Aus dem Experimental and Clinical Research Center (ECRC) der
Medizinischen Fakultät Charité – Universitätsmedizin Berlin

DISSERTATION

Effects of fasting and a ketogenic diet on neuropsychiatric outcomes in multiple sclerosis patients – a randomized controlled trial

Auswirkungen von Fasten und ketogener Ernährung auf neuropsychiatrische Symptome bei Multipler Sklerose – eine randomisierte kontrollierte Studie

zur Erlangung des akademischen Grades

Doctor rerum medicinalium (Dr. rer. medic.)

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von

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I. List of figures and tables

II. List of abbreviations

ANOVA – Analysis of variance
ATP – Adenosine triphosphate
BDI-II – Beck Depression Inventory-II
BHB – Beta-hydroxybutyrate
BMI – Body mass index
CI – Confidence interval
CNS – Central nervous system
DGE – Deutsche Gesellschaft für Ernährung
DMT – Disease modifying therapy
EAE – Experimental autoimmune encephalomyelitis
EDSS – Expanded Disability Status Scale
FD – Fasting diet
FSS – Fatigue Severity Scale
GM-CSF – Granulocyte-macrophage colony-stimulating factor
IFN – Interferon
IL – Interleukin
KD – Ketogenic diet
MDA – Malondialdehyde
MRI – Magnetic resonance imaging
MS – Multiple sclerosis
NAMS – Nutritional Approaches in Multiple Sclerosis
PBMC – Peripheral blood mononuclear cell
RCT – Randomized controlled trial
ROS – Reactive oxygen species
RRMS – Relapsing-remitting multiple sclerosis
SD – Standard healthy diet
SDMT – Symbol Digit Modalities Test
StD – Standard deviation
TNF – Tumor necrosis factor
VEGF-A – Vascular endothelial growth factor A
V1 – Baseline
V2 – After nine months intervention

III. Glossary

Fasting = extreme caloric restriction: very low-calorie intake due to voluntary abstinence from solid food for a restricted period; leads to a scarcity of glucose availability and hence to a metabolic switch from mostly glucose utilization to the generation of ketones as a substitutional energy source for, e.g., the brain

Intermittent fasting: a time restricted eating pattern (e.g., restricted food intake for 10 h/day, followed by abstinence of solid foods for 14 h/day)

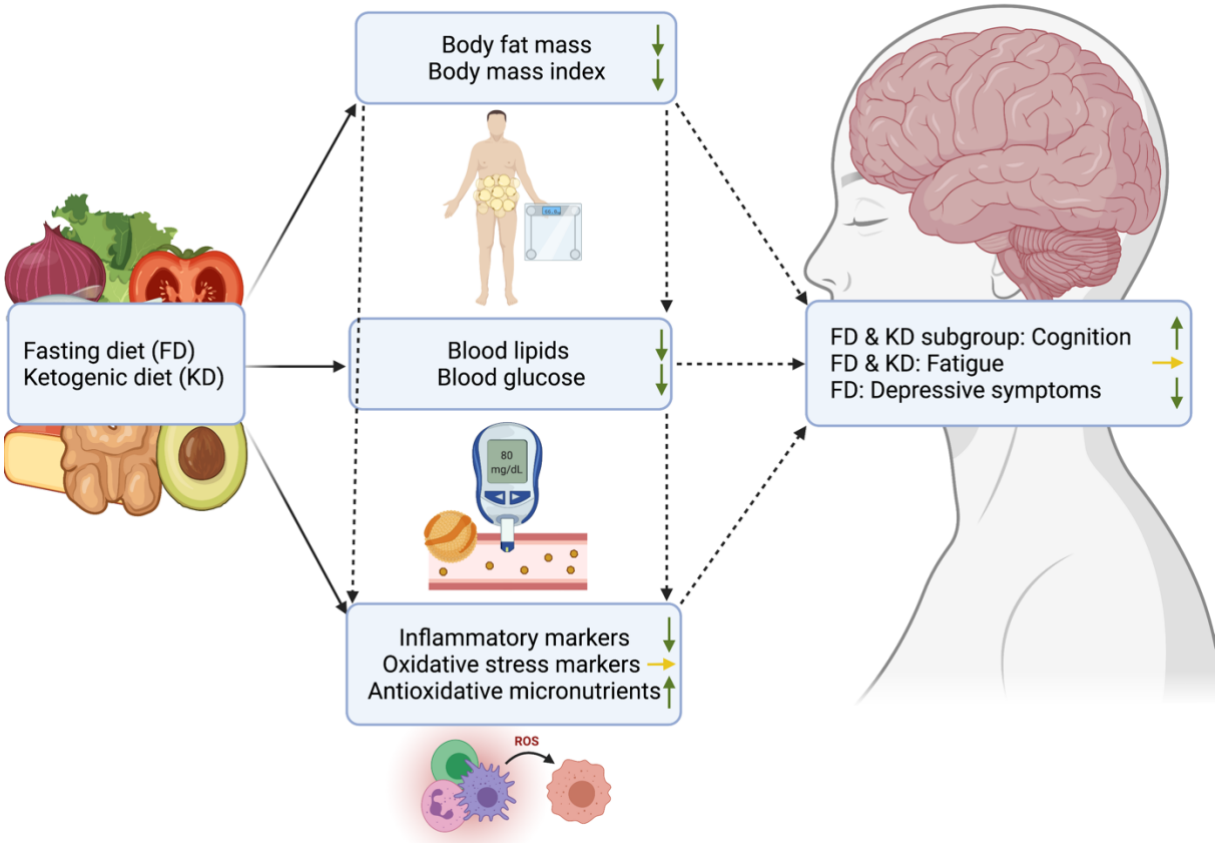
Ketogenic diet = extreme carbohydrate restriction: a diet mimicking a fasting state through a very low-carbohydrate intake; leads to a scarcity of glucose availability and hence to a metabolic switch from mostly glucose utilization to the generation of ketones as a substitutional energy source for, e.g., the brain

Beta-hydroxybutyrate (BHB): main circulating ketone body of the three metabolically related molecules (acetone, acetoacetate, and beta-hydroxybutyrate) that can be used as an alternative energy source by, e.g., the brain, when blood glucose concentrations are low; elevated levels of BHB in the blood are indicative of a state of ketosis

Oxidative stress: an imbalance of reactive oxygen species (ROS) productions and antioxidants causes oxidative stress; oxidative stress is suggested to play a major role in chronic inflammation, neurodegeneration, and aging; ROS physiologically function as signalling molecules and defence against pathogens

Inflammatory cytokines: cytokines are secreted proteins with activator or suppressor functions that regulate immune responses; cytokines may play an important role in the development of MS lesions, and probably in peripheral immune activation in MS; peripheral pro- and anti-inflammatory cytokine concentrations were shown to correlate with MS disease activity

IV. Graphical and written abstract



Introduction: Multiple sclerosis (MS) is the most common non-traumatic, debilitating disease of the central nervous system in young adults, with neuropsychiatric symptoms among the most disabling symptoms. Existing pharmacological treatments are not consistently effective, have side effects, and rarely target neuropsychiatric symptoms. In contrast, new approaches such as specific diets have been shown to improve neuropsychiatric symptoms including fatigue, cognitive impairment, and depressive symptoms in some neurodegenerative diseases. Examples of these dietary approaches are ketone-based diets. Ketone bodies are generated during low-carbohydrate intake or very low-calorie intake also referred to as ketogenic diet (KD) respectively fasting (FD). They deliver an alternative energy substrate for the brain. This work investigates the effects of a KD and FD on neuropsychiatric outcomes compared to a standard healthy diet (SD) and examines the potential mediation of effects via changes in nutritional status, inflammation, and oxidative stress.

Methods: This is an exploratory sub-cohort analysis of the NAMS study (**N**utritional **A**pproaches in **MS,** NCT03508414), a single-center, randomized controlled study. This analysis investigates data of 71 patients over 9 months who were included in the larger

NAMS study (105 patients randomized, study duration 18 months). Patients were randomized to 1 of 3 dietary interventions: a KD with a restricted carbohydrate intake of 20-50 g/day, a FD with 7-day fasts every 6 months and, 14-h daily intermittent fasting in between, and a SD as recommended by the German Nutrition Society.

Results: Body fat mass and body mass index (BMI) improved in the KD and FD group. Blood lipids improved in different ways in all groups. Pro-inflammatory leptin decreased significantly, and blood glucose decreased slightly in the KD and FD group. Plasma micronutrients increased in the KD and FD group, while oxidative stress markers did not change. Minimal depressive symptoms improved by 17% in the FD group (higher baseline values in the FD group must be considered). There was an increase of 2.5 points in the cognition score in the FD group. In the KD group, the cognition score increased by 3.9 points, but only in patients who had a relevant ketosis. Certain plasma micronutrients were positively, blood lipids and blood glucose inversely, associated with cognition, suggesting mediation of effects. The diets did not have a relevant effect on fatigue symptoms. Adherence in the KD group was low compared to the FD group.

Conclusion: A KD and FD were efficient in improving BMI, body composition, inflammatory and metabolic markers, parameters that are commonly altered in MS patients and associated with a poor disease course. The KD was less feasible and improved cognition only in a subgroup of patients. The FD was feasible and improved cognition and minimal depressive symptoms in our cohort. A healthy diet combined with fasting interventions may be a low-risk, multi-target approach that could be recommended to MS patients to improve their cardiometabolic risk profile and mild neuropsychiatric symptoms, and thus complement MS treatment.

V. Zusammenfassung

Einleitung: Multiple Sklerose (MS) ist die häufigste nichttraumatische Erkrankung des zentralen Nervensystems bei jungen Erwachsenen. Neuropsychiatrische Symptome gehören zu den am stärksten beeinträchtigenden Symptomen. Pharmakologische Therapien sind nicht gleichermaßen wirksam, haben Nebenwirkungen und zielen kaum auf neuropsychiatrische Symptome ab. Diätetische Ansätze haben neuropsychiatrische Symptome wie Fatigue, kognitive Beeinträchtigung und depressive Symptome bei einzelnen neurodegenerativen Erkrankungen verbessern

können. Ketonkörper, die während einer kohlenhydratreduzierten ketogenen Diät (KD) und bei drastischer Kalorienreduktion, dem Fasten (FD), gebildet werden, stellen eine alternative Energiequelle für das Gehirn dar. Hier werden die Effekte einer KD und FD im Vergleich zu einer Standarddiät (SD) auf neuropsychiatrische Symptome untersucht, und die mögliche Vermittlung über Ernährungsstatus, Inflammation und oxidativen Stress.

Methoden: Es handelt sich um eine explorative Sub-Kohorten Analyse der NAMS-Studie (**N**utritional **A**pproaches in **MS**, NCT03508414), einer monozentrischen, randomisierten kontrollierten Studie. Die Analyse umfasst 71 Patienten über 9 Monate, die in die größere NAMS-Studie (105 Patienten, Dauer 18 Monate) eingeschlossen wurden. Die Patienten wurden in eine der drei Ernährungsinterventionen randomisiert: eine KD mit einer Kohlenhydratzufuhr von 20-50 g/Tag, eine FD mit 7-tägigem Fasten alle 6 Monate und 14-h intermittierendem Fasten sowie eine SD nach Empfehlungen der Deutschen Gesellschaft für Ernährung.

Ergebnisse: Körperfettanteil und Body-Mass-Index verbesserten sich in der KD- und FD-Gruppe. Blutfette verbesserten sich in allen Gruppen auf unterschiedliche Weise. Leptin und Blutglukose verringerten sich in der KD- und FD-Gruppe. Einige Plasmamikronährstoffe stiegen in der KD- und FD-Gruppe an, während sich Marker für oxidativen Stress nicht veränderten. In der FD-Gruppe verbesserten sich minimal-ausgeprägte depressive Symptome um 17 % (höhere Ausgangswerte in der FD-Gruppe müssen berücksichtigt werden). Der Kognitionswert stieg in der FD-Gruppe um 2,5 Punkte an. In der KD-Gruppe stieg der Kognitionswert um 3,9 Punkte, jedoch nur in Patienten, die eine relevante Ketose aufwiesen. Einige Plasmamikronährstoffe waren positiv, Blutfette und -glukose invers, mit der Kognition assoziiert, was auf eine Mediation der Effekte hindeutet. Die Diäten zeigten keinen relevanten Effekt auf Fatigue Symptome. Die Adhärenz in der KD-Gruppe war im Vergleich zur FD-Gruppe gering.

Schlussfolgerung: KD und FD verbesserten BMI, Körperzusammensetzung, inflammatorische und metabolische Marker - Faktoren, die bei MS oft ungünstig verändert und mit einem progressiveren Krankheitsverlauf assoziiert sind. Die KD war weniger praktikabel und verbesserte die Kognition nur in einer Subgruppe. Die FD war praktikabel und verbesserte Kognition sowie depressive Symptome. Eine gesunde Ernährung mit regelmäßigem Fasten ist ein risikoarmer Multi-Target Ansatz der MS-

Patienten empfohlen werden kann, um ihr kardiometabolisches Risikoprofil sowie milde neuropsychiatrische Symptome zu verbessern und so das Behandlungs-Angebot zu ergänzen.

1 Introduction

1.1 Burden of disease

Multiple sclerosis (MS) is a chronic disease of the central nervous system (CNS), and one major cause of gradually progressive neurological impairment as well as early retirement in young adults (1-3). Prevalence is estimated at 2.8 million worldwide and has risen in every world region since 2013 (4). With a mean age of 32 years, the disease onset usually occurs in the most productive phase of life, imposing a high financial burden and loss of quality of life. Further, there is a strong female preponderance, affecting women in child-bearing age and family planning (5). Most patients initially have reversible symptoms, the relapsing-remitting MS (RRMS), with progression of symptoms developing over time, leading to non-reversible symptoms (secondary-progressive MS). A minority of patients suffers from non-reversible symptoms from the onset of the disease (primary-progressive MS) (6).

Neuropsychiatric symptoms such as fatigue, cognitive impairment and depressive symptoms occur in more than 60% of MS patients (7) and are a relevant reason for early retirement and unemployment (8-10). By now, there are over a dozen different disease modifying therapies (DMT) available, which are associated with improved clinical and radiological outcomes (11). However, all of them have substantial side effects, barely target neuropsychiatric symptoms and in the case of the widely used DMT interferon-beta, associations with depressive symptoms were even found (12). One problem that hampers the development of more targeted therapies is the fact that the initiating antigen and other etiological factors are still unknown or incompletely understood. Hence, many MS patients are interested in complementary therapies such as diets. A review suggests that up to 70% of MS patients have tried one or more complementary treatments to improve their MS including diets and nutritional supplements (13). A survey of 416 MS patients showed that low-fat, low-sugar and gluten-free diets are the most applied dietary approaches (14).

1.2 MS etiology, environment, and diet

Even though the exact MS disease etiology remains unknown, it is widely accepted that a combination of factors contributes to MS pathology: immune dysregulation due to a combination of genetic susceptibility and environmental factors appears central

(15, 16). Genetic susceptibility must occur simultaneously with non-genetic factors to trigger the disease onset. Evidence comes from epidemiological studies showing that MS is not evenly distributed across regions and ethnicities worldwide (15). There is a geographical gradient: disease prevalence is higher in northern and moderate climate zones, and decreases as the equator is approached (15). Further, migration studies have shown that people migrating from a high-risk to a low-risk area before adolescence, assume the lower risk of their destination. In contrast, adults continue to carry the risk of the country they originated from, indicating a role of environmental factors in disease etiology (17).

More evidence for a genetic and environmental component is coming from twin studies. Compared to dizygotic twins, the concordance rate in monozygotic twins is 30% (compared to 5% in dizygotic twins)(18). The elevated concordance rate in monozygotic twins compared to dizygotic twins, on the one hand emphasizes genetic risk factors, but on the other hand, as about 70% of monozygotic twins are discordant, stresses the role of non-genetic environmental risk factors (15, 18).

Environmental influences contributing to disease formation besides latitude, include smoking, viruses (16) and nutritional factors (19). A review by Riccio et al. describes the plausible involvement of dietary and lifestyle factors in the MS etiology. Apart from lower vitamin D availability in latitudes, were MS is more prevalent (20), higher serum levels of vitamin D were shown to be associated with a decreased risk for MS (21). Moreover, higher vitamin D levels are associated with lower disease activity (22). However, to this date, randomized-controlled trials (RCTs) failed to prove a beneficial effect of vitamin D supplementation on the disease course of MS patients (23, 24). The geographical distribution shows the highest prevalence in high-income countries that are most distant from the equator. Riccio and Rossano conclude that typical “features of these countries are sedentary lifestyle, high-calorie diet rich in saturated fats of animal origin (Western diet) and low sunshine exposure” (20). Typical Western diets are indeed characterized by high intakes of animal fats and high intakes of refined carbohydrates that may drive postprandial inflammation and insulin resistance (25, 26). In line with this, a high body mass index (BMI) of > 27 kg/m^2 before the age of 20 has been shown to double the risk for MS development (27). Moreover, a recent Mendelian randomization study including 14.498 cases and 24.091 controls concluded that there are several nutrition- and lifestyle-related modifiable risk factors for MS: “Childhood

and adulthood BMI were positively associated with MS, whereas physical activity and serum 25-hydroxyvitamin D levels were inversely associated with MS. There was evidence of possible associations of type 2 diabetes, waist circumference, body fat percentage (…) and high-density lipoprotein cholesterol (28).” Riccio and Rossano suggest dietary components to trigger inflammatory processes in MS, especially saturated fats, unsaturated trans fats, red meat, sweetened drinks, increased salt intake and cow’s milk proteins and anti-inflammatory components such as polyunsaturated fats, polyphenols, antioxidants and certain dietary regimens to be potentially useful in MS (20). To this date there is, however, no proof for causality of diets to directly affect MS incidences (29, 30).

1.2.1 Suspected pathogenesis

MS is characterized by neuroinflammation, demyelination and axonal degeneration in the central nervous system (CNS) (16). MS is classified as an autoimmune disease, with autoreactive T cells crossing the leaky blood-brain-barrier and attacking the neuron-encasing myelin sheaths that are essentially required for a rapid signal transduction. Further, T cells, via interacting with microglia - the CNS-based immune cells - are initiating the release of cytokines and induce auto-antibody production by B cells, promoting an inflammatory environment (Figure 1) (16).

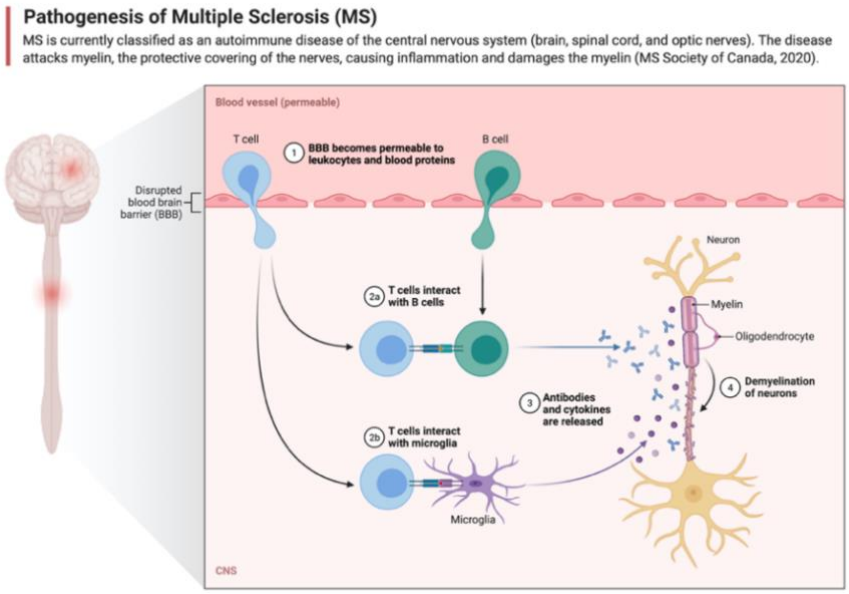


Figure 1: Reprinted from “pathogenesis of multiple sclerosis (MS)”, by Biorender.com 2021. Retrieved from https://app.biorender.com/biorender-templates

Autoantigen specificity of the misguided immune response remains unknown. Further, whether the antigen-priming occurs in the periphery and a molecular mimicry takes place in the CNS or the antigen is presented in the CNS and leads to an activation and migration of T cells into the CNS, is not completely understood (31).

1.2.2 The role of oxidative stress and antioxidants

Studies in mice and humans demonstrate that oxidative stress or reduced antioxidative responses, play an important role in MS progression (32) and MS pathogenesis (33, 34). Even in the early stage of the disease - the Clinical Isolated Syndrome – plasma markers of oxidative stress such as advanced oxidation protein products were found to be elevated (35). A recent meta-analysis of 31 studies including 2001 MS patients and 2212 healthy controls concluded that MS patients had significantly increased blood concentrations of oxidative stress markers, above all malondialdehyde (MDA) (36). Even in the saliva of MS patients, elevated markers of oxidative stress and lower levels of antioxidants, compared to healthy controls, were found (37). MS has been characterized as a mainly inflammatory disease and neurodegenerative processes in the disease development have been neglected in the past. This view has been challenged by observations, arguing that reducing inflammation and even relapses, does not stop disease progression (38).

Just recently, a study confirmed that oxidative stress markers, known also to be involved in Alzheimer's disease, are elevated up to five years before disease onset, indicating that they are rather a cause of neurodegenerative processes than a consequence (39).

Generally, a state of oxidative stress is reached if reactive oxygen species (ROS) outbalance neutralizing antioxidant agents. ROS are generated physiologically and function as a substantial defense mechanism against pathogens. In case of an increased generation of these ROS, oxidative stress responses are, among other pathways, controlled by the counter-regulative antioxidative Nrf2 pathway (40). Interestingly, activation of Nrf2 and its mediation of mitochondrial biogenesis is one main mechanism of action of the well-established DMT agent dimethyl fumarate (41). As pointed out in the next chapter, ROS regulate immune responses and may further play a role in inducing a functional hypometabolism and mitochondrial

dysfunction, which may have implications in MS pathophysiology, described later in 1.4. ROS such as superoxides and hydroxyl radicals are, as the name implies, very reactive, due to unpaired electrons (40). Hence, they are unstable and can hardly be measured. Oxidation-induced damage to lipids, proteins, carbohydrates are therefore typically assessed as indirect markers of oxidative stress.

1.2.3 The role of peripheral and central inflammation

Inflammation and oxidative stress are closely linked. A review describes that inflammatory diseases rapidly deteriorate if mice were lacking antioxidant defense mechanisms such as Nrf2 (40). With regards to MS, a dysregulation of pro-inflammatory responses via Th1 and Th17 cells and anti-inflammatory responses via regulatory T cells or Th2 cells appears central for disease pathogenesis: Increased Th1 cytokines are present in peripheral blood mononuclear cells (PBMCs) from MS patients during relapses, while increased Th2 cytokines are found during remission (42). Similar to the balance of ROS and antioxidant agents, to maintain homeostasis, a balance between pro- and anti-inflammatory cytokines must be maintained (43). Pathologically reduced concentrations of anti-inflammatory cytokines can result in an imbalance in favour of pro-inflammatory responses, which may drive autoimmune diseases. In contrast, excessive anti-inflammatory cytokine concentrations may dampen defence against pathogens (44).

Generally, studies on cytokines in MS patients deliver indistinct results, e.g., TNF-alpha, a pro-inflammatory cytokine, was shown to be elevated in MS (43). Moreover, high numbers of TNF-alpha, interferon-gamma and interleukin-6 (IL-6) are present in active lesions (45) (46) and increased expressions of TNF-alpha mRNA were found in active demyelinating lesions from brain biopsies, compared to remyelinating lesions (47). In line with this, cytokines, including interferon-gamma, TNF-alpha and IL-1 can activate the CNS-based immune cells microglia and macrophages that can in turn further damage myelin (43). However, in contrast to another autoimmune disease, rheumatoid arthritis, a RCT with a TNF-alpha inhibitor was not successful in MS patients (48).

Even though, there is evidence for a role of cytokines in the MS pathogenesis, studies deliver heterogenous results on the relationship between clinical disease outcomes and cytokines, even in untreated MS patients (49). This may be influenced by

methodological issues, biological complexity of cytokines and a short half-life of cytokines (49), but also due to the complexity of predicting the right time point for cytokine assessment (43). However, studies *do* suggest associations between cytokine concentrations in the cerebrospinal fluid or periphery with the MS disease course (50, 51).

1.2.4 The role of the gut-immune-brain axis

To date, the initial trigger of a potential peripheral autoimmune activation remains unknown. Within the last decade, the gut-immune-brain axis has gained attention and importance. It was shown that gut dysbiosis plays a role in enhancing inflammatory immune responses in Alzheimer's disease and MS (52). A review by Rossi et al. concluded that the gut microbiome along with its produced metabolites further regulates T cell-mediated immune responses, e.g., distinct bacterial species correlate with MS and induce a pro-inflammatory T cell environment that preserves autoimmunity (52). In contrast, neuroinflammation and demyelination during the MS-like experimental autoimmune encephalomyelitis (EAE) is repressed by the gut microbiome enriched in certain bacteria (53). Remarkably, human-derived gut microbiota from for MS discordant identical twins transplanted into germ-free mice were able to spontaneously induce the MS-like autoimmune encephalomyelitis, significantly more often in the mice that received the microbiota from the MS-affected twin (54). Moreover, defects in gut barrier function were described to play a role in MS pathophysiology (55). Increased intestinal permeability, due to tight junction dysfunction among epithelial cells, may lead to a low-grade bacterial translocation from the gut to the periphery which may trigger misguided immune responses. An increase in intestinal permeability, the so-called leaky gut syndrome, has been found to be common in MS – as evidenced by increased levels of bacterial lipopolysaccharides, non-metabolized sugars and the tight junction regulating protein zonulin in urine, plasma or sera of MS patients (56-58). Lipopolysaccharides further stimulate the generation of inflammatory cytokines (59). Finally, zonulin was even associated with MS disease progression in a clinical trial and suggested to mediate disruption in both the intestinal barrier and blood-brain barrier (58).

1.3 History of nutritional research in MS

Diet, especially dietary fiber and dietary fats, as well as antibiotics and other environmental factors shape the gut microbiota and their associated microbial metabolites as well as intestinal permeability. Ultimately, specifically Western diets were shown to induce gut microbiome dysbiosis (19). Moreover, postprandial glycemic peaks, increased caloric intake and specific lipid patterns were shown to further drive inflammation and oxidative stress, and via these mechanisms suggested to play a role in MS pathophysiology (20, 60). The role of diet has therefore been subject of research since the 1950s (61-63) and several dietary factors were found to be associated with MS incidence, disease course and symptomatology (20, 64-66).

1.3.1 Nutritional neuroepidemiology

Swank et al. described already in 1952 the incidence of MS to be varying across different regions in Norway (61). They found a higher incidence in inland areas compared to coastal areas, where more fish and less butterfat were consumed than in inland areas. Further, Agranoff and Goldberg described dairy consumption to be correlated positively with MS, whereas vegetable fat consumption correlated inversely with MS (67). Alter et al. looked at epidemiological data of 22 countries and found MS prevalence to be strongly positively associated with animal fat consumption (68). Moreover, Esparza et al. analysed epidemiological data from 36 countries and concluded high animal fat (minus fish fat) and saturated fatty acid consumption to correlate independently and positively with MS mortality (69). In line with this, a positive correlation between MS prevalence and total fat, meat and pork consumption was further found by Nanji and Narod (70), between MS prevalence and milk as well as butter consumption in data obtained from 27 countries by Malosse et al. (71) and between MS prevalence and pork meat consumption by Lauer (72).

Summarizing the results of studies, there is, however, no consistence: whereas there are numerous indications of an association between the disease risk and the consumption of saturated fats from animal origin from epidemiological studies, a review from 2005 reports that most case-control studies could not confirm these results (30), concluding that there is no proof of evidence for a causal relationship between MS prevalence and diet, yet.

1.3.2 The role of the nutritional status in MS

Studies on nutritional status in MS patients are showing heterogeneous results, depending on the assessment method and patient population. In a study population with many newly diagnosed patients, the prevalence of malnutrition was rather low (73). In contrast, other studies with MS patients show a higher prevalence of poor diets (74) and intake deficiency of polyunsaturated fatty acids, fiber and vitamin D (75), especially in more severely affected MS patients. The BMI was found to be lower during the course of the disease compared to healthy controls in a meta-analysis (76). However, it was found that the BMI seems to underestimate obesity in MS (77). One study showed that body composition and adipose tissue distribution was unfavorably altered in male MS patients, compared to age-matched healthy controls (78) and total body fat was associated with greater disability in another study with MS patients (79). In line with this, another study reported that body composition showed the same strong associations with disability as functional assessments such as the six-minute walk test, while the BMI did not (80). As mentioned in 1.2.2, micronutrient deficiency, reflected by a reduced antioxidative capacity or elevated oxidative stress parameters, was found to be prevalent in the sera, plasma and cerebrospinal fluid of many MS patients (35, 81, 82) and was even predictive for MS disease progression (83).

Many diet-related cardiovascular disease (CVD) risk factors such as hypercholesterinemia and hyperinsulinemia are common among MS patients (84-86). Moreover, diet-related comorbidities such as obesity, insulin resistance and other CVD risk factors are associated with increased MS susceptibility and/or poor disease course (85, 87, 88). Growing evidence further suggests that CVD risk factors increase the risk of disability progression and present associations with increased lesion burden and brain atrophy (89-91). In line with this, a 5-year study proposed that overweight and obesity significantly elevate the risk of MS progression (92). In accordance, a potential role for leptin has emerged. The adipocyte-derived hormone regulating energy expenditure and satiety, was found to have pro-inflammatory functions and was suggested to be an important link between nutrition and immune function, balancing Th1/Th2 immune homeostasis (93). Conformingly, Matarese et al. found high levels of leptin to be associated with reduced regulatory T cells in sera of MS patients (94) - cells that are crucial to keep autoreactive T cells at bay.

1.3.3 Dietary approaches in MS

A recent Cochrane review concludes that evidence for efficacy of dietary interventions in MS is still uncertain (95). However, the idea that a modification of dietary intake may have a beneficial effect on the course of the disease or comorbidities appears plausible against the body of evidence from epidemiological studies and is attractive to many patients. A recent survey with 1014 MS patients concludes that 81% used complementary medicine and 41% followed a specific diet to manage their MS symptoms (96). Current “MS-diets” are based on pathophysiological considerations and on findings from animal or epidemiological studies as described above.

