description above the tables. Tables with all p-values are shown in the supplement (section 9.2.2, tables 31 and 32).

Table 15: Numbers of total monocytes, classical ($CD14^{++}CD16^{-}$), intermediate ($CD14^{++}CD16^{+}$) and non-classical ($CD14^{+}CD16^{++}$) monocytes in [mean±SD] at baseline (L0), timepoint L2, timepoint L6 and the washout timepoint (WO) for pwMS.

*p(L0 vs. L2, total monocytes)=0.0507, *** p(L0 vs. WO, total monocytes) = 0.0004, p (L6 vs. WO, total monocytes) = 0.0501by Wilcoxon matched-pairs signed rank test (non-parametric) or paired t-test (parametric).*

	pwMS			
cell population	**L0**	**L2**	**L6**	**WO**
total monocytes	6.35 ± 1.94	5.42 ± 3.67	5.70 ± 2.40	4.51 ± 2.64
$CD14^{++}CD16^{-}$	76.74 ± 11.85	75.53 ± 6.50	75.61 ± 13.07	74.12 ± 9.57
$CD14^{++}CD16^{+}$	6.1 ± 5.66	6.0 ± 3.20	6.5 ± 4.73	6.5 ± 4.73
$CD14^{+}CD16^{++}$	1.98 ± 1.51	2.73 ± 2.58	2.57 ± 2.98	2.24 ± 1.41

Table 16: Numbers of total monocytes, classical ($CD14^{++}CD16^{-}$), intermediate ($CD14^{++}CD16^{+}$) and non-classical ($CD14^{+}CD16^{++}$) monocytes in [mean±SD] at baseline (L0), timepoint L2, timepoint L6 and the washout timepoint (WO) for pwMS under Ocrelizumab therapy.
** p(L0 vs. WO, total monocytes) = 0.0131by paired t-test (parametric).*

	pwMS under Ocrelizumab therapy			
cell population	**L0**	**L2**	**L6**	**WO**
total monocytes	6.19 ± 1.82	5.6 ± 4.37	5.57 ± 2.04	4.43 ± 2.65
$CD14^{++}CD16^{-}$	77.53 ± 13.83	77.31 ± 6.92	78.54 ± 14.09	76.99 ± 10.30
$CD14^{++}CD16^{+}$	6.38 ± 6.36	5.66 ± 3.58	4.51 ± 2.86	5.45 ± 4.49
$CD14^{+}CD16^{++}$	1.76 ± 1.79	1.96 ± 0.87	1.61 ± 0.81	2.0 ± 1.58

Table 17: Numbers of total monocytes, classical ($CD14^{++}CD16^{-}$), intermediate ($CD14^{++}CD16^{+}$) and non-classical ($CD14^{+}CD16^{++}$) monocytes in [mean±SD] at baseline (L0), timepoint L2, timepoint L6 and the washout timepoint (WO) for HC.

	HC			
cell population	**L0**	**L2**	**L6**	**WO**
total monocytes	3.65 ± 1.65	3.60 ± 1.8	3.67 ± 1.96	4.06 ± 2.98
$CD14^{++}CD16^{-}$	76.60 ± 7.55	70.44 ± 19.83	77.55 ± 12.36	77.57 ± 14.18
$CD14^{++}CD16^{+}$	2.46 ± 1.28	2.12 ± 1.18	4.27 ± 6.28	3.27 ± 2.52
$CD14^{+}CD16^{++}$	1.84 ± 0.82	1.45 ± 0.66	1.48 ± 0.86	1.36 ± 1.03

6.4.4 No major effects on $CD4^+$, $CD8^+$ and $CD4^+CD8^+$ T cells in pwMS under Vivomixx®

We additionally investigated $CD4^+$, $CD8^+$ and $CD4^+CD8^+$ T cells as well as memory ($CD45RO^-CCR7^+$) and effector ($CD45RO^+CCR7^-$) subsets of these cells as important contributors to MS immunopathology.

We observed no effect of probiotic Vivomixx® intake in pwMS and HC for $CD4^+$ T cells, neither after two nor after six weeks (tables 18 to 20). In depth analysis of memory ($CD45RO^-CCR7^+$) and effector ($CD45RO^+CCR7^-$) $CD4^+$ T cells discovered no effect of Vivomixx® intake either (tables 18 to 20). Interestingly, there was a significant decrease in $CD4^+$ T cells when comparing timepoint L6 and the washout timepoint in pwMS and baseline and the washout timepoint in pwMS under Ocrelizumab therapy (tables 18 and 19). Tables with all p-values are shown in the supplement (section 9.2.3, tables 33 and 34).

Table 18: Numbers of $CD4^+$ T cells and memory ($CD45RO^-CCR7^+$) and effector ($CD45RO^+CCR7^-$) $CD4^+$ T cells in [mean±SD] at baseline (L0), timepoint L2, timepoint L6 and the washout timepoint (WO) for pwMS.

**p(L6 vs. WO, $CD4^+$ T cells)=0.0334 by Wilcoxon matched-pairs signed rank test (non-parametric).*

	pwMS			
cell population	**L0**	**L2**	**L6**	**WO**
$CD4^+$ T cells	62.95 ± 20.18	59.71 ± 21.43	63.10 ± 19.72	61.18 ± 21.31
$CD45RO^-CCR7^+$ $CD4^+$ T cells	20.98 ± 11.68	28.93 ± 17.40	19.35 ± 8.64	23.33 ± 10.75
$CD45RO^+CCR7^-$ $CD4^+$ T cells	48.06 ± 18.18	42.89 ± 15.24	49.93 ± 15.88	48.34 ± 16.45

Table 19: Numbers of $CD4^+$ T cells and memory ($CD45RO^-CCR7^+$) and effector ($CD45RO^+CCR7^-$) $CD4^+$ T cells in [mean±SD] at baseline (L0), timepoint L2, timepoint L6 and the washout timepoint (WO) for pwMS under Ocrelizumab therapy.
p(L0 vs. WO, $CD4^+$ T cells)=0.0576 by paired t-test (parametric).

	pwMS under Ocrelizumab therapy			
cell population	**L0**	**L2**	**L6**	**WO**
$CD4^+$ T cells	72.85 ± 9.26	69.98 ± 9.86	72.65 ± 8.85	71.07 ± 9.37
$CD45RO^-CCR7^+$ $CD4^+$ T cells	22.74 ± 11.27	26.60 ± 11.71	20.55 ± 8.95	24.20 ± 10.90
$CD45RO^+CCR7^-$ $CD4^+$ T cells	40.15 ± 11.25	39.77 ± 9.21	42.95 ± 11.38	41.71 ± 11.05

Table 20: Numbers of $CD4^+$ T cells and memory ($CD45RO^-CCR7^+$) and effector ($CD45RO^+CCR7^-$) $CD4^+$ T cells in [mean±SD] at baseline (L0), timepoint L2, timepoint L6 and the washout timepoint (WO) for HC.

	HC			
cell population	**L0**	**L2**	**L6**	**WO**
$CD4^+$ T cells	60.96 ± 9.11	60.51 ± 9.34	61.51 ± 9.44	60.19 ± 10.92
$CD45RO^-CCR7^+$ $CD4^+$ T cells	21.75 ± 12.04	20.78 ± 10.18	18.23 ± 6.37	24.43 ± 20.25
$CD45RO^+CCR7^-$ $CD4^+$ T cells	41.28 ± 14.60	41.75 ± 12.98	43.62 ± 13.51	43.60 ± 15.95

$CD8^+$ T cells showed a significant decrease from baseline (L0) to timepoint L6 in pwMS and pwMS under Ocrelizumab therapy (tables 21 and 22). $CD8^+$ effector ($CD45RO^+CCR7^-$) T cells showed a significant increase from baseline and timepoint L2 to the washout timepoint in HC (table 23). There were no other significant results detectable for $CD8^+$ T cells at any other timepoints when analysing pwMS, pwMS under Ocrelizumab therapy and HC. Tables with all p-values are shown in the supplement (section 9.2.3, tables 35 and 36).

Table 21: Numbers of $CD8^+$ T cells and memory ($CD45RO^-CCR7^+$) and effector ($CD45RO^+CCR7^-$) $CD8^+$ T cells in [mean±SD] at baseline (L0), timepoint L2, timepoint L6 and the washout timepoint (WO) for pwMS.

**p(L0 vs. L6, $CD8^+$ T cells)=0.0251 by paired t-test (parametric).*

	pwMS			
cell population	**L0**	**L2**	**L6**	**WO**
$CD8^+$ T cells	25.08 ± 11.59	26.02 ± 13.74	23.98 ± 11.95	23.48 ± 9.15
$CD45RO^-CCR7^+$ $CD8^+$ T cells	21.95 ± 10.84	25.58 ± 11.86	20.88 ± 15.69	20.22 ± 13.98
$CD45RO^+CCR7^-$ $CD8^+$ T cells	38.86 ± 17.27	38.03 ± 14.91	40.02 ± 17.18	44.08 ± 20.26

Table 22: Numbers of $CD8^+$ T cells and memory ($CD45RO^-CCR7^+$) and effector ($CD45RO^+CCR7^-$) $CD8^+$ T cells in [mean±SD] at baseline (L0), timepoint L2, timepoint L6 and the washout timepoint (WO) for pwMS under Ocrelizumab therapy.

**p(L0 vs. L6, $CD8^+$ T cells)=0.0420 by paired t-test (parametric).*

	pwMS under Ocrelizumab therapy			
cell population	**L0**	**L2**	**L6**	**WO**
$CD8^+$ T cells	19.54 ± 6.83	20.77 ± 7.35	18.42 ± 6.34	19.39 ± 6.73
$CD45RO^-CCR7^+$ $CD8^+$ T cells	22.98 ± 10.22	23.90 ± 12.36	19.94 ± 15.38	22.54 ± 13.62
$CD45RO^+CCR7^-$ $CD8^+$ T cells	31.90 ± 12.91	31.82 ± 11.78	33.40 ± 15.61	33.89 ± 12.65

Table 23: Numbers of $CD8^+$ T cells and memory (CD45RO-CCR7+) and effector (CD45RO+CCR7-) $CD8^+$ T cells in [mean±SD] at baseline (L0), timepoint L2, timepoint L6 and the washout timepoint (WO) for HC.

***p(L0 vs. WO, $CD45RO^+CCR7^-$ $CD8^+$ T cells)=0.0027, *p(L2 vs. WO, $CD45RO^+CCR7^-$ $CD8^+$ T cells)=0.0306 by paired t-test (parametric).*

	HC			
cell population	**L0**	**L2**	**L6**	**WO**
$CD8^+$ T cells	25.56 ± 6.93	25.99 ± 6.25	25.48 ± 5.65	25.54 ± 7.06
$CD45RO^-CCR7^+$ $CD8^+$ T cells	23.26 ± 20.17	21.68 ± 18.96	20.66 ± 17.33	21.00 ± 21.94
$CD45RO^+CCR7^-$ $CD8^+$ T cells	30.29 ± 12.00	31.98 ± 13.26	33.52 ± 12.66	36.70 ± 15.09

Probiotic intake of Vivomixx® had no significant effects on $CD4^+CD8^+$ T cells in pwMS, pwMS under Ocrelizumab therapy and HC (tables 24 to 26). However, further analysis of $CD4^+CD8^+$ memory ($CD45RO^-CCR7^+$) T cells revealed a significant increase after two weeks of Vivomixx® intake and a following significant decrease from timepoint L2 to the washout timepoint in pwMS (table 24). Additionally, $CD4^+CD8^+$ effector ($CD45RO^+CCR7^-$) T cells showed a significant decrease after two weeks of Vivomixx® intake but no significant change to the washout timepoint (table 24). These effects could not be found for pwMS under Ocrelizumab therapy and HC (tables 25 and 26). Tables with all p-values are shown in the supplement (section 9.2.3, tables 37 and 38).

Table 24: Numbers of $CD4^+CD8^+$ T cells and memory ($CD45RO^-CCR7^+$) and effector ($CD45RO^+CCR7$) $CD4^+CD8^+$ T cells in [mean±SD] at baseline (L0), timepoint L2, timepoint L6 and the washout timepoint (WO) for pwMS.

***p(L0 vs. L2, $CD45RO^-CCR7^+$ $CD4^+CD8^+$ T cells)=0.0063, p(L0 vs. L2, $CD45RO^+CCR7^-$ $CD4^+CD8^+$ T cells)=0.0507, *p(L2 vs. WO, $CD45RO^-CCR7^+$ $CD4^+CD8^+$ T cells)=0.0250 by Wilcoxon matched-pairs signed rank test (non-parametric).*

	pwMS			
cell population	**L0**	**L2**	**L6**	**WO**
$CD4^+CD8^+$ T cells	1.13 ± 0.66	1.47 ± 1.29	1.27 ± 0.71	1.15 ± 0.69
$CD45RO^-CCR7^+$ $CD4^+CD8^+$ T cells	13.36 ± 12.91	24.61 ± 22.49	13.67 ± 16.35	16.89 ± 16.96
$CD45RO^+CCR7^-$ $CD4^+CD8^+$ T cells	76.09 ± 13.84	66.86 ± 21.01	79.18 ± 16.98	73.07 ± 17.02

Table 25: Numbers of $CD4^+CD8^+$ T cells and memory ($CD45RO^-CCR7^+$) and effector ($CD45RO^+CCR7$) $CD4^+CD8^+$ T cells in [mean±SD] at baseline (L0), timepoint L2, timepoint L6 and the washout timepoint (WO) for pwMS under Ocrelizumab therapy.

	pwMS under Ocrelizumab therapy			
cell population	**L0**	**L2**	**L6**	**WO**
$CD4^+CD8^+$ T cells	1.02 ± 0.67	1.11 ± 0.75	1.10 ± 0.73	1.05 ± 0.71
$CD45RO^-CCR7^+$ $CD4^+CD8^+$ T cells	16.22 ± 13.99	22.42 ± 17.08	17.13 ± 18.70	21.48 ± 18.55
$CD45RO^+CCR7^-$ $CD4^+CD8^+$ T cells	72.83 ± 14.89	69.66 ± 16.42	76.05 ± 19.34	67.63 ± 17.55

Table 26: Numbers of $CD4^+CD8^+$ T cells and memory ($CD45RO^-CCR7^+$) and effector ($CD45RO^+CCR7$) $CD4^+CD8^+$ T cells in [mean±SD] at baseline (L0), timepoint L2, timepoint L6 and the washout timepoint (WO) for HC.

	HC			
cell population	**L0**	**L2**	**L6**	**WO**
$CD4^+CD8^+$ T cells	1.05 ± 1.00	1.17 ± 1.12	0.89 ± 0.39	1.01 ± 0.70
$CD45RO^-CCR7^+$ $CD4^+CD8^+$ T cells	17.74 ± 20.28	17.95 ± 20.31	11.60 ± 8.54	14.76 ± 20.83
$CD45RO^+CCR7^-$ $CD4^+CD8^+$ T cells	73.95 ± 18.59	73.67 ± 19.47	75.83 ± 21.09	76.80 ± 19.44

6.4.5 Increased Treg cell frequencies in pwMS under Vivomixx® intake

Flow cytometry analysis revealed a significant increase of $FoxP3^+$ Treg cells in pwMS after two weeks of Vivomixx® intake compared to baseline (figure 8A). This increase remained stable after six weeks of Vivomixx® intake. However, Treg frequencies decreased during the washout period to baseline levels.

Interestingly, depicting only B-cell-depleted pwMS also showed a significant increase after two weeks of Vivomixx® intake but not after six weeks (figure 8B). The reduction of $FoxP3^+$ Treg cells from timepoint L2 to the washout timepoint was also found for the subgroup of pwMS under Ocrelizumab therapy.

There was no significant effect of probiotic intake on $FoxP3^+$ Treg frequencies in HC. However, $FoxP3^+$ Treg cells also showed an increase (figure 8C).

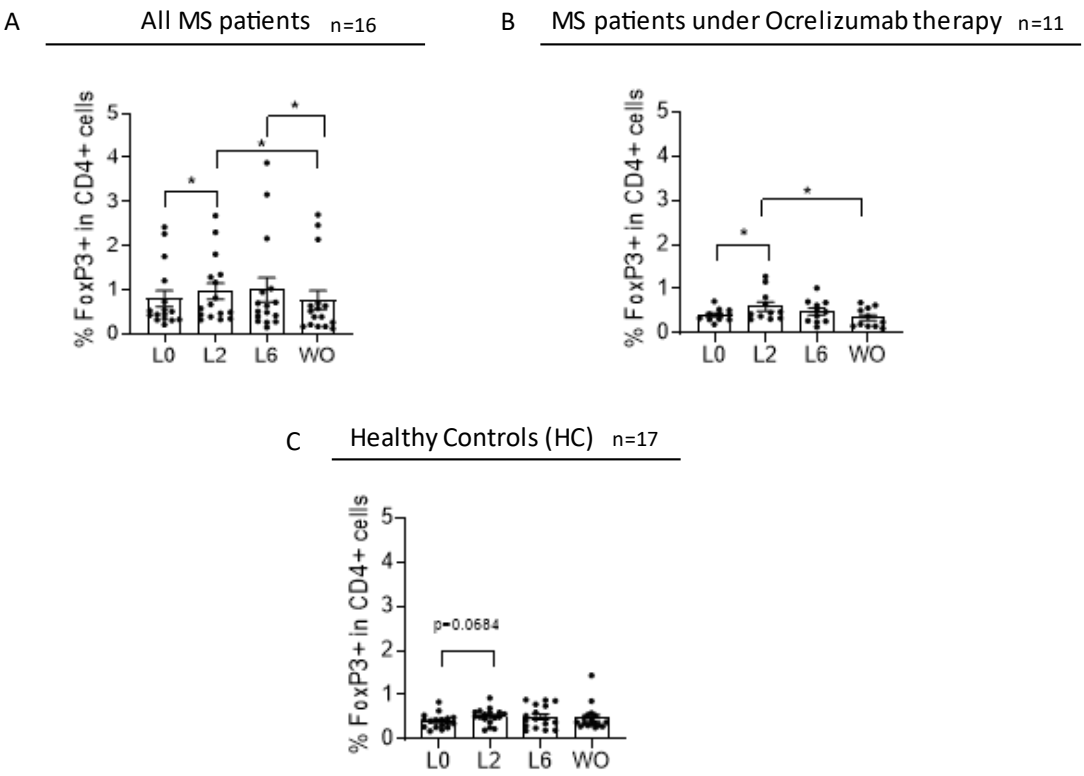

Figure 8: Vivomixx® intake significantly increased Treg frequencies in pwMS but not in HC.

Flow cytometry analysis of FoxP3$^+$ Treg cells in pwMS and HC at baseline (L0) and after two (L2) and six (L6) weeks of Vivomixx® intake as well as four to six weeks after the last intake (washout timepoint, WO).

(A) Frequencies of FoxP3$^+$ Treg cells in all pwMS (n=16 per timepoint). (B) FoxP3$^+$ Treg cell frequencies in pwMS treated with Ocrelizumab (n=11 per timepoint). (C) FoxP3$^+$ Treg cell frequencies in HC (n=17 per timepoint).

**p<0.05, **p<0.01, ***p<0.001 by Wilcoxon matched-pairs signed rank test (non-parametric) or paired t-test (parametric).*

**p(A, L0-L2)=0.0430, *p(A, L2-WO)=0.0323, *p(A, L6-WO)=0.0148*

**p(B, L0-L2)=0.0410, *p(B, L2-WO)=0.0244*

6.4.6 Effects of Vivomixx® intake on Th17 cells

Flow cytometry analysis of Th17 cells showed increased frequencies. This effect was statistically significant when comparing timepoints before and while taking Vivomixx® with the washout timepoint in pwMS and pwMS under Ocrelizumab (figures 9A and B).

In HC, frequencies of Th17 cells already increased statistically significant after six weeks of Vivomixx® intake and further increased during the washout phase (figure 9C).

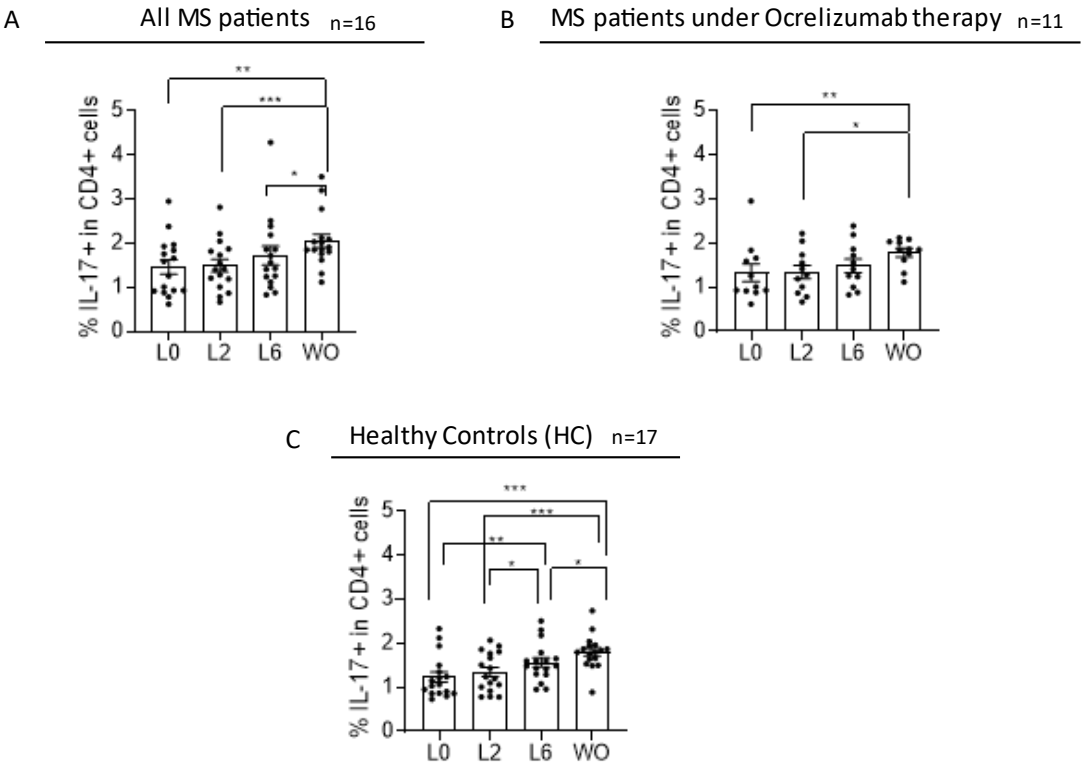

Figure 9: Vivomixx® intake increased Th17 cell frequencies in pwMS and HC during the washout phase. Flow cytometry analysis of IL-17 producing Th17 cells in pwMS and HC before (L0), two (L2) or six (L6) weeks after Vivomixx® intake and at the washout timepoint four to six weeks after finishing Vivomixx® intake.