Dietary polyunsaturated fatty acids

The strong associations between dietary fats and MS risk have put a spotlight on dietary fats. Polyunsaturated fatty acids, especially omega-3 fatty acids, have numerous anti-inflammatory effects, which may have an impact on autoimmunity, a review concludes (97). A small clinical study has shown that supplementation with omega-3 fatty acids decreases cytokines in the PBMCs of MS patients (98). In line with this, a recent systematic review concluded that omega-3 fatty acid supplementation improves quality of life, inflammatory markers and even reduces relapse rates in MS patients (99). However, other recent systematic reviews including a Cochrane review concluded that there may be no clinically relevant impact of omega-3 fatty acid supplementation on disability or inflammatory markers (100) and there may be little to no effect of polyunsaturated fats with regards to improving MS outcomes (95).

The Fratzer diet

The so-called Fratzer diet, invented by the German physician Uwe Fratzer, also focused on the modification of dietary fats (101). The diet aims to change the relation of omega-3 and omega-6 fatty acids, to reduce the generation of pro-inflammatory arachidonic acid: to achieve that, dietary linoleic acid intake is aimed to be reduced - in order to enhance compensatory endogenous production of the competing omega-3 fatty acids, which are thought to be generated in greater amounts, in dependence of omega-6 linoleic acid reduction (102). Furthermore, supplementation with cost-intensive fish oil capsules and corticoids is recommended to build up an anti-

inflammatory environment (30). Dietary linoleic acid and arachidonic acid are supposed to be drastically reduced by limiting food intake of plant fats, oily fruits, meat, and milk products. A self-reported survey with 414 MS patients following the diet claims that there was no disease progression in 82% and improvement in neurological disability in 36% of the surveyed within 5.6 years of dietary treatment (103). However, there is no rigorous scientific evidence for the diet to improve MS outcomes. Schwarz and Leweling further emphasize in their review that the simplified view on the essential omega-6 fatty acids to function entirely pro-inflammatory cannot be upheld (30).

The Swank diet

Roy Swank observed that a diet with high amounts of saturated fats is associated with an accelerated disease progression. He followed 146 patients with mild to more severe disability over in average 17.1 years. They consumed the Swank diet containing less than 20 g of saturated fats per day. Swank compared the 146 patients regarding their disease course with untreated cases from the literature (104). In this study, the number of relapses and disability progression correlated strongly with intake of saturated fats. It was claimed that fatigue has improved substantially during the third year of dietary adherence. Further, according to the author, there was a significant reduction in death rates, frequency of relapses and disability in the treated group. Moreover, it was stated that many early treated patients remained stable for up to 20 years (104). The Swank study, however, did neither run independently nor rigorously. A randomized control group design is missing, and the lack of brain imaging is another limitation. Moreover, a selection bias must be considered - as patients, who did not improve on the diet, most probably did not continue the study. A study, just recently published, found clinically significant within-group improvements regarding fatigue in MS patients following a Swank diet. However, the study found similar fatigue improvements in the control group that followed a paleo diet (105).

Vegetarian diets

In 2016, a very low-fat plant-based diet was shown to improve blood lipids, insulin, BMI, and fatigue in a RCT with MS patients (106), but did not show effects on brain magnetic resonance imaging (MRI) outcomes, relapses, or disability. The authors of the study claimed to interpret the improved fatigue outcomes with caution because the

applied waitlist-control design does not fully prevent placebo effects. Further, baseline differences of questionnaire-derived fatigue scores between the groups were observed (106). However, semi-vegetarian diets have been recommended for other chronic inflammatory diseases, such as rheumatoid arthritis and inflammatory bowel disease before (107, 108). A lacto-vegetarian diet to specifically reduce dietary intake of the pro-inflammatory arachidonic acid, which is present in animal-products, has been developed by the German physician Olaf Adam (109), who on the same basis wrote popular dietary recommendations for MS patients, first published in 2003 (110). Olaf Adam and colleagues, however, have studied the effects of this anti-inflammatory diet only in patients with rheumatoid arthritis (109), showing ameliorated clinical signs of inflammation in the diet group.

Ketogenic diets

Ketogenic diets became very popular within MS communities recently. This may be due to successful single case studies of MS patients who improved disability and other symptoms rapidly on a ketogenic diet (111). Another reason for the rise in popularity of ketogenic diets might be popular-science media promoting books such as “The Wahls Protocol”, a book written by an MS-affected physician who self-claimed that she reversed her symptoms by a ketogenic-like paleolithic diet among other interventions and on this basis ran a first pilot study in 2015 (112). In the medical community, ketogenic diets are known for their 100-year-old history of successful application especially in children suffering from epilepsy (113-115) and the interest in ketogenic diets for treatments of other neurodegenerative diseases is rising. Recently, ketone-based therapies have been shown to successfully improve cognition in Alzheimer’s disease, mild cognitive impairment (116, 117) and Parkinson’s disease (118). Some pilot studies have investigated ketogenic diets and the related paleo diet in MS and reported improved fatigue, depressive symptoms, oxidative stress, and inflammatory markers (119-121). However, validity of these studies is limited due to small sample sizes or missing control groups.

Fasting diets

Besides ketogenic diets, fasting interventions have been successfully applied in chronic inflammatory diseases such as psoriasis, psoriatic arthritis or asthma in some studies (122-124). The strongest evidence is coming from clinical studies with rheumatoid arthritis, in which fasting reduced pain symptoms, tender/swollen joints, white blood counts, pro-inflammatory IL-6, and levels of C-reactive protein (125-127). Different intermittent fasting regimens aiming to mimic fasting effects have been investigated in MS patients and showed that restricted caloric intake is less feasible compared to time-restricted eating and intermittent drastic caloric restriction (75% reduction of daily caloric needs at 2 days a week) to be less feasible compared to daily moderate caloric restriction (22% reduction of daily caloric needs) (128, 129). The study by Fitzgerald et al. shows, however, improvements in depressive symptoms in MS patients, in both fasting regimens, intermittent and daily caloric restriction (129).

As MS is a disease with chronic inflammatory and neurodegenerative features, ketogenic diets and fasting diets that are both based on ketone body generation may be promising approaches.

1.4 Ketone-based diets in MS

1.4.1 Evolutionary aspects of the state of ketosis

Ketogenic diets and fasting, through either carbohydrate or caloric restriction, both lead to a hepatic production of ketone bodies from fats. Specifically, low insulin and high glucagon levels initiate lipolysis and ketone production via beta-oxidation of free fatty acids in the liver to acetyl-coA, which is the precursor of ketone bodies (130).

Upon fasting, glycogen storages in muscle and liver are depleted first and then fatty acids are mobilized and transported to the liver for ketone body production. Ketone bodies are then circulated and allocated to metabolically active tissues. At the target organs, the ketone bodies are converted back to acetyl-CoA (131).

Ketone bodies provide an alternative energy source for the brain if glucose is scarce and can compensate for up to 60% of the brain's energy needs (132). The brain, even though just 2% of the adult body weight, demands 20% of the body's energy (133). Under usual carbohydrate-containing dietary intake conditions, glucose is the main

physiological fuel for the brain and is required for the highly efficient energy-demanding synaptic transmission (134).

However, episodic starvation was a common occurrence during human evolution, especially in hunter-gatherer times when food availability was limited. The production of ketone bodies is a critical evolutionary survival mechanism to spare muscle mass and provide energy to the brain from energy-dense storage fats when external nutrient availability is disrupted. As ketone bodies provide more energy per unit oxygen compared to glucose, they are a very efficient fuel (135).

There are three ketone bodies: beta-hydroxybutyrate (BHB), acetoacetate and acetone with BHB constituting 70% of available circulating ketone bodies (136). In humans, blood ketone concentrations are normally at around 0.1 mmol/l and begin to rise after 12 hours of fasting to up to 0.3 mmol/l (137). Blood ketones can reach 2-3 mmol/l after 2 days of fasting or during liberalized ketogenic diets and can increase to about 5-7 mmol/l during prolonged fasting or starvation (137-139). Under pathological conditions such as untreated diabetes, when insulin is deficient, ketones can rise up to 25 mmol/l, inducing a diabetic ketoacidosis (137).

Ketogenic diets are not necessarily energy-restricted but mimic fasting states and share many biochemical characteristics with caloric restriction such as cell- and neuroprotective pathways. Fasting therapies have been applied as anticonvulsive therapies since ancient times with early recordings in the Hippocratic collection (140), and the ketogenic diet has been in clinical use for epilepsy patients for over a century (115).

1.4.2 Main cellular mechanisms of action of ketone bodies

Ketone bodies do not only provide an alternative energy substrate for the “starving” brain when blood glucose is scarce, but may directly ameliorate energy production within the neuron, by enhancing adenosine triphosphate (ATP) production, decreasing ATP degradation products, enhancing mitochondrial biogenesis and stabilizing mitochondrial membranes by inhibiting the formation of mitochondrial permeability transition pores (38, 141-143). Further, ketones may increase mitochondrial uncoupling proteins that correspond with a reduction of oxidative stress (144). Moreover, the ketone body BHB is suggested to improve the mitochondrial redox state

via activating the antioxidative Nrf2-pathway (145), inhibiting the pro-inflammatory NF-kB-pathway (146) and NLRP3 inflammasome (147) as well as their associated pro-inflammatory cytokine secretion (38) (Figure 2).

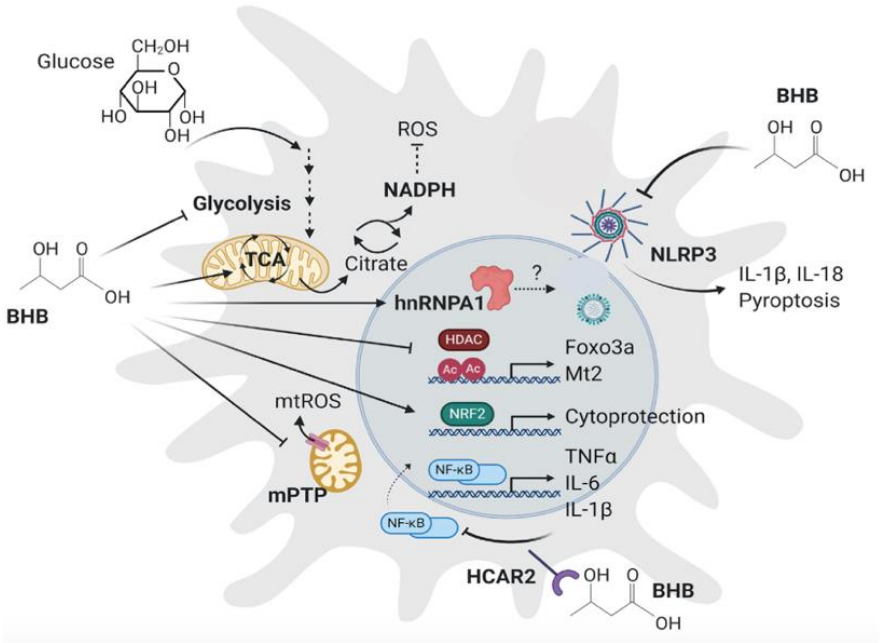


Figure 2: Anti-inflammatory properties of the main circulating ketone body beta-hydroxybutyrate (BHB), figure by Stubbs et al. Investigating Ketone Bodies as Immunometabolic Countermeasures against Respiratory Viral Infections. Med. 2020 (148). License for reprint provided by Elsevier and Copyright Clearance Center.

Caloric restriction and ketogenic diets may also have effects on the main cellular recycling mechanisms - autophagy and the proteasome. The diets were shown to influence autophagy and proteasome pathways in studies with rodents (149-152).

The proteasome degrades more than 80% of the cellular damaged and unneeded proteins. Moreover, the proteasome highly depends on ATP and its function may be diminished when mitochondria function is impaired (153). Impaired autophagy and proteasomal activity have been suggested to play a role in the pathogenesis of neurodegenerative diseases (154).

Another mechanism of action of ketones may be via BHB influencing epigenetic processes by inhibiting histone deacetylases, regulating a variety of pathways implicated in longevity and age-related disease (38, 155).

Ultimately, it is important to note that fasting and ketogenic diets are not entirely anti-inflammatory or immune suppressive in their mechanisms of action. A recent review described that ketone-based interventions can rather modulate protective and pathogenic immune functions and improve chronic inflammation without impairing the host defense against parasitic or viral infections (156).

1.4.3 Potential targets of ketone-based diets in MS

Studies have shown that MS patients, as well as patients with other neurodegenerative diseases, have a decreased cerebral glucose metabolism, which was even partly correlated with lesion load and cognitive dysfunction in MS patients (157-161). Specifically in MS patients, glucose hypometabolism was associated with cognitive impairment compared to non-impaired MS patients (162). Moreover, fatigue symptoms of MS patients were strongly associated with a reduced cerebral metabolism in one study (163). Furthermore, signs of an impaired peripheral glucose metabolism, such as increased lactate productions, were found to be prevalent in MS patients compared to healthy controls (164).

Increased lactate concentrations may be indicators for a mitochondrial dysfunction. Lactate concentrations in the serum and in the cerebrospinal fluid have even been suggested to serve as biomarkers as they were found to be associated with clinical outcomes in MS (165-167). Lactate production occurs when the pyruvate from glycolysis is unable to be oxidized in the mitochondria by oxidative phosphorylation, e.g., when oxygen is lacking. If the mitochondria can no longer oxidize glycolysis-derived pyruvate, the cell is forced to reduce pyruvate to lactate via lactate dehydrogenase – a process referred to as lactate fermentation (168). In some cases, however, a metabolic switch to the less efficient lactate fermentation occurs, even when enough oxygen is present for oxidative phosphorylation - a condition which is also known as “Warburg effect” or aerobic glycolysis (169).

During lactate fermentation, only two molecules of ATP are produced for every molecule of glucose metabolized anaerobically in glycolysis to lactate, compared to 36 ATP molecules that are produced through efficient mitochondrial oxidative phosphorylation (170). As lactate fermentation is relatively inefficient, the Warburg effect may be a result of a pathological mitochondrial dysfunction (171). Further indicators of an impaired mitochondrial metabolism are elevated markers of oxidative

stress such as oxidized molecules due to reactive oxygen species reflecting a disturbed oxidative phosphorylation (172).

Neurodegenerative processes are highly driven by oxidative stress and mitochondrial dysfunction (173) and ketone bodies were suggested to improve several relevant pathways regarding cellular energy production (38). Specifically, ketone-based diets have been suggested to decrease oxidative stress in cell, animal, and small pilot studies with MS patients (120, 174, 175), possibly mostly via mechanisms described in 1.4.2. Further, ketones were suggested to have the potential to compensate for a deteriorating cerebral glucose hypometabolism in Alzheimer's disease (176-178).

1.4.4 Ketone-based diets in experimental murine MS models

Animal studies with the well-established MS-model EAE have shown that fasting interventions can slow disease progression via reducing the infiltration and amount of autoreactive T cells, stimulating remyelination through promoting proliferation of myelin-producing oligodendrocytes and increasing anti-inflammatory pathways such as corticosterone production and enhancing regulatory T cell function (179, 180). One study further showed that EAE mice fed a ketogenic diet had fewer lesions, less brain atrophy and decreased production of ROS (174). Moreover, studies with cuprizone-induced mouse models investigating a ketogenic diet showed reductions of oxidative stress parameters such as MDA, upregulation of antioxidative glutathione, while enhancing expression of oligodendrocytes, decreasing autoreactive astrocytes, and improving motor abnormalities (181, 182).

1.4.5 Effects of ketone-based diets on gut, metabolism and body weight

Ketogenic diets have been shown to reduce Th17 cell produced IL-17 (183) probably via modulating the gut microbiome by reducing Bifidobacteria (184). Intermittent fasting and fasting mimicking diets have been shown to similarly affect the microbiome: both regimens ameliorated the clinical course of EAE by reducing IL-17 producing T cells and increasing protective regulatory T cells (179, 185). Specifically, "fecal microbiome transplantation from mice on intermittent fasting ameliorated EAE in immunized

recipient mice on a normal diet, suggesting that intermittent fasting effects are at least partially mediated by the gut flora" (185).

Ketogenic diets and fasting diets have a strong potential to reduce body weight, lower blood lipids, blood glucose and insulin. A recent meta-analysis including 14 RCTs with overweight patients, with or without diabetes type 2, undergoing a ketogenic diet, concludes that ketogenic diets "were more effective in improving metabolic parameters associated with glycaemic, weight, and lipid controls in patients with overweight or obesity, especially those with pre-existing diabetes, as compared to low-fat diets" (186). Ketogenic diets have been shown to even improve blood lipids long term (> 12 months) in obese subjects (187). Similar results were found for different fasting interventions that have also been shown to be effective regarding weight loss, improving blood lipids and body composition in obese adults (188). One study shows improvements in cardiovascular biomarkers in normal weight persons and independent of weight loss due to a ketogenic diet (189). Fasting interventions, on the other hand, have shown beneficial effects regarding optimizing blood pressure and body weight (190) and even intermittent fasting to be sufficient to improve overweight (191).

1.4.6 Effects of ketone-based diets on neuropsychiatric symptoms

Neuropsychiatric symptoms, such as fatigue, impaired cognition, and depressive symptoms, are prevalent in MS and among the most disabling symptoms - severely affecting employment and quality of life. Neuropsychiatric symptoms are difficult to diagnose and treat as symptoms often overlap. As of today there is no efficient treatment for fatigue and impaired cognition, while treatment for depression is accompanied by side effects (192).

Ketogenic diets or ketone-supplements have been investigated for their effects on cognition in several neurodegenerative diseases. A recent systematic review has investigated ketogenic diets in Alzheimer's disease, Parkinson's disease, refractory epilepsy, and type 1 glucose deficiency syndrome and concludes that ketogenic diets are effective in improving cognitive impairment, even though the authors concede that many studies lack scientific rigor (193). Another review concluded that ketogenic diets have a positive effect on cognitive function in children and adolescents with refractory epilepsy. Specifically improved mood, sustained attention, and improved social interactions were observed (194). Supplementation with ketones or ketogenic middle

chain triglyceride oil was shown to improve cognition in patients with Alzheimer's disease (116, 195).

Moreover, fatigue is currently discussed to be a potential target for ketone-based interventions. Ketone-based diets have been suggested for chronic fatigue syndrome (196) and investigated in fatigued cancer patients (197, 198), patients with Parkinson's disease suffering from fatigue (118) and MS patients with fatigue (105, 119). As described in 1.4.3, studies have shown that patients with certain neurodegenerative diseases may have a decreased cerebral glucose metabolism, which is correlated with MRI changes. In MS, a glucose hypometabolism was found to be associated with fatigue (163). Ketones may be able to compensate for an insufficient cerebral glucose uptake (176) and improve brain energy metabolism as shown in Alzheimer's disease patients (178).

Furthermore, ketone-based approaches are widely discussed as potential additional therapies for depressive symptoms – which are highly prevalent in MS patients (199). Small RCTs have already shown improved mood or depressive symptoms due to fasting and low-carbohydrate diets (200-202).

Remarkably, it was shown that depressive symptoms, fatigue, and cognitive impairment are accompanied by peripheral inflammation and/or oxidative stress in MS (203-207) and ketone-based diets were shown to address these targets. A detailed overview of all potentially relevant effects of ketone-based diets are displayed below in Figure 3.

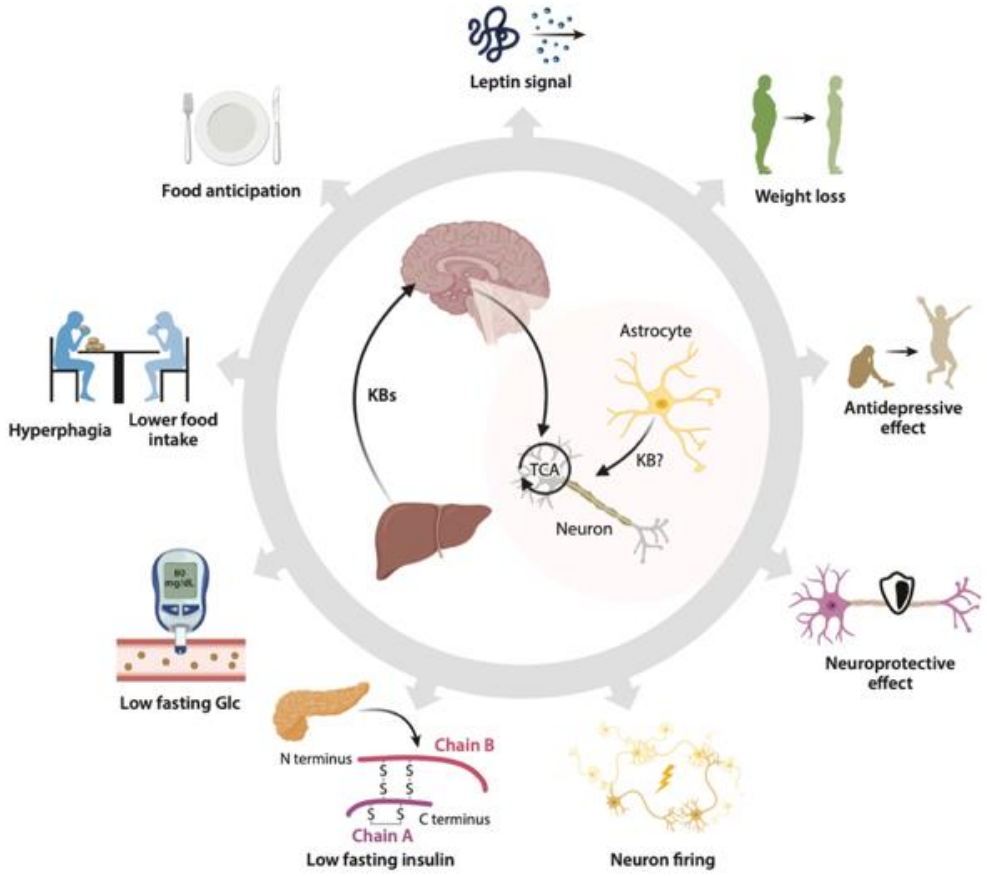


Figure 3: Observed effects of ketones in the nervous system. Figure by Puchalska P, Crawford PA. Metabolic and Signaling Roles of Ketone Bodies in Health and Disease (208). Annu Rev Nutr. 2021 Oct. Figure adapted from images created with Biorender.com. Glc = glucose, KB = ketone body, TCA = tricarboxylic acid. License for reprint provided.

1.4.7 Important preliminary work at our study center

The “IGEL study”, a RCT that was published in 2016, was the pilot study of the NAMS (**N**utritional **A**pproaches in **MS**) study, that is subject of this dissertation. The IGEL study was also conducted at our study center at Charité – Universitätsmedizin Berlin. The study proved that a ketogenic and a fasting diet are safe and feasible over six months and that both diets improved health-related quality of life, including physical and mental health, in 60 MS patients. Further, in the fasting and ketogenic diet group, compared to a control group, a mild reduction of the Expanded Disability Status Scale (EDSS) score was observed (179).

The NAMS study is the first RCT to investigate ketone-based dietary interventions in MS patients with a medium sample size and with disease-relevant MRI endpoints over a long period of time of 9 and 18 months (209). Moreover, this analysis, which follows patients for 9 months, aims to investigate the patient-relevant neuropsychiatric outcomes fatigue, depression, and cognition. Further, this analysis intents to investigate mechanisms of action of the ketone-based diets, especially potentially mediative nutrition-related, inflammatory, and oxidative stress pathways.

1.5 Study hypotheses

Against this body of evidence, I hypothesize

1. that the ketone-based dietary interventions, fasting and ketogenic diet, are effective in improving neuropsychiatric outcomes including fatigue, depressive symptoms, and cognition in our MS cohort.
2. that effects of fasting and ketogenic diets are driven by changes in nutritional status, inflammatory cytokines, and oxidative stress.

2 Ethics, recruitment, study design and study conduction

This dissertation is based on a sub-cohort of the NAMS study, a single center, randomized, controlled, three-armed study conducted at Charité – Universitätsmedizin Berlin. The study protocol has been published (209). Patient recruitment started in April 2017 and last patient out was in October 2021. The NAMS study has been registered at ClinicalTrials.gov (identifier: NCT03508414). Patients were recruited via flyer distribution in medical practices, social media presentations and information lectures. Patients were further informed about the study via the website of the German Multiple Sclerosis Society (DMSG) as well as the Charité website. The study has been approved by the institutional review board of the Charité – Universitätsmedizin Berlin. The study followed the currently applicable form of the Declaration of Helsinki, the guidelines of the International Conference on Harmonization of Good Clinical Practice (ICH-GCP) and relevant German laws.

Due to the Corona virus pandemic and the associated protective measures in clinical research and governmental-ordered travel restrictions, recruitment stopped prematurely in March 2020, which resulted in an overall reduced sample size.

2.1 Participants, inclusion, and exclusion criteria

According to the guidelines of the institutional review board of Charité – Universitätsmedizin Berlin, study material including study information and informed consent was provided. The study documents were handed out to potential participants before the consultation with a study physician. After explanation of the study procedures by the study physician, the potential participant had time for questions. If willing to participate, a written informed consent was given, and inclusion and exclusion criteria were queried. Relevant inclusion criteria were a definite diagnosis of RRMS according to the 2017 revised McDonald criteria (210) and a stable DMT or no DMT for at least six months at study entry. Further, the EDSS score had to be below 4.5 (211). Moreover, a disease activity defined as at least one new lesion in the brain MRI or at least one relapse within the last two years prior to enrolment, had to be ensured. Participants were recruited in cohorts of 15 - 25 patients, to keep group sizes for the nutritional counselling relatively small. The complete list of inclusion and exclusion criteria is listed below and was published within the study protocol publication (209):

Inclusion criteria

- Diagnosis of RRMS according to revised McDonald criteria
- 18 - 65 years of age
- Disability status according to EDSS < 4.5
- Stable DMT or no DMT ≥ 6 months before study entry
- ≥ 1 relapse or ≥ 1 new T2 lesion or ≥ 1 contrast-enhancing lesion on MRI within the last 2 years prior enrolment
- Agreement that possible incidental findings will be communicated
- BMI between 19 and 45 kg/m^2
- Ability to give verbal and written consent
- Health insurance

Exclusion criteria

- Start or change of DMT < 6 months before or during the study
- Relapse or cortisone treatment within 30 days before study entry
- Clinically relevant metabolic, progressive, or malignant diseases
- Intake of > 1 g/day omega-3 fatty acid supplements
- Significant cognitive-cooperative impairment
- Insulin-dependent diabetes mellitus
- Participation in other interventional studies
- Weight loss diet or loss of more than 5 kg within 2 months before study entry
- Insufficient mental ability for cooperation
- Eating disorders
- Kidney stones
- Therapy with oral anticoagulants
- Pregnancy and breastfeeding
- Suspected lack of compliance
- Smokers (> 5 cigarettes per day)
- Known alcohol and drug abuse
- Inability to give informed consent or to adhere to the study protocol
- Contraindications for MRI

2.2 Randomization

Randomization was applied considering three strata to distribute potential confounders evenly to the three dietary interventional groups. The chosen strata were sex (male or female), DMT (yes or no) and lesion load (low / < 15 or high / ≥ 15 T2 lesions) according to the baseline MRI scan. An external statistician carried out the randomization, using a block randomization with a variable block length for a homogeneous distribution (209).

2.3 Study design and dietary interventions

Patients were recruited from all over Germany. In this analysis 98 RRMS patients were randomized to one of the following dietary interventions that were also described in the study protocol publication before (209):

- a ketogenic diet (KD) with a carbohydrate intake restricted to 20 - 50 g/day
- a fasting diet (FD) with a 7-day fast every 6 months and 14-h daily intermittent fasting in between at 6 out of 7 days a week
- a standard healthy diet (SD) with a vegetarian focus as recommended by the German Nutrition Society (DGE)

Nutritional counseling was run in seven sessions within the nine months study duration (five sessions in the first three months, two sessions in month four to nine). The group setting was chosen to encourage exchange of experiences between patients, and to carefully observe compliance as well as to document dietary challenges and adverse events. Apart from the group sessions, study participants were encouraged to contact their nutritional counselor at any time.

▪ ***KD*.** The KD was initiated by instructing the patients to reduce their carbohydrate intake to 20 g/day for four weeks. Afterwards, patients were allowed to increase their carbohydrate intake by 5 g each week up to a maximum of 40 - 50 g/day depending on the individual carbohydrate intake limit that still allowed a stable ketosis. Ketosis was defined as values ≥ 0.5 mmol/l of blood ketone BHB. Specifically, carbohydrates that elevate blood glucose – meaning, excluding fibers and sugar alcohols – were supposed to be limited to 40 - 50 g/day. Patients had to research their foods for their carbohydrate content in online databases. Foods were weighed and calculations were performed to not exceed the daily carbohydrate-allowance. To determine the individual carbohydrate intake limit, participants received a hand-held ketone-meter to document blood ketone concentrations at given intervals (data not presented). Ketones were supposed to be measured in the late morning to bypass a potential *Cortisol Awakening Response*. Ketones were additionally measured at the regular study visits in the accredited central laboratory (Labor Berlin - Charité Vivantes GmbH) of the study center. This KD design follows a traditional KD pattern, but with a more flexible macronutrient composition of 70 - 80% fats, 15 - 20% proteins and 5 - 10% carbohydrates (a strict traditional KD contains of 90% fat, 6% proteins and 4%

carbohydrates). Due to this liberalized approach with up to 40 - 50 g carbohydrates/day, this KD was more similar to a modified Atkins diet or low glycemic index treatment (212). Similar to the FD and SD group, a vegetarian focus was set in the KD group (reduced meat consumption of 1 - 2 portions/week). Patients were allowed to have two “cheat days” per month at which they followed the rules less strictly.