(A) Frequencies of Th17 cells in all pwMS (n=16 per timepoint). (B) Th17 cell frequencies in pwMS treated with Ocrelizumab (n=11 per timepoint). (C) Frequencies of Th17 cells in HC (n=17 per timepoint).

**p<0.05, **p<0.01, ***p<0.001 by Wilcoxon matched-pairs signed rank test (non-parametric) or paired t-test (parametric).*

***p(A, L0-WO)=0.0092, ***p(A, L2-WO)=0.0004, *p(A, L6-WO)=0.0386*

***p(B, L0-WO)=0.0092, *p(B, L2-WO)=0.00121*

***p(C, L0-L6)=0.0076, *p(C, L2-L6)=0.0230, ***p(C, L0-WO)=0.0003, ***p(C, L2-WO)=0.0003, *p (C, L6-WO)=0.0497*

6.4.7 Decreased Th1 cells in pwMS but not in HC under Vivomixx®

Flow cytometry analysis of Th1 cells revealed a statistically significant decrease of Th1 cells in pwMS comparing timepoint L2 versus timepoint L6 and the washout timepoint. Moreover, there was a numeric decrease of Th1 cells after six weeks of Vivomixx® intake and when comparing baseline and the washout period (figure 10A).

Investigating only B-cell-depleted pwMS revealed a significant decrease of Th1 cells comparing the frequencies at baseline and at the washout phase (figure 10B).

There was no significant effect on Th1 cell frequencies in HC at any timepoint of the study (figure 10C).

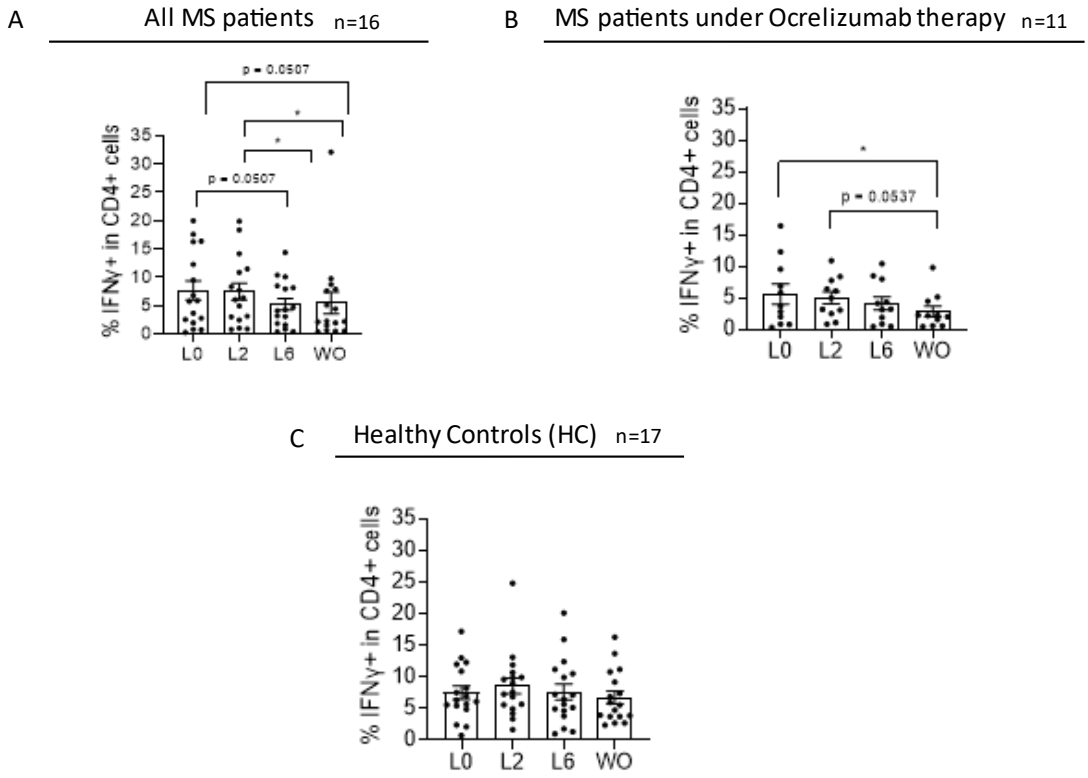

Figure 10: Vivomixx® intake decreased Th1 cells in pwMS but not in HC.

Flow cytometry analysis of IFNy producing Th1 cells in pwMS and HC before (L0), two (L2) or six (L6) weeks after Vivomixx® intake and at the washout timepoint four to six weeks after finishing Vivomixx® intake. (A) Frequencies of Th1 cells in all pwMS (n=16 per timepoint). (B) Th1 cell frequencies in pwMS treated with Ocrelizumab (n=11 per timepoint). (C) Frequencies of Th1 cells in HC (n=17).

**p<0.05, **p<0.01, ***p<0.001 by Wilcoxon matched-pairs signed rank test (non-parametric) or paired t-test (parametric).*

**p(A, L2-L6)=0.0264, *p(A,L2-WO)=0.0443*

**p(B, L0-WO)=0.0137*

6.4.8 No effect on Th2 cells under Vivomixx®

Further analysis of Th2 cells did not show any significant effects of Vivomixx® intake, neither in pwMS (figure 11A, B) nor in HC (figure 11C).

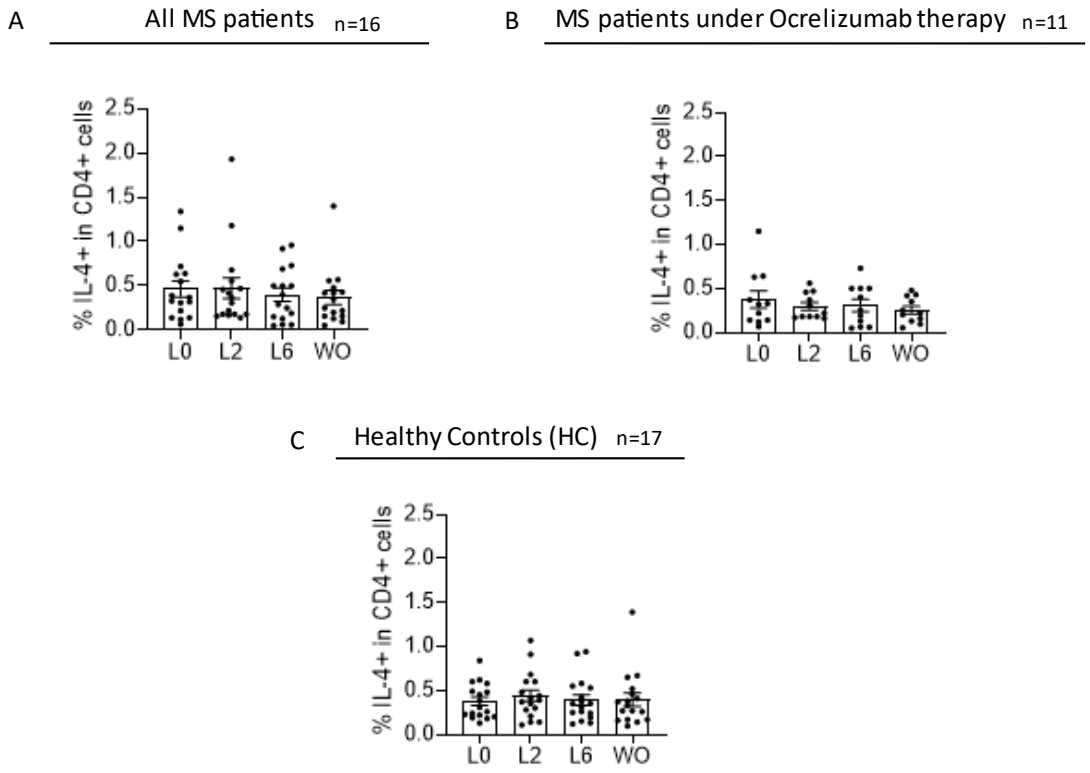

Figure 11: Vivomixx® intake did not significantly affect Th2 cell frequencies in pwMS.
*Flow cytometry analysis of IL-4 producing Th2 cells in pwMS and HC before (L0), two (L2) or six (L6) weeks after Vivomixx® intake and at the washout timepoint four to six weeks after finishing Vivomixx® intake. (A) Frequencies of Th2 cells in all pwMS (n=16 per timepoint). (B) Th2 cell frequencies in pwMS treated with Ocrelizumab (n=11 per timepoint). (C) Th2 cell frequencies in HC (n=17 per timepoint) *p<0.05, **p<0.01, ***p<0.001 by Wilcoxon matched-pairs signed rank test (non-parametric) or paired t-test (parametric).*

.

6.4.9 Effects on pwMS with high or low Th17 baseline levels under Vivomixx®

Interestingly, we observed a clustering of Th17 cell frequencies in pwMS treated with Ocrelizumab. Therefore, we divided the pwMS in "high" (> 1,01 % Th17 cells at baseline) and "low" (< 1.01 % Th17 cells at baseline) and investigated the effect of Vivomixx® intake. PwMS with high Th17 frequencies at baseline showed a significant decrease of Th17 cells from baseline (L0) to timepoint L2 (figure 12A) and a trend towards decreased Th17 cells after six weeks of Vivomixx® intake (figure 12B). In contrast, pwMS with low Th17 numbers at baseline displayed a trend towards increased Th17 cells between baseline (L0) and timepoint L2 (figure 12C) and a significant increase of Th17 frequencies after six weeks of Vivomixx® intake (figure 12D). Yet, more samples are needed to validate these findings.

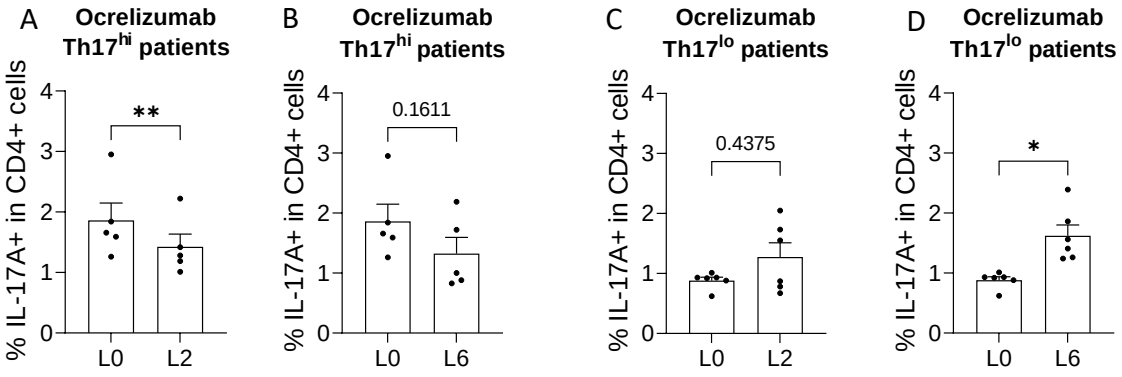

Figure 12: Effects of Vivomixx® intake in pwMS with high or low Th17 baseline levels.
Flow cytometry analysis of IL-17A producing Th17 cells in pwMS under Ocrelizumab therapy with high (>1.01%) Th17 baseline levels. (C-D) Flow cytometry analysis of IL-17A producing Th17 cells in pwMS under Ocrelizumab therapy with low (<1.01%) Th17 baseline levels.
**p<0.05, **p<0.01, ***p<0.001 by Wilcoxon matched-pairs signed rank test (non-parametric) or paired t-test (parametric).*
***p(A)=0.0053, *p(D)=0.0313*

6.4.10 Effects on T cell subsets in pwMS treated with another therapy than Ocrelizumab under Vivomixx®

The data presented in 6.4.1 to 6.4.9 focused on all pwMS and pwMS treated with Ocrelizumab. However, a small population of pwMS also received other immune modulating therapies (Fingolimod: n=2, Natalizumab: n=1) or were therapy-naïve (n=2) during probiotic supplementation. To investigate potential effects of the patients´ immune medication, the flow cytometry data of these study participants were analysed separately (figure 13). Yet, there was no significant effect of Vivomixx® intake on FoxP3^{+} Treg cells (figure 13A) and Th2 cells (figure 13C), although there was a trend towards increased FoxP3^{+} Treg cells in patients under Fingolimod therapy ingesting Vivomixx® for six weeks (figure 15A, blue lines). There was a significant increase in Th17 cells between timepoint L2 and the washout timepoint (figure 13B). Concerning Th1 cells, there was a significant decrease between timepoint L2 and L6 (figure 13D).

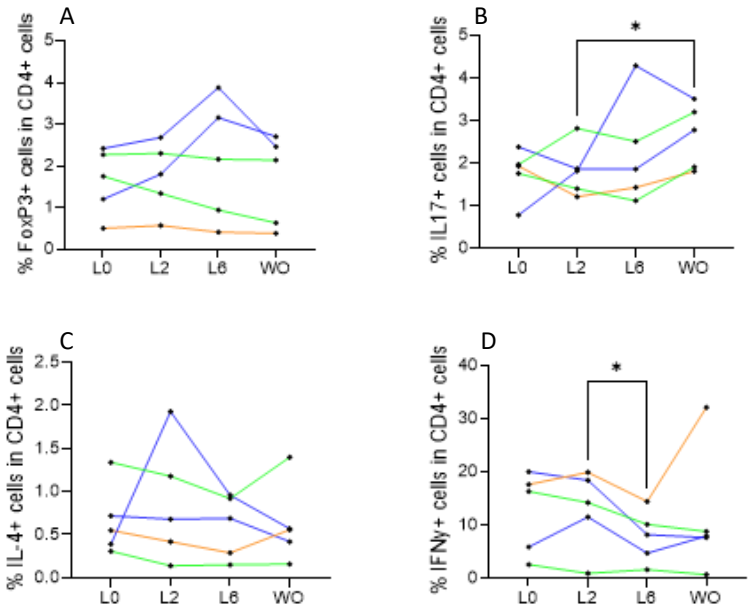

Figure 13: The effect of Vivomixx® intake on T cell subsets in pwMS treated with another therapy than Ocrelizumab.

Flow cytometry analysis of (A) Treg frequencies, (B) Th17 cells, (C) Th2 cells and (D) Th1 cells in pwMS under another therapy than Ocrelizumab before (L0), two (L2) and six (L6) weeks after probiotic Vivomixx® intake and at the washout timepoint.

Blue: Fingolimod therapy, orange: Natalizumab therapy, green: therapy naïve patients.

**p<0.05, **p<0.01, ***p<0.001 by paired t-test. *p(B)=0.0253, *p(D)=0.0442*

7 Discussion

This study investigated the potential immunomodulatory effect of a probiotic *lactobacilli* intake in pwMS and HC. Flow cytometry analysis after two and six weeks of Vivomixx® intake suggests a potential shift in the immune cell compartment towards a regulatory phenotype. These data indicate a potential benefit of probiotic intake in pwMS.

7.1 Routine blood parameter analysis

Within this study, routine blood parameter analysis revealed only few significant changes after two weeks of Vivomixx® intake compared to baseline, for instance a decrease in sodium and γ-GT levels in pwMS or a decrease in haematocrit and urea in HC. However, these changes were not the same in pwMS and HC and rather seem to be fluctuations and not due to Vivomixx® intake.

Interestingly, a significant decrease in cholesterol from baseline to timepoint L2 was found in pwMS and HC. It may be discussed if Vivomixx® intake could have any direct impact on blood cholesterol levels. *Lactobacilli* have previously been credited with decreasing blood cholesterol levels (59). However, we can only cautiously confirm such observations in our cohorts, because pwMS were older than HC and cholesterol levels rise with age. Moreover, our participants were not empty stomached when blood was drawn, limiting the validity of the results.

Comparing pwMS and HC at baseline, pwMS showed lower numbers of lymphocytes and higher numbers of monocytes and neutrophiles, which may refer to the effect of therapy of MS.

7.2 Food questionnaire

Experimental studies demonstrated a direct effect of high-salt intake on the gut microbiome (2). This study was independent from the participants´ diet. However, probands filled in a food questionnaire once during the whole study flow. The analysis showed that pwMS had an average higher energy intake than HC. The trend of a higher energy intake by pwMS occurred in many of the measured nutrients. Differences in the diet between pwMS and HC may be related to the age difference between the groups and a possible difference in age-dependent lifestyle. Further studies focusing on the diet, especially on the different nutrients and their impact on the gut microbiome and the immune system in pwMS are needed.

7.3 Immunophenotyping

7.3.1 Vivomixx® intake had no major effect on B cells, NK cells, monocytes, $CD4^+$, $CD8^+$ and $CD4^+CD8^+$ T cells

Vivomixx® intake did not show any significant effect on B cells and NK cells. There was no major effect of Vivomixx® on monocytes, $CD4^+$, $CD8^+$ and $CD4^+CD8^+$ T cells given the fact that the significant results concerning these cell types could not be verified in all their subpopulations or for all participating groups. Yet, total $CD8^+$ T cell numbers significantly decreased from baseline to timepoint L6 in pwMS, while $CD8^+$ effector cells increased in HC from baseline and timepoint L2 to the washout timepoint. MS is classically regarded as a $CD4^+$ T cells mediated disease. At present, several studies also suggest a crucial role of $CD8^+$ T cells (60). For instance, post mortem analysis of brains of pwMS showed higher numbers of $CD8^+$ T cell infiltration than $CD4^+$ T cell infiltration (61). Additionally, another study showed higher numbers of $CD8^+$ T cells than $CD4^+$ T cells in the cerebrospinal fluid in patients with a first episode of MS, especially in patients with RRMS (62). Moreover, some $CD8^+$ T cells co-label for IL-17 in MS lesions (63). Additionally, a study on probiotic intake of *L. johnsonii* in healthy human beings showed a significant decrease of $CD8^+$ T cells as well as a significant increase of effector and memory $CD8^+$ T cells (49). However, to verify any effect of Vivomixx® on $CD8^+$ T cells, further studies with larger groups are required.

7.3.2 Effects of Vivomixx® on $FoxP3^+$ Treg-, Th17-, Th1- and Th2 cells

We observed a significant increase of $FoxP3^+$ Treg cells after two weeks of Vivomixx® intake in pwMS, which decreased to baseline levels four to six weeks after the last probiotic intake. The same results were found for pwMS under Ocrelizumab therapy. HC showed an increase of $FoxP3^+$ Treg cells from baseline to timepoint L2. These findings indicate a potential anti-inflammatory effect of Vivomixx®. In line with our observations, a study on EAE diseased mice showed an increase in regulatory T cells under probiotic treatment with “Lactibiane iki” (50). Probiotic supplementation with *L. johnsonii* in healthy individuals led to an increased activation of memory regulatory T cells in the washout period (49). Concerning the anti-inflammatory Th2 cells no effect of Vivomixx® has been found in our study.

Pro-inflammatory Th17 cells showed an increase under Vivomixx® intake, which led to statistically significant results when comparing the first three timepoints with the washout timepoint

for pwMS, pwMS under Ocrelizumab therapy and HC. This observation is in contrast to the observations in experimental models, where levels of Th17 cells decreased in HSD fed mice when concurrently receiving *L. murinus* (2). Further studies with larger numbers of participants and more homogenous groups are needed to investigate the effect of probiotic supplementation on Th17 cells. Interestingly, pwMS under Ocrelizumab therapy showed a clustering of cells in the cell types Th17, Th1, Th2 and $FoxP3^+$ Treg cells. Especially pwMS with high or low Th17 numbers at baseline showed significant changes to timepoints L2 or L6. Interestingly, high and low numbers at baseline changed oppositely, which is not explainable so far. When comparing clinical parameters of pwMS showing high or low numbers of any of the four immune cell-types, no clear association was found so far. Higher case numbers will be helpful to strengthen the hypothesis of distinct impacts of a probiotic Vivomixx® intake on different therapies of MS.

Upon analysis of pro-inflammatory Th1 cells, a significant decrease from timepoint L2 to L6 and L2 to the washout timepoint was seen in pwMS. PwMS under Ocrelizumab therapy also showed the same trend. Yet, HC did not show any significant changes in Th1 cells. In summary, the decrease of Th1 cells in pwMS underlines a potential anti-inflammatory effect of Vivomixx®.

7.3.3 Investigations of a potential therapy-dependent effect of Vivomixx®

Eleven out of sixteen pwMS in our study were treated with Ocrelizumab. However, we also investigated the effect of Vivomixx® on pwMS treated with another therapy than Ocrelizumab. There were no indications for any numerical difference in the Vivomixx® effect between pwMS on Ocrelizumab therapy and pwMS treated with another immunotherapy than Ocrelizumab. Therefore, the effects of probiotic Vivomixx® intake may not to be only due to B-cell-depletion. However, our findings on pwMS treated with another therapy than Ocrelizumab should not yet be generalised because of the small number of participants.

7.4 Study Limitations

First, it should be considered that this was a pilot study with broad inclusion criteria. Therefore, the study population was quite inhomogeneous, especially concerning age, gender and BMI. The significant difference in age raises a discussion about different lifestyles possibly affecting nutritional intake, compliance and dealing with infections. Due to the given time for the project, it was not possible to investigate larger groups of participants which would render the results

more reliable. In the future, larger study populations and more differentiated recruitment may be considered in follow-up studies.

Furthermore, infections and vaccinations throughout the study flow are one likely essential influence. One exclusion criterium was a timeline less than four to six weeks between vaccinations and appointments. However, we observed high rates of influenza vaccination, the upcoming vaccination against Covid-19 and vaccination counselling before receiving Ocrelizumab in our pwMS cohort. Moreover, acute infections – while an exclusion criterium – need attention in the study. Here, the Covid-19 pandemic may constitute a possible confounder.