- ***FD*.** Patients in the FD group fasted for seven days every six months. Unsweetened tea and water consumption were not restricted. Patients were not allowed to eat solid foods but were supposed to consume liquids such as juices or broth, not exceeding a daily calorie intake of 200 - 350 kcal. Two days before and three days after fasting, patients were instructed to follow a low-calorie vegetarian-based diet to ensure a gradual preparation and aftercare. Before fasting, an intestinal cleansing with laxatives (e.g., Glauber’s Salt by FX-Passage) was recommended. Between the fasting weeks, patients ate ad libitum for 8 - 10 h and fasted for 14 - 16 h on six days per week. Further, patients were instructed to adhere to a diet following the recommendations of the DGE, equivalently to the SD group. Patients had to attend dietary counseling within group sessions every second day during the fasting periods that were monitored by a physician and a dietician. Blood ketones were not self-measured during the study, as it is not expected that patients have a relevant ketosis due to intermittent fasting (137). Ketones most certainly would have been increased during the 7-day fasting periods but were not measured during this time. However, blood ketones were assessed at the study center during regular study visits. To enhance compliance, participants were allowed to take one “cheat day” per week at which they were allowed to skip intermittent fasting.
- ***SD*.** Patients were instructed to adhere to a standard healthy diet according to the recommendations of the DGE and according to epidemiological results on dietary fats as described under 1.3.1 and 1.3.3. This diet had a vegetarian focus. Patients were instructed to reduce consumption of meat, animal fats, eggs, and egg products, similar to a Mediterranean diet. Dairy products were recommended to prevent deficient calcium intake. Dietary recommendations further included modifications of the omega-6 to omega-3 fatty acid ratio to achieve an approximate 5:1 ratio to also set an anti-inflammatory approach (monitoring based on food records, data not shown).

Study visits were conducted at baseline and after nine months (Figure 4).

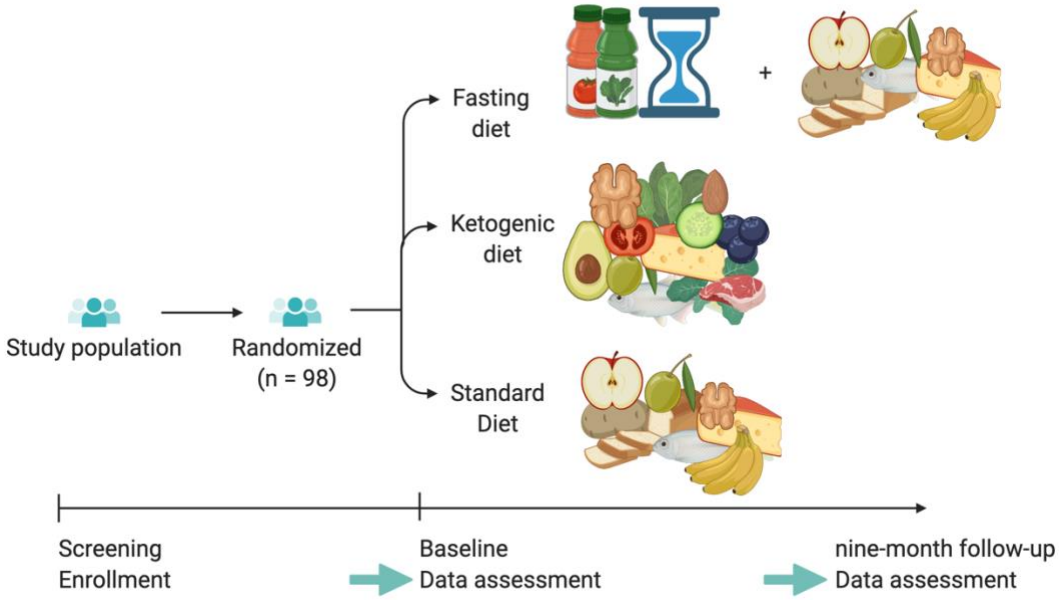


Figure 4: Study design of this sub-cohort of the NAMS study. All data assessments and blood sampling took place in a standardized way at baseline and after nine months. Figure created with Biorender.com.

2.4 Protocol deviations, compliance, and adverse events

No major risks were expected to be caused by participating in this study. All adverse events were continuously and thoroughly documented during study visits and group sessions. Retention of patients was actively encouraged by the study team via several channels including telephone, email, and during study visits. Discontinuation criteria were subsequently arising exclusion criteria (start or change of DMT, pregnancy), suspected or proven lack of compliance or medical reasons for discontinuing the dietary intervention. Patients who attended at least eight of ten group sessions and each of the main study visits, were defined as compliant to the study protocol. Additionally, in the KD group, 75% of the self-measured blood ketone concentrations were supposed to be ≥ 0.5 mmol/l. Patients in the FD group had additional group meetings during their two 7-day fasting periods (209). This investigation is based on a sub-cohort of 71 patients who followed the study protocol (per protocol analysis) and analysed the longitudinal data from baseline to the nine-month follow-up visit (Figure 5).

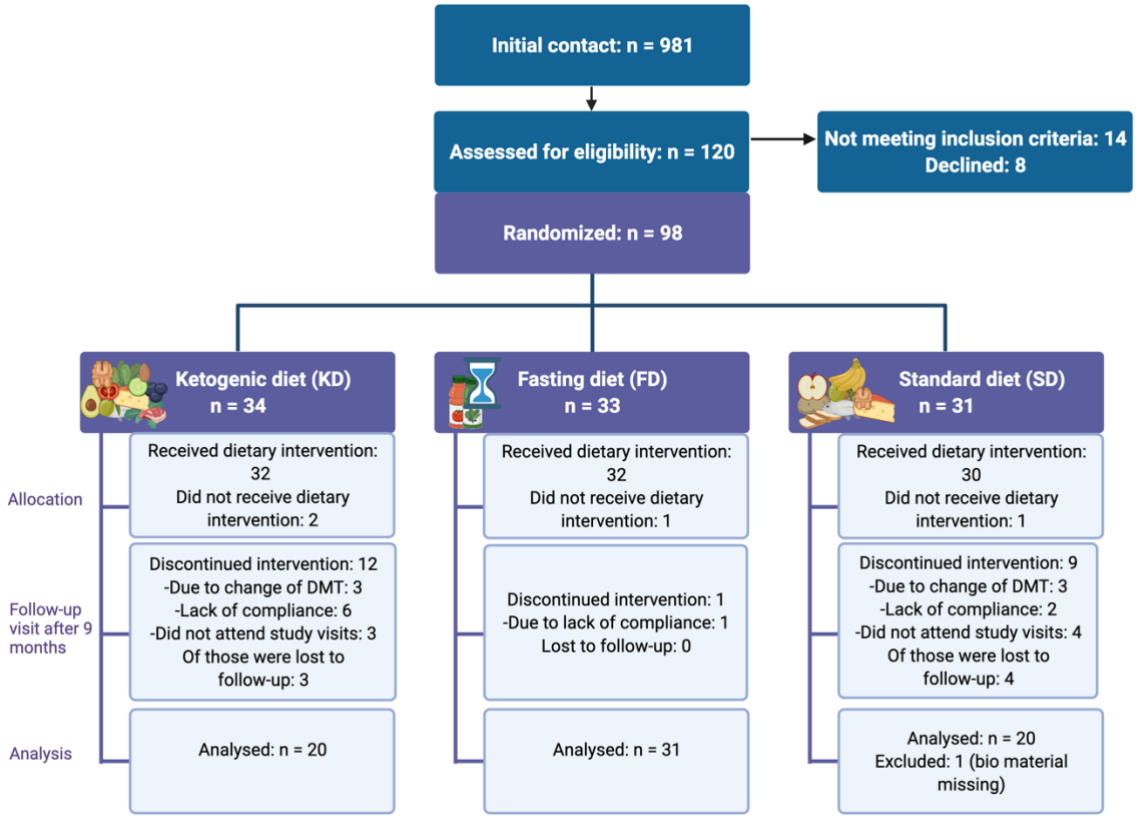


Figure 5: CONSORT flow diagram of this sub-cohort analysis of the NAMS study. Figure created with biorender.com, DMT=disease modifying therapy

3 Applied methods

This analysis investigated secondary endpoints of the NAMS study, such as neuropsychiatric outcomes, nutritional status, inflammatory status, and oxidative stress parameters.

3.1 Assessment of anthropometry and body composition

Body weight was assessed on a standard scale (seca GmbH and Co.kg, Hamburg, Germany). Body height was asked at baseline and the value transferred to the follow-up study visit. BMI was calculated from body weight divided by height squared in meters. BMI is divided into five categories according to the World Health Organization classification: underweight (< 18.5 kg/m^2), normal weight (18.5 - 24.9 kg/m^2), overweight (25 - 29.9 kg/m^2), obesity (30 - 39.9 kg/m^2), morbid obesity (≥ 40 kg/m^2) (213). Body composition was measured by bioelectrical impedance analysis

(BIACORPUS RX 4004M, MEDICAL HealthCare GmbH, measuring accuracy 3% for reactance, 1% for resistance)(214). Body fat and fat-free mass were calculated with the associated software (Body Comp 9.0, MEDICAL Healthcare Gmbh) from the measured raw data according to the formula by Sun et al. (215).

3.2 Assessment of metabolic parameters

Metabolic parameters such as blood glucose, insulin, adiponectin, leptin, haptoglobin, BHB and blood lipids were measured after an overnight-fast in the accredited central laboratory Labor Berlin - Charité Vivantes GmbH of the Charité – Universitätsmedizin Berlin, using standard procedures. Blood lipids (total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides) were measured using a homogeneous enzymatic color test. Blood glucose was measured photometrically, BHB potentiometrically and insulin with an electro chemiluminescence immuno-assay. Haptoglobin was measured by using turbidimetry. All markers were assessed via the Roche Cobas systems c501/c701 and c8000 by Roche diagnostics (216, 217). Leptin and adiponectin were measured in the collaborating laboratory Labor 28 (Medizinisches Versorgungszentrum Labor 28 GmbH) using enzymatic immunoadsorption and radioimmunoassay methods. Further, in a subgroup of patients, we evaluated daily blood glucose fluctuations by applying continuous glucose monitoring sensors (FreeStyle Libre Sensors; Abbott) that assessed glucose concentrations in the interstitial over the first 14 days after onset of the dietary interventions (218).

3.3 Assessment of plasma micronutrients and oxidative stress

Markers of oxidative stress, respectively reaction products of antioxidative enzymes, status of antioxidative micronutrients and oxidized molecules were analysed in plasma samples and PBMC samples in collaboration with the molecular toxicology group of the German institute of nutritional research (Deutsches Institut für Ernährungsforschung). Oxidative stress marker MDA and the micronutrients retinol, alpha-tocopherol, gamma-tocopherol, alpha-carotene, beta-carotene, lycopene, lutein/zeaxanthin, and beta-cryptoxanthin in plasma samples were analysed by high-performance liquid chromatography described here (219, 220). Moreover, the markers of oxidative stress protein carbonyl and protein-bound 3-nitrotyrosin in plasma samples

were analysed by using non-commercial in-house ELISA methods as described by Weber et al. (220, 221). Plasma 3-methlyhistidine and 1-methlyhistidine concentrations were assessed by ultra-performance liquid chromatography-tandem mass spectrometry (222). 25-Hydroxy-Vitamin D3 was measured by isotope-dilution liquid chromatography tandem-mass spectrometry as described by van den Ouweland et al. with minor modifications concerning sample volume (50 µL) and solid phase extraction cartridge used (Oasis Prime HLB 1cc) (223). 26S-proteasomal activity was measured in PBMCs in a 96-well format via fluorescence detection as described by Reeg et al. with modifications concerning incubation time (60 minutes) and adjusted for protein concentration (224).

3.4 Assessment of plasma cytokines

Cytokine concentrations in plasma samples were assessed in collaboration with the immunological study laboratory at Charité – Universitätsmedizin Berlin. The following cytokine concentrations were measured with multiplex technology (Luminex Bio-Plex® 200) from 600 microliter plasma samples at baseline and after nine months: GM-CSF, IL-12p40, IL-15, IL-16, IL-17, IL-1alpha, IL-5, IL-7, TNF-beta, VEGF, Interferon-gamma, IL-10, IL-12p70, IL-13, IL-1beta, IL-2, IL-4, IL-6, IL-8, TNF-alpha. The cytokines were analysed in parallel on a bead-based sandwich ELISA principle, described in detail elsewhere (225).

3.5 Assessment of fatigue symptoms

Fatigue was assessed with the Fatigue Severity Scale (FSS) that was developed in 1989 (226). The FSS is a nine-item self-report questionnaire evaluating the severity of MS fatigue symptoms. Study participants rate nine fatigue-related items on a seven-point scale with response options between “strongly disagree” [1] and “strongly agree” [7] depending on their experiences in the previous week. The total fatigue score is calculated as the mean of the summed item scores and can lay between one and seven. A score of four or above implies moderate to severe MS-related fatigue (227). 1.5 - 2.0 points were defined as the minimum detectable change respectively as a clinically important change, irrespective from time frame (228).

3.6 Assessment of depressive symptoms

Depressive symptoms were assessed with the Beck Depression Inventory-II (BDI-II) that was developed in 1961 and revised in 1996 (229). The BDI-II is a 21-item self-report inventory evaluating depressive symptoms. Individual items are rated from zero to three depending on symptom severity. Scores from 0 - 8 indicate no depression; 9 - 13 minimal depression; 14 - 19 mild depression; 20 - 28 moderate depression; and 29 - 63 indicate severe depression (230). The BDI-II was evaluated as a reliable and valid tool to detect and grade depressive symptoms in MS patients (231). Clinically relevant changes depend on baseline severity. In patients with moderate baseline severity a threshold of -3.5 points or 20% reduction of their baseline scores was associated with reported improvement. The authors note that “patients with milder symptoms require much larger reductions as percentage of their baseline to endorse improvement” (232). However, another study suggests a minimal clinically important difference of 17.5% from baseline values (233), noting the opposite: that patients with higher baseline scores require larger changes in BDI-II scores to feel better.

3.7 Assessment of cognition

Cognition was assessed with the Symbol Digit Modalities Test (SDMT) that was first published in 1982 (234). The SDMT score aims to reflect information processing speed and efficiency by performing a symbol-digit substitution task: study participants had 90 seconds to match specific symbols with assigned digits and verbally translate them by using a reference key. For every substitution of a symbol-to-digit (Figure 6), a point was given, and the number of correct substitutions was summed to the score. The SDMT has been evaluated as sensitive as well as superior to other established cognition-tools in terms of feasibility and validity. It was recommended to be used in clinical trials with MS patients (235, 236). Parmenter et al. describe a total score of 55 or lower to classify cognitive impairment in MS patients (237). A responder definition of a clinically meaningful improvement was defined as ≥ 4 points or 10% increases of the baseline score (236), respectively a stable cognitive status with < 4 points of change and cognitive deterioration with ≥ 4 points decrease (238). Another study defined a change of 3 points as a clinically meaningful change within a 96-weeks pharmaceutical intervention in MS patients (239).

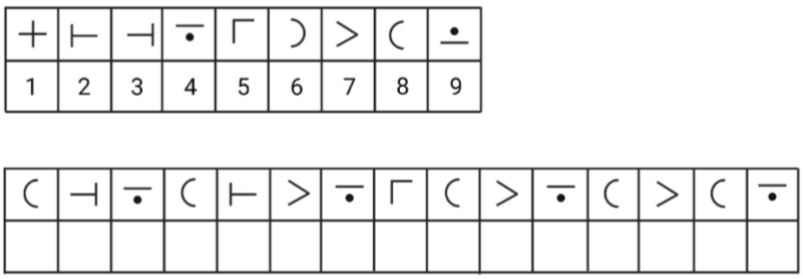


Figure 6: Symbol Digit Modalities Test excerption. Figure created with Biorender.com

3.8 Assessment of disability status

Disability was assessed with the Expanded Disability Status Scale (EDSS) (211). The EDSS scale ranges from 0 to 10 in 0.5 units with higher scores reflecting more severe disability. The scoring is carried out by neurologists during a clinical examination. The EDSS will not be analysed as an outcome parameter but will be referred to, in order to compare baseline disease severity between the groups, and for correlations respectively as a covariate in this analysis.

4 Data analysis

This analysis is based on a sub-cohort of 71 patients of the per-protocol population of the NAMS study. Descriptives are given using the mean value and standard deviation for normally distributed metric data. Skewed metric or ordinal data are presented with the median and interquartile range ((IQR): 25th and 75th percentiles). Absolute and relative frequencies were considered for count data. T-tests have been applied to detect within-group differences over time and the analysis of variance (ANOVA) to detect between-group differences, when assumptions including normal distributions of data were fulfilled. Otherwise, the non-parametric Wilcoxon test and Kruskal-Wallis tests have been applied. To investigate influencing factors for health outcomes, linear regressions were applied, based on normally distributed continuous data. The 95% confidence interval was given for unstandardized coefficients. For correlations, depending on the distribution of data, Spearman or Pearson correlations were used. All correlations were applied in all patients regardless of the interventions, except explained otherwise.

A level of significance with 5% was applied for all statistical tests. Data analysis has been conducted with SPSS (version 26) and graphics in the results section were created with GraphPad prism (version 9.0.0). This analysis is exploratory and aims to investigate secondary endpoints. No sample size calculation or associated power analysis was done in advance on this sub-cohort; hence p-values must be interpreted with caution. No complete adjustment regarding multiple testing was done. To account for missing values, adapted sample size numbers are displayed. Missing values were excluded in a pairwise manner in statistical tests.

5 Results

5.1 Baseline characteristics

There was no relevant age-difference between the interventional groups. In the SD group, there were about 10% more male participants, compared to the KD and FD group. There is a relevant gap, comparing disease duration since first manifestation vs. since diagnosis, with a longer disease duration in the KD group, when looking at disease duration since manifestation. There was no difference between the groups regarding disability status (EDSS). Half of the patients did not receive a DMT. There were no relevant baseline differences between the groups regarding blood pressure, BMI, and most metabolic parameters. There were slight differences regarding blood lipids between the groups, with the FD group showing slightly higher values of total cholesterol, LDL-cholesterol, and triglycerides, compared to the KD and SD group. At baseline, 38% of the study participants had high total cholesterol (> 200 mg/dl) (Figure 7), even though patients with clinically significant hyperlipidaemia were excluded from the study.

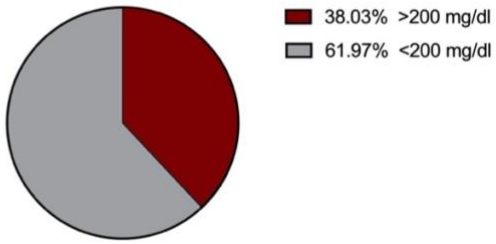


Figure 7: Prevalence of high total cholesterol (> 200 mg/dl) at baseline in the study population (n = 71)

The cognition score (SDMT score) was slightly higher in the KD group, compared to the FD and SD group. The fatigue score (FSS score) was slightly higher in the FD group, reflecting moderate fatigue (a score of four is the cut-off). The FD group showed higher median baseline values for depressive symptoms, indicating minimal depressive symptoms (a score of nine is the cut-off), compared to the KD and SD group, showing no depressive symptoms (Table 1).

Table 1: Baseline characteristics of study participants

Baseline characteristics		**Total n = 71**	**KD n = 20**	**FD n = 31**	**SD n = 20**
Age (years)	**Mean (StD)**	42 (10)	42 (11)	42 (9)	43 (10)
Gender (female)	**n (%)**	58 (82)	17 (85)	26 (84)	15 (75)
MS duration since manifestation (years)	**Mean (StD)**	9 (7)	11 (9)	8 (6)	8 (7)
MS duration since diagnosis (years)	**Median (IQR)**	3 (10 - 1)	3 (12 - 1)	4 (11 - 2)	2 (5 - 1)
Disability status (EDSS score)	**Median (IQR)**	2.0 (2.5 - 1.5)	2.0 (3.4 -1.5)	2.0 (2.5 - 1.5)	2.0 (2.5 - 1.5)
DMT (yes)	**n (%)**	37 (52)	9 (45)	18 (58)	10 (50)
Interferon beta (1a/1b)		7 (10)	3 (15)	3 (10)	1 (5)
Glatiramer acetate		7 (10)	1 (5)	2 (7)	4 (20)
Dimethyl fumarate		11 (16)	5 (25)	5 (16)	1 (5)
Fingolimod		5 (7)		5 (16)	
Teriflunomide		2 (3)			2 (10)
Natalizumab		3 (4)		2 (7)	1 (5)
Cladribine		1 (1)		1 (3)	
Immunoglobulins		1 (1)			1 (5)
Blood pressure					

Systolic (mmHg)	**Mean (StD)**	123.4 (15.7)	125.5 (18.0)	121.2 (14.9)	124.5 (14.8)
Diastolic (mmHg)	**Mean (StD)**	80.2 (10.4)	79.4 (11.8)	80.9 (11.0)	80.0 (8.3)
BMI					
BMI (kg/m^2)	**Median (IQR)**	23.7 (26.3 - 22.3)	23.8 (26.3 - 22.5)	23.6 (26.3 - 21.6)	23.9 (26.8 - 21.9)
18.0 - 24.9 / normal weight	**n (%)**	45 (63)	13 (65)	20 (65)	12 (60)
25.0 - 29.9 / overweight		19 (27)	4 (20)	8 (26)	7 (35)
> 30 / obese		7 (10)	3 (15)	3 (10)	1 (5)
Metabolism					
Blood glucose (mg/dl)	**Mean (StD)**	83.5 (8.4)	82.8 (8.9)	82.4 (8.1)	85.6 (8.4)
Insulin (mg/dl)	**Median (IQR)**	7.1 (10.1 - 5.5)	6.7 (10.0 - 6.1)	7.4 (9.6 - 4.6)	7.8 (10.9 - 5.4)
Total cholesterol (mg/dl)	**Median (IQR)**	195.5 (213.5 - 176.8)	186.0 (204.3 - 157.3)	202.0 (221.3 - 177.3)	189.0 (215.3 - 179.8)
HDL-cholesterol (mg/dl)	**Mean (StD)**	72.1 (16.3)	69.6 (15.1)	74.1 (19.2)	72.0 (13.4)
LDL-cholesterol (mg/dl)	**Median (IQR)**	123.0 (144.5 - 99.5)	120.5 (142.3 - 95.0)	127.0 (145.3 - 99.8)	110.0 (145.0 - 101.5)
Triglycerides (mg/dl)	**Median (IQR)**	80.0 (102.5 - 64.5)	75.0 (83.0 - 61.0)	91.0 (127.3 - 66.3)	78.0 (107.5 - 59.3)
Leptin (µg/l)	**Median (IQR)**	9.4 (14.3 - 4.4)	9.1 (17.0 - 3.4)	9.6 (15.0 - 4.8)	8.1 (11.9 - 4.4)
Adiponectin (µg/ml)	**Median (IQR)**	8.4 (11.4 - 5.6)	8.9 (12.9 - 5.9)	8.7 (11.3. - 5.2)	7.1 (10.1 - 5.0)
Neuropsychiatric assessment					
Fatigue (FSS score)	**Mean (StD)**	3.7 (1.7)	3.2 (1.5)	4.0 (1.7)	3.6 (1.8)
Portion of moderately to severely fatigued patients (FSS score ≥ 4)	**n (%)**	26 (37)	4 (20)	14 (45)	8 (40)

Depressive symptoms (BDI-II score)	**Median (IQR)**	7.0 (12.0 - 3.8)	5.5 (9.8 - 2.3)	9.0 (13.0 - 5.8)	6.5 (14.3 - 2.3)
Portion of minimally to severely depressed patients (BDI-II score ≥ 9)	**n (%)**	31 (44)	6 (30)	16 (52)	9 (45)
Cognition (SDMT score)	**Mean (StD)**	57.0 (11.2)	58.0 (13.6)	56.3 (10.0)	57.0 (10.6)
Portion of cognitively impaired patients (SDMT score ≤ 55)	**n (%)**	32 (44)	8 (40)	14 (45)	10 (50)

BDI-II = Beck Depression Inventory-II, BMI = body mass index, DMT = disease modifying therapy, EDSS = Expanded Disability Status Scale, FD = fasting diet, FSS = Fatigue Severity Scale, HDL = high-density lipoprotein, IQR = interquartile range, KD = ketogenic diet, LDL = low-density lipoprotein, MS = multiple sclerosis, SD = standard healthy diet, SDMT = Symbol Digit Modalities Test, StD = standard deviation

5.2 Dietary adherence

5.2.1 Protocol deviations and adverse events

Adherence differed between the groups: twelve participants in the KD group deviated from the study protocol, nine in the SD group and one participant in the FD group (Figure 5). Number of adverse events differed between the groups, with 52 in the SD, 64 in the KD group and 100 in the FD group. Adverse events were mostly mild. There was one unrelated serious adverse event in the FD group (hospitalisation). The most frequent symptoms were the common cold, especially in the SD group (13 cases vs. 9 in the KD and 7 in FD group). Nausea and herpes labialis (occurrence mainly in one patient) as well as vertigo were frequent in the FD group and headache in the KD group. All adverse events were transient. Generally, infections were more frequent in the SD, compared to the KD and FD group. Pain and perceptual disturbances were frequent in the KD and FD group compared to almost non-existent in the SD group. Vision impairment only occurred in the FD group. Depressive symptoms occurred in the KD and SD, but not in the FD group. A detailed overview of all adverse events can be found in Table 9 in the appendix.

5.2.2 Ketone elevation

Adherence in the KD group was assessed by monitoring blood ketone bodies. Plasma blood ketones (BHB) were significantly higher after nine months compared to baseline in the KD group: interquartile range was within a physiological range, between 0.3 and 0.9 mmol/l with a median at 0.7 mmol/l. In the SD and FD group median BHB concentrations did not change as expected and remained below 0.1 mmol/l (Figure 8). Twelve of twenty patients (60%) in the KD group achieved a relevant ketosis – defined as BHB concentrations ≥ 0.5 mmol/l – at the nine-month study visit.

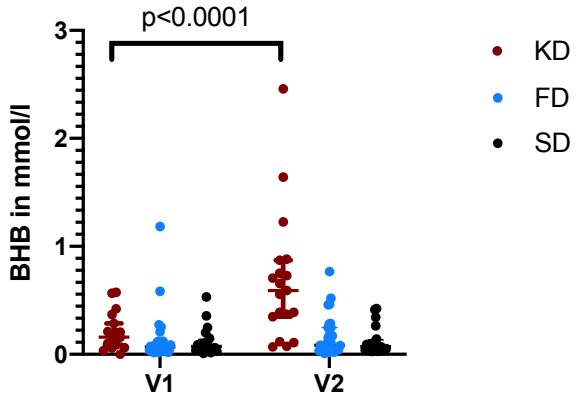


Figure 8: Plasma beta-hydroxybutyrate (BHB) concentrations before (V1) and after nine months (V2) of a ketogenic diet (KD, n = 19), fasting diet (FD, n = 30) and standard healthy diet (SD, n = 20). Single values, median and interquartile range. P-value by Wilcoxon test.

BHB values in the KD group after nine months were positively associated with leptin, but not with leptins counterpart adiponectin. Further, BHB values were positively correlated with body fat mass, but not with body weight (Figure 9).

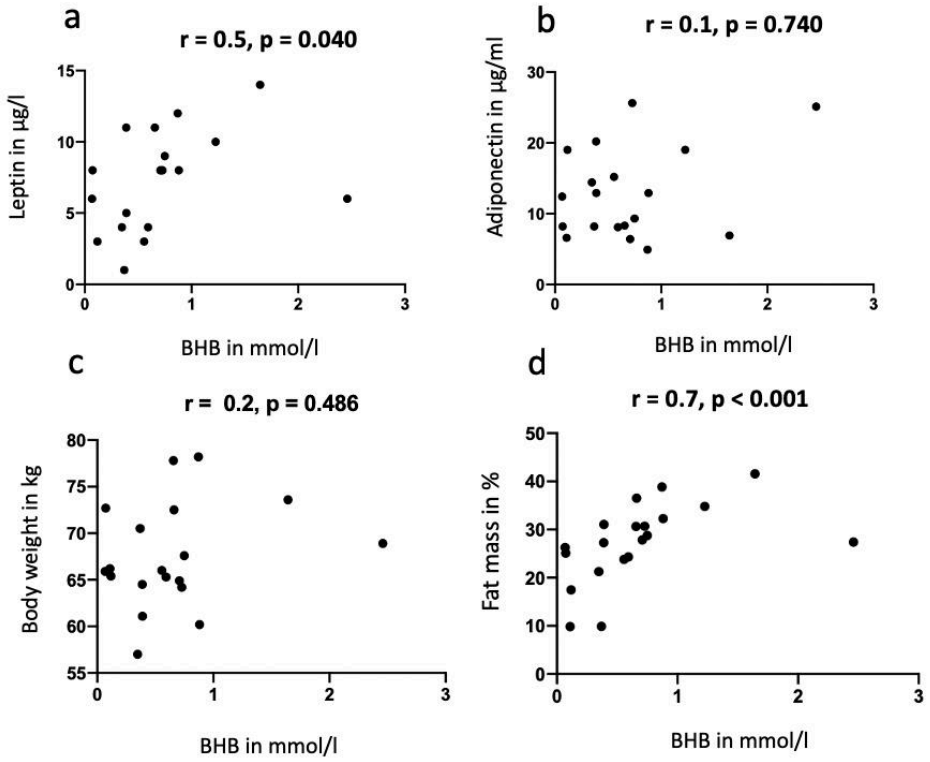


Figure 9: Correlations of plasma beta-hydroxybutyrate (BHB) with a) leptin, b) adiponectin, c) body weight, and d) fat mass after nine months in the ketogenic group (n = 20). Single values. P-values by Spearman correlations, r = correlation coefficient.

Further, bivariate Spearman correlations have shown that blood glucose concentrations (r = -0.528, p = 0.017), but not insulin concentrations (r = 0.381, p = 0.108), were inversely correlated with BHB concentrations after nine months of a KD.

5.3 Effects of the diets on BMI, body composition and metabolism

While the median BMI in the SD group slightly increased, the BMI decreased significantly in the FD and KD group (Table 2). Fifty percent of participants in the SD, 60% in the KD and 67% in the FD group experienced weight loss (= more than 1 kg reduction of weight during nine months). Weight loss was highest in the KD group with a mean (and standard deviation) of 6.9 (5.6) kg, followed by the FD group with 5.6 (5.0) kg and SD group with 2.9 (1.1) kg. In the KD and FD group, percentage of body fat mass decreased significantly (Table 2) while there was no change in the SD group. However, patients in the KD and FD group started with slightly higher baseline values

compared to the SD (29.6% and 30.0% vs. 27.4% in the SD group). In the KD and FD group 85% and 84% were female vs. only 75% in the SD group (Table 1), which could have contributed to the overall lower body fat mass in the SD group at baseline. Mean change (and standard deviation) of body fat mass in the KD and FD group was -2.7% (2.9) and -2.0% (3.6) compared to -0.5% (2.5) in the SD group. However, these between-group differences missed statistical significance (Figure 10).