8 Summary and Prospects

In summary, some results suggest a potential benefit of the probiotic Vivomixx® on FoxP3$^+$ Treg cells as well as on Th1 and Th17 cell frequencies in pwMS. It remains interesting to investigate, how strong the effects of a *Lactobacillus* supplementation on pwMS actually are. This project provides a basis for further investigations on the impact of probiotics, especially those containing *Lactobacillus spp.*, on patients with autoimmune diseases like MS, with the aim of a better understanding and treatment of the disease. Further studies are necessary to investigate the effects of probiotic Vivomixx® intake on the immune system, the gut microbiome and the clinical outcome of pwMS in more detail. Therefore, larger and more homogenous cohorts, longer study periods and further analysis of blood and stool samples are required.
Serum-, stool- and RNA samples collected within this pilot study are planned to be investigated on pwMS under Ocrelizumab therapy versus HC at baseline and timepoint L2 to amend metabolomic aspects and the composition of the gut microbiome into the results. In addition, given the increased levels of FoxP3$^+$ Treg cells under Vivomixx® intake, functionality of FoxP3$^+$ Treg cells may be analysed as well. Here, suppression assays which analyse e.g. the cytokine IL-10 from serum- and RNA samples may be of interest. Moreover, microbiota metabolites may be investigated. Here, tryptophan may be of special interest. It is metabolized into immune-modulating factors like indoles by microbes as *lactobacilli* (33). If differences in the metabolites are detected, their direct influence on T cells may be tested *in vitro* in cell culture.

9 Supplement

9.1 Routine blood parameter analysis

Table 27: Routine blood parameters of pwMS at baseline, timepoint L2, L6 and WO. Parameters are shown as [mean ± SD].
At baseline there are missing some values of the first two pwMS, because of initial ambiguities what should be analysed (deviating "n" are listed in the table).

Parameters	**MS (L0)**	**MS (L2)**	**MS (L6)**	**MS (WO)**
Individuals, n	16	16	16	16
CRP [mg/l]	1.4±1.7	1.0±1.2	1.1±1.2	1.1±1.0
Leukocytes/BB [x10^3/µl]	6.8±3.2	6.3±2.1	6.6±2.5	6.1±2.1
Lymphocytes [%]	21.7±8.9 (n=15)	21.3±8.9	20.0±8.4	21.5±9.6
Neutrophils [%]	65.1±8.7 (n=15)	65.9±8.4	66.7±8.7	65.1±9.5
Monocytes [%]	10.8±2.5 (n=15)	10.0±3.1	10.4±4.2	10.5±2.8
Basophils [%]	0.7±0.7 (n=15)	0.9±0.6	0.8±0.7	1.0±0.5
Eosinophils [%]	2.0±1.3 (n=15)	2.3±1.5	2.2±1.6	2.4±1.7
Erythrocytes/BB [/pl]	4.9±0.5	4.8±0.5	4.9±0.6	4.9±0.5
Haemoglobin [g/dl]	14.8±1.5	14.6±1.3	15.0±1.4	14.9±1.4
Haematocrit [%]	42.5±3.7	42.3±3.5	43.5±3.5	43.3±3.4
medium cell volume [fl]	88.4±5.9	88.6±5.4	87.6±6.0	88.4±6.5
medium cell-HB [pg]	30.6±2.1	30.7±2.2	30.5±2.6	30.7±2.5
medium. HB-conc. [g Hb/dl Ery]	34.7±1.1	34.7±0.9	34.6±1.0	34.5±1.0
Thrombocytes [x10^3/µl]	260.6±52.9	258.5±48.9	258.6±51.4	254.1±52.3
Sodium [mmol/l]	140.8±1.7	139.7±1.7	138.9±1.8	139.3±2.1
Potassium [mmol/l]	4.3±0.3	4.3±0.2	4.2±0.3	4.2±0.3
Calcium [mmol/l]	2.4±0.1 (n=14)	2.4±0.1	2.4±0.1	2.4±0.1
GOT [U/l]	23.6±5.8	22.6±5.8	23.4±7.1	25.8±7.6
GPT [U/l]	30.9±33.2	25.1±13.6	23.6±17.3	25.8±15.3
γ-GT [U/l]	55.4±50.2 (n=14)	43.9±32.5	44.4±35.5	42.9±30.1
Creatinine [mg/dl]	0.9±0.2	0.9±0.2	0.9±0.1	0.9±0.1
Urea [mg/dl]	14.3±5.9	14.5±6.3	13.9±4.6	14.3±3.9
GFR [ml/min]	89.2±22.7	87.4±20.6	88.1±18.4	86.1±16.6
Cholesterol [mg/dl]	213.9±44.0 (n=14)	193.6±39.2	199.3±33.9	205.8±42.0
Triglycerides [mg/dl]	145.0±84.8 (n=14)	132.3±97.3	124.4±78.9	124.8±91.1
HbA1c [%]	5.6±0.3 (n=14)	5.6±0.4	5.4±0.3	5.4±0.3

List of abbreviations: CRP=C-reactive protein, HB=haemoglobin, conc.=concentration, GOT=Glutamate-Oxalacetate-Transaminase, GPT=Glutamate-Pyruvate-Transaminase, GFR=glomerular filtration rate.

Table 28: Routine blood parameters of HC at baseline, timepoint L2, L6 and WO. Parameters are shown as [mean ± SD].

Parameters	**HC (L0)**	**HC (L2)**	**HC (L6)**	**HC (WO)**
Individuals, n	17	17	17	17
CRP [mg/l]	1.4±2.0	1.0±1.1	1.4±2.4	0.7±0.8
Leukocytes/BB [x10^3/µl]	5.8±1.2	5.8±0.9	6.1±1.5	5.7±1.4
Lymphocytes [%]	33.2±7.1	32.7±6.1	31.1±6.0	34.1±8.2
Neutrophiles [%]	55.8±7.8	56.2±7.2	57.9±7.5	54.7±8.9
Monocytes [%]	7.9±2.2	8.0±2.3	8.1±1.5	7.7±1.8
Basophiles [%]	0.9±0.2	0.9±0.3	0.9±0.3	0.8±0.4
Eosinophiles [%]	2.6±2.0	2.6±1.7	2.5±2.2	2.9±2.7
Erythrocytes/BB [/pl]	4.8±0.5	4.7±0.4	4.8±0.5	4.8±0.5
Haemoglobin [g/dl]	14.4±1.3	14.2±1.3	14.2±1.2	14.5±1.3
Haematocrite [%]	41.3±3.7	40.5±3.5	40.7±3.0	41.5±3.5
medium cell volume [fl]	86.7±3.2	86.4±3.8	85.9±3.4	85.9±3.3
medium cell-HB [pg]	30.3±1.2	30.2±1.3	30.0±1.4	30.0±1.4
medium HB-conc. [g Hb/dl Ery]	34.9±0.7	35.0±0.8	35.0±0.9	35.0±0.8
Thrombocytes [x10^3/µl]	245.7±48.4	246.1±55.3	250.6±45.6	245.2±55.8
Sodium [mmol/l]	139.4±2.5	139.0±1.8	138.8±1.3	137.9±1.7
Potassium [mmol/l]	4.2±0.3	4.2±0.3	4.1±0.3	4.1±0.2
Calcium [mmol/l]	2.4±0.1	2.4±0.1	2.4±0.1	2.4±0.1
GOT [U/l]	23.9±11.9	22.8±7.0	20.4±3.7	21.1±6.1
GPT [U/l]	17.7±6.2	20.9±11.1	17.6±6.9	16.8±7.3
γ-GT [U/l]	16.1±5.2	16.3±7.2	15.6±6.4	15.4±5.3
Creatinine [mg/dl]	0.8±0.2	0.8±0.2	0.8±0.2	0.8±0.2
Urea [mg/dl]	11.6±3.3	9.5±2.2	11.0±2.9	10.2±1.9
GFR [ml/min]	98.4±20.3	97.9±15.6	96.4±16.9	99.5±16.5
Cholesterol [mg/dl]	178.1±39.7	170.5±35.5	169.6±37.5	179.8±38.5
Triglycerides [mg/dl]	95.1±40.6	99.7±38.1	106.6±55.8	99.2±44.3
HbA1c [%]	5.2±0.2	5.2±0.3	5.1±0.4	5.1±0.3

List of abbreviations: CRP=C-reactive protein, HB=haemoglobin, conc.=concentration, GOT=Glutamate-Oxalacetate-Transaminase, GPT=Glutamate-Pyruvate-Transaminase, GFR=glomerular filtration rate.

9.2 Statistical analysis of blood immunophenotyping in pwMS and HC

9.2.1 $CD19^+$ B cells and $CD56^{bright}CD16^{dim}$-, $CD56^{bright}CD16^-$- and $CD56^{dim}CD16^{bright}$ NK cells

Table 29: p-values of $CD19^+$ B cells and $CD56^{bright}CD16^{dim}$ ($CD16^{dim}$)-, $CD56^{bright}CD16^-$ ($CD16^-$)- and $CD56^{dim}CD16^{bright}$ ($CD16^+$) NK cells when comparing baseline with timepoints L2 and L6 and timepoints L2 and L6 for pwMS, pwMS under Ocrelizumab therapy and HC.
**p<0.05, **p<0.01, ***p<0.001 by Wilcoxon matched-pairs signed rank test (non-parametric) or paired t-test (parametric).*
Abbreviations: Ocreli=Ocrelizumab, np=non-parametric statistical analysis, p=parametric statistical analysis.

cell population	L0 vs. L2	L0 vs. L6	L2 vs. L6
CD19 + B-cells_MS	p = 0.8209 -np	p = 0.1591 -np	p = 0.2522 -np
CD19 + B-cells_MS_Ocreli	p = > 0.9999 -np	p = 0.5771 -np	p = > 0.9999 -np
CD19+ B-cells_HC	p = 0.9364 -p	p = 0.7125 -p	p = 0.6635 -p
NK cells CD16-_MS	p = 0.9399 -np	p = 0.6322 -np	p = 0.8503 -np
NK cells CD16-_MS_Ocreli	p = 0.7105 -p	p = 0.8779 -p	p = 0.6164 -p
NK cells CD16-_HC	p = 0.8132 -p	p = 0.3335 -p	p = 0.2667 -p
NK cells CD16dim_MS	p = 0.3894 -np	p = 0.1754 -np	p = 0.8503 -np
NK cells CD16dim_MS_Ocreli	p = 0.3750 -np	p = 0.2402 -np	p = 0.9658 -np
NK cells CD16dim_HC	p = 0.9799 -np	p = 0.0720 -np	p = 0.1454 -np
NK cells CD16+_MS	p = 0.7206 -p	p = 0.4733 -p	p = 0.2450 -p
NK cells CD16+_MS_Ocreli	p = 0.9084 -p	p = 0.3403 -p	p = 0.1680 -p
NK cells CD16+_HC	p = 0.7819 -np	p = >0.9999 -np	p = 8808 -np

Table 290: p-values of $CD19^+$ B cells and $CD56^{bright}CD16^{dim}$ ($CD16^{dim}$)-, $CD56^{bright}CD16^-$ ($CD16^-$)- and $CD56^{dim}CD16^{bright}$ ($CD16^+$) NK cells when comparing baseline, timepoints L2 and L6 with WO for pwMS, pwMS under Ocrelizumab therapy and HC.

**p<0.05, **p<0.01, ***p<0.001 by Wilcoxon matched-pairs signed rank test (non-parametric) or paired t-test (parametric).*

Abbreviations: Ocreli=Ocrelizumab, np=non-parametric statistical analysis, p=parametric statistical analysis.

cell population	L0 vs. WO	L2 vs. WO	L6 vs. WO
CD19 + B-cells_MS	p = 0.3484 -np	p = 0.5966 -np	p = 0.5619 -np
CD19 + B-cells_MS Ocreli	p = 0.6377 -np	p = 0.7646 -np	p = 0.8984 -np
CD19+ B-cells_HC	p = 0.4586 -np	p = 0.3529 -np	p = 0.2842 -np
NK cells CD16-_MS	p = 0.5619 -np	p = 0.7092 -p	p = 0.4284 -p
NK cells CD16-_MS Ocreli	p = 0.6323 -p	p = 0.9782 -p	p = 0.3542 -p
NK cells CD16-_HC	p = 0.5198 -p	p = 0.4324 -p	p = 0.5787 -p
NK cells CD16dim_MS	p = 0.9298 -np	p = 0.4332 -np	p = 0.1754 -np
NK cells CD16dim_MS Ocreli	p = 0.8984 -np	p = 0.4131 -np	p = 0.3103 -p
NK cells CD16dim _ HC	p = 0.0654 -np	p = 0.1928 -np	p = 0.5091 -np
NK cells CD16+_MS	p = 0.5583 -p	p = 0.7115 -p	p = 0.2694 -p
NK cells CD16+_MS Ocreli	p = 0.8467 -p	p = 0.9143 -p	p = 0.2545 -p
NK cells CD16+_HC	p = 0.9632 -np	p = 0.7908 -np	p = 0.8270 -np

9.2.2 Total monocytes, classical ($CD14^{++}CD16^{-}$), intermediate ($CD14^{++}CD16^{+}$) and non-classical ($CD14^{+}CD16^{++}$) monocytes

Table 31: p-values of total monocytes, $CD14^{++}CD16^{-}$ classical-, $CD14^{++}CD16^{+}$ intermediate- and $CD14^{+}CD16^{++}$ non-classical monocytes when comparing baseline with timepoints L2 and L6 and timepoints L2 and L6 for pwMS, pwMS under Ocrelizumab therapy and HC. Significant decrease in total monocytes in pwMS from baseline to timepoint L2.

**p<0.05, **p<0.01, ***p<0.001 by Wilcoxon matched-pairs signed rank test (non-parametric) or paired t-test (parametric).*

Abbreviations: Ocreli=Ocrelizumab, CM=Classical Monocytes, IM=Intermediate Monocytes, NCM=Non-Classical Monocytes, np=non-parametric statistical analysis, p=parametric statistical analysis.

cell population	L0 vs. L2	L0 vs. L6	L2 vs. L6
Monocytes_MS	***p = 0.0507 -np***	p = 0.2423 -p	p = 0.3755 -np
Monocytes_MS_Ocreli	p = 0.2783 -np	p = 0.2787 -p	p = 0.5771 -np
Monocytes_HC	p = 0.9345 -p	p = 0.7467 -np	p = 0.8209 -np
CM_MS	p = 0.7000 -p	p = 0.4718 -np	p = 0.6590 -np
CM_MS_Ocreli	p = 0.9606 -p	p = 0.9854 -np	p = 0.4648 -np
CM_HC	p = 0.3755 -np	p = 0.3231 -np	p = 0.2788 -np
IM_MS	p = 0.6058 -np	p = 0.8603 -np	p = 0.5282 -np
IM_MS_Ocreli	p = > 0.9999 -np	p = 0.7646 -np	p = 0.3994 -np
IM_HC	p = 0.3428 -p	p = 0.2633 -np	p = 0.0797 -np
NCM_MS	p = 0.0674 -np	p = 0.3824 -np	p = 0.2801 -np
NCM_MS_Ocreli	p = 0.1230 -np	p = 0.5195 -np	p = 0.0674 -np
NCM_HC	p = 0.1781 -np	p = 0.1901 -np	p = 0.8820 -p

*Table 32: p-values of total monocytes, $CD14^{++}CD16^{-}$ classical-, $CD14^{++}CD16^{+}$ intermediate- and $CD14^{+}CD16^{++}$ non-classical monocytes when comparing baseline, timepoints L2 and L6 with WO for pwMS, pwMS under Ocrelizumab therapy and HC. Significant decrease in total monocytes from baseline and timepoint L6 to WO in pwMS and from baseline to WO in pwMS under Ocrelizumab therapy. *p<0.05, **p<0.01, ***p<0.001 by Wilcoxon matched-pairs signed rank test (non-parametric) or paired t-test (parametric).*

Abbreviations: Ocreli=Ocrelizumab, CM=Classical Monocytes, IM=Intermediate Monocytes, NCM=Non-Classical Monocytes, np=non-parametric statistical analysis, p=parametric statistical analysis.

cell population	L0 vs. WO	L2 vs. WO	L6 vs. WO
Monocytes_MS	***** p = 0.0004 -p**	p = 0.4037 -np	***p = 0.0501 -p***
Monocytes_MS_Ocreli	*** p = 0.0131 -p**	p = 0.5195 -np	p = 0.0996 -p
Monocytes_HC	p = >0.9999 -np	p = 0.7467 -np	p = >0.9999 -np
Classical Monocytes (CM)_MS	p = 0.3954 -p	p = 0.4415 -p	p = 0.3965 -np
CM_MS_Ocreli	p = 0.5771 -np	p = 0.8457 -np	p = 0.2783 -np
CM_HC	p = 0.4307 -np	p = 0.1439 -np	p = 0.9265 -np
Intermediate Monocytes (IM)_MS	p = 0.5619 -np	p = 0.7436 -np	p = 0.5966 -np
IM_MS_Ocreli	p = >0.9999 -np	p = 0.3652 -np	p = 0.5195 -np
IM_HC	p = 0.3289 -np	p = 0.2684 -np	p = 0.6692 -np
Non-Classical Moncytes (NCM)_MS	p = 0.5679 -p	p = 0.3288 -np	p = 0.8209 -np
NCM_MS_Ocreli	p = 0.4316 -np	p = 0.3203 -np	p = 0.9658 -np
NCM_HC	p = 0.0656 -np	p = 0.6736 -p	p = 0.3726 -p

9.2.3 $CD4^+$, $CD8^+$ and $CD4^+CD8^+$ T cells and memory ($CD45RO^-CCR7^+$) and effector ($CD45RO^+CCR7^-$) subsets of these cells

Table 3330: p-values of $CD4^+$ T cells and memory ($CD45RO^-CCR7^+$) and effector ($CD45RO^+CCR7^-$) $CD4^+$ T cells when comparing baseline with timepoints L2 and L6 and timepoints L2 and L6 for pwMS, pwMS under Ocrelizumab therapy and HC.

**p<0.05, **p<0.01, ***p<0.001 by Wilcoxon matched-pairs signed rank test (non-parametric) or paired t-test (parametric).*

Abbreviations: Ocreli=Ocrelizumab, np=non-parametric statistical analysis, p=parametric statistical analysis.

cell population	L0 vs. L2	L0 vs. L6	L2 vs. L6
CD4+ T cells_MS	p = 0.1297 -np	p = 0.7530 -np	p = 0.1754 -np
CD4+ T cells_MS_Ocreli	p = 0.2051 -p	p = 0.7274 -p	p = 0.2043 -p
CD4+ T cells_HC	p = 0.4021 -p	p = 0.2314 -p	p = 0.1445 -p
CD4+ memory T cells_MS	p = 0.0934 -np	p = 0.5854 -p	p = 0.0934 -np
CD4+ memory T cells_MS_Ocreli	p = 0.2648 -p	p = 0.5891 -p	p = 0.1265 -p
CD4+ memory T cells_HC	p = 0.3778 -np	p = 0.1898 -np	p = 0.4873 -np
CD4+ effector T cells_MS	p = 0.2801 -p	p = 0.4844 -p	p = 0.1507 -p
CD4+ effector T cells_MS_Ocreli	p = 0.8707 -p	p = 0.4210 -p	p = 0.2384 -p
CD4+ effector T cells_HC	p = 0.8115 -p	p = 0.2763 -p	p = 0.3388 -p

Table 34: p-values of $CD4^+$ T cells and memory ($CD45RO^-CCR7^+$) and effector ($CD45RO^+CCR7^-$) $CD4^+$ T cells when comparing baseline (L0), timepoints L2 and L6 with WO for pwMS, pwMS under Ocrelizumab therapy and HC. Significant decreases of CD4+ T cells from L6 to WO in pwMS and from baseline to WO in pwMS under Ocrelizumab.

**p<0.05, **p<0.01, ***p<0.001 by Wilcoxon matched-pairs signed rank test (non-parametric) or paired t-test (parametric).*

Abbreviations: Ocreli=Ocrelizumab, np=non-parametric statistical analysis, p=parametric statistical analysis.

cell population	L0 vs. WO	L2 vs. WO	L6 vs. WO
CD4+ T cells_MS	p = 0.0636 -np	p = 0.8143 -np	*** p = 0.0334 -np**
CD4+ T cells_MS_Ocreli	***p = 0.0576 -p***	p = 0.5742 -p	p = 0.0708 -p
CD4+ T cells_HC	p = 0.5818 -p	p = 0.7958 -p	p = 0.3304 -p
CD4+ memory T cells_MS	p = 0.4679 -p	p = 0.5533 -np	p = 0.1677 -p
CD4+ memory T cells_MS_Ocreli	p = 0.7505 -p	p = 0.5921 -p	p = 0.3694 -p
CD4+ memory T cells_HC	p = 0.6194 -np	p = 0.2842 -np	p = 0.3529 -np
CD4+ effector T cells_MS	p = 0.9089 -p	p = 0.2622 -p	p = 0.3818 -p
CD4+ effector T cells_MS_Ocreli	p = 0.6415 -p	p = 0.4965 -p	p = 0.6130 -p
CD4+ effector T cells_HC	p = 0.2974 -p	p = 0.3512 -p	p = 0.9951 -p

Table 35: p-values of $CD8^+$ T cells and memory ($CD45RO^-CCR7^+$) and effector ($CD45RO^+CCR7^-$) $CD8^+$ T cells when comparing baseline with timepoints L2 and L6 and timepoints L2 and L6 for pwMS, pwMS under Ocrelizumab therapy and HC. Significant decreases from baseline to timepoint L6 in pwMS and pwMS under Ocrelizumab therapy.