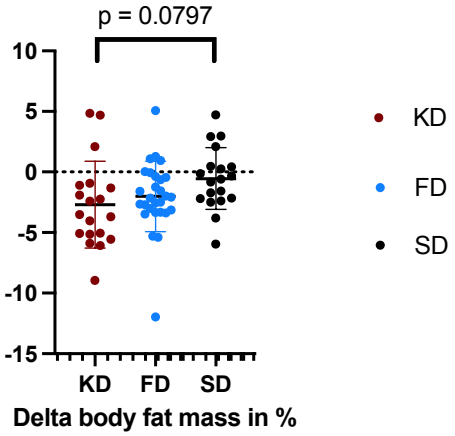


Figure 10: Delta of body fat mass in % between baseline and after nine months of a ketogenic diet (KD, n = 19), fasting diet (FD, n = 30) and standard healthy diet (SD, n = 20), assessed with bioimpedance analysis. Lower values indicate greater loss of body fat mass. Single values, mean value, and standard deviation. P-value by one-way analysis of variance.

Fat-free mass decreased only in the FD group (Table 2), while remaining stable in the KD and SD group.

To evaluate to what extent the KD and FD influenced metabolism, the effects on blood glucose, blood lipids and adipokines were assessed. Comparing the overnight-fasted glucose concentrations at baseline with the concentrations after nine months, there were no significant within-group changes in the KD and FD group, but a statistically significant increase in the SD group (Table 2).

A subgroup of 15 study participants agreed on wearing a continuous glucose monitoring (CGM) sensor within the first 14 days of the dietary interventions to assess short-term effects of the diets. The CGM showed distinctive lower median glucose values in the KD and FD group compared to the SD group, with a significant between-group difference comparing FD and SD group, and a trend to significance comparing KD and SD (Figure 11a). It is important to note that the CGM sensor was applied during the actual fasting week, while the blood samples for assessing long-term blood glucose

concentrations (Figure 11b) (Table 2) were collected three months after the last fasting week, at the nine-month study visit. Comparing the deltas of these blood glucose concentrations, a slight decrease of blood glucose in the KD and FD group can be observed, while there was an increase in the SD group (Figure 11b). In line with the CGM results, the deltas also show a significant between-group difference comparing FD and SD group (Figure 11b).

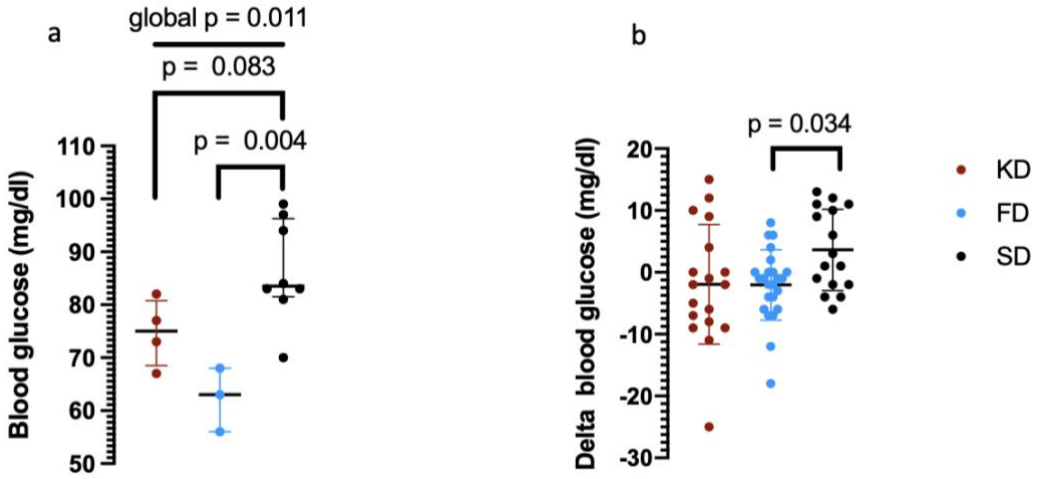


Figure 11: a) Median glucose concentrations in the interstitial obtained from a subgroup of 15 patients with a continuous glucose monitoring sensor that was worn the first 14 days of a ketogenic diet (KD, n = 4), fasting diet (FD, n = 3), and standard healthy diet (SD, n = 8). Single values, group median value and interquartile range. Global p-value by Kruskal-Wallis test. P-values of pairwise comparison after Bonferroni correction for multiple tests. **b)** Delta of glucose concentrations between baseline and after nine months of a ketogenic diet (KD, n = 18), fasting diet (FD, n = 24) and standard healthy diet (SD, n = 16). Lower values indicate a decrease, higher values an increase of glucose concentrations over time. Single values, mean value, and standard deviation. Adjusted p-value by one-way analysis of variance, Tukey's multiple comparison test.

A linear regression (adjusted R square: 0.232, p (ANOVA): 0.016) showed that the baseline BMI (B = 0.921, p = 0.043), and proteasomal activity (B = 0.055, p = 0.005) were predictive for glucose concentrations at nine months. IL-13 (B = 2.341, p = 0.073), an anti-inflammatory cytokine, which is involved in glucose metabolism, was borderline-significantly predictive for glucose concentrations at nine months.

Total cholesterol and LDL-cholesterol significantly decreased in the FD and SD group, and non-significantly in the KD group. There was a slight increase in HDL-cholesterol in the KD and no change in the FD group. In the SD group, HDL-cholesterol unfavorably significantly decreased (Table 2). Comparing the mean changes in HDL-cholesterol between baseline and after nine months, there is a significant between-

group difference comparing the KD and SD group (Figure 12), mostly due to the unfavourable decrease of HDL-cholesterol in the SD group.

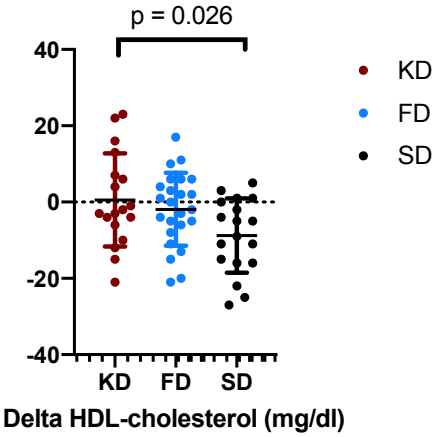


Figure 12: Delta of HDL-cholesterol concentrations between baseline and after nine months of a ketogenic diet (KD, n = 18), fasting diet (FD, n = 24) and standard healthy diet (SD, n = 18). Lower values indicate a greater decrease of HDL-cholesterol. Single values, mean value, and standard deviation. Adjusted p-value according to one-way analysis of variance including Tukey's multiple comparison test. HDL = high-density lipoprotein.

Triglycerides significantly decreased in the KD group and borderline-significantly in the FD group, while there were no changes in the SD group. Triglycerides were strongly correlated with body weight (r = 0.32, p = 0.007) after nine months in a Spearman correlation, whereas total cholesterol, LDL-cholesterol and HDL-cholesterol were not. KD and FD have shown significant effects on the inflammatory adipokines leptin and adiponectin. Pro-inflammatory leptin decreased significantly in the KD and borderline-significantly in the FD group, while there was no change in the SD group. Adiponectin significantly increased in the KD and FD group and did not change in the SD group (Table 2). There was a strong positive correlation between leptin and body fat mass at nine months (Spearman correlation, r = 0.793, p < 0.001). Insulin did not relevantly change in any of the groups. The acute-phase protein haptoglobin decreased equally and significantly in all three groups. All parameters are displayed in Table 2.

Table 2: Changes of nutritional status - BMI, body composition, blood lipids, metabolic parameters

Nutritional status	KD	FD	SD
Mean (StD) / Median (IQR)			

Paired-sample t-test[a] / related-samples Wilcoxon test[b]									
	V1	**V2**	**p**	**V1**	**V2**	**p**	**V1**	**V2**	**p**
BMI (kg/m²)[b]	23.8 (26.3 - 22.5) n = 19	22.9 (24.1 - 22.2) n = 19	**0.005**	23.6 (26.3 - 21.6) n = 31	23.1 (24.7 - 21.0) n = 31	**<0.001**	23.9 (26.8 - 21.9) n = 20	24.4 (26.6 - 22.3) n = 20	0.279
Body fat mass (%)[a]	29.6 (9.3) n = 19	27.3 (8.3) n = 19	**0.004**	30.0 (6.4) n = 28	27.9 (5.4) n = 28	**0.001**	27.4 (7.6) n = 19	27.0 (6.7) n = 19	0.366
Fat-free mass (kg/m²)[b]	17.0 (17.9 - 16.3) n = 19	16.9 (17.4 - 16.0) n = 20	0.376	17.0 (18.2 - 16.2) n = 28	16.2 (17.5 - 15.7) n = 31	**0.040**	17.3 (19.8 - 15.8) n =19	17.0 (19.7 - 16.3) n = 20	0.936
Total cholesterol (mg/dl)[b]	186.0 (204.3 - 157.3) n = 18	175.5 (206.3 - 160.0) n = 18	0.586	202.0 (219.5 - 178.5) n = 24	189.5 (213.0 - 168.0) n = 24	**0.012**	189.0 (212.0 - 180.0) n = 18	172.0 (189.0 - 156.0) n = 18	**0.004**
HDL-cholesterol (mg/dl)[a]	69.6 (15.1) n = 18	73.7 (23.7) n = 18	0.849	74.1 (19.2) n = 24	72.2 (18.0) n = 24	0.347	72.2 (13.4) n = 18	63.2 (9.2) n = 18	**0.001**
LDL-cholesterol (mg/dl)[b]	120.5 (141.0 - 95.0) n = 18	111.5 (131.0 - 98.0) n = 18	0.647	127.0 (144.5 - 100.5) n = 24	109.5 (128.5 - 91.0) n = 24	**0.003**	110.0 (145.0 - 103.0) n = 18	102.0 (123.0 - 93.0) n = 18	**0.008**
Triglycerides (mg/dl)[b]	75.0 (82.0 - 64.0) n = 18	59.5 (79.0 - 49.0) n = 18	**0.021**	91.0 (124.5 - 66.5) n = 24	79.5 (109.0 - 64.0) n = 24	0.061	78.0 (103.0 - 60.0) n = 18	78.5 (90.0 - 68.0) n = 18	0.177
Glucose (mg/dl)[a]	82.8 (8.9) n = 18	80.8 (6.5) n = 18	0.405	82.4 (8.1) n = 24	80.4 (9.0) n = 24	0.093	85.6 (8.9) n = 16	89.3 (6.5) n = 16	**0.044**
Leptin (µg/l)[b]	9.5 (17.0 - 3.6) n = 18	7.6 (9.9 - 4.2) n = 18	**0.024**	9.6 (14.8 - 5.0) n = 29	5.9 (12.6 - 4.4) n = 29	0.063	8.1 (11.8 - 4.6) n = 20	7.7 (11.9 - 4.4) n = 20	0.911
Adiponectin (µg/ml)[b]	8.9 (12.5 - 6.3) n = 19	12.4 (17.1 - 8.2) n = 19	**0.041**	8.7 (11.3 - 5.4) n = 30	10.1 (11.8 - 6.5) n = 30	**0.012**	7.1 (10.0 - 5.3) n = 20	7.8 (9.9 - 6.0) n = 20	0.881
Insulin (mg/dl)[b]	6.5 (9.1 - 6.2) n = 19	7.1 (10.5 - 5.9) n = 19	0.717	7.4 (9.3 - 4.8) n = 31	6.8 (8.9 - 4.6) n = 31	0.126	8.5 (10.8 - 5.5) n = 19	8.2 (11.0 - 5.5) n = 19	0.777
Haptoglobin (g/l)[a]	74.9 (5.6) n = 17	71.8 (5.1) n = 17	**0.040**	74.1 (5.1) n = 22	71.9 (3.5) n = 22	**0.002**	75.2 (3.1) n = 18	71.5 (3.1) n = 18	**0.001**

BMI = body mass index, FD = fasting diet, HDL = high-density lipoprotein, IQR = interquartile range, KD = ketogenic diet, LDL = low-density lipoprotein, SD = standard healthy diet, StD = standard deviation, V1 = baseline, V2 = after nine months

5.4 Effects of the diets on plasma micronutrients and oxidative stress

The antioxidant cryptoxanthin, a carotenoid found in fruits and vegetables, decreased in the KD group, and increased in the FD group. Lutein/zeaxanthin, an antioxidative secondary phytochemical and marker for consumption of vegetables, increased in the KD and FD group, but did not change in the SD group.

3-methylhistidine, a marker for protein-turnover, decreased in the FD group, only. Plasma 3-methylhistidine after nine months was positively correlated with fat-free mass in a Spearman correlation (r = 0.288, p = 0.015) and with body weight (r = 0.287, p = 0.016) at nine months in all patients. Further, after nine months, 3-methylhistidine was positively associated with baseline proteasomal activity (r = 0.234, p = 0.064). 3-methylhistidine at nine months was further correlated with leptin (r = 0.229, p = 0.058), LDL-cholesterol (r = 0.281, p = 0.018), total cholesterol (r = 0.262, p = 0.028) and the oxidative stress marker MDA at nine months (r = 0.313, p = 0.008) in Spearman correlations in all patients.

MDA and plasma protein concentrations increased in the SD group only and remained stable in the KD and FD group. Antioxidative gamma-tocopherol increased, while alpha-tocopherol decreased in the SD group only. MDA (r = 0.301, p = 0.011) and plasma protein concentrations (r = 0.222, p = 0.063) at baseline correlated positively with the EDSS disability score at baseline in Spearman correlations in all patients. All plasma micronutrients and markers of oxidative stress are displayed in Table 3.

Table 3: Changes of plasma micronutrients and oxidative stress

Micronutrients, Oxidative stress	**KD n = 20**			**FD n = 31**			**SD n = 20**		
Mean (StD) / Median (IQR) Paired-sample t-test[a] / related-samples Wilcoxon test[b]									
	V1	**V2**	**p**	**V1**	**V2**	**p**	**V1**	**V2**	**p**
Cryptoxanthin (µmol/l)[b]	0.23 (0.36 - 0.18)	0.16 (0.24 - 0.11)	**0.004**	0.25 (0.31 - 0.18)	0.31 (0.38 - 0.21)	**0.041**	0.25 (0.33 - 0.16)	0.23 (0.35 - 0.17)	0.765
Lutein/zeaxanthin (µmol/l)[b]	0.34 (0.38 - 0.25)	0.37 (0.47 - 0.24)	**0.012**	0.30 (0.39 - 0.22)	0.35 (0.44 - 0.27)	**0.009**	0.29 (0.43 - 0.23)	0.33 (0.43 - 0.27)	0.185
Alpha-carotene (µmol/l)[b]	0.15 (0.19 - 0.09)	0.08 (0.15 - 0.06)	**0.005**	0.13 (0.20 - 0.10)	0.15 (0.22 - 0.10)	0.624	0.17 (0.24 - 0.11)	0.17 (0.30 - 0.11)	0.550
Beta-carotene (µmol/l)[b]	0.64 (0.96 - 0.41)	0.52 (1.00 - 0.38)	0.086	0.65 (0.89 - 0.48)	0.63 (1.00 - 0.50)	0.210	0.93 (1.07 - 0.56)	0.92 (1.16 - 0.66)	0.737
Lycopene (µmol/l)[b]	0.52 (0.65 - 0.45)	0.51 (0.71 - 0.33)	0.881	0.58 (0.77 - 0.44)	0.58 (0.78 - 0.45)	0.860	0.58 (0.70 - 0.43)	0.51 (0.70 - 0.42)	0.911

Vitamin D (n/M)[b]	85.25 (115.10 - 69.50)	98.25 (134.30 - 78.55)	0.332	98.70 (127.05 - 66.00)	100.00 (132.50 - 78.80)	0.739	101.10 (119.20 - 83.50)	102.90 (119.40 - 85.50)	0.240
Retinol (µmol/l)[a]	1.55 (0.25)	1.36 (0.24)	**0.003**	1.80 (0.43)	1.76 (0.38)	0.425	1.72 (0.37)	1.73 (0.30)	0.821
Gamma-tocopherol (µmol/l)[b]	2.76 (3.23 - 1.78)	2.16 (3.18 - 1.82)	0.627	2.01 (2.65 - 1.72)	2.38 (2.93 - 1.93)	0.248	2.10 (2.86 - 1.73)	2.44 (2.90 - 2.07)	**0.040**
Alpha-tocopherol (µmol/l)[b]	31.69 (35.23 - 29.96)	30.46 (35.06 - 27.50)	0.709	32.02 (37.22 - 28.46)	32.85 (36.69 - 28.77)	0.638	31.12 (34.36 - 27.61)	28.71 (30.71 - 26.37)	**0.006**
3-methylhistidine (µmol/l)[b]	2.67 (3.19 - 2.22)	2.52 (3.26 - 1.99)	0.709	2.92 (3.36 - 2.33)	2.53 (3.10 - 2.02)	**0.013**	2.80 (3.48 - 2.15)	2.67 (3.58 - 2.22)	0.575
1-methylhistidine (µmol/l)[b]	0.64 (2.07 - 0.41)	1.22 (3.67 - 0.61)	0.179	0.68 (2.13 - 0.34)	1.13 (3.00 - 0.26)	0.597	0.84 (2.71 - 0.47)	2.08 (4.03 - 0.32)	0.526
MDA (µmol/l)[b]	0.50 (0.59 - 0.41)	0.59 (0.78 - 0.42)	0.117	0.55 (0.65 - 0.45)	0.60 (0.65 - 0.50)	0.465	0.53 (0.71 - 0.43)	0.66 (1.01 - 0.54)	**0.001**
Protein (mg/ml)[b]	98.00 (106.75 - 93.25)	99.10 (103.20 - 93.70)	0.681	96.00 (100.45 - 90.40)	96.30 (99.40 - 91.25)	0.695	96.35 (99.60 - 90.30)	99.40 (105.50 - 94.70)	**0.006**
Proteincarbonyl (nmol/mg)[a]	1.35 (0.16)	1.41 (0.13)	0.097	1.35 (0.17)	1.33 (0.11)	0.250	1.29 (0.12)	1.30 (0.09)	0.384
3-Nitrotyrosin (pmol/mg)[b]	5.35 (7.56 - 3.09)	6.04 (9.39 - 2.48)	0.502	5.43 (10.61 - 3.40)	5.95 (11.56 - 3.95)	0.953	4.36 (6.20 - 2.25)	4.79 (7.02 - 2.75)	0.198
Proteasomal activity [nM/(mg*min)][b]	198.89 (224.70 - 157.35)	176.32 (287.54 - 135.15)	0.877	178.95 (226.73 - 152.29)	181.04 (248.55 - 145.86)	0.820	199.84 (225.09 - 166.36)	167.19 (197.77 - 147.52)	0.159

FD = fasting diet, IQR = interquartile range, KD = ketogenic diet, MDA = malondialdehyde, SD = standard healthy diet, StD = standard deviation, V1 = baseline, V2 = after nine months

5.5 Effects of the diets on plasma cytokines

To assess the effects of the dietary interventions on peripheral inflammation, plasma cytokine concentrations were analysed at baseline and after nine months. The dietary interventions did not show major significant effects on most of the anti- and pro-inflammatory cytokines. Green-marked cytokines represent rather anti-inflammatory cytokines, yellow-marked cytokines rather pro-inflammatory ones. There was a significant decrease of IL-4 and IL-13 in the KD group. According to a Kruskal-Wallis test, the changes of IL-13 between the groups are statistically significant (Figure 13). IL-7 and VEGF-A decreased significantly in the SD group and the two cytokines were strongly correlated in a Spearman correlation ($r = 0.570$, $p < 0.001$). There was a slight trend of a decrease of IL-7 in the KD group. There were within-group changes regarding IL-16 and IL-12 in all three groups and decreases of pro-inflammatory IL-1a in the KD group (Figure 13), however, all non-significant. Interestingly, there were no statistically significant changes in the FD group. All plasma cytokine changes are displayed in Table 4.

Table 4: Changes of plasma cytokines

Plasma cytokines	KD n = 20			FD n = 31			SD n = 20		
	Median (IQR) related-samples Wilcoxon test								
in pg/ml	V1	V2	p	V1	V2	p	V1	V2	p
IFN-γ	2.334 (3.396 - 1.750)	2.967 (4.038 - 1.896)	0.243	3.477 (4.533 - 2.599)	4.014 (5.199 - 2.857)	0.153	3.867 (5.551 - 2.304)	3.845 (6.158 - 2.722)	1.000
IL-2	0.141 (0.251 - 0.064)	0.144 (0.213 - 0.067)	0.861	0.171 (0.243 - 0.127)	0.119 (0.248 - 0.058)	0.184	0.349 (0.416 - 0.094)	0.149 (0.280 - 0.069)	0.158
IL-4	0.010 (0.013 - 0.005)	0.006 (0.010 - 0.002)	**0.007**	0.007 (0.013 - 0.003)	0.008 (0.011 - 0.006)	0.986	0.011 (0.014 - 0.008)	0.009 (0.011 - 0.005)	0.109
IL-6	0.319 (0.442 - 0.232)	0.338 (0.516 - 0.231)	1.000	0.558 (0.877 - 0.339)	0.713 (0.870 - 0.326)	0.230	0.508 (0.707 - 0.233)	0.499 (0.769 - 0.386)	1.000
IL-8	2.667 (3.103 - 1.894)	2.902 (3.151 - 2.144)	0.520	2.164 (3.437 - 1.885)	2.561 (3.105 - 1.972)	0.813	2.818 (4.588 - 2.114)	2.061 (2.785 - 1.956)	0.070
IL-13	0.758 (1.614 - 0.272)	0.481 (0.724 - 0.250)	**0.008**	0.707 (0.829 - 0.371)	0.627 (0.955 - 0.368)	0.407	0.612 (1.473 - 0.317)	0.439 (1.532 - 0.119)	0.173
TNF-α	2.427 (2.810 - 1.050)	1.917 (2.573 - 1.393)	0.107	1.483 (2.588 - 1.165)	1.615 (2.497 - 1.207)	0.734	1.870 (2.624 - 1.467)	1.953 (2.593 - 1.373)	0.936
IL-1b	0.161 (0.302 - 0.039)	0.110 (0.234 - 0.031)	0.285	0.130 (0.285 - 0.359)	0.173 (0.475 - 0.025)	0.925	0.098 (0.259 - 0.034)	0.039 (0.124 - 0.021)	0.173
IL-10	0.228 (0.335 - 0.189)	0.219 (0.265 - 0.184)	0.573	0.188 (0.295 - 0.122)	0.195 (0.309 - 0.138)	0.871	0.236 (0.363 - 0.172)	0.234 (0.276 - 0.148)	1.000
IL-12p70	0.128 (0.120 - 0.073)	0.101 (0.204 - 0.053)	0.845	0.106 (0.166 - 0.064)	0.100 (0.185 - 0.052)	0.572	0.157 (0.242 - 0.069)	0.090 (0.248 - 0.061)	0.420
GM-CSF	0.295 (0.407 - 0.199)	0.284 (0.386 - 0.206)	0.334	0.242 (0.312 - 0.192)	0.244 (0.351 - 0.206)	0.572	0.265 (0.365 - 0.206)	0.252 (0.334 - 0.206)	0.396
IL-12/IL-23p40	104.368 (115.611 - 64.666)	96.136 (108.128 - 56.572)	0.243	92.857 (156.001 - 61.496)	93.330 (147.172 - 59.901)	0.280	106.689 (136.733 - 77.851)	88.357 (125.190 - 77.035)	0.334
IL-15	1.975 (2.188 - 1.535)	1.940 (2.116 - 1.528)	0.604	1.974 (2.298 - 2.496)	1.959 (2.496 - 1.586)	0.754	2.067 (2.454 - 1.458)	2.068 (2.346 - 1.490)	0.421
IL-16	311.297 (344.289 - 235.885)	309.694 (377.555 - 269.912)	0.658	287.823 (324.508 - 250.781)	288.034 (324.087 - 229.060)	0.517	328.397 (405.697 - 275.645)	326.646 (436.852 - 276.861)	0.841
IL-17A	1.192 (1.404 - 1.049)	1.154 (1.304 - 1.015)	0.546	1.192 (1.387 - 1.065)	1.241 (1.490 - 1.102)	0.262	1.243 (1.550 - 1.154)	1.215 (1.366 - 1.068)	0.314
IL-1a	0.585 (7.501 - 0.145)	0.359 (1.237 - 0.069)	0.285	1.502 (5.675 - 0.437)	0.583 (1.050 - 0.245)	0.182	2.342 (4.050 - 0.633)	0.735 (2.118 - 0.129)	0.169
IL-5	0.420 (0.635 - 0.268)	0.398 (0.481 - 0.278)	0.778	0.373 (0.471 - 0.294)	0.390 (0.545 - 0.256)	0.861	0.361 (0.556 - 0.311)	0.378 (0.465 - 0.332)	0.936
IL-7	3.605 (5.166 - 2.290)	2.623 (3.739 - 1.757)	0.099	2.580 (5.444 - 2.329)	3.245 (4.423 - 2.215)	0.845	3.637 (5.024 - 2.606)	2.866 (4.390 - 1.844)	**0.040**
TNF-ß	0.163 (0.186 - 0.122)	0.142 (0.162 - 0.121)	0.355	0.130 (0.160 - 0.118)	0.141 (0.153 - 0.120)	0.688	0.147 (0.196 - 0.129)	0.142 (0.176 - 0.128)	0.446

VEGF-A	20.666 (34.306 - 17.692)	20.480 (33.185 - 18.663)	0.494	25.062 (40.080 - 16.826)	27.537 (38.773 - 15.557)	0.910	30.351 (39.811 - 21.205)	25.865 (34.756 - 13.905)	**0.027**

FD = fasting diet, GM-CSF = granulocyte macrophage colony-stimulating factor, IFN = interferon, IL = interleukin, IQR = interquartile range, KD = ketogenic diet, SD = standard healthy diet, TNF = tumor necrosis factor, VEGF = vascular endothelial growth factor, V1 = baseline, V2 = after nine months

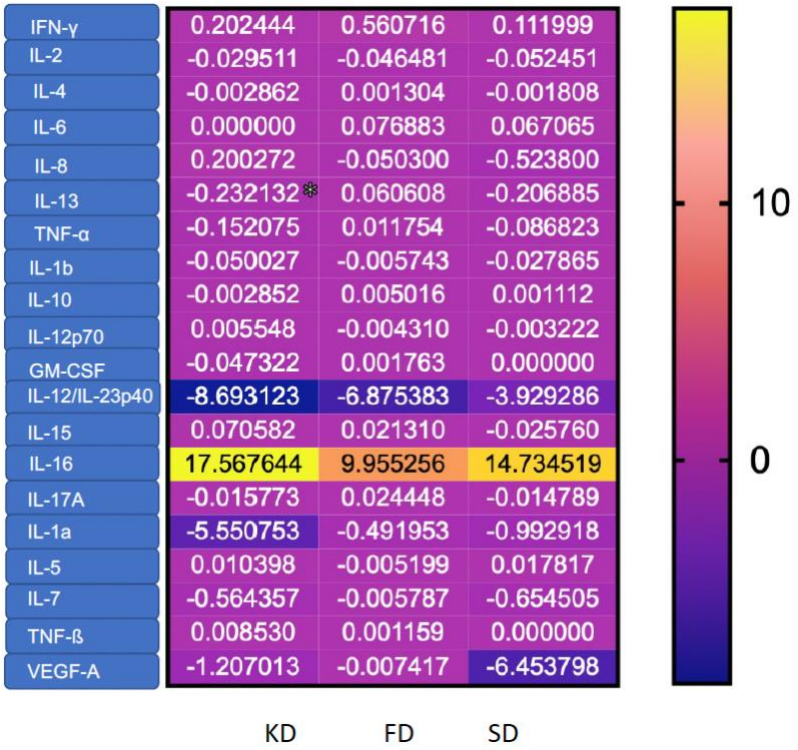


Figure 13: Heatmap presenting deltas of plasma cytokine concentrations (in pg/ml) between baseline and after nine months of a ketogenic diet (KD, n = 20), fasting diet (FD, n = 31) and standard healthy diet (SD, n = 20) with a significant difference (*, p = 0.005) between the KD vs. FD group regarding IL-13. P-value by Kruskal-Wallis test. GM-CSF = granulocyte macrophage colony-stimulating factor, IFN = interferon, IL = interleukin, TNF = tumor necrosis factor, VEGF = vascular endothelial growth factor

5.6 Effects of the diets on neuropsychiatric outcomes

5.6.1 Fatigue symptoms

When comparing the mean fatigue scores, there was no significant difference within any of the groups after nine months (Figure 14). Comparing the deltas of fatigue scores between the groups, no significant differences were seen either according to a one-way ANOVA (p = 0.402).

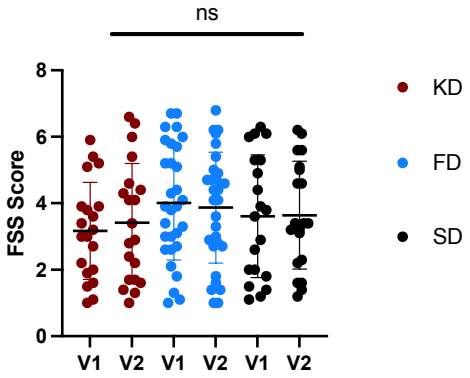


Figure 14: Fatigue scores according to Fatigue Severity Scale (FSS) at baseline (V1) and after nine months (V2) of a ketogenic diet (KD, n = 20), fasting diet (FD, n = 29) and standard healthy diet (SD, n = 20). Higher values at V2 indicate deteriorated fatigue symptoms. Single values, mean value, and standard deviation. Paired sample t-test. Ns = non-significant.

When analyzing only patients who had moderate to severe fatigue at baseline (FSS score at baseline ≥ 4), there was a trend for improved fatigue in the FD group (Figure 15a). The strongly reduced sample size, however, needs to be considered here (KD group, n = 4; SD group, n = 8), which also emphasizes that the majority of patients in the KD and SD group were not fatigued at baseline, compared to the FD group (n = 14). In contrast, filtering the patients according to no or mild fatigue symptoms at baseline (FSS score at baseline < 4), there was no relevant change observed after nine months in the KD and FD group, but a significant increase of the FSS score in the SD group. This reflects a deterioration of fatigue symptoms in the SD group, even though the mean value remains under the cut-off of 4 (Figure 15b).