**p<0.05, **p<0.01, ***p<0.001 by Wilcoxon matched-pairs signed rank test (non-parametric) or paired t-test (parametric).*

Abbreviations: Ocreli=Ocrelizumab, np=non-parametric statistical analysis, p=parametric statistical analysis.

cell population	L0 vs. L2	L0 vs. L6	L2 vs. L6
CD8+ T cells_MS	p = 0.8899 -np	*** p = 0.0251 -p**	p = 0.1828 -np
CD8+ T cells_MS_Ocreli	p = 0.3745 -p	*** p = 0.0420 -p**	p = 0.0981 -p
CD8+ T cells_HC	p = 0.5579 -p	p = 0.9219 -np	p = 0.3257 -p
CD8+ memory T cells_MS	p = 0.2308 -p	p = 0.7039 -p	p = 0.2700 -p
CD8+ memory T cells_MS_Ocreli	p = 0.7662 -p	p = 0.2061 -np	p = 0.3007 -p
CD8+ memory T cells_HC	p = 0.2029 -np	p = 0.6196 -np	p = 0.4874 -np
CD8+ effector T cells_MS	p = 0.3230 -p	p = 0.9590 -p	p = 0.6347 -p
CD8+ effector T cells_MS_Ocreli	p = 0.9706 -p	p = 0.5487 -p	p = 0.4882 -p
CD8+ effector T cells_HC	p = 0.2014 -p	p = 0.1017 -p	p = 0.3695 -p

Table 36: p-values of $CD8^+$ T cells and memory ($CD45RO^-CCR7^+$) and effector ($CD45RO^+CCR7^-$) $CD8^+$ T cells when comparing baseline (L0), timepoints L2 and L6 with WO for pwMS, pwMS under Ocrelizumab therapy and HC. Significant increase of CD8+ effector T cells in HC.

**p<0.05, **p<0.01, ***p<0.001 by Wilcoxon matched-pairs signed rank test (non-parametric) or paired t-test (parametric).*

Abbreviations: Ocreli=Ocrelizumab, np=non-parametric statistical analysis, p=parametric statistical analysis.

cell population	L0 vs. WO	L2 vs. WO	L6 vs. WO
CD8+ T cells_MS	p = 0.2250 -p	p = 0.7340 -np	p = 0.7335 -p
CD8+ T cells_MS_Ocreli	p = 0.7761 -p	p = 0.3060 -p	p = 0.2263 -p
CD8+ T cells_HC	p = 0.9861 -p	p = 0.6038 -p	p = 0.9332 -p
CD8+ memory T cells_MS	p = 0.4511 -p	p = 0.1687 -p	p = 0.8508 -p
CD8+ memory T cells_MS_Ocreli	p = 0.8745 -p	p = 0.7356 -p	p = 0.5771 -np
CD8+ memory T cells_HC	p = 0.2633 -np	p = 0.5477 -np	p = 0.5171 -np
CD8+ effector T cells_MS	p = 0.1344 -p	p = 0.0738 -p	p = 0.2661 -p
CD8+ effector T cells_MS_Ocreli	p = 0.5346 -p	p = 0.5138 -p	p = 0.8628 -p
CD8+ effector T cells_HC	**** p = 0.0072 -p**	*** p = 0.0306 -p**	p = 0.2905 -p

Table 37: p-values of $CD4^+CD8^+$ T cells and memory ($CD45RO^-CCR7^+$) and effector ($CD45RO^+CCR7$) $CD4^+CD8^+$ T cells when comparing baseline with timepoints L2 and L6 and timepoints L2 and L6 for pwMS, pwMS under Ocrelizumab therapy and HC. Significant effects of Vivomixx® intake on CD4+CD8+ memory and effector T cells in pwMS from baseline to timepoint L2.

**p<0.05, **p<0.01, ***p<0.001 by Wilcoxon matched-pairs signed rank test (non-parametric) or paired t-test (parametric).*

Abbreviations: Ocreli=Ocrelizumab, np=non-parametric statistical analysis, p=parametric statistical analysis.

cell population	L0 vs. L2	L0 vs. L6	L2 vs. L6
CD4+CD8+ T cells_MS	p = 0.3683 -np	p = 0.1056 -p	p = 0.6591 -np
CD4+CD8+ T cells_MS_Ocreli	p = 0.7822 -np	p = 0.2139 -np	p = 0.8818 -np
CD4+CD8+ T cells_HC	p = 0.3590 -np	p = 0.9908 -np	p = 0.5553 -np
CD4+CD8+ memory T cells_MS	**** p = 0.0063 -np**	p = 0.8999 -np	p = 0.0634 -np
CD4+CD8+ memory T cells_MS_Ocreli	p = 0.0782 -p	p = 0.8311 -np	p = 0.2061 -np
CD4+CD8+ memory T cells_HC	p = 0.2842 -np	p = 0.1901 -np	p = 0.2247 -np
CD4+CD8+ effector T cells_MS	***p = 0.0507 -np***	p = 0.1928 -np	p = 0.0654 -np
CD4+CD8+ effector T cells_MS_Ocreli	p = 0.3192 -p	p = 0.3652 -np	p = 0.2402 -np
CD4+CD8+ effector T cells_HC	p = 0.5477 -np	p = 0.3842 -np	p = 0.7550 -np

Table 31: p-values of $CD4^+CD8^+$ T cells and memory ($CD45RO^-CCR7^+$) and effector ($CD45RO^+CCR7$) $CD4^+CD8^+$ T cells when comparing baseline (L0), timepoints L2 and L6 with WO for pwMS, pwMS under Ocrelizumab therapy and HC. Significant decrease of $CD4^+CD8^+$ memory (CD45RO-CCR7+) T cells in pwMS.

**p<0.05, **p<0.01, ***p<0.001 by Wilcoxon matched-pairs signed rank test (non-parametric) or paired t-test (parametric).*

Abbreviations: Ocreli=Ocrelizumab, np=non-parametric statistical analysis, p=parametric statistical analysis.

cell population	L0 vs. WO	L2 vs. WO	L6 vs. WO
CD4+CD8+ T cells_MS	p = 0.7508 -np	p = 0.1710 -np	p = 0.4130 -np
CD4+CD8+ T cells_MS_Ocreli	p = 0.4219 -np	p = 0.6855 -np	p = 0.4766 -np
CD4+CD8+ T cells_HC	p = 0.7379 -np	p = 0.3965 -np	p = <0.9999 -np
CD4+CD8+ memory T cells_MS	p = 0.7722 -np	*** p = 0.0250 -np**	p = 0.5966 -np
CD4+CD8+ memory T cells_MS_Ocreli	p = 0.3211 -p	p = 0.8742 -p	p = 0.8311 -np
CD4+CD8+ memory T cells_HC	p = 0.2435 -np	p = 0.3004 -np	p = 0.5791 -np
CD4+CD8+ effector T cells_MS	p = 0.4448 -p	p = 0.0634 -np	p = 0.1203 -np
CD4+CD8+ effector T cells_MS_Ocreli	p = 0.3692 -p	p = 0.7143 -p	p = 0.1748 -np
CD4+CD8+ effector T cells_HC	p = 0.4874 -np	p = 0.6532 -np	p = 0.4038 -np

10 References

1. Pantazou V, Schluep M, Du Pasquier R. Environmental factors in multiple sclerosis. Presse Med 2015; 44(4 Pt 2):e113-20. doi: 10.1016/j.lpm.2015.01.001.
2. Wilck N, Matus MG, Kearney SM, Olesen SW, Forslund K, Bartolomaeus H et al. Salt-responsive gut commensal modulates TH17 axis and disease. Nature 2017; 551(7682):585–9. doi: 10.1038/nature24628.
3. Compston A, Coles A. Multiple sclerosis. Lancet 2008; 372(9648):1502–17. doi: 10.1016/S0140-6736(08)61620-7.
4. Doshi A, Chataway J. Multiple sclerosis, a treatable disease. Clin Med (Lond) 2016; 16(Suppl 6):s53-s59. doi: 10.7861/clinmedicine.16-6-s53.
5. Danikowski KM, Jayaraman S, Prabhakar BS. Regulatory T cells in multiple sclerosis and myasthenia gravis. J Neuroinflammation 2017; 14(1):117. doi: 10.1186/s12974-017-0892-8.
6. Yamout BI, Alroughani R. Multiple Sclerosis. Semin Neurol 2018; 38(2):212–25. doi: 10.1055/s-0038-1649502.
7. Smolders J, Damoiseaux J. Vitamin D as a T-cell modulator in multiple sclerosis. Vitam Horm 2011; 86:401–28. doi: 10.1016/B978-0-12-386960-9.00018-6.
8. Bjornevik K, Cortese M, Healy BC, Kuhle J, Mina MJ, Leng Y et al. Longitudinal analysis reveals high prevalence of Epstein-Barr virus associated with multiple sclerosis. Science 2022; 375(6578):296–301. doi: 10.1126/science.abj8222.
9. Rosso M, Chitnis T. Association Between Cigarette Smoking and Multiple Sclerosis: A Review. JAMA Neurol 2020; 77(2):245–53. doi: 10.1001/jamaneurol.2019.4271.
10. Ramanujam R, Hedström A-K, Manouchehrinia A, Alfredsson L, Olsson T, Bottai M et al. Effect of Smoking Cessation on Multiple Sclerosis Prognosis. JAMA Neurol 2015; 72(10):1117–23. doi: 10.1001/jamaneurol.2015.1788.
11. Hollenbach JA, Oksenberg JR. The immunogenetics of multiple sclerosis: A comprehensive review. J Autoimmun 2015; 64:13–25. doi: 10.1016/j.jaut.2015.06.010.
12. Patsopoulos NA, Barcellos LF, Hintzen RQ, Schaefer C, van Duijn CM, Noble JA et al. Fine-mapping the genetic association of the major histocompatibility complex in multiple sclerosis: HLA and non-HLA effects. PLoS Genet 2013; 9(11):e1003926. doi: 10.1371/journal.pgen.1003926.
13. Martin R, Sospedra M, Eiermann T, Olsson T. Multiple sclerosis: doubling down on MHC. Trends Genet 2021; 37(9):784–97. doi: 10.1016/j.tig.2021.04.012.
14. Hafler DA, Compston A, Sawcer S, Lander ES, Daly MJ, Jager PL de et al. Risk alleles for multiple sclerosis identified by a genomewide study. N Engl J Med 2007; 357(9):851–62. doi: 10.1056/NEJMoa073493.
15. International Multiple Sclerosis Genetics Consortium. Multiple sclerosis genomic map implicates peripheral immune cells and microglia in susceptibility. Science 2019; 365(6460). doi: 10.1126/science.aav7188.
16. Hemmer B, Kerschensteiner M, Korn T. Role of the innate and adaptive immune responses in the course of multiple sclerosis. Lancet Neurol 2015; 14(4):406–19. doi: 10.1016/S1474-4422(14)70305-9.
17. Haase S, Linker RA. Inflammation in multiple sclerosis. Ther Adv Neurol Disord 2021; 14:17562864211007687. doi: 10.1177/17562864211007687.
18. Kunkl M, Frascolla S, Amormino C, Volpe E, Tuosto L. T Helper Cells: The Modulators of Inflammation in Multiple Sclerosis. Cells 2020; 9(2). doi: 10.3390/cells9020482.
19. Viglietta V, Baecher-Allan C, Weiner HL, Hafler DA. Loss of functional suppression by CD4+CD25+ regulatory T cells in patients with multiple sclerosis. J Exp Med 2004; 199(7):971–9. doi: 10.1084/jem.20031579.
20. Thursby E, Juge N. Introduction to the human gut microbiota. Biochem J 2017; 474(11):1823–36. doi: 10.1042/BCJ20160510.
21. David LA, Maurice CF, Carmody RN, Gootenberg DB, Button JE, Wolfe BE et al. Diet rapidly and reproducibly alters the human gut microbiome. Nature 2014:559–63. doi: 10.1038/nature12820.
22. Wu GD, Chen J, Hoffmann C, Bittinger K, Chen Y-Y, Keilbaugh SA et al. Linking long-term dietary patterns with gut microbial enterotypes. Science 2011; 334(6052):105–8. doi: 10.1126/science.1208344.