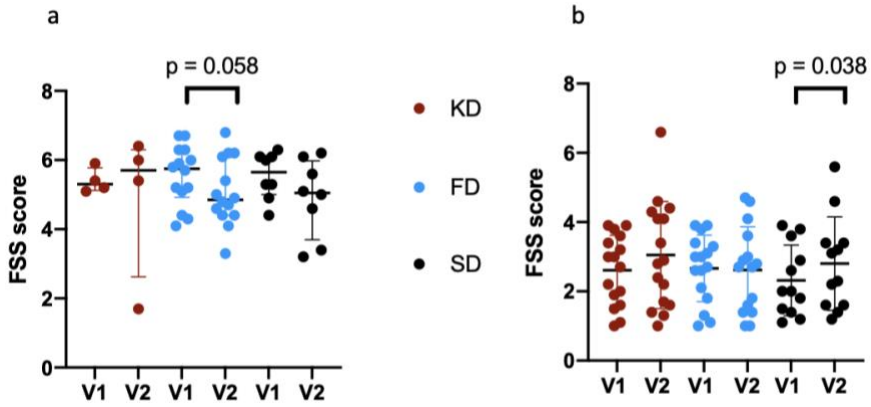


Figure 15: a) Fatigue scores of patients reporting moderate to severe fatigue symptoms (FSS scores ≥ 4) at baseline (V1) and their fatigue scores after nine months (V2) of a ketogenic diet (KD, n = 4), fasting diet (FD, n = 14) and standard healthy diet (SD, n = 8). Lower values at V2 indicate improvement. Single values, median and interquartile range. P-value by related-samples Wilcoxon test. **b)** Fatigue scores of patients reporting no or mild fatigue symptoms (FSS scores < 4) at baseline (V1) and their fatigue scores after nine months (V2) of a ketogenic diet (KD, n = 16), fasting diet (FD, n = 15) and standard healthy

diet (SD, n = 12). Higher values at V2 indicate deterioration. Single values, mean value, and standard deviation. P-value by paired-sample t-test. Fatigue scores derived by FSS (Fatigue Severity Scale questionnaire).

Predictors for fatigue symptoms

As none of the interventions showed a major effect on fatigue, regressions were applied in all study participants, regardless of the dietary intervention, to identify potential determinants. A linear regression showed that 36% of the changes in the fatigue score after nine months depend on the disability status (assessed with the EDSS), depressive symptoms (assessed with the BDI-II) and on taking DMT at baseline (Table 5).

Table 5: Linear regression analysis with the fatigue score at nine months as a dependent variable and different independent variables at baseline

Dependent variable (outcome): Fatigue symptoms at V2 (FSS score)	**Unstandardized Beta (95% CI)**	**p-value**
Independent variables (predictors):		
Depressive symptoms at V1 (BDI-II score)	0.107 (0.064 - 0.154)	**< 0.001**
Disability status at V1 (EDSS score)	0.573 (0.139 - 0.994)	**0.010**
DMT at V1 (yes)	-0.710 (-1.312 - (-0.004))	**0.039**
Cognition at V1 (SDMT score)	0.018 (-0.019 - 0.049)	0.300
Age at V1 (years)	0.014 (-0.028 - 0.055)	0.512
Disease duration since first manifestation (years)	-0.009 (-0.070 - 0.45)	0.757

Linear regression. Adjusted R-square: 0.356, p < 0.001, n = 68, BDI-II = Beck Depression Inventory-II, CI = 95% confidence interval, DMT = disease modifying therapy, EDSS = Expanded Disability Status Scale, FSS = Fatigue Severity Scale, SDMT = Symbol Digit Modalities Test, V1 = baseline, V2 = after nine months

Further regression analyses showed that neither oxidative stress parameters nor micronutrient levels, BMI, body composition or inflammatory cytokines were associated with the fatigue score (data not shown). However, adiponectin, which increased in the KD and FD group (Table 2), was inversely associated with the fatigue score after nine months. Interestingly, insulin was also inversely associated with fatigue symptoms.

Haptoglobin, which decreased in all groups (Table 2), was positively associated with the fatigue score after nine months (Table 6).

Table 6: Linear regression analysis with the fatigue score at nine months as a dependent variable and different independent variables at nine months

Dependent variable (outcome): Fatigue symptoms at V2 (FSS score)	**Unstandardized Beta (95% CI)**	**p-value**
Independent variables (determinants):		
Haptoglobin at V2 (g/l)	0.164 (0.060 - 0.269)	**0.003**
BHB at V2 (mmol/l)	0.157 (-0.901 - 1.216)	0.767
Glucose at V2 (mg/dl)	-0.006 (-0.063 - 0.052)	0.842
Adiponectin at V2 (µg/ml)	-0.075 (-0.148 - (-0.001))	**0.046**
3-methylhistidine at V2 (µmol/l)	0.228 (-0.181 - 0.637)	0.270
Leptin at V2 (µg/l)	0.040 (-0.048 - 0.127)	0.372
Insulin at V2 (mU/l)	-0.142 (-0.265 - (-0.019))	**0.024**

Linear regression. Adjusted R-square: 0.196, p = 0.006, n = 64, BHB = beta-hydroxybutyrate, CI = 95% confidence interval, FSS = Fatigue Severity Scale, V2 = after nine months

5.6.2 Depressive symptoms

Depressive symptoms were more frequent in the FD group at baseline, compared to the other groups (Figure 16a). The BDI-II score decreased significantly in the FD group after nine months (Figure 16b). The BDI-II score decreased by 1.5 points (17%) from a baseline median of 9.0 (IQR: 13.0 - 5.8) to 7.5 (IQR: 12.3 - 2.8) in the FD group and did not change in the KD group (baseline: 5.5 (IQR: 9.8 - 2.3), after nine months: 5.5 (IQR: 13.8 - 1.3)). However, the BDI-II score increased by 2 points (31%) from a baseline median of 6.5 (IQR: 14.3 - 2.3) to 8.5 (IQR: 11.0 - 2.0) in the SD group, reflecting deterioration. Comparing the median changes between the groups, the difference is borderline-significant between KD and FD group (Figure 16c). The change of the BDI-II score from a median of 9.0 to 7.5 in the FD group describes an improvement from minimal depressive symptoms to none.

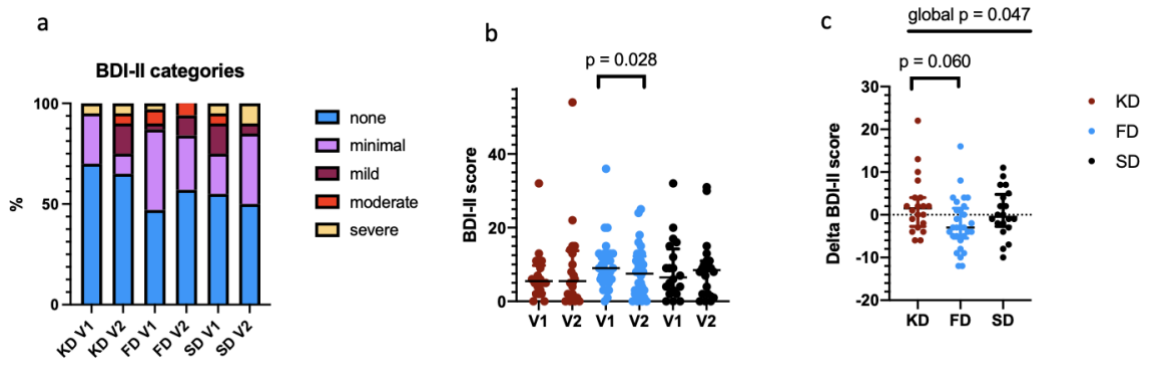


Figure 16: a) Frequency of different BDI-II severity categories of depressive symptoms at baseline (V1) and after nine months (V2) on a ketogenic diet (KD, n = 20), fasting diet (FD, n = 29) and standard healthy diet (SD, n = 20), presenting a higher frequency of depressive symptoms in the FD group at baseline. **b)** Within-group changes of depressive symptoms according to BDI-II scores between baseline (V1) and after nine months dietary interventions (V2). Decreased values at V2 in the FD group indicate improved depressive symptoms. Single values, median and interquartile range. P-value by related-samples Wilcoxon test. **c)** Delta BDI-II scores. Decreased values indicate improved depressive symptoms. Single values, median and interquartile range. Global p-value by Kruskal-Wallis test, p-value of pairwise comparison after Bonferroni correction for multiple tests. BDI-II = Beck Depression Inventory-II.

Predictors for depressive symptoms

The BDI-II score at nine months was strongly inversely correlated with plasma VEGF-A at nine months, which decreased significantly in the SD group only (Spearman correlation, r = -0.319, p = 0.009). The CGM-measured glucose concentrations and the baseline glucose concentrations were significantly (r = 0.845, p = 0.034) respectively borderline significantly (r = 0.227, p = 0.082) correlated with the BDI-II score at baseline in a Spearman correlation. However, looking at the BDI-II score after nine months, these correlations lost significance (CGM: r = 0.314, p = 0.544, baseline glucose concentrations: r = -0.029, p = 0.830). The BDI-II score was positively associated with body fat mass (r = 0.461, p = 0.010), but not with body weight at nine months in the FD group in a Pearson correlation. The strongest correlation remained between the FSS score at baseline with the BDI-II score at baseline (r = 0.509, p < 0.001) and the BDI-II score at nine months (r = 0.325, p = 0.006). All correlations were applied in all patients regardless of the interventions, except explained otherwise.

5.6.3 Cognition

Although, the mean SDMT score, reflecting cognitive processing speed, slightly increased over time in all three groups, the increase was statistically significant in the FD group only (Figure 17) with a mean increase of 2.52 points. An ANOVA revealed that this is no significant between-group effect (data not shown).

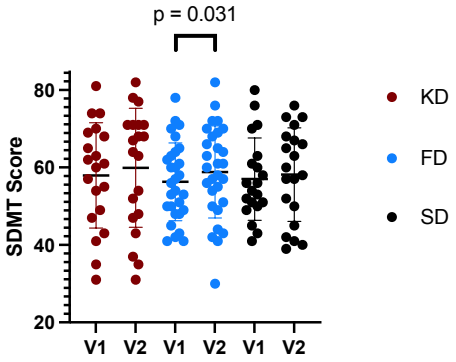


Figure 17: Changes in cognition (SDMT score) between baseline (V1) and after nine months (V2) of a ketogenic diet (KD, n = 20), fasting diet (FD, n = 29) and standard healthy diet (SD, n = 20). Increased values indicate improved cognition. Single values, mean value, and standard deviation. P-value by paired sample t-test. SDMT = Symbol Digit Modalities Test.

Not all patients achieved a relevant ketosis in the KD group – 40% of the KD group patients did not show relevantly elevated plasma BHB concentrations at the nine-month study visit (5.2.2). When considering only patients in the KD group who achieved a relevant ketosis – defined as BHB concentrations ≥ 0.5 mmol/l at nine months – the mean SDMT score increased borderline-significantly by 3.9 points from 57.7 (12.8) to 61.6 (15.1) (Figure 18).

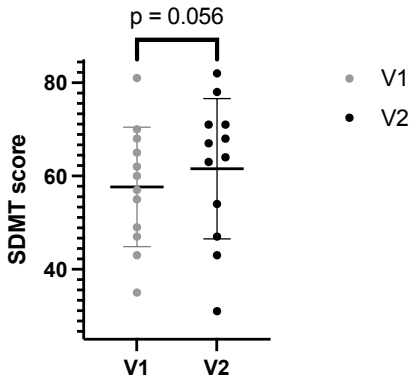


Figure 18: Changes in cognition (SDMT score) between baseline (V1) and after nine months (V2) of a ketogenic diet (KD, n = 12) in patients with successful ketosis, defined as plasma beta-hydroxybutyrate concentrations ≥ 0.5 mmol/l at V2. Increased values indicate improved cognition. Single values, mean value, and standard deviation. P-value by paired sample t-test. SDMT = Symbol Digit Modalities Test.

Micronutrients as predictors for cognition

Several baseline antioxidative micronutrients were predictive for the SDMT score after nine months. Lutein/zeaxanthin (significant) and cryptoxanthin (trend to significance) were positively correlated with cognition according to a linear regression (Table 7) and both parameters increased in the FD group (Table 3). 3-methylhistidine, which decreased in the FD group only (Table 3), showed a tendency of an inverse relationship with the cognition score. Alpha-tocopherol and alpha-carotene showed inverse correlations with cognition. Most of the correlations remained stable, when looking at the same micronutrients after nine months in a linear regression, with significant correlations of cognition and alpha-tocopherol (B = -0.651, p = 0.013, 95% CI: -1.161 - (-0.141)), lutein/zeaxanthin (B = 18.346, p = 0.032, 95% CI: 1.670 - 35.022), and alpha-carotene (B = -32.022, p = 0.016, 95% CI: -57.854 - (-6.190)).

Table 7: Linear regression analysis with the cognition score at nine months as a dependent variable and micronutrients as independent variables at baseline

Dependent variable (outcome): Cognition at V2 (SDMT score)	**Unstandardized Beta (95% CI)**	**p-value**
Independent variables (predictors):		
3-methyldistidine at V1 (µmol/l)	-2.297 (-4.942 - 0.349)	0.088
Alpha-tocopherol at V1 (µmol/l)	-1.055 (-1.590 - (-0.520))	**< 0.001**
Gamma-tocopherol at V1 (µmol/l)	-1.699 (-4.968 - 1.570)	0.303
Retinol at V1 (µmol/l)	-0.071 (-8.769 - 8.628)	0.987
Lycopene at V1 (µmol/l)	6.594 (-7.629 - 20.817)	0.358
Lutein/zeaxanthin at V1 (µmol/l)	30.254 (10.568 - 49.940)	**0.003**
Cryptoxanthin at V1 (µmol/l)	16.858 (-1.044 - 34.759)	0.064
Alpha-carotene at V1 (µmol/l)	-41.244 (-74.641 - (-7.848))	**0.016**

Linear regression. Adjusted R-square: 0.280, p < 0.001, n = 71, CI = 95% confidence interval, SDMT = Symbol Digit Modalities Test, V1 = baseline, V2 = after nine months

Metabolic predictors for cognition

To understand the role of metabolic parameters in cognition, another linear regression was applied. Baseline blood lipids and blood glucose concentrations showed significant inverse correlations with the SDMT cognition score after nine months (Table 8).

Table 8: Linear regression analysis with the cognition score at nine months as a dependent variable and metabolic parameters as independent variables at baseline

Dependent variable (outcome): Cognition at V2 (SDMT score)	**Unstandardized Beta (95% CI)**	**p-value**
Independent variables (predictors):		
Triglycerides at V1 (mg/dl)	-0.128 (-0.205 - (-0.050))	**0.002**
LDL-cholesterol at V1 (mg/dl)	-0.069 (-0.177 - 0.040)	0.210
Blood glucose at V1 (mg/dl)	-0.535 (-0.938 - (-0.132))	**0.010**
HDL-cholesterol at V1 (mg/dl)	-0.259 (-0.450 - 0.068)	**0.009**
Insulin at V1 (mU/L)	0.467 (-0.351 - 1.285)	0.258

Linear regression. Adjusted R-square: 0.253, p = 0.001, n = 60, CI = 95% confidence interval, HDL = high-density lipoprotein, LDL = low-density lipoprotein, SDMT = Symbol Digit Modalities Test, V1 = baseline, V2 = after nine months

When running a linear regression with the same independent variables from the nine-month time point, the ANOVA remains significant (p = 0.010): Blood glucose remains significantly predictive (B = -0.402, p = 0.042, 95% CI: -0.789 - (-0.015)). LDL-cholesterol at the nine-month visit inversely correlates with the SDMT score (B = -0.119, p = 0.015, 95% CI: -0.213 - (-0.024)). In contrast, in a further linear regression, body weight and body composition were not associated with the cognition score at nine months. Baseline body weight showed only a tendency for a significant inverse Pearson correlation with the cognition score at nine months (r = -0.204, p = 0.088). Inflammatory plasma cytokines did not have any predictive value regarding cognition.

6 Discussion

This sub-cohort analysis investigates effects of a KD and FD compared to a SD on neuropsychiatric outcomes and potential nutritional, inflammatory and oxidative stress mediators, over a nine-month study duration in MS patients.

6.1 Feasibility and adherence to the diets

6.1.1 Compliance and anecdotal evidence

Adherence to the diets differed between the groups, which is reflected by the study protocol deviations: KD group with twelve, SD group with nine and FD group with one (Figure 5). Reasons were lack of dietary compliance, lack of compliance to adhere to the study procedures and change of DMT. In the KD group, the radical food choice restrictions were present long-term over the whole study duration, while in the FD group the radical changes were rather short-term (every six months, seven-day fasts). Further, to follow an intermittent fasting pattern such as time-restricted eating, without changing the actual dietary habits in a restrictive way – as done in the KD group – appears more feasible and integrable into family and social life according to patient reports. Time restricted eating patterns were shown before to be more feasible in studies with MS patients and adherence to be decreased with increasing the number of daily calories that are restricted (128, 129).

In the KD group, the demanded effort to follow the diet was high and laborious: meal preparation was time-consuming, but essential, as it is quite difficult to purchase ready-made suitable ketogenic foods. Further, foods had to be researched for their carbohydrate content, weighed and calculations had to be performed to not exceed the daily carbohydrate allowance. Some patients in the KD group with families had to cook twice, which they reported to be exhaustive. Further, the social acceptability was a major challenge. Patients in the KD group explained that they felt a social pressure to justify their low-carbohydrate meal choices in groups and felt excluded at work and social meetings when mostly carbohydrate-rich food was offered. Patients in the KD group also reported to experience increased mental load due to the dietary restrictions. In the SD group, the opposite seemed to be the case. Since many participants already had a healthy habitual diet, they perceived the intervention as rather insufficient. Hence, participants in the SD group were looking for more options to optimize their

diets, e.g., with nutritional supplements or adding intermittent fasting, for which we had to exclude them from this per-protocol analysis.

6.1.2 Adverse events

Regarding adverse events, we must consider a high risk for nocebo effects especially for radical changes in diets that were tried out the first time such as a KD and FD: side effects had to be explained in detail in advance and therefore might have been expected by our study participants (240). Further, the higher number of adverse events in the FD group could be due to frequent evaluations during fasting weeks. Adverse events indeed occurred mostly during the fasting weeks and were transient. The proven compliance in the FD group otherwise shows feasibility of a FD in our cohort.
Frequent expected adverse events were headaches in the KD group (241) and nausea, vertigo, and temporary vision impairment in the FD group (242). Interestingly, pain and perception disturbances such as paraesthesia were much more frequent in the ketone-based diet groups compared to the SD group. Infections, on the other hand, were more frequent in the SD group compared to the ketone-based diet groups (Table 9). Apart from anecdotal evidence from internet forums, perception disturbances and pain as side effects following a KD or FD have not been described yet and may be disease specific. Airway infections, such as the common cold, were the most frequent adverse events in this cohort as well as in the precursor IGEL study (179).

6.1.3 Ketone elevation

The blood ketone BHB increased significantly in the KD group with a median of 0.7 mmol/l, with an interquartile range of 0.3 - 0.9 mmol/l (Figure 8). These values reflect a physiological nutritional ketosis and prove a certain adherence to the diet in the KD group. In the future, it would be meaningful to use continuous ketone sensors that were recently developed (243) to reduce measurement burden for study participants and to better understand daily fluctuations as well as individual responses to carbohydrate-rich foods. As expected, 14 hours of daily fasting were not sufficient to raise BHB concentrations in the FD group (Figure 8). Patients in the KD group, who presented both low glucose and ketone concentrations, reported a subjective lack of energy. Forty percent of the patients in the KD group showed BHB values < 0.5 mmol/l at the nine-

month study visit, showing that not all patients achieved a stable and relevant ketosis, although they reported thorough dietary adherence. In our cohort, body fat mass, but not body weight; leptin, but not adiponectin; and glucose, but not insulin was significantly predictive for ketosis after nine months of a KD (chapter 5.2.2). We suggest using these variables to predict individual success and extent of ketosis induction in dietary counselling.

6.2 Effects of the diets on BMI and body composition

BMI and body fat mass decreased in the KD and FD group

Median BMI decreased in the KD and FD, but not in the SD group. Further, there was a reduction in body fat mass in the KD and FD group, but not in the SD group. However, in our study neither BMI nor body fat were relevantly predictive for clinically outcomes such as cognition, fatigue, or depressive symptoms, while baseline body weight showed only a tendency of an inverse correlation with the cognition score (chapter 5.6.3). In the literature, especially central body fat has been shown to be associated with disability in MS (244) and may be a better marker than BMI to estimate obesity in MS patients (77). As shown by our data and supported by current literature, ketone-based diets are suitable and efficient to achieve sustainable weight loss and to improve body composition in MS (119, 120). As obesity and obesity-related metabolic alterations are not only common in MS but also associated with a poor disease course, optimizing body weight and body composition must be seen as highly desirable.

Fat-free mass decreased only in the FD group

In the FD group, there was a significant reduction of fat-free mass with a mean change of almost 5% compared to baseline. A reduction of fat-free mass by 3 - 6% over a longer study duration of six months due to different regimens of caloric restriction, with or without exercise interventions, was demonstrated in a study before (245). During ketone-based diets mitochondrial oxidative phosphorylation increases (246) for which fatty acids are the main substrate. However, blood glucose homeostasis must be maintained as well. As our data show, the FD induces greater reductions in blood

glucose concentrations than the KD – which may be the reason for greater glycogen storage depletion in the FD group, reflected by reduced fat-free mass.

During total caloric restriction, muscle glycogen breakdown may provide substrate for maintaining blood glucose homeostasis to support liver glycogen breakdown. In contrast, in the KD group, a critical amount of dietary glucose and glucose-forming amino acids are supplied continuously, which may prevent muscle breakdown. A reduction of fat-free mass due to prolonged fasting via greater muscle breakdown and due to an overall energy deficit is unfavourable but was expected as described before (247). In contrast - a KD was shown to prevent muscle loss (248).

MS can lead to muscle weakness and balance disturbances. Additionally, glucocorticoid use, which is the established acute therapy during relapses, is associated with a reduced bone and fat-free mass in MS patients (249). For those reasons, fasting may be rather contraindicated in case of patients are already suffering from muscle weakness and/or having a low BMI (< 18.5 kg/m^2) and/or low fat-free mass. Prevention of muscle loss should be in focus, e.g., by adding strategies such as physical exercise to fasting regimens that may reduce the risk of muscle loss (247).

6.3 Effects of the diets on metabolism

Blood lipids improved in all groups

Total cholesterol was elevated in almost 40% of the study population at baseline, even though patients with clinically significant hyperlipidaemia were excluded from the study. High total cholesterol is common among MS patients and associated with disease activity (88). Interestingly, greater increases in HDL-cholesterol were suggested to be neuroprotective in MS, whereas greater increases in LDL-cholesterol were associated with increases in new lesions in a 5-year longitudinal study (250). In line with this, an adverse lipid profile was shown to correlate with increased inflammation and was suggested to mediate negative effects of obesity on the clinical course of MS (251). Total cholesterol decreased in our FD and SD group, while in the KD group only triglycerides decreased significantly. HDL-cholesterol remained stable in the FD group, slightly increased in the KD group, but unfavourably decreased in the SD group. That a high-fat KD can actually improve HDL-cholesterol levels was shown before as concluded by a meta-analysis (252). However, patients in most of the

analysed studies were obese and lost a substantial amount of weight due to the intervention. In line with this, our data show a strong correlation between body weight and triglycerides, but interestingly not with total, LDL- and HDL-cholesterol (chapter 5.3), implying that weight loss is only a partial mediator of the diets blood lipid-reducing effects. Here, composition of diets may play an important role. The KD in our study – even though a high-fat diet – was counselled with a vegetarian focus: specifically, a reduced meat consumption to 1 - 2 portions/week and increased fatty fish and plant oil consumption was recommended, explaining the slightly increased HDL-cholesterol and the slightly decreased LDL-cholesterol concentrations in the KD group. Due to a very low-carbohydrate intake, a significant decrease of triglycerides was expected in the KD group. Blood lipids were inversely correlated with the cognition score in our cohort, explained in detail further down (chapter 6.7).

Already a 1% reduction in LDL-cholesterol or 1% increase of HDL-cholesterol is each associated with a 1% risk reduction for cardiovascular events (253). Hence, a LDL-cholesterol reduction of 14% in the FD group, 7% in the SD group and 8% in the KD group as well as a 6% increase of HDL-cholesterol in the KD group and a 13% decrease of HDL-cholesterol in the SD group (Table 2), must be interpreted as clinically relevant.

Leptin decreased in the KD and FD group

Leptin, an adipocyte-secreted hormone that plays a dual role as a hormone and as a pro-inflammatory cytokine, decreased significantly in the KD group and borderline-significantly in the FD group. Leptin was found to be increased in MS patients compared to healthy controls according to a meta-analysis (254). Further, leptin was reported to be an independent risk factor for MS among young adults, suggested as a biomarker for disease course in paediatric MS patients (255, 256) and expression of leptin receptors to be involved in clinical relapses (257). That a KD has the potential to lower leptin concentrations in MS is supported by results from a previous study (119). Since KD and FD significantly decreased body fat, and body fat was strongly associated with leptin (chapter 5.3), the improvement of body composition may have mediated the reduction of leptin levels. In line with this, adiponectin, the counterpart of leptin, was significantly elevated after nine months in the KD and FD group, but not in

the SD group. However, the inflammatory potential of adiponectin and its role in MS is less clear and has been discussed controversially. Recent studies suggest high adiponectin levels to be more prevalent in MS compared to healthy controls and high levels to be associated with MS progression (258, 259). Other studies, however, have proposed lower concentrations of adiponectin to be prevalent in MS patients (260). A review describes adiponectin to play a dual role in MS, exerting anti-inflammatory and pro-inflammatory effects. The review also reports that EAE mice deficient of adiponectin develop a more severe disease type (261). Systematic reviews conclude that higher adiponectin concentrations are associated with a lower risk for metabolic diseases such as diabetes (262) and with lower circulating pro-inflammatory cytokines (263), indicating increased concentrations to be desirable. Adiponectin, but not leptin, was associated with fatigue symptoms in our cohort (chapter 5.6.1), described in detail further down (chapter 6.5). The role of obesity in MS severity and especially the mechanistic role of obesity-associated factors such as body fat and adipokines needs more attention in future research.

KD and FD lowered blood glucose concentrations

A subgroup of 15 patients wore a CGM sensor over the first 14 days of the dietary interventions. They showed significantly distinct blood glucose concentrations, allowing to differentiate the groups, and allowing to evaluate acute effects of the diets on blood glucose: the FD group with a median value of 63 mg/dl had the lowest blood glucose concentrations, followed by the KD group with a median value of 75 mg/dl and the SD group with 84 mg/dl. Even though the small sample size must be considered, especially in the FD group (n = 3), low blood glucose concentrations due to a FD and KD were expected. Comparing the deltas - baseline vs. nine months - of the blood glucose concentrations, a significant between-group difference was shown between the SD (increased blood glucose concentrations) and the FD group (decreased blood glucose concentrations) (Figure 11b), emphasizing long-term effects of the diets on blood glucose concentrations. Lower blood glucose concentrations were expected in the ketone-based diet groups and may be desirable, especially since glucose concentrations were inversely associated with clinical outcomes such as cognition in our data (chapter 5.6.3). Dietary interventions that regulate blood glucose

concentrations are favourable for MS patients, since insulin resistance is prevalent in this population and might contribute to metabolic alterations and disability (85). A study concluded that even “early markers of dysglycemia should be sought for in these patients to avoid additional deterioration of their quality of life” (264).

6.4 Effects of the diets on oxidative stress, plasma micronutrients, and plasma cytokines

KDs and FDs have been shown to ameliorate inflammatory responses and oxidative stress. Changes to these assumably pathological aspects comprise the main suggested mechanisms of action of KDs and FDs.

Markers of oxidative stress remained stable in the KD and FD group, but deteriorated in the SD group

Concentrations of the oxidative stress marker MDA were elevated in all three groups within a similar range at baseline, compared to a study with 170 MS patients and 163 healthy controls (265). Interestingly, in the KD and FD group, MDA did not change over the study period, while increasing significantly in the SD group. A study including 212 MS patients and 249 healthy controls aimed to investigate oxidative stress parameters as potential biomarkers for disease progression. The authors found oxidative stress markers to be predictive for cerebellar symptoms and other aspects of disease progression (266). Against this background, stabilized markers of oxidative stress as observed in the KD and FD group in our study, may be seen as favourable, even though we could not confirm the often-proposed oxidative stress reducing effect of ketone-based diets (38, 120, 174).

However, antioxidative micronutrients increased in the KD and FD group (but also partly in the SD group) and were associated with clinical outcomes such as cognition (chapter 6.7). Further, in line with previous data that show oxidative stress markers to be associated with disability (267), MDA concentrations were strongly positively correlated with the EDSS score in our cohort (chapter 5.4).

Moreover, proteasomal activity that accounts for degradation of unnecessary and damaged proteins by proteolysis, decreased in the KD and to a greater extent – but non-significantly – in the SD group (Table 3). In the FD group, proteasomal activity

slightly increased (Table 3). Proteasome dysfunction has been discussed to play a role in neurodegenerative diseases and the proteasome even suggested to be a major autoantigen in MS (268, 269). Furthermore, activation of proteasomal activity through glucose restriction and caloric restriction was shown before (270, 271). Whether a rescue of proteasomal dysfunction or stabilizing the proteasome by, for instance a KD/FD, may be beneficial regarding ameliorating MS disease activity, remains elusive. In our cohort, proteasomal activity was not correlated with clinical outcomes. However, proteasomal activity was significantly positively correlated with glucose concentrations (chapter 5.3) and according to a study, high glucose concentrations may diminish the proteasome (272). Against this background the blood glucose reducing effects of a KD/FD appear desirable. The main NAMS study will additionally analyse the effects of the KD and FD on autophagy inducing polyamines and hence will shed more light on the diets potential to regulate cell-regenerative pathways.

FD and KD change plasma micronutrient concentrations

In the KD and FD group, plasma lutein/zeaxanthin, a dietary carotenoid derived from dark green leafy vegetables, significantly increased. Lutein/zeaxanthin was shown to have important antioxidative properties (273). In the FD group, cryptoxanthin also increased significantly. The increased lutein/zeaxanthin and cryptoxanthin imply higher fruit and vegetable intake in the KD and especially FD group (274). Lutein/zeaxanthin and cryptoxanthin may be essential for long-term health and adequate levels might play a role in preventing chronic disease and premature aging (275). In line with this, both lutein/zeaxanthin and cryptoxanthin showed positive associations with cognition outcomes (Table 7), referred to in detail in chapter 6.7. Of note, the antioxidants cryptoxanthin, retinol and alpha carotene decreased in the KD group. This might be due to inadequate micronutrient intake. Previous reports already discussed the necessity of close monitoring of several micronutrients during KD interventions (276, 277).

FD decreased 3-methylhistidine

Plasma 3-methylhistidine is a post-translationally amino acid and is a sensitive biomarker for protein degradation and skeletal muscle protein turnover (278). Even

though dietary intake may have an influence on plasma 3-methylhistidine concentrations, it was suggested that about 80% of plasma 3-methylhistidine, assessed after an overnight fast, can be considered endogenous (279). According to a review, there are some studies with chronic fatigue syndrome patients, a neuroinflammatory disease that has many overlaps with MS (280), showing 3-methylhistidine in the urine to be significantly elevated (281).

Interestingly, 3-methylhistidine only decreased significantly in our FD group. Studies on the effects of a FD and KD on this biomarker are scarce. One study that has investigated a three-week caloric restriction and has taken blood samples after four weeks found – in contrast to our data – increased 3-methylhistidine (282), indicating that the caloric restriction induced skeletal muscle protein turnover. Short-term effects of caloric restriction may be indeed an enhanced skeletal muscle protein turnover reflected by the increased 3-methylhistidine in this study. This may have occurred in our study as well, as reflected by the reduced fat-free mass, which may be a direct result of the prolonged fasting during the fasting weeks. Intermittent fasting alone, in contrast, was shown to even prevent muscle loss, in a study (247).

Of note, our study data show long-term effects of fasting as blood samples were taken three months after the last fasting episode. Hence, our data may rather show a condition reflecting post-fasting compensatory growth where muscle protein degradation is already downregulated (283) and therefore reduced 3-methylhistidine concentrations.

Moreover, a study with Alzheimer's disease patients showed increased levels of 3-methylhistidine compared to healthy controls (284).

Comparing the 3-methylhistidine baseline values from our cohort with the study's healthy controls and Alzheimer's disease patients (as both our studies were using liquid chromatography techniques), elevated plasma levels of 3-methylhistidine in our cohort must be noted: the baseline 3-methylhistidine values from our cohort are three times higher, compared with healthy controls from this study, but still around 20% lower compared to the Alzheimer's disease patients (284).

Interestingly, 3-methlyhistidine was also borderline-significantly correlated inversely with cognition after nine months in our data (Table 7), indicating that reduced concentrations - as occurred in the FD group - may be rather beneficial.

Plasma 3-methylhistidine after nine months was positively correlated with fat-free mass, body weight, leptin, LDL-cholesterol, and total cholesterol at nine months and baseline proteasomal activity in Spearman correlations in all patients (chapter 5.4).
Thus, 3-methylhistidine appears to be a sensitive marker for fasting-induced outcomes in our cohort. Moreover, 3-methylhistidine was positively associated with oxidative stress marker MDA after nine months. Whether 3-methylhistidine additionally reflects oxidative stress and/or inflammation is not certain but plausible, since oxidative stress was discussed to be a relevant potential inducer of protein degradation (285) and 3-methyhistidine has been shown to positively correlate with systemic inflammation (286).
Interestingly, while fat-free mass and 3-methylhistidine decreased in the FD group only, proteasomal activity was preserved in the FD group only.
It appears that in short-term, muscle protein degradation was probably increased due to prolonged fasting - reflected as a sustained lower fat-free mass in the FD group. In long-term, effects of fasting may be that protein degradation is downregulated - reflected as preserved proteasomal activity and decreased 3-methylhistidine in the FD group.

IL-13 and IL-4 decreased in the KD group

In our cohort, IL-13 and IL-4 significantly decreased only in the KD group. IL-13 and IL-4 are two physiologically connected cytokines that have rather anti-inflammatory properties. Increased expression of IL-13 and IL-4 plays an important role in type-2 immune responses, especially allergic inflammation (287). IL-13 is considered a Th2-type cytokine, however, it was shown that IL-13 can be generated by Th1 cells and IL-17+ Th17 cells (288), both that were described to have a pathogenic role in MS (289). As the two interleukins IL-13 and IL-4 decreased simultaneously in our cohort, a true effect of the KD on the two cytokines or their secretory T cells appears plausible. In the current literature, a KD affecting IL-13 has not been described yet.
However, IL-13 has been shown to play a role in glucose metabolism and in cognitive function (290). Our data also show a borderline-significant positive correlation of IL-13 with glucose concentrations and significant inverse correlations of glucose concentrations with the cognition score (chapter 5.3 and Table 8). It appears plausible that the KD lowered IL-13 concentrations via glucose reductions, as increased IL-13

strongly correlated with hyperglycaemia, insulin resistance and even hypertriglyceridemia in one study (291). In this study, IL-13 appeared to be a biomarker for metabolic alterations, but was not associated with low-grade inflammation, emphasizing a rather metabolic function of this cytokine.

However, the role of IL-13 in MS is inconclusive. A study comparing 57 MS patients with healthy controls, described IL-13 to be increased (and adiponectin decreased) and suggested that these parameters might be involved in MS pathogenesis (260). Another study found that patients during relapses had higher frequencies of IL-13 producing T cells than those in remission and these cells were positively correlated with disability (EDSS score) (292). In line with this, a KD-mediated decrease of IL-13 and increase of adiponectin, as occurred in our study, appears beneficial. In contrast, however, IL-13 was reported to be neuroprotective (293). Specifically, a correlation analysis showed that levels of IL-13 in the cerebrospinal fluid were associated with better scoring in the Multiple Sclerosis Functional Composite as well as with neuronal integrity, concretely the retinal nerve fiber layer thickness (293).

Studies in animal models also show conflicting results. Whereas IL-13 seemed to be neuroprotective in a rat model (294), an IL-13 knockout was associated with decreased disease susceptibility in female EAE mice. IL-13 was suggested to be one pro-inflammatory factor mediating gender differences: in the study, “females lacking IL-13 displayed lower incidence and milder EAE disease severity than males after immunization with myelin oligodendrocyte glycoprotein (MOG)-35–55 peptide/CFA/pertussis toxin” (295). This shows that more studies are needed to characterize the effects of IL-13 and to reproduce investigations of potentially IL-13-lowering effects of KDs. These results could also be relevant for type 2 immunity-related diseases.

The KD also lowered IL-4 concentrations in our cohort. In line with our results, IL-4 was reduced in mice fed a KD (296). However, there was no change in psoriasis patients on a KD regarding IL-4 concentrations (297). Interestingly, IL-4 was found to be elevated in MS and neuromyelitis optica patients (298). Remarkably, a gender-dependent effect, such as just described for IL-13, was also observed for IL-4 in this study, displayed as increased IL-4 serum levels in females with neuromyelitis optica (298).

In summary, even though IL-13 and IL-4 were suggested to act mainly anti-inflammatory, most anti-inflammatory cytokines have at least some pro-inflammatory properties and vice versa (43). The role of IL-13 and IL-4, especially with regards to a potentially gender-specific susceptibility in neuroinflammation and the potential of dietary modification of the cytokines needs more attention in future research.

IL-7 and VEGF-A decreased in the SD group

IL-7 has been suggested to play a role in MS pathogenesis and even as a response marker of the DMT interferon-beta (299). A study investigating an anti-inflammatory Mediterranean diet - comparable to our SD - also showed a reduction of IL-7 in 90 metabolic syndrome patients (300). In line with this, pro-inflammatory diets have been shown to be associated with increased IL-7 concentrations (301). This implies that we see a true cytokine-lowering effect of the SD in our cohort.

Vascular endothelial growth factor A (VEGF-A) activates angiogenesis and was shown to have pro-inflammatory properties. In a recent study, VEGF-A was upregulated in MS patients compared to healthy controls, suggesting a role in MS pathogenesis (302). A Japanese study showed an upregulation of VEGF in MS patients during relapses, compared to patients in remission and controls, suggesting a contribution to spinal cord lesion formation by increasing vascular permeability (303).

Interestingly, VEGF-A and IL-7 showed a strong positive correlation in the SD group. Further, VEGF-A was inversely correlated with the depression score after nine months in our cohort - an association that has been investigated previously (304). In the SD group, one major goal was to improve the relation of omega-3/omega-6 fatty acids, as in Western diets a high consumption of omega-6 fatty acids and low consumption of omega-3 fatty acids is common. The VEGF-A reduction in the SD group might be due to increased omega-3 fatty acid consumption, as suggested by a mouse and a clinical study (305, 306). Considering the existing literature, the joint decrease of IL-7 and VEGF-A in our SD group appears to be an anti-inflammatory effect of the diet.

It remains questionable why these changes were not seen in the KD and FD group, as they received – besides from the focus on caloric/carbohydrate restriction – the same dietary recommendations as the SD group. Furthermore, as due to the inverse correlation of VEGF-A with depression scores, and due to deteriorated depressive

symptoms simultaneously with reduced VEGF-A in the SD group, the role of VEGF-A remains unclear.

Surprisingly, there were no changes of plasma cytokines in the FD group. However, blood samples were drawn three months after the last fasting week. It would be interesting in the future to measure plasma cytokines within and shortly after the actual fasting weeks and/or measure cytokine secretion of stimulated PBMCs. Cytokines are usually produced in small amounts and “act mainly in a paracrine and autocrine manner such that they are released and consumed locally at the site where the immune reaction occurs. Therefore, they are seldom detectable in peripheral blood” (307), which is why PBMC stimulation may be more relevant. Further, postprandial cytokine response may differ from fasting states (308). We took blood samples after a 12-h overnight fast, which precludes extrapolation to the postprandial phase. In our MS cohort, there were hardly any consistent associations between cytokines and clinical outcomes. However, a characteristic feature of MS is its clinical and immunopathological diversity resulting in multiple, partly unpredictable disease stages. This could lead to regular changes in local cytokine productions, which are not necessarily detectable in the periphery. In contrast to our results, studies do show associations between sera or plasma inflammatory cytokine levels and MS disease severity (309, 310). Furthermore, there are DMTs targeting specific cytokines or their receptors - such as interferon-beta - emphasizing the role of cytokines in MS. At the same time, intake of the well-established DMT interferon-beta could have confounded our cytokine results here.

6.5 Effects of the diets on fatigue symptoms

Although several patients reported improved fatigue during their counselling sessions, we could not confirm this with the applied questionnaires. One reason for this might be that the majority showed no or only mild fatigue symptoms at baseline according to their FSS scores, leaving rather little room for improvement. When we excluded patients with mild fatigue and only analysed those with moderate to severe baseline fatigue, sample sizes were rather small, especially in the KD group (n = 4; Figure 15a). However, we saw a trend for improved fatigue in the FD group in this adjusted analysis (Figure 15a). Interestingly, patients in the SD group, who had only mild fatigue

symptoms at baseline, rated that they were more fatigued after nine months (Figure 15b). A study by Tellez et al. including 227 MS patients found that 87% of the patients who had fatigue at baseline still had fatigue at their 1-year-follow-up visit. Moreover, 25% of patients, who were not fatigued at baseline, had developed fatigue within 1 year (311). This raises the question if the increased fatigue score in the SD group reflects disease progression. A study by Fox et al. confirms that fatigue progresses over the life span of MS patients (312).

Patients, in particular in the KD group, anecdotally reported improved fatigue, specifically described as prolonged attentiveness and mental performance. However, the tool that assessed fatigue in our study, the FSS, even though being a well-established tool for MS-related fatigue, focuses strongly on physical fatigue and depression-like symptoms. This is corroborated by the strong correlations between FSS and BDI-II scores in our cohort (chapter 5.6.2).

Cognitive fatigue, however, is a severe symptom that is as debilitating for MS patients as physical fatigue. Most importantly, cognitive fatigue remains poorly understood and cannot be assessed with the FSS (313). A review by Hanken et al. further describes that there is strong evidence for fatigue to be associated with a dysfunction of alertness and vigilance, while there is no evidence for a relation with other cognitive dimensions such as memory performance or cognitive processing speed – explaining why cognitive fatigue cannot be assessed with the usual cognition tools either. The authors further state that “areas with brain atrophy in fatigue patients overlap with brain regions activated in healthy controls performing alerting/vigilance tasks”. Thus, the authors suggest to include alerting/vigilance assessment into clinical routine of MS patients who suffer from fatigue symptoms (314), to better account for cognitive fatigue.

As fatigue symptoms are associated with a reduced central glucose metabolism in MS (315) and KDs possibly improve central hypometabolism (316), changes may rather be detectable on a scale assessing cognitive fatigue rather than physical fatigue, hence not by the FSS.

Since fatigue appears to be a progressive symptom (312) and may reflect disease progression, it is relevant that fatigue symptoms did not deteriorate in the KD and FD group within nine months and even slightly improved in patients in the FD group, who were moderately to severely fatigued at baseline. However, the observed changes are not considered clinically relevant, as a clinically relevant change has been defined as

1.5 - 2 points change on the FSS before (228). The main NAMS study, which additionally assessed fatigue symptoms after 18 months, will provide more insights on this.

Further studies should use multidimensional fatigue scales that include cognitive fatigue such as the Modified Fatigue Impact Scale or Fatigue Scale for Motor and Cognitive Functions (313). Assessment of fatigue symptoms further needs to be controlled for symptoms of sleep disorders, non-fatigue related tiredness and fatigability, symptoms that cannot be distinguished by the FSS (317).

Predictors for fatigue

Depression and disability scores at baseline were strongly predictive for fatigue symptoms after nine months study duration: the stronger the depressive symptoms and the worse the disability status, the more severe the fatigue score (Table 5). Depressive symptoms and disability were previously described to be reliable predictors for increases of fatigue over time in MS patients (311, 318). Further, our patients on DMT had lower fatigue scores compared to those without DMT (Table 5). Interestingly, the highly sensitive acute-phase protein haptoglobin that decreased in all three groups, was significantly predictive for the fatigue score after nine months (Table 6). Associations between fatigue and haptoglobin are not yet described in the literature. Contrarily to our data, a study investigating effects of Ramadan fasting even showed a haptoglobin increase (319).

Interestingly, the metabolic cytokine adiponectin, which significantly increased in the KD and FD group, was inversely associated with the fatigue score: every unit increase of adiponectin was associated with a decrease on the FSS (Table 6). However, as adiponectin significantly increased in the KD group as well, it must be assumed that fatigue-improving effects are not primarily mediated via adiponectin, as fatigue scores did not change relevantly in the KD group.

Research for improving fatigue, which is one of the most disabling symptoms for MS patients, should receive more attention in the future, especially the mentioned overlap of depressive and fatigue symptoms as well as cognitive fatigue. Haptoglobin and adiponectin should be further investigated as possible biomarkers.

6.6 Effects of the diets on depressive symptoms

Depressive symptoms according to the BDI-II score improved significantly in the FD group (1.5 points decrease), did not change in the KD group, and deteriorated (2 points increase) in the SD group. Previous literature proposes a clinically relevant change of 17.5% or 19% from baseline within six months (233, 320). Thus, a 1.5-point and 17% decrease in our cohort represents a borderline clinically relevant improvement, and the 31% increase in the SD group, a clinically relevant deterioration of depressive symptoms.

These tendencies deserve attention as depression is one major symptom of MS with more than 30% of patients affected (199). Since the early 1970ies, FDs and KDs have been investigated for their stabilizing potential in mood disorders including depression. Interestingly, potential anti-depressive effects are thought to be mediated via leptin reduction (321), which occurred in our KD and FD group. A RCT with 36 MS patients investigated different intermittent fasting regimens and observed clinically meaningful improvements regarding emotional health including depressive symptoms (129). Another RCT with healthy older humans investigated the effect of a calorie-restricted dietary regimen with intermittent fasting and has also shown improvements in different mood states simultaneously with nutritional status (200). A review by Manchishi et al. concluded that dietary supplementation produced “additive or synergistic effects when administered with conventional drugs or in combination with other interventions” and emphasised ketone body production, reduction of inflammatory cytokines and an improved nutritional status among the main mechanisms of action (322). This can be only partly confirmed by our data, as VEGF-A showed associations with the BDI-II score as well as body fat mass, but not ketosis (chapter 5.6.2).

Importantly, our FD group reported more depressive symptoms at baseline, which may have biased the result. In general, the prevalence of mild, moderate and severe depressive symptoms was very low in the majority of our patients (Figure 16a). The baseline median BDI-II score of the FD group was highest with a score of nine among the three groups, but still only indicated minimal depressive symptoms. We assume that studies with mildly, moderately, or severely depressed patients might provide more information on the anti-depressant potential of ketone-based diets.

Considering the median changes of depressive symptoms, it appears that in the KD group depressive symptoms rather increased, while decreasing in the FD group and

there was a trend for a significant group difference between the KD and FD group (Figure 16c). Looking at the adverse events (Table 9), depressive symptoms were reported twice in the KD and once in the SD group. It must be noted that we only analysed the total BDI-II score. However, the BDI-II includes diet-specific questions, such as questions on decreasing appetite. Choosing response options on decreased appetite increases the BDI-II score by 1-3 points – but probably does not reflect deteriorated depressive symptoms – instead rather shows a physiological effect of a KD. That a KD decreases appetite was shown before and is an expected and usually desired effect (323).

Similarly, the BDI-II includes questions on sleep: response options such as “I sleep much more than usual” or “I sleep much less than usual” increase the BDI-II score by up to 2 points (229). However, it must be considered that ketone-based diets directly influence sleep irrespective of depressive symptoms (324), which may lead to a false interpretation of BDI-II score changes.

Additionally, it must be considered that patients in the KD group answered the BDI-II questionnaire during their actual intervention, whereas patients in the FD group answered three months after their fasting week. Thus, immediate post-fasting BDI-II responses might have been different, especially responses on appetite and sleep.

In summary, the BDI-II tool could have biased the outcome of depressive symptoms, especially in the KD group, and future studies with dietary interventions should rather employ depression scales without diet-related questions.

It is possible that the larger sample size of the main NAMS study will have a more balanced distribution of baseline depressive symptoms over the groups and, therefore, allow for conclusions that are more robust. In addition, the 18-month time point will allow the evaluation of possible fluctuations of depressive symptoms, which are common in MS. In contrast to fatigue, depressive symptoms do not typically progress over time and do not necessarily reflect disease progression (312, 325).

Predictors for depressive symptoms

Body weight, micronutrients, and most cytokines did not show predictive value for the depression score. As mentioned above, VEGF-A concentrations, which may have decreased due to higher vegetable oil consumption in the SD group, were inversely correlated with the depression score after nine months.

Interestingly, glucose concentrations were correlated with the BDI-II score at baseline but lost significance at nine months looking at the total study population. Body fat was positively associated with the depression score after nine months in the FD group. The strongest relation remains the one between fatigue and depressive symptoms, highlighting their pathological overlap as well as overlaps due to assessment tools. The potential marker properties of VEGF-A, body fat and glucose concentrations will be further elucidated in the analysis of the main NAMS study.

6.7 Effects of the diets on cognition

The SDMT cognition score increased slightly over time in all three groups, which might be the result of a practice effect (326). The FD group showed a significant increase of 2.52 points from baseline. According to a study by Benedict et al., a clinically meaningful change can be defined as a three-point change from baseline (239). However, the study duration of this reference study was 22 months, so an improvement of 2.52 points within nine months could be interpreted as clinically meaningful. Furthermore, it is important to note that – in contrast to normative age-matched data presenting mean scores of ≥ 59 (327) – our cohort shows slightly lower baseline scores across all three groups. Parmenter et al. describes a cut-off score of 55 or lower on the SDMT to classify cognitive impairment in MS patients (237). Forty to fifty percent of our patients had a SDMT score of 55 or lower at baseline among all three groups (Table 1), indicating that mild cognitive impairment was prevalent in half of the patients.

In the KD group, the SDMT score did not increase significantly. However, it must be noted that not all patients developed a relevant ketosis – 40% of the KD group patients did not show relevantly elevated BHB concentrations at the nine-month study visit (5.2.2). When considering only patients in the KD group who did achieve a relevant ketosis – defined as plasma BHB concentrations ≥ 0.5 mmol/l at nine months – the SDMT score increased by 3.9 points from a mean of 57.7 to 61.6 (Figure 18). Hence, it can be assumed that ketosis has been the driver of improved cognition in the KD subgroup. Moreover, the improved cognition in patients presenting ketosis may be explained by simultaneously increased brain ketone uptake that correlates with peripheral plasma ketones, as shown before (178).

It must be noted that – even though the per protocol population has been analysed here – the 40% not developing a relevant ketosis at the time of their study visit,

otherwise showed good dietary adherence. We therefore must critically appraise that our carbohydrate recommendation of up to 50 g per day yielding ketone median levels of 0.7 mmol/l might have been too liberal to achieve relevant sustained peripheral and associated central ketone concentrations in all patients. Furthermore, the question is raised, whether beneficial effects of KDs on cognition are as fluctuant and temporary as ketosis itself. If so, a long-term application does not seem to be reasonable for stable and reliable outcome improvement for many patients who have difficulties developing ketosis or show high fluctuations.

Ketone-based therapies have been shown to improve cognition in Alzheimer's disease, epilepsy, and Parkinson's disease, before (116, 118, 328, 329). It was further shown that plasma ketones directly correlate with glucose utilization, showing linearly decreased utilization of glucose with increased blood ketones (330). In patients with mild cognitive impairment on a low-carbohydrate diet, urine ketones were positively associated with memory performance. In this study, patients were only allowed to consume 20 g of carbohydrates per day (331), compared to up to 50 g in our study.

It must be noted that anecdotal patient reports substantiate the common notion that long-term adherence to a KD is challenging. This can be confirmed by the reduced adherence to the KD as shown in our cohort (Figure 5). As an alternative for a restrictive KD, the consumption of medium-chain triglycerides or ketone esters has been investigated, specifically in Alzheimer's disease patients, for whom a KD is supposed to be unreasonable. Those studies showed that supplementation-induced increases of blood ketones improved cognitive dysfunction in Alzheimer's disease patients (116, 195, 328).

However, these studies also showed that a ketosis of approximately 0.5 mmol/l BHB concentrations was sufficient to improve cognitive impairment, confirming our results. It must be noted that our KD group had relatively high SDMT baseline values with a mean SDMT score of 58, so the question arises whether KD-induced improvements in cognition would have been more prominent with more pronounced cognitive impairment at baseline. Ultimately, as in the FD group, ketone levels did not elevate relevantly, the question is raised whether there are other factors that may have affected cognition.

Blood lipids and blood glucose as predictors for cognition

The analysis of predictors for cognition at follow-up showed strong inverse correlations with HDL-cholesterol and triglycerides at baseline as well as with LDL-cholesterol after nine months (chapter 5.6.3), i.e., lower blood lipids were associated with better cognitive performance. Recent studies corroborate these results, showing inverse correlations between triglycerides and cholesterol with cognition scores in MS patients (332, 333).

Surprisingly, HDL-cholesterol was also inversely associated with cognition in our data. In line with this, higher HDL-cholesterol was found to be associated with cognitive decline in one longitudinal cohort study with 2514 participants, showing an inverted U-shaped relation between cognitive processing speed and HDL-cholesterol (334). However, there are other large cohort studies showing positive associations of higher HDL-cholesterol with better cognitive function and inverse associations of higher HDL-cholesterol levels with dementia and mild cognitive impairment (335, 336). Due to these controversial findings, it was recently suggested to differentiate HDL-cholesterol into subclasses to consider lipoprotein particle characteristics of HDL-cholesterol in future studies (334). Furthermore, a wide confidence interval given for the coefficient regarding HDL-cholesterol must be considered here, especially as the confidence interval includes 0 (Table 8). Hence, the result of the main study needs to be waited for more validity.

Interestingly, we found that blood glucose concentrations were inversely associated with the cognition score (Table 8). Lower blood glucose concentrations at baseline and after nine months were associated with a higher SDMT cognition score after nine months. Associations between peripheral glucose concentrations and cognition scores have not been investigated yet in MS. One study that investigates glucose tolerance and cognition in MS is underway (NCT04748302). Accordingly, this is the first report of an inverse association of blood glucose concentrations with cognition in MS patients. In line with this, insulin resistance was shown to be more prevalent in MS patients compared to healthy controls and to be associated with adverse MS outcomes (85). In addition, insulin resistance is associated with cognitive impairment in different diseases and age groups (337). A 4.4-year study with 3369 community-dwelling men aged 40 – 79 years showed that glycemia, but not the metabolic syndrome or inflammation, was inversely associated with cognition, in particular processing speed (338).

It may be valuable to assess blood glucose and cytokine concentrations postprandially in future studies, as postprandial responses may provide insights on potentially subclinical glycemia and inflammation (339, 340). Additionally, the hypothesis of central glucose hypometabolism in MS patients and a potential peripheral reflection via blood glucose concentrations requires more attention in the future. In conclusion, our data show that a FD improved cognition and mildly lowered blood glucose and blood lipids which is associated with improved cognition. A KD mildly lowered blood glucose and triglycerides while improving cognition only in a subgroup of patients in dependence of ketosis induction.

Body composition and BMI are no predictors for cognition

BMI and body fat were not predictive for cognition, implying that dietary effects on cognition are not entirely mediated by weight loss. Another study confirmed that body composition might not be a target for improving cognition in MS (341). Targeting blood lipids may be more promising, even though evidence thus far only originates from observational studies (332, 333, 342). However, there was a trend for an inverse correlation between baseline body weight and the cognition score after nine months in our data.

Plasma micronutrients as predictors for cognition

All three diets had effects on plasma micronutrients. Both ketone-based interventions increased lutein/zeaxanthin (Table 3), an antioxidant carotenoid derived from dark green leafy vegetables. Lutein/zeaxanthin was positively correlated with cognition (Table 7). The role of lutein/zeaxanthin regarding cognition has been investigated since 1998 (343) and higher plasma lutein/zeaxanthin or higher estimated lutein/zeaxanthin dietary intake was independently associated with better cognition in large studies (344-346). RCTs confirmed causality and provided a rationale for lutein/zeaxanthin supplementation for the improvement of cognition in young healthy adults and older adults (347, 348). Lutein/zeaxanthin might enhance neural efficiency (349) and influence white matter integrity, especially in regions that are susceptible to age-related decline (350).

Moreover, cryptoxanthin, which decreased in the KD group and increased in the FD group, was borderline-significantly positively associated with cognition - even though relatively wide confidence intervals must be considered (Table 7). Plasma cryptoxanthin levels have been shown to be positively associated with cognitive function in an untargeted metabolomics study with 736 adults (351). Remarkably, a cohort study with 927 participants who were followed up for a mean of seven years and who were free from Alzheimer's disease at baseline, showed high carotenoid intake including cryptoxanthin and lutein/zeaxanthin, to be associated with a 48% risk reduction of developing Alzheimer's disease, even after controlling for several risk factors (352). Increased plasma micronutrients further imply a tendency to general healthier dietary habits initiated through the dietary counselling. The observed improvements of plasma micronutrients, adipokines, blood lipids, body weight and body composition underline the efficacy of dietary counselling to improve nutritional status in MS patients and shed light on effects of ketone-based diets besides ketone production.

7 Limitations of the NAMS study and this sub-cohort

The NAMS study remains as of today the largest rigorously designed study investigating ketone-based interventions in MS patients. Further, the study is not only a controlled but also a randomized study, which is especially important, as most dietary interventions cannot be blinded. MS is a rather slowly progressing disease and the long study duration of nine months gives a realistic opportunity to follow-up the disease course and enclose potential disease activity. However, the already mentioned bias factors concerning the outcome tools FSS and BDI-II must be considered, as the tools do not distinguish between MS-related symptoms such as fatigue and depression and, e.g., sleep disorders. The FSS further does not appear suitable for assessing cognitive fatigue. The BDI-II that includes diet-specific items does not appear suitable for dietary interventional studies. Further, wide confidence intervals in our regression analyses must be considered when interpreting results. Regarding the SDMT, practice effects might have appeared and may have led to an increase in scores at the follow-up visit among all three groups (326). Placebo and nocebo effects in the FD and KD group might have influenced the results as the diets cannot be blinded.

Evaluating the dietary adherence of patients remained somewhat challenging in outpatient settings. As the patients in the KD group did not respond in the same way as the patients in the FD group, it is questionable if dietary adherence was sufficient over the complete study duration. However, such long intervention periods cannot be conducted in inpatient settings and *real-world* application is more relevant for transfer into dietary recommendations. Especially in the KD group, however, the dietary adherence was difficult to assess within the outpatient setting and could be only reviewed at fixed time points. App-supported dietary tracking for compliance monitoring and continuous ketone/glucose monitoring may play a role in the future, possibly improving compliance and monitoring in dietary studies as well as reducing measurement burden for patients. Another limitation of the study is the long duration between study visits. Relevant short-term effects of the diets may have occurred during the first three to six months of the study and may have been missed by our study design. Further, as mentioned above, the study visits including data assessment and blood draws were not applied during the actual fasting weeks, but three months after the last fasting week. Hence, acute fasting or temporary post-fasting effects are not covered by our data.

The higher number of adverse events in the FD group may be biased as there were quantitatively more group meetings during the fasting periods in the FD group compared to KD and SD group, so more opportunities for reporting adverse events were given. Due to the selection of the per-protocol population, insufficient randomization as shown in the baseline table (Table 1) was caused: higher blood lipid concentrations, and distinct baseline values that are indicative of more progressed cognitive impairment (comparing the mean SDMT baseline scores between the three groups), fatigue and depressive symptoms can be seen in the FD group, compared to the KD and SD group. It is therefore not fully clear, whether the FD was superior to the KD, or whether the FD appears more efficient due to its more impaired study population. Besides, a healthy participant bias population must be presumed, meaning that usually high proportions of healthier and well-educated patients enrol into clinical studies who are already practicing a healthier lifestyle (353). This may explain why the prevalence of *severe* fatigue and *mild, moderate,* and *severe* depression was low in our cohort, compared to the general MS population. Hence, the evaluation of the diet's efficacy in improving these symptoms is limited as room for improvement has been

rather low. Eventually, the exploratory approach of this analysis must be considered when interpreting the results.