23. Wang Y, Kasper LH. The role of microbiome in central nervous system disorders. Brain Behav Immun 2014; 38:1–12. doi: 10.1016/j.bbi.2013.12.015.

24. Rhee SH, Pothoulakis C, Mayer EA. Principles and clinical implications of the brain-gut-enteric microbiota axis. Nat Rev Gastroenterol Hepatol 2009; 6(5):306–14. doi: 10.1038/nrgastro.2009.35.

25. Braniste V, Al-Asmakh M, Kowal C, Anuar F, Abbaspour A, Tóth M et al. The gut microbiota influences blood-brain barrier permeability in mice. Sci Transl Med 2014; 6(263):263ra158. doi: 10.1126/scitranslmed.3009759.

26. Bercik P, Denou E, Collins J, Jackson W, Lu J, Jury J et al. The intestinal microbiota affect central levels of brain-derived neurotropic factor and behavior in mice. Gastroenterology 2011; 141(2):599-609, 609.e1-3. doi: 10.1053/j.gastro.2011.04.052.

27. Farshim P, Walton G, Chakrabarti B, Givens I, Saddy D, Kitchen I et al. Maternal Weaning Modulates Emotional Behavior and Regulates the Gut-Brain Axis. Sci Rep 2016; 6:21958. doi: 10.1038/srep21958.

28. Berer K, Mues M, Koutrolos M, Rasbi ZA, Boziki M, Johner C et al. Commensal microbiota and myelin autoantigen cooperate to trigger autoimmune demyelination. Nature 2011:538–41. doi: 10.1038/nature10554.

29. Shahi SK, Freedman SN, Mangalam AK. Gut microbiome in multiple sclerosis: The players involved and the roles they play. Gut Microbes 2017; 8(6):607–15. doi: 10.1080/19490976.2017.1349041.

30. Jangi S, Gandhi R, Cox LM, Li N, Glehn F von, Yan R et al. Alterations of the human gut microbiome in multiple sclerosis. Nat Commun 2016:12015. doi: 10.1038/ncomms12015.

31. Ochoa-Repáraz J, Mielcarz DW, Ditrio LE, Burroughs AR, Foureau DM, Haque-Begum S et al. Role of gut commensal microflora in the development of experimental autoimmune encephalomyelitis. J Immunol 2009:6041–50. doi: 10.4049/jimmunol.0900747.

32. Berer K, Gerdes LA, Cekanaviciute E, Jia X, Xiao L, Xia Z et al. Gut microbiota from multiple sclerosis patients enables spontaneous autoimmune encephalomyelitis in mice. Proc Natl Acad Sci U S A 2017:10719–24. doi: 10.1073/pnas.1711233114.

33. Haase S, Haghikia A, Wilck N, Müller DN, Linker RA. Impacts of microbiome metabolites on immune regulation and autoimmunity. Immunology 2018; 154(2):230–8. doi: 10.1111/imm.12933.

34. Sugimoto S, Naganuma M, Kanai T. Indole compounds may be promising medicines for ulcerative colitis. J Gastroenterol 2016:853–61. doi: 10.1007/s00535-016-1220-2.

35. Zelante T, Iannitti RG, Cunha C, Luca A de, Giovannini G, Pieraccini G et al. Tryptophan catabolites from microbiota engage aryl hydrocarbon receptor and balance mucosal reactivity via interleukin-22. Immunity 2013; 39(2):372–85. doi: 10.1016/j.immuni.2013.08.003.

36. Duscha A, Gisevius B, Hirschberg S, Yissachar N, Stangl GI, Dawin E et al. Propionic Acid Shapes the Multiple Sclerosis Disease Course by an Immunomodulatory Mechanism. Cell 2020; 180(6):1067-1080.e16. doi: 10.1016/j.cell.2020.02.035.

37. Haase S, Mäurer J, Duscha A, Lee D-H, Balogh A, Gold R et al. Propionic Acid Rescues High-Fat Diet Enhanced Immunopathology in Autoimmunity via Effects on Th17 Responses. Front Immunol 2021; 12:701626. doi: 10.3389/fimmu.2021.701626.

38. Manzel A, Muller DN, Hafler DA, Erdman SE, Linker RA, Kleinewietfeld M. Role of "Western diet" in inflammatory autoimmune diseases. Curr Allergy Asthma Rep 2014; 14(1):404. doi: 10.1007/s11882-013-0404-6.

39. Munger KL. Childhood obesity is a risk factor for multiple sclerosis. Mult Scler 2013; 19(13):1800. doi: 10.1177/1352458513507357.

40. Haase S, Haghikia A, Gold R, Linker RA. Dietary fatty acids and susceptibility to multiple sclerosis. Mult Scler 2018; 24(1):12–6. doi: 10.1177/1352458517737372.

41. Haghikia A, Jörg S, Duscha A, Berg J, Manzel A, Waschbisch A et al. Dietary Fatty Acids Directly Impact Central Nervous System Autoimmunity via the Small Intestine. Immunity 2015; 43(4):817–29. doi: 10.1016/j.immuni.2015.09.007.

42. Kleinewietfeld M, Manzel A, Titze J, Kvakan H, Yosef N, Linker RA et al. Sodium chloride drives autoimmune disease by the induction of pathogenic TH17 cells. Nature 2013:518–22. doi: 10.1038/nature11868.

43. Shapiro L, Dinarello CA. Osmotic regulation of cytokine synthesis in vitro. Proc Natl Acad Sci U S A 1995; 92(26):12230–4. doi: 10.1073/pnas.92.26.12230.

44. Farez MF, Fiol MP, Gaitán MI, Quintana FJ, Correale J. Sodium intake is associated with increased disease activity in multiple sclerosis. J Neurol Neurosurg Psychiatry 2015:26–31. doi: 10.1136/jnnp-2014-307928.

45. Fitzgerald KC, Munger KL, Hartung H-P, Freedman MS, Montalbán X, Edan G et al. Sodium intake and multiple sclerosis activity and progression in BENEFIT. Ann Neurol 2017; 82(1):20–9. doi: 10.1002/ana.24965.

46. McDonald J, Graves J, Waldman A, Lotze T, Schreiner T, Belman A et al. A case-control study of dietary salt intake in pediatric-onset multiple sclerosis. Mult Scler Relat Disord 2016; 6:87–92. doi: 10.1016/j.msard.2016.02.011.

47. Huhn K, Linz P, Pemsel F, Michalke B, Seyferth S, Kopp C et al. Skin sodium is increased in male patients with multiple sclerosis and related animal models. Proc Natl Acad Sci U S A 2021; 118(28). doi: 10.1073/pnas.2102549118.

48. Wilkins T, Sequoia J. Probiotics for Gastrointestinal Conditions: A Summary of the Evidence. Am Fam Physician 2017; 96(3):170–8.

49. Marcial GE, Ford AL, Haller MJ, Gezan SA, Harrison NA, Cai D et al. Lactobacillus johnsonii N6.2 Modulates the Host Immune Responses: A Double-Blind, Randomized Trial in Healthy Adults. Front Immunol 2017; 8:655. doi: 10.3389/fimmu.2017.00655.

50. Calvo-Barreiro L, Eixarch H, Ponce-Alonso M, Castillo M, Lebrón-Galán R, Mestre L et al. A Commercial Probiotic Induces Tolerogenic and Reduces Pathogenic Responses in Experimental Autoimmune Encephalomyelitis. Cells 2020; 9(4). doi: 10.3390/cells9040906.

51. Mestre L, Carrillo-Salinas FJ, Feliú A, Mecha M, Alonso G, Espejo C et al. How oral probiotics affect the severity of an experimental model of progressive multiple sclerosis? Bringing commensal bacteria into the neurodegenerative process. Gut Microbes 2020; 12(1):1813532. doi: 10.1080/19490976.2020.1813532.

52. Snydman DR. The safety of probiotics. Clin Infect Dis 2008; 46 Suppl 2:S104-11; discussion S144-51. doi: 10.1086/523331.

53. Alipour B, Homayouni-Rad A, Vaghef-Mehrabany E, Sharif SK, Vaghef-Mehrabany L, Asghari-Jafarabadi M et al. Effects of Lactobacillus casei supplementation on disease activity and inflammatory cytokines in rheumatoid arthritis patients: a randomized double-blind clinical trial. Int J Rheum Dis 2014:519–27. doi: 10.1111/1756-185X.12333.

54. Zhang X, Zhang D, Jia H, Feng Q, Wang D, Di Liang et al. The oral and gut microbiomes are perturbed in rheumatoid arthritis and partly normalized after treatment. Nat Med 2015:895–905. doi: 10.1038/nm.3914.

55. Khalesi S, Sun J, Buys N, Jayasinghe R. Effect of probiotics on blood pressure: a systematic review and meta-analysis of randomized, controlled trials. Hypertension 2014:897–903. doi: 10.1161/HYPERTENSIONAHA.114.03469.

56. Mähler A, Wilck N, Rauch G, Dechend R, Müller DN. Effect of a probiotic on blood pressure in grade 1 hypertension (HYPRO): protocol of a randomized controlled study. Trials 2020; 21(1):1032. doi: 10.1186/s13063-020-04973-0.

57. Tao Y-W, Gu Y-L, Mao X-Q, Zhang L, Pei Y-F. Effects of probiotics on type II diabetes mellitus: a meta-analysis. J Transl Med 2020; 18(1):30. doi: 10.1186/s12967-020-02213-2.

58. RKI - DEGS1: Ernährung - DEGS-Ernährungsfragebogen; 2022 [Stand: 16.06.2022]. Verfügbar unter: https://www.rki.de/DE/Content/Gesundheitsmonitoring/Studien/Methodik/Befragungsmethoden/ernaehrung/DEGS_FFQ_inhalt.html.

59. Costabile A, Buttarazzi I, Kolida S, Quercia S, Baldini J, Swann JR et al. An in vivo assessment of the cholesterol-lowering efficacy of Lactobacillus plantarum ECGC 13110402 in normal to mildly hypercholesterolaemic adults. PLoS One 2017; 12(12):e0187964. doi: 10.1371/journal.pone.0187964.

60. Kaskow BJ, Baecher-Allan C. Effector T Cells in Multiple Sclerosis. Cold Spring Harb Perspect Med 2018; 8(4). doi: 10.1101/cshperspect.a029025.

61. Hauser SL, Bhan AK, Gilles F, Kemp M, Kerr C, Weiner HL. Immunohistochemical analysis of the cellular infiltrate in multiple sclerosis lesions. Ann Neurol 1986; 19(6):578–87. doi: 10.1002/ana.410190610.

62. Jilek S, Schluep M, Rossetti AO, Guignard L, Le Goff G, Pantaleo G et al. CSF enrichment of highly differentiated CD8+ T cells in early multiple sclerosis. Clin Immunol 2007; 123(1):105–13. doi: 10.1016/j.clim.2006.11.004.

63. Tzartos JS, Friese MA, Craner MJ, Palace J, Newcombe J, Esiri MM et al. Interleukin-17 production in central nervous system-infiltrating T cells and glial cells is associated with active disease in multiple sclerosis. Am J Pathol 2008; 172(1):146–55. doi: 10.2353/ajpath.2008.070690.