8 Main Conclusions

The ketone-based dietary regimens KD and FD improved body weight, body fat, inflammatory adipokines, micronutrients, blood glucose, and blood lipids. Both interventions can be considered safe. Although most adverse events occurred in the FD group, the number of protocol deviations in the FD group was the lowest. Remarkably, the FD ameliorated minimal depressive symptoms and cognition. Some plasma micronutrients were positively, and metabolic markers (blood lipids, blood glucose) were inversely associated with cognition, suggesting mediation of dietary effects. Changes in markers for vegetable consumption and plasma micronutrients further suggest that due to the FD, healthier eating patterns may have developed, which in turn may have promoted these beneficial effects aside from caloric restriction. The low adherence in the KD group indicates limited feasibility of this diet and even in the patients who did adhere, following the KD did not necessarily induce substantial ketosis at the nine-month study visit. However, ketosis induction appears to be the driving mechanism of improving cognitive function due to KDs, as shown in previous research, and confirmed by our results: in the KD group, only patients showing a successful ketosis induction at their study visit, presented significant improvements in cognition and their mean increase of 3.9 points on the SDMT score is clinically relevant. Due to imbalanced baseline values in the relevant outcome parameters cognition, fatigue, depressive symptoms, and potential mediators (blood lipid concentrations) between FD and KD group - with the FD group including more progressed patients - conclusions on the superiority of the FD against the KD cannot be drawn without uncertainty. However, within-group effects in the FD group and KD subgroup regarding cognition may be each clinically relevant. Furthermore, the improvements of minimal depressive symptoms as observed in the FD group only, may be clinically relevant. In line with these results, in at baseline moderately fatigued patients, a trend of improvement was seen after nine months in the FD group, only. Ultimately, the greater feasibility of the FD compared to the KD indicates a certain superiority of the FD.

Clinical implications: Further rigorous interventional studies with greater sample sizes and neuropsychiatric outcomes as primary endpoints are necessary to build on our results. Additionally, the choice of outcome measuring tools, proper randomization, and the role of other potential bias factors need to be considered in further studies.
However, against the background that pharmacological therapies have substantial side effects and are not sufficient to improve neuropsychiatric symptoms in all MS patients, regular fasting episodes combined with intermittent fasting may be a safe, feasible, low-cost, multi-target approach. KDs, in contrast, appear more challenging, difficult to adhere to, and may address only a narrow patient population. However, it must be noted that patients who did manage to adhere and achieve relevant ketosis, benefited from a KD. Nevertheless, fasting interventions are more feasible long-term, less restrictive, and therefore more suitable for a broader population. Especially MS patients with refractory obesity, metabolic alterations, and mild neuropsychiatric symptoms, who are free from muscle weakness symptoms or other motor system impairments might benefit from fasting. Of note, suitable patients should be identified by trained physicians, who are qualified to instruct fasting interventions and guide patients.
Conclusion: A healthy diet combined with regular prolonged fasting episodes with subsequent intermittent fasting might be recommended to MS patients to improve their cardiometabolic risk profile and mild neuropsychiatric symptoms, and thus complement treatment options for MS.

9 Appendix

Table 9: Adverse events

Adverse events and organ systems / affected areas	KD n = 20	FD n = 31	SD n = 20
Total number of adverse events	64	100	52
Number of serious adverse events	none	1 (unrelated to the dietary intervention)	none
Dermatological			
Furuncle			1
Eczema on the neck			1
Urticaria		1	
Skin changes		1	
Gastrointestinal			
Bloated stomach		1	
Flatulence			1
Nausea		6	2
Stomach pain	1	1	2
Stomach cramps		1	
Gastritis	2		1
Diarrhoea	3	1	1
Reflux	1		
Vomiting		3	
Other gastrointestinal symptoms	3	3	
Infectious			
Flu		1	
Fever			3
Sinusitis			1
Nail mycosis			1
Pinworms			1
Cold	9	7	13
Vaginal mycosis			2
Rota virus infection			1
Infection of upper airways		1	1
Bronchitis	3	1	
Herpes labialis		7	
Musculoskeletal			
Head trauma		1	
Muscle weakness		1	
Osteoporosis	1		
Radius node fracture			1
External ligament rupture			1
Tendinitis	1		

Lateral stitch		1	
Rip bruise		1	
Teeth			
Teeth root treatment			1
Tooth root inflammation			1
Neurological			
Impaired walking ability			1
Fatigue			1
Vertigo/dizziness	2	6	
Increased perspiration	1		
Tremor		1	
Migraine		1	
Disorders of the ear			
Tinnitus		2	1
Hearing impairment			1
Otitis media	2		
Cotton wool feeling	1		
Psychiatric			
Loss of drive/difficulties concentrating		1	
Depressive symptoms	2		1
Sleep disruption		2	
Inner unrest and shaking		1	
Bladder disorder			
Urinary tract infection	1	3	
Miction disorder	1		
Pollakiuria			1
Urinary incontinence			1
Micronutrients			
Folic acid deficiency		1	
Hypervitaminosis D	1		
Hypovitaminosis D		1	
Hypopotassemia		1	
Vitamin B12 deficiency		1	1
Blood system and metabolism			
Microhaematuria		1	1
Elevated uric acid			1
Elevated creatinine			1
Leukopenia	1	1	1
Hypoglycaemia	1		1
Lymphocytopenia	1	1	
Eosinophilia	1		
Pain			
Facial pain		1	

Burning feeling on scalp		1	
Pain in elbow		1	
Pain at multiple sites		3	
Pain after tooth extraction		1	
Leg pain		1	
Thoracic pain	1		
Pain in the liver region	1		
Neck pain	1		
Headache	4	3	
Trigeminal pain	1	1	
Kidney pain	1		
Shoulder pain		1	1
Backpain (prolapse)	2	3	
Pain after twisted knee		1	
Allergies			
Allergic symptoms	1	1	1
Perceptual disturbances			
Feeling of heat in legs		1	
Tingling in limbs	1	1	
Stiffness in legs and arms	2	1	
Heavy feeling of the legs	1	3	
Feeling of tension in limbs		1	
Paraesthesia		1	
Lhermitte sign			1
Numbness of both hands	1		
Cardiovascular			
Tachycardia	1	1	
Hypotonia	1		
Hypercholesterinemia	1	1	
Vision and eyes			
Vision impairments		2	
Flashlight vision		1	
Itchy eyes		1	
Retinal tear		1	
Other			
Lower appetite	1		
Weight loss	1		
Hair loss	1		
Sweating	2	2	
Appendicitis		1	
Uterus myoma		1	

FD = fasting diet, KD = ketogenic diet, SD = standard healthy diet

10 References

1. Pfleger CC, Flachs EM, Koch-Henriksen N. Social consequences of multiple sclerosis (1): early pension and temporary unemployment--a historical prospective cohort study. Mult Scler. 2010;16(1):121-6.

2. Flachenecker P, Stuke K, Elias W, Freidel M, Haas J, Pitschnau-Michel D, Schimrigk S, Zettl UK, Rieckmann P. Multiple sclerosis registry in Germany: results of the extension phase 2005/2006. Dtsch Arztebl Int. 2008;105(7):113-9.

3. Reich DS, Lucchinetti CF, Calabresi PA. Multiple Sclerosis. N Engl J Med. 2018;378(2):169-80.

4. Walton C, King R, Rechtman L, Kaye W, Leray E, Marrie RA, Robertson N, La Rocca N, Uitdehaag B, van der Mei I, Wallin M, Helme A, Angood Napier C, Rijke N, Baneke P. Rising prevalence of multiple sclerosis worldwide: Insights from the Atlas of MS, third edition. Mult Scler. 2020;26(14):1816-21.

5. Gold SM, Willing A, Leypoldt F, Paul F, Friese MA. Sex differences in autoimmune disorders of the central nervous system. Semin Immunopathol. 2019;41(2):177-88.

6. Multiple sclerosis. Nat Rev Dis Primers. 2018;4(1):44.

7. Marrie RA, Horwitz R, Cutter G, Tyry T, Campagnolo D, Vollmer T. The burden of mental comorbidity in multiple sclerosis: frequent, underdiagnosed, and undertreated. Mult Scler. 2009;15(3):385-92.

8. Gerhard L, Dorstyn DS, Murphy G, Roberts RM. Neurological, physical and sociodemographic correlates of employment in multiple sclerosis: A meta-analysis. J Health Psychol. 2020;25(1):92-104.

9. Clemens L, Langdon D. How does cognition relate to employment in multiple sclerosis? A systematic review. Mult Scler Relat Dis. 2018;26:183-91.

10. Penner IK, Paul F. Fatigue as a symptom or comorbidity of neurological diseases. Nature Reviews Neurology. 2017;13(11):662-75.

11. Jalkh G, Abi Nahed R, Macaron G, Rensel M. Safety of Newer Disease Modifying Therapies in Multiple Sclerosis. Vaccines (Basel). 2020;9(1).

12. Alba Pale L, Leon Caballero J, Samso Buxareu B, Salgado Serrano P, Perez Sola V. Systematic review of depression in patients with multiple sclerosis and its relationship to interferonbeta treatment. Mult Scler Relat Disord. 2017;17:138-43.

13. Kes VB, Cesarik M, Matovina LZ, Zavoreo I, Coric L, Drnasin S, Demarin V. The role of complementary and alternative medicine in therapy of multiple sclerosis. Acta Clin Croat. 2013;52(4):464-71.

14. Leong EM, Semple SJ, Angley M, Siebert W, Petkov J, McKinnon RA. Complementary and alternative medicines and dietary interventions in multiple sclerosis: What is being used in South Australia and why? Complement Ther Med. 2009;17(4):216-23.

15. Wekerle H. Nature, nurture, and microbes: The development of multiple sclerosis. Acta neurologica Scandinavica. 2017;136 Suppl 201:22-5.

16. Kamm CP, Uitdehaag BM, Polman CH. Multiple sclerosis: current knowledge and future outlook. Eur Neurol. 2014;72(3-4):132-41.

17. Simon KC, Munger KL, Ascherio A. XVI European Charcot Foundation lecture: nutrition and environment: can MS be prevented? Journal of the neurological sciences. 2011;311(1-2):1-8.

18. Sadovnick AD, Armstrong H, Rice GP, Bulman D, Hashimoto L, Paty DW, Hashimoto SA, Warren S, Hader W, Murray TJ, Seland TP, Metz L, Bell R, Duquette P, Gray T, Nelson R, Weinshenkar B, Brunt D, Ebers GC. A population-based study of multiple sclerosis in twins: update. Ann Neurol. 1993;33(3):281-5.

19. Riccio P, Rossano R. Diet, Gut Microbiota, and Vitamins D + A in Multiple Sclerosis. Neurotherapeutics : the journal of the American Society for Experimental NeuroTherapeutics. 2018;15(1):75-91.

20. Riccio P, Rossano R. Nutrition facts in multiple sclerosis. ASN Neuro. 2015;7(1).

21. Munger KL, Levin LI, Hollis BW, Howard NS, Ascherio A. Serum 25-hydroxyvitamin D levels and risk of multiple sclerosis. JAMA. 2006;296(23):2832-8.

22. Ascherio A, Munger KL, White R, Kochert K, Simon KC, Polman CH, Freedman MS, Hartung HP, Miller DH, Montalban X, Edan G, Barkhof F, Pleimes D, Radu EW, Sandbrink R, Kappos L, Pohl C. Vitamin D as an Early Predictor of Multiple Sclerosis Activity and Progression. Jama Neurology. 2014;71(3):306-14.

23. Zheng C, He L, Liu L, Zhu J, Jin T. The efficacy of vitamin D in multiple sclerosis: A meta-analysis. Mult Scler Relat Disord. 2018;23:56-61.

24. Doosti-Irani A, Tamtaji OR, Mansournia MA, Ghayour-Mobarhan M, Ferns G, Daneshvar Kakhaki R, Rezaei Shahmirzadi A, Asemi Z. The effects of vitamin D supplementation on expanded disability status scale in people with multiple sclerosis: A critical, systematic review and metaanalysis of randomized controlled trials. Clinical neurology and neurosurgery. 2019;187:105564.

25. Erridge C, Attina T, Spickett CM, Webb DJ. A high-fat meal induces low-grade endotoxemia: evidence of a novel mechanism of postprandial inflammation. Am J Clin Nutr. 2007;86(5):1286-92.

26. Ghanim H, Abuaysheh S, Sia CL, Korzeniewski K, Chaudhuri A, Fernandez-Real JM, Dandona P. Increase in plasma endotoxin concentrations and the expression of Toll-like receptors and suppressor of cytokine signaling-3 in mononuclear cells after a high-fat, high-carbohydrate meal: implications for insulin resistance. Diabetes Care. 2009;32(12):2281-7.

27. Hedstrom AK, Olsson T, Alfredsson L. High body mass index before age 20 is associated with increased risk for multiple sclerosis in both men and women. Mult Scler J. 2012;18(9):1334-6.

28. Yuan S, Xiong Y, Larsson SC. An atlas on risk factors for multiple sclerosis: a Mendelian randomization study. J Neurol. 2021;268(1):114-24.

29. Coo H, Aronson KJ. A systematic review of several potential non-genetic risk factors for multiple sclerosis. Neuroepidemiology. 2004;23(1-2):1-12.

30. Schwarz S, Leweling H. [Diet and multiple sclerosis]. Nervenarzt. 2005;76(2):131-42.

31. Stys PK, Zamponi GW, van Minnen J, Geurts JJ. Will the real multiple sclerosis please stand up? Nat Rev Neurosci. 2012;13(7):507-14.

32. Oliveira SR, Kallaur AP, Simao AN, Morimoto HK, Lopes J, Panis C, Petenucci DL, da Silva E, Cecchini R, Kaimen-Maciel DR, Reiche EM. Oxidative stress in multiple sclerosis patients in clinical remission: association with the expanded disability status scale. Journal of the neurological sciences. 2012;321(1-2):49-53.

33. Gilgun-Sherki Y, Melamed E, Offen D. The role of oxidative stress in the pathogenesis of multiple sclerosis: the need for effective antioxidant therapy. J Neurol. 2004;251(3):261-8.

34. Pegoretti V, Swanson KA, Bethea JR, Probert L, Eisel ULM, Fischer R. Inflammation and Oxidative Stress in Multiple Sclerosis: Consequences for Therapy Development. Oxid Med Cell Longev. 2020;2020:7191080.

35. Ljubisavljevic S, Stojanovic I, Vojinovic S, Stojanov D, Stojanovic S, Cvetkovic T, Savic D, Pavlovic D. The patients with clinically isolated syndrome and relapsing remitting multiple sclerosis show different levels of advanced protein oxidation products and total thiol content in plasma and CSF. Neurochemistry international. 2013;62(7):988-97.

36. Zhang SY, Gui LN, Liu YY, Shi S, Cheng Y. Oxidative Stress Marker Aberrations in Multiple Sclerosis: A Meta-Analysis Study. Front Neurosci. 2020;14:823.

37. Karlik M, Valkovic P, Hancinova V, Krizova L, Tothova L, Celec P. Markers of oxidative stress in plasma and saliva in patients with multiple sclerosis. Clin Biochem. 2015;48(1-2):24-8.

38. Storoni M, Plant GT. The Therapeutic Potential of the Ketogenic Diet in Treating Progressive Multiple Sclerosis. Mult Scler Int. 2015;2015:681289.

39. Ben Khedher MR, Haddad M, Laurin D, Ramassamy C. Effect of APOE epsilon4 allele on levels of apolipoproteins E, J, and D, and redox signature in circulating extracellular vesicles from cognitively impaired with no dementia participants converted to Alzheimer's disease. Alzheimers Dement (Amst). 2021;13(1):e12231.

40. Ohl K, Tenbrock K, Kipp M. Oxidative stress in multiple sclerosis: Central and peripheral mode of action. Experimental neurology. 2016;277:58-67.

41. Hayashi G, Jasoliya M, Sahdeo S, Sacca F, Pane C, Filla A, Marsili A, Puorro G, Lanzillo R, Brescia Morra V, Cortopassi G. Dimethyl fumarate mediates Nrf2-dependent mitochondrial biogenesis in mice and humans. Hum Mol Genet. 2017;26(15):2864-73.

42. Imam SA, Guyton MK, Haque A, Vandenbark A, Tyor WR, Ray SK, Banik NL. Increased calpain correlates with Th1 cytokine profile in PBMCs from MS patients. J Neuroimmunol. 2007;190(1-2):139-45.

43. Ozenci V, Kouwenhoven M, Link H. Cytokines in multiple sclerosis: methodological aspects and pathogenic implications. Mult Scler. 2002;8(5):396-404.

44. Opal SM, DePalo VA. Anti-inflammatory cytokines. Chest. 2000;117(4):1162-72.

45. Cannella B, Raine CS. The adhesion molecule and cytokine profile of multiple sclerosis lesions. Ann Neurol. 1995;37(4):424-35.

46. Maimone D, Guazzi GC, Annunziata P. IL-6 detection in multiple sclerosis brain. Journal of the neurological sciences. 1997;146(1):59-65.

47. Bitsch A, Kuhlmann T, Da Costa C, Bunkowski S, Polak T, Bruck W. Tumour necrosis factor alpha mRNA expression in early multiple sclerosis lesions: correlation with demyelinating activity and oligodendrocyte pathology. Glia. 2000;29(4):366-75.

48. TNF neutralization in MS: results of a randomized, placebo-controlled multicenter study. The Lenercept Multiple Sclerosis Study Group and The University of British Columbia MS/MRI Analysis Group. Neurology. 1999;53(3):457-65.

49. Link H. The cytokine storm in multiple sclerosis. Mult Scler. 1998;4(1):12-5.

50. Harris VK, Sadiq SA. Disease biomarkers in multiple sclerosis: potential for use in therapeutic decision making. Molecular diagnosis & therapy. 2009;13(4):225-44.

51. Schluep M, van Melle G, Henry H, Stadler C, Roth-Wicky B, Magistretti PJ. In vitro cytokine profiles as indicators of relapse activity and clinical course in multiple sclerosis. Multiple Sclerosis. 1998;4(3):198-202.

52. Rossi B, Santos-Lima B, Terrabuio E, Zenaro E, Constantin G. Common Peripheral Immunity Mechanisms in Multiple Sclerosis and Alzheimer's Disease. Front Immunol. 2021;12:639369.

53. Wang Y, Telesford KM, Ochoa-Reparaz J, Haque-Begum S, Christy M, Kasper EJ, Wang L, Wu Y, Robson SC, Kasper DL, Kasper LH. An intestinal commensal symbiosis factor controls neuroinflammation via TLR2-mediated CD39 signalling. Nat Commun. 2014;5:4432.

54. Berer K, Gerdes LA, Cekanaviciute E, Jia X, Xiao L, Xia Z, Liu C, Klotz L, Stauffer U, Baranzini SE, Kumpfel T, Hohlfeld R, Krishnamoorthy G, Wekerle H. Gut microbiota from multiple sclerosis patients enables spontaneous autoimmune encephalomyelitis in mice. Proc Natl Acad Sci U S A. 2017;114(40):10719-24.

55. Buscarinu MC, Fornasiero A, Romano S, Ferraldeschi M, Mechelli R, Renie R, Morena E, Romano C, Pellicciari G, Landi AC, Salvetti M, Ristori G. The Contribution of Gut Barrier Changes to Multiple Sclerosis Pathophysiology. Front Immunol. 2019;10:1916.

56. Buscarinu MC, Cerasoli B, Annibali V, Policano C, Lionetto L, Capi M, Mechelli R, Romano S, Fornasiero A, Mattei G, Piras E, Angelini DF, Battistini L, Simmaco M, Umeton R, Salvetti M, Ristori G. Altered intestinal permeability in patients with relapsing-remitting multiple sclerosis: A pilot study. Mult Scler. 2017;23(3):442-6.

57. Teixeira B, Bittencourt VCB, Ferreira TB, Kasahara TM, Barros PO, Alvarenga R, Hygino J, Andrade RM, Andrade AF, Bento CAM. Low sensitivity to glucocorticoid inhibition of in vitro Th17-related cytokine production in multiple sclerosis patients is related to elevated plasma lipopolysaccharide levels. Clin Immunol. 2013;148(2):209-18.

58. Camara-Lemarroy CR, Silva C, Greenfield J, Liu WQ, Metz LM, Yong VW. Biomarkers of intestinal barrier function in multiple sclerosis are associated with disease activity. Mult Scler. 2020;26(11):1340-50.

59. Ghareghani M, Reiter RJ, Zibara K, Farhadi N. Latitude, Vitamin D, Melatonin, and Gut Microbiota Act in Concert to Initiate Multiple Sclerosis: A New Mechanistic Pathway. Front Immunol. 2018;9:2484.

60. Esposito S, Bonavita S, Sparaco M, Gallo A, Tedeschi G. The role of diet in multiple sclerosis: A review. Nutritional neuroscience. 2018;21(6):377-90.

61. Swank RL, Lerstad O, Strom A, Backer J. Multiple sclerosis in rural Norway its geographic and occupational incidence in relation to nutrition. N Engl J Med. 1952;246(19):722-8.

62. Wullstein O. [Results of Evers' dietetic treatment of multiple sclerosis]. Med Klin. 1951;46(31):843-5.

63. Destunis G. [Vitamin therapy of multiplesclerosis]. Dtsch Med J. 1952;3(21-22):546-7.

64. Katz Sand I. The Role of Diet in Multiple Sclerosis: Mechanistic Connections and Current Evidence. Curr Nutr Rep. 2018;7(3):150-60.

65. Bagur MJ, Murcia MA, Jimenez-Monreal AM, Tur JA, Bibiloni MM, Alonso GL, Martinez-Tome M. Influence of Diet in Multiple Sclerosis: A Systematic Review. Adv Nutr. 2017;8(3):463-72.

66. Jakimovski D, Guan Y, Ramanathan M, Weinstock-Guttman B, Zivadinov R. Lifestyle-based modifiable risk factors in multiple sclerosis: review of experimental and clinical findings. Neurodegener Dis Manag. 2019;9(3):149-72.

67. Agranoff BW, Goldberg D. Diet and the geographical distribution of multiple sclerosis. Lancet. 1974;2(7888):1061-6.

68. Alter M, Yamoor M, Harshe M. Multiple sclerosis and nutrition. Archives of neurology. 1974;31(4):267-72.

69. Esparza ML, Sasaki S, Kesteloot H. Nutrition, latitude, and multiple sclerosis mortality: an ecologic study. Am J Epidemiol. 1995;142(7):733-7.

70. Nanji AA, Narod S. Multiple-Sclerosis, Latitude and Dietary-Fat - Is Pork the Missing Link. Medical hypotheses. 1986;20(3):279-82.

71. Malosse D, Perron H, Sasco A, Seigneurin JM. Correlation between Milk and Dairy Product Consumption and Multiple-Sclerosis Prevalence - a Worldwide Study. Neuroepidemiology. 1992;11(4-6):304-12.

72. Lauer K. The food pattern in geographical relation to the risk of multiple sclerosis in the Mediterranean and Near East region. J Epidemiol Community Health. 1991;45(3):251-2.

73. Thomas FJ, Wiles CM. Dysphagia and nutritional status in multiple sclerosis. J Neurol. 1999;246(8):677-82.

74. Slawta JN, Wilcox AR, McCubbin JA, Nalle DJ, Fox SD, Anderson G. Health behaviors, body composition, and coronary heart disease risk in women with multiple sclerosis. Arch Phys Med Rehabil. 2003;84(12):1823-30.

75. Redondo Robles L, Pintor de la Maza B, Tejada Garcia J, Garcia Vieitez JJ, Fernandez Gomez MJ, Barrera Mellado I, Ballesteros Pomar MD. Nutritional profile of multiple sclerosis. Nutr Hosp. 2019;36(2):340-9.

76. Dardiotis E, Tsouris Z, Aslanidou P, Aloizou AM, Sokratous M, Provatas A, Siokas V, Deretzi G, Hadjigeorgiou GM. Body mass index in patients with Multiple Sclerosis: a meta-analysis. Neurol Res. 2019;41(9):836-46.

77. Pilutti LA, Motl RW. Body Mass Index Underestimates Adiposity in Persons With Multiple Sclerosis. Arch Phys Med Rehabil. 2016;97(3):405-12.

78. Wingo BC, Young HJ, Motl RW. Body composition differences between adults with multiple sclerosis and BMI-matched controls without MS. Disabil Health J. 2018;11(2):243-8.

79. Pilutti LA, Motl RW. Body composition and disability in people with multiple sclerosis: A dual-energy x-ray absorptiometry study. Mult Scler Relat Disord. 2019;29:41-7.

80. Matusik E, Augustak A, Durmala J. Functional Mobility and Basic Motor Skills in Patients with Multiple Sclerosis and Its Relation to the Anthropometrical Status and Body Composition Parameters. Medicina (Kaunas). 2019;55(12).

81. Armon-Omer A, Waldman C, Simaan N, Neuman H, Tamir S, Shahien R. New Insights on the Nutrition Status and Antioxidant Capacity in Multiple Sclerosis Patients. Nutrients. 2019;11(2).

82. Salemi G, Gueli MC, Vitale F, Battaglieri F, Guglielmini E, Ragonese P, Trentacosti A, Massenti MF, Savettieri G, Bono A. Blood lipids, homocysteine, stress factors, and vitamins in clinically stable multiple sclerosis patients. Lipids in health and disease. 2010;9:19.

83. Lutsky MA, Zemskov AM, Razinkin KA. [Biochemical markers of oxidative stress in different forms and phases of multiple sclerosis]. Zh Nevrol Psikhiatr Im S S Korsakova. 2014;114(11):74-7.

84. Wens I, Dalgas U, Stenager E, Eijnde BO. Risk factors related to cardiovascular diseases and the metabolic syndrome in multiple sclerosis - a systematic review. Mult Scler. 2013;19(12):1556-64.

85. Oliveira SR, Simao AN, Kallaur AP, de Almeida ER, Morimoto HK, Lopes J, Dichi I, Kaimen-Maciel DR, Reiche EM. Disability in patients with multiple sclerosis: influence of insulin resistance, adiposity, and oxidative stress. Nutrition (Burbank, Los Angeles County, Calif). 2014;30(3):268-73.

86. Penesova A, Vlcek M, Imrich R, Vernerova L, Marko A, Meskova M, Grunnerova L, Turcani P, Jezova D, Kollar B. Hyperinsulinemia in newly diagnosed patients with multiple sclerosis. Metabolic brain disease. 2015;30(4):895-901.

87. Ascherio A, Munger KL. Epidemiology of Multiple Sclerosis: From Risk Factors to Prevention-An Update. Semin Neurol. 2016;36(2):103-14.

88. Zhornitsky S, McKay KA, Metz LM, Teunissen CE, Rangachari M. Cholesterol and markers of cholesterol turnover in multiple sclerosis: relationship with disease outcomes. Mult Scler Relat Disord. 2016;5:53-65.

89. Marrie RA, Rudick R, Horwitz R, Cutter G, Tyry T, Campagnolo D, Vollmer T. Vascular comorbidity is associated with more rapid disability progression in multiple sclerosis. Neurology. 2010;74(13):1041-7.

90. Kappus N, Weinstock-Guttman B, Hagemeier J, Kennedy C, Melia R, Carl E, Ramasamy DP, Cherneva M, Durfee J, Bergsland N, Dwyer MG, Kolb C, Hojnacki D, Ramanathan M, Zivadinov R. Cardiovascular risk factors are associated with increased lesion burden and brain atrophy in multiple sclerosis. J Neurol Neurosur Ps. 2016;87(2):181-7.

91. Weinstock-Guttman B, Zivadinov R, Mahfooz N, Carl E, Drake A, Schneider J, Teter B, Hussein S, Mehta B, Weiskopf M, Durfee J, Bergsland N, Ramanathan M. Serum lipid profiles are associated with disability and MRI outcomes in multiple sclerosis. J Neuroinflammation. 2011;8:127.

92. Ben-Zacharia A. The Effects of Body Mass Index (BMI) on Multiple Sclerosis (MS) Progression. Nurs Res. 2015;64(2):E49-E.

93. Matarese G, La Cava A, Sanna V, Lord GM, Lechler RI, Fontana S, Zappacosta S. Balancing susceptibility to infection and autoimmunity: a role for leptin? Trends Immunol. 2002;23(4):182-7.

94. Matarese G, Carrieri PB, La Cava A, Perna F, Sanna V, De Rosa V, Aufiero D, Fontana S, Zappacosta S. Leptin increase in multiple sclerosis associates with reduced number of CD4(+)CD25+ regulatory T cells. Proc Natl Acad Sci U S A. 2005;102(14):5150-5.

95. Parks NE, Jackson-Tarlton CS, Vacchi L, Merdad R, Johnston BC. Dietary interventions for multiple sclerosis-related outcomes. Cochrane Database Syst Rev. 2020;5:CD004192.

96. Silbermann E, Senders A, Wooliscroft L, Rice J, Cameron M, Waslo C, Orban A, Chase E, Yadav V, Bourdette D, Spain RI. Cross-sectional survey of complementary and alternative medicine used in Oregon and Southwest Washington to treat multiple sclerosis: A 17-Year update. Mult Scler Relat Dis. 2020;41.

97. Harbige LS. Dietary n-6 and n-3 fatty acids in immunity and autoimmune disease. Proc Nutr Soc. 1998;57(4):555-62.

98. Gallai V, Sarchielli P, Trequattrini A, Franceschini M, Floridi A, Firenze C, Alberti A, Di Benedetto D, Stragliotto E. Cytokine secretion and eicosanoid production in the peripheral blood mononuclear cells of MS patients undergoing dietary supplementation with n-3 polyunsaturated fatty acids. J Neuroimmunol. 1995;56(2):143-53.

99. AlAmmar WA, Albeesh FH, Ibrahim LM, Algindan YY, Yamani LZ, Khattab RY. Effect of omega-3 fatty acids and fish oil supplementation on multiple sclerosis: a systematic review. Nutritional neuroscience. 2021;24(7):569-79.

100. Sedighiyan M, Djafarian K, Dabiri S, Abdolahi M, Shab-Bidar S. The Effects of Omega-3 Supplementation on the Expanded Disability Status Scale and Inflammatory Cytokines in Multiple Sclerosis Patients: A Systematic Review and Meta-Analysis. CNS & neurological disorders drug targets. 2019;18(7):523-9.

101. Fratzer U. Multiple Sklerose: Eine neue Therapie vor der Blut-Hirn-Schranke. . VitaMinSpur 1992:3:142–7.

102. Simopoulos AP. An Increase in the Omega-6/Omega-3 Fatty Acid Ratio Increases the Risk for Obesity. Nutrients. 2016;8(3):128.

103. Hebener O, Ackermann H, Kappel U, Kramer J. Empirische Untersuchungen zur Progressionsminderung der Multiplen Sklerose durch eine ergänzende bilanzierte Diät. . Erfahrungsheilkunde 51:675–681. 2002.

104. Swank RL. Multiple sclerosis: twenty years on low fat diet. Archives of neurology. 1970;23(5):460-74.

105. Wahls TL, Titcomb TJ, Bisht B, Eyck PT, Rubenstein LM, Carr LJ, Darling WG, Hoth KF, Kamholz J, Snetselaar LG. Impact of the Swank and Wahls elimination dietary interventions on fatigue and quality of life in relapsing-remitting multiple sclerosis: The WAVES randomized parallel-arm clinical trial. Multiple Sclerosis Journal– Experimental, Translational and Clinical. July–September 2021, 1–11.

106. Yadav V, Marracci G, Kim E, Spain R, Cameron M, Overs S, Riddehough A, Li DK, McDougall J, Lovera J, Murchison C, Bourdette D. Low-fat, plant-based diet in multiple sclerosis: A randomized controlled trial. Mult Scler Relat Disord. 2016;9:80-90.

107. Chehade L, Jaafar ZA, El Masri D, Zmerly H, Kreidieh D, Tannir H, Itani L, El Ghoch M. Lifestyle Modification in Rheumatoid Arthritis: Dietary and Physical Activity Recommendations Based on Evidence. Curr Rheumatol Rev. 2019;15(3):209-14.

108. Hsieh MS, Hsu WH, Wang JW, Wang YK, Hu HM, Chang WK, Chen CY, Wu DC, Kuo FC, Su WW. Nutritional and dietary strategy in the clinical care of inflammatory bowel disease. J Formos Med Assoc. 2020;119(12):1742-9.

109. Adam O, Beringer C, Kless T, Lemmen C, Adam A, Wiseman M, Adam P, Klimmek R, Forth W. Anti-inflammatory effects of a low arachidonic acid diet and fish oil in patients with rheumatoid arthritis. Rheumatol Int. 2003;23(1):27-36.

110. Olaf Adam TA.

Ernährungsrichtlinien bei Multipler Sklerose: Ein Leitfaden: DMV Deutscher Medizin Verlag;; 2003.

111. Nathan J, Khedekar Kale D, Naik VD, Thakker F, Bailur S. Dietary Therapy in Secondary Progressive Multiple Sclerosis: A Case Report. Cureus. 2019;11(8):e5341.

112. Bisht B, Darling WG, Shivapour ET, Lutgendorf SK, Snetselaar LG, Chenard CA, Wahls TL. Multimodal intervention improves fatigue and quality of life in subjects with progressive multiple sclerosis: a pilot study. Degener Neurol Neuromuscul Dis. 2015;5:19-35.

113. Levy RG, Cooper PN, Giri P. Ketogenic diet and other dietary treatments for epilepsy. Cochrane Database Syst Rev. 2012(3):CD001903.

114. Martin K, Jackson CF, Levy RG, Cooper PN. Ketogenic diet and other dietary treatments for epilepsy. Cochrane Database Syst Rev. 2016;2:CD001903.

115. A DB. The Ketogenic Diet in Epilepsy. Can Med Assoc J. 1931;24(1):106-7.

116. Henderson ST, Vogel JL, Barr LJ, Garvin F, Jones JJ, Costantini LC. Study of the ketogenic agent AC-1202 in mild to moderate Alzheimer's disease: a randomized, double-blind, placebo-controlled, multicenter trial. Nutr Metab (Lond). 2009;6:31.

117. Reger MA, Henderson ST, Hale C, Cholerton B, Baker LD, Watson GS, Hyde K, Chapman D, Craft S. Effects of beta-hydroxybutyrate on cognition in memory-impaired adults. Neurobiol Aging. 2004;25(3):311-4.

118. Phillips MCL, Murtagh DKJ, Gilbertson LJ, Asztely FJS, Lynch CDP. Low-fat versus ketogenic diet in Parkinson's disease: A pilot randomized controlled trial. Mov Disord. 2018;33(8):1306-14.

119. Brenton JN, Banwell B, Bergqvist AGC, Lehner-Gulotta D, Gampper L, Leytham E, Coleman R, Goldman MD. Pilot study of a ketogenic diet in relapsing-remitting MS. Neurol Neuroimmunol Neuroinflamm. 2019;6(4):e565.

120. Benlloch M, Lopez-Rodriguez MM, Cuerda-Ballester M, Drehmer E, Carrera S, Ceron JJ, Tvarijonaviciute A, Chirivella J, Fernandez-Garcia D, de la Rubia Orti JE. Satiating Effect of a Ketogenic Diet and Its Impact on Muscle Improvement and Oxidation State in Multiple Sclerosis Patients. Nutrients. 2019;11(5).

121. Lee JE, Titcomb TJ, Bisht B, Rubenstein LM, Louison R, Wahls TL. A Modified MCT-Based Ketogenic Diet Increases Plasma beta-Hydroxybutyrate but Has Less Effect on Fatigue and Quality of Life in People with Multiple Sclerosis Compared to a Modified Paleolithic Diet: A Waitlist-Controlled, Randomized Pilot Study. J Am Coll Nutr. 2020:1-13.

122. Wolters M. Diet and psoriasis: experimental data and clinical evidence. Br J Dermatol. 2005;153(4):706-14.

123. Adawi M, Damiani G, Bragazzi NL, Bridgewood C, Pacifico A, Conic RRZ, Morrone A, Malagoli P, Pigatto PDM, Amital H, McGonagle D, Watad A. The Impact of Intermittent Fasting (Ramadan Fasting) on Psoriatic Arthritis Disease Activity, Enthesitis, and Dactylitis: A Multicentre Study. Nutrients. 2019;11(3).

124. Han K, Nguyen A, Traba J, Yao X, Kaler M, Huffstutler RD, Levine SJ, Sack MN. A Pilot Study To Investigate the Immune-Modulatory Effects of Fasting in Steroid-Naive Mild Asthmatics. J Immunol. 2018;201(5):1382-8.

125. Kjeldsen-Kragh J, Haugen M, Borchgrevink CF, Laerum E, Eek M, Mowinkel P, Hovi K, Forre O. Controlled trial of fasting and one-year vegetarian diet in rheumatoid arthritis. Lancet. 1991;338(8772):899-902.

126. Skoldstam L, Larsson L, Lindstrom FD. Effect of fasting and lactovegetarian diet on rheumatoid arthritis. Scand J Rheumatol. 1979;8(4):249-55.

127. Fraser DA, Thoen J, Djoseland O, Forre O, Kjeldsen-Kragh J. Serum levels of interleukin-6 and dehydroepiandrosterone sulphate in response to either fasting or a ketogenic diet in rheumatoid arthritis patients. Clinical and experimental rheumatology. 2000;18(3):357-62.

128. Roman SN, Fitzgerald KC, Beier M, Mowry EM. Safety and feasibility of various fasting - mimicking diets among people with multiple sclerosis. Mult Scler Relat Dis. 2020;42.

129. Fitzgerald KC, Vizthum D, Henry-Barron B, Schweitzer A, Cassard SD, Kossoff E, Hartman AL, Kapogiannis D, Sullivan P, Baer DJ, Mattson MP, Appel LJ, Mowry EM. Effect of intermittent vs. daily calorie restriction on changes in weight and patient-reported outcomes in people with multiple sclerosis. Mult Scler Relat Disord. 2018;23:33-9.

130. Laffel L. Ketone bodies: a review of physiology, pathophysiology and application of monitoring to diabetes. Diabetes Metab Res Rev. 1999;15(6):412-26.

131. Berg JM. Biochemistry, revised edition. Chem Eng News. 2001;79(13):130-.

132. Maurer GD, Brucker DP, Bahr O, Harter PN, Hattingen E, Walenta S, Mueller-Klieser W, Steinbach JP, Rieger J. Differential utilization of ketone bodies by neurons and glioma cell lines: a rationale for ketogenic diet as experimental glioma therapy. Bmc Cancer. 2011;11.

133. Geissler C, Powers HJ. Human nutrition. Thirteenth edition. ed. Oxford: Oxford University Press; 2017. xiii, 769 pages p.

134. Harris JJ, Jolivet R, Attwell D. Synaptic Energy Use and Supply. Neuron. 2012;75(5):762-77.

135. Veech RL, Chance B, Kashiwaya Y, Lardy HA, Cahill GF, Jr. Ketone bodies, potential therapeutic uses. IUBMB Life. 2001;51(4):241-7.

136. Dedkova EN, Blatter LA. Role of beta-hydroxybutyrate, its polymer poly-beta-hydroxybutyrate and inorganic polyphosphate in mammalian health and disease. Frontiers in physiology. 2014;5:260.

137. Robinson AM, Williamson DH. Physiological Roles of Ketone-Bodies as Substrates and Signals in Mammalian-Tissues. Physiol Rev. 1980;60(1):143-87.

138. Cahill GF, Jr. Fuel metabolism in starvation. Annu Rev Nutr. 2006;26:1-22.

139. Kim DY, Rho JM. The ketogenic diet and epilepsy. Curr Opin Clin Nutr Metab Care. 2008;11(2):113-20.

140. Temkin O. The falling sickness: a history of epilepsy from the Greeks to the beginnings of modern neurology: JHU Press; 1994.

141. Bough KJ, Wetherington J, Hassel B, Pare JF, Gawryluk JW, Greene JG, Shaw R, Smith Y, Geiger JD, Dingledine RJ. Mitochondrial biogenesis in the anticonvulsant mechanism of the ketogenic diet. Ann Neurol. 2006;60(2):223-35.

142. Kim DY, Davis LM, Sullivan PG, Maalouf M, Simeone TA, van Brederode J, Rho JM. Ketone bodies are protective against oxidative stress in neocortical neurons. J Neurochem. 2007;101(5):1316-26.

143. Lestan B, Walden K, Schmaltz S, Spychala J, Fox IH. beta-Hydroxybutyrate decreases adenosine triphosphate degradation products in human subjects. J Lab Clin Med. 1994;124(2):199-209.

144. Sullivan PG, Rippy NA, Dorenbos K, Concepcion RC, Agarwal AK, Rho JM. The ketogenic diet increases mitochondrial uncoupling protein levels and activity. Ann Neurol. 2004;55(4):576-80.

145. Milder JB, Liang LP, Patel M. Acute oxidative stress and systemic Nrf2 activation by the ketogenic diet. Neurobiol Dis. 2010;40(1):238-44.

146. Jeong EA, Jeon BT, Shin HJ, Kim N, Lee DH, Kim HJ, Kang SS, Cho GJ, Choi WS, Roh GS. Ketogenic diet-induced peroxisome proliferator-activated receptor-gamma activation decreases neuroinflammation in the mouse hippocampus after kainic acid-induced seizures. Experimental neurology. 2011;232(2):195-202.

147. Youm YH, Nguyen KY, Grant RW, Goldberg EL, Bodogai M, Kim D, D'Agostino D, Planavsky N, Lupfer C, Kanneganti TD, Kang S, Horvath TL, Fahmy TM, Crawford PA, Biragyn A, Alnemri E, Dixit VD. The ketone metabolite beta-hydroxybutyrate blocks NLRP3 inflammasome-mediated inflammatory disease. Nat Med. 2015;21(3):263-9.

148. Stubbs BJ, Koutnik AP, Goldberg EL, Upadhyay V, Turnbaugh PJ, Verdin E, Newman JC. Investigating Ketone Bodies as Immunometabolic Countermeasures against Respiratory Viral Infections. Med-Cambridge. 2020;1(1):43-65.

149. Hepple RT, Qin M, Nakamoto H, Goto S. Caloric restriction optimizes the proteasome pathway with aging in rat plantaris muscle: implications for sarcopenia. Am J Physiol Regul Integr Comp Physiol. 2008;295(4):R1231-7.

150. Wallace MA, Aguirre NW, Marcotte GR, Marshall AG, Baehr LM, Hughes DC, Hamilton KL, Roberts MN, Lopez-Dominguez JA, Miller BF, Ramsey JJ, Baar K. The ketogenic diet preserves skeletal muscle with aging in mice. Aging Cell. 2021;20(4):e13322.

151. McCarty MF, DiNicolantonio JJ, O'Keefe JH. Ketosis may promote brain macroautophagy by activating Sirt1 and hypoxia-inducible factor-1. Medical hypotheses. 2015;85(5):631-9.

152. Liskiewicz D, Liskiewicz A, Grabowski M, Nowacka-Chmielewska MM, Jablonska K, Wojakowska A, Marczak L, Barski JJ, Malecki A. Upregulation of hepatic autophagy under nutritional ketosis. J Nutr Biochem. 2021;93:108620.

153. Wang Y, Le WD. Autophagy and Ubiquitin-Proteasome System. Adv Exp Med Biol. 2019;1206:527-50.

154. Lilienbaum A. Relationship between the proteasomal system and autophagy. Int J Biochem Mol Biol. 2013;4(1):1-26.

155. Newman JC, Verdin E. Ketone bodies as signaling metabolites. Trends Endocrin Met. 2014;25(1):42-52.

156. Wilhelm C, Surendar J, Karagiannis F. Enemy or ally? Fasting as an essential regulator of immune responses. Trends in Immunology. 2021;42(5):389-400.

157. Bakshi R, Miletich RS, Kinkel PR, Emmet ML, Kinkel WR. High-resolution fluorodeoxyglucose positron emission tomography shows both global and regional cerebral hypometabolism in multiple sclerosis. J Neuroimaging. 1998;8(4):228-34.

158. Blinkenberg M, Jensen CV, Holm S, Paulson OB, Sorensen PS. A longitudinal study of cerebral glucose metabolism, MRI, and disability in patients with MS. Neurology. 1999;53(1):149-53.

159. Bateman RJ, Xiong C, Benzinger TL, Fagan AM, Goate A, Fox NC, Marcus DS, Cairns NJ, Xie X, Blazey TM, Holtzman DM, Santacruz A, Buckles V, Oliver A, Moulder K, Aisen PS, Ghetti B, Klunk WE, McDade E, Martins RN, Masters CL, Mayeux R, Ringman JM, Rossor MN, Schofield PR, Sperling RA, Salloway S, Morris JC, Dominantly Inherited Alzheimer N. Clinical and biomarker changes in dominantly inherited Alzheimer's disease. N Engl J Med. 2012;367(9):795-804.

160. Borghammer P, Chakravarty M, Jonsdottir KY, Sato N, Matsuda H, Ito K, Arahata Y, Kato T, Gjedde A. Cortical hypometabolism and hypoperfusion in Parkinson's disease is extensive: probably even at early disease stages. Brain Struct Funct. 2010;214(4):303-17.

161. Blinkenberg M, Rune K, Jensen CV, Ravnborg M, Kyllingsbaek S, Holm S, Paulson OB, Sorensen PS. Cortical cerebral metabolism correlates with MRI lesion load and cognitive dysfunction in MS. Neurology. 2000;54(3):558-64.

162. Paulesu E, Perani D, Fazio F, Comi G, Pozzilli C, Martinelli V, Filippi M, Bettinardi V, Sirabian G, Passafiume D, Anzini A, Lenzi GL, Canal N, Fieschi C. Functional basis of memory impairment in multiple sclerosis: a[18F]FDG PET study. Neuroimage. 1996;4(2):87-96.

163. Derache N, Grassiot B, Mezenge F, Emmanuelle Dugue A, Desgranges B, Constans JM, Defer GL. Fatigue is associated with metabolic and density alterations of cortical and deep gray matter in Relapsing-Remitting-Multiple Sclerosis patients at the earlier stage of the disease: A PET/MR study. Mult Scler Relat Disord. 2013;2(4):362-9.

164. Regenold WT, Phatak P, Makley MJ, Stone RD, Kling MA. Cerebrospinal fluid evidence of increased extra-mitochondrial glucose metabolism implicates mitochondrial dysfunction in multiple sclerosis disease progression. Journal of the neurological sciences. 2008;275(1-2):106-12.

165. Haarmann A, Hahnel L, Schuhmann MK, Buttmann M. Age-adjusted CSF beta 2-microglobulin and lactate are increased and ACE is decreased in patients with multiple sclerosis, but only lactate correlates with clinical disease duration and severity. Journal of Neuroimmunology. 2018;323:19-27.

166. Albanese M, Zagaglia S, Landi D, Boffa L, Nicoletti CG, Marciani MG, Mandolesi G, Marfia GA, Buttari F, Mori F, Centonze D. Cerebrospinal fluid lactate is associated with multiple sclerosis disease progression. J Neuroinflamm. 2016;13.

167. Amorini AM, Nociti V, Petzold A, Gasperini C, Quartuccio E, Lazzarino G, Di Pietro V, Belli A, Signoretti S, Vagnozzi R, Lazzarino G, Tavazzi B. Serum lactate as a novel potential biomarker in multiple sclerosis. Biochim Biophys Acta. 2014;1842(7):1137-43.

168. Melkonian EA, Schury MP. Biochemistry, Anaerobic Glycolysis. StatPearls. Treasure Island (FL)2021.

169. Warburg O. Über den Stoffwechsel der Carcinomzelle. Naturwissenschaften 12. 1924:1131–7.

170. Lehninger AL ND, Cox MM. Principles of Biochemistry. : Worth; New York; 1993.

171. Barcelos IP, Troxell RM, Graves JS. Mitochondrial Dysfunction and Multiple Sclerosis. Biology (Basel). 2019;8(2).

172. Pieczenik SR, Neustadt J. Mitochondrial dysfunction and molecular pathways of disease. Exp Mol Pathol. 2007;83(1):84-92.

173. Guo C, Sun L, Chen X, Zhang D. Oxidative stress, mitochondrial damage and neurodegenerative diseases. Neural Regen Res. 2013;8(21):2003-14.

174. Kim DY, Hao J, Liu R, Turner G, Shi FD, Rho JM. Inflammation-mediated memory dysfunction and effects of a ketogenic diet in a murine model of multiple sclerosis. PLoS One. 2012;7(5):e35476.

175. Maalouf M, Sullivan PG, Davis L, Kim DY, Rho JM. Ketones inhibit mitochondrial production of reactive oxygen species production following glutamate excitotoxicity by increasing NADH oxidation. Neuroscience. 2007;145(1):256-64.

176. Cunnane SC, Courchesne-Loyer A, St-Pierre V, Vandenberghe C, Pierotti T, Fortier M, Croteau E, Castellano CA. Can ketones compensate for deteriorating brain glucose uptake during aging? Implications for the risk and treatment of Alzheimer's disease. Annals of the New York Academy of Sciences. 2016;1367(1):12-20.

177. Castellano CA, Nugent S, Paquet N, Tremblay S, Bocti C, Lacombe G, Imbeault H, Turcotte E, Fulop T, Cunnane SC. Lower brain 18F-fluorodeoxyglucose uptake but normal 11C-acetoacetate metabolism in mild Alzheimer's disease dementia. J Alzheimers Dis. 2015;43(4):1343-53.

178. Croteau E, Castellano CA, Richard MA, Fortier M, Nugent S, Lepage M, Duchesne S, Whittingstall K, Turcotte EE, Bocti C, Fulop T, Cunnane SC. Ketogenic Medium Chain Triglycerides Increase Brain Energy Metabolism in Alzheimer's Disease. J Alzheimers Dis. 2018;64(2):551-61.

179. Choi IY, Piccio L, Childress P, Bollman B, Ghosh A, Brandhorst S, Suarez J, Michalsen A, Cross AH, Morgan TE, Wei M, Paul F, Bock M, Longo VD. A Diet Mimicking Fasting Promotes Regeneration and Reduces Autoimmunity and Multiple Sclerosis Symptoms. Cell Rep. 2016;15(10):2136-46.

180. Bai M, Wang Y, Han R, Xu L, Huang M, Zhao J, Lin Y, Song S, Chen Y. Intermittent caloric restriction with a modified fasting-mimicking diet ameliorates autoimmunity and promotes recovery in a mouse model of multiple sclerosis. J Nutr Biochem. 2021;87:108493.

181. Liu CH, Zhang N, Zhang RY, Jin L, Petridis AK, Loers G, Zheng XX, Wang ZP, Siebert HC. Cuprizone-Induced Demyelination in Mouse Hippocampus Is Alleviated by Ketogenic Diet. J Agr Food Chem. 2020;68(40):11215-28.

182. Zhang N, Liu CH, Zhang RY, Jin L, Yin XH, Zheng XX, Siebert HC, Li YB, Wang ZP, Loers G, Petridis AK. Amelioration of clinical course and demyelination in the cuprizone mouse model in relation to ketogenic diet. Food & Function. 2020;11(6):5647-63.

183. Ni FF, Li CR, Liao JX, Wang GB, Lin SF, Xia Y, Wen JL. The effects of ketogenic diet on the Th17/Treg cells imbalance in patients with intractable childhood epilepsy. Seizure. 2016;38:17-22.

184. Ang QY, Alexander M, Newman JC, Tian Y, Cai J, Upadhyay V, Turnbaugh JA, Verdin E, Hall KD, Leibel RL, Ravussin E, Rosenbaum M, Patterson AD, Turnbaugh PJ. Ketogenic Diets Alter the Gut Microbiome Resulting in Decreased Intestinal Th17 Cells. Cell. 2020;181(6):1263-75 e16.

185. Cignarella F, Cantoni C, Ghezzi L, Salter A, Dorsett Y, Chen L, Phillips D, Weinstock GM, Fontana L, Cross AH, Zhou Y, Piccio L. Intermittent Fasting Confers Protection in CNS Autoimmunity by Altering the Gut Microbiota. Cell Metab. 2018;27(6):1222-35 e6.

186. Choi YJ, Jeon SM, Shin S. Impact of a Ketogenic Diet on Metabolic Parameters in Patients with Obesity or Overweight and with or without Type 2 Diabetes: A Meta-Analysis of Randomized Controlled Trials. Nutrients. 2020;12(7).

187. Dashti HM, Al-Zaid NS, Mathew TC, Al-Mousawi M, Talib H, Asfar SK, Behbahani AI. Long term effects of ketogenic diet in obese subjects with high cholesterol level. Mol Cell Biochem. 2006;286(1-2):1-9.

188. Catenacci VA, Pan Z, Ostendorf D, Brannon S, Gozansky WS, Mattson MP, Martin B, MacLean PS, Melanson EL, Troy Donahoo W. A randomized pilot study comparing zero-calorie alternate-day fasting to daily caloric restriction in adults with obesity. Obesity (Silver Spring). 2016;24(9):1874-83.

189. Sharman MJ, Kraemer WJ, Love DM, Avery NG, Gomez AL, Scheett TP, Volek JS. A ketogenic diet favorably affects serum biomarkers for cardiovascular disease in normal-weight men. J Nutr. 2002;132(7):1879-85.

190. Maifeld A, Bartolomaeus H, Lober U, Avery EG, Steckhan N, Marko L, Wilck N, Hamad I, Susnjar U, Mahler A, Hohmann C, Chen CY, Cramer H, Dobos G, Lesker TR, Strowig T, Dechend R, Bzdok D, Kleinewietfeld M, Michalsen A, Muller DN, Forslund SK. Fasting alters the gut microbiome reducing blood pressure and body weight in metabolic syndrome patients. Nat Commun. 2021;12(1):1970.

191. Harris L, Hamilton S, Azevedo LB, Olajide J, De Brun C, Waller G, Whittaker V, Sharp T, Lean M, Hankey C, Ells L. Intermittent fasting interventions for treatment of overweight and obesity in adults: a systematic review and meta-analysis. JBI Database System Rev Implement Rep. 2018;16(2):507-47.

192. Silveira C, Guedes R, Maia D, Curral R, Coelho R. Neuropsychiatric Symptoms of Multiple Sclerosis: State of the Art. Psychiatry Investig. 2019;16(12):877-88.

193. Pavon S, Lazaro E, Martinez O, Amayra I, Lopez-Paz JF, Caballero P, Al-Rashaida M, Luna PM, Garcia M, Perez M, Berrocoso S, Rodriguez AA, Perez-Nunez P. Ketogenic diet and cognition in neurological diseases: a systematic review. Nutr Rev. 2021;79(7):802-13.

194. Garcia-Penas JJ. [Epilepsy, cognition and ketogenic diet]. Rev Neurol. 2018;66(S01):S71-S5.

195. Ota M, Matsuo J, Ishida I, Takano H, Yokoi Y, Hori H, Yoshida S, Ashida K, Nakamura K, Takahashi T, Kunugi H. Effects of a medium-chain triglyceride-based ketogenic formula on cognitive function in patients with mild-to-moderate Alzheimer's disease. Neurosci Lett. 2019;690:232-6.

196. Craig C. Mitoprotective dietary approaches for Myalgic Encephalomyelitis/Chronic Fatigue Syndrome: Caloric restriction, fasting, and ketogenic diets. Medical hypotheses. 2015;85(5):690-3.

197. Cohen CW, Fontaine KR, Arend RC, Soleymani T, Gower BA. Favorable Effects of a Ketogenic Diet on Physical Function, Perceived Energy, and Food Cravings in Women with Ovarian or Endometrial Cancer: A Randomized, Controlled Trial. Nutrients. 2018;10(9).

198. Plotti F, Terranova C, Luvero D, Bartolone M, Messina G, Feole L, Cianci S, Scaletta G, Marchetti C, Di Donato V, Fagotti A, Scambia G, Benedetti Panici P, Angioli R. Diet and Chemotherapy: The Effects of Fasting and Ketogenic Diet on Cancer Treatment. Chemotherapy. 2020;65(3-4):77-84.

199. Boeschoten RE, Braamse AMJ, Beekman ATF, Cuijpers P, van Oppen P, Dekker J, Uitdehaag BMJ. Prevalence of depression and anxiety in Multiple Sclerosis: A systematic review and meta-analysis. Journal of the neurological sciences. 2017;372:331-41.

200. Hussin NM, Shahar S, Teng NIMF, Ngah WZW, Das SK. Efficacy of Fasting and Calorie Restriction (FCR) on mood and depression among ageing men. J Nutr Health Aging. 2013;17(8):674-80.

201. Ren MX, Zhang HY, Qi JD, Hu AN, Jiang Q, Hou YY, Feng QQ, Ojo O, Wang XH. An Almond-Based Low Carbohydrate Diet Improves Depression and Glycometabolism in Patients with Type 2 Diabetes through Modulating Gut Microbiota and GLP-1: A Randomized Controlled Trial. Nutrients. 2020;12(10).

202. Maniaci G, La Cascia C, Giammanco A, Ferraro L, Chianetta R, Di Peri R, Sardella Z, Citarrella R, Mannella Y, Larcan S, Montana S, Mirisola MG, Longo V, Rizzo M, La Barbera D. Efficacy of a fasting-mimicking diet in functional therapy for depression: A randomised controlled pilot trial. J Clin Psychol. 2020;76(10):1807-17.

203. Heesen C, Nawrath L, Reich C, Bauer N, Schulz KH, Gold SM. Fatigue in multiple sclerosis: an example of cytokine mediated sickness behaviour? Journal of neurology, neurosurgery, and psychiatry. 2006;77(1):34-9.

204. Patejdl R, Penner IK, Noack TK, Zettl UK. Multiple sclerosis and fatigue: A review on the contribution of inflammation and immune-mediated neurodegeneration. Autoimmun Rev. 2016;15(3):210-20.

205. Morris G, Reiche EMV, Murru A, Carvalho AF, Maes M, Berk M, Puri BK. Multiple Immune-Inflammatory and Oxidative and Nitrosative Stress Pathways Explain the Frequent Presence of Depression in Multiple Sclerosis. Mol Neurobiol. 2018;55(8):6282-306.

206. Katarina V, Gordana T, Svetlana MD, Milica B. Oxidative stress and neuroinflammation should be both considered in the occurrence of fatigue and depression in multiple sclerosis. Acta Neurol Belg. 2020;120(4):853-61.

207. Wilson CJ, Finch CE, Cohen HJ. Cytokines and cognition--the case for a head-to-toe inflammatory paradigm. J Am Geriatr Soc. 2002;50(12):2041-56.

208. Puchalska P, Crawford PA. Metabolic and Signaling Roles of Ketone Bodies in Health and Disease. Annu Rev Nutr. 2021;41:49-77.

209. Bahr LS, Bock M, Liebscher D, Bellmann-Strobl J, Franz L, Pruss A, Schumann D, Piper SK, Kessler CS, Steckhan N, Michalsen A, Paul F, Mahler A. Ketogenic diet and fasting diet as Nutritional Approaches in Multiple Sclerosis (NAMS): protocol of a randomized controlled study. Trials. 2020;21(1):3.

210. Thompson AJ, Banwell BL, Barkhof F, Carroll WM, Coetzee T, Comi G, Correale J, Fazekas F, Filippi M, Freedman MS, Fujihara K, Galetta SL, Hartung HP, Kappos L, Lublin FD, Marrie RA, Miller AE, Miller DH, Montalban X, Mowry EM, Sorensen PS, Tintore M, Traboulsee AL, Trojano M, Uitdehaag BMJ, Vukusic S, Waubant E, Weinshenker BG, Reingold SC, Cohen JA. Diagnosis of multiple sclerosis: 2017 revisions of the McDonald criteria. Lancet Neurol. 2018;17(2):162-73.

211. Kurtzke JF. Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). Neurology. 1983;33(11):1444-52.

212. Schoeler NE, Cross JH. Ketogenic dietary therapies in adults with epilepsy: a practical guide. Pract Neurol. 2016;16(3):208-14.

213. Seidell JC, Flegal KM. Assessing obesity: classification and epidemiology. Br Med Bull. 1997;53(2):238-52.

214. Kreissl A, Jorda A, Truschner K, Skacel G, Greber-Platzer S. Clinically relevant body composition methods for obese pediatric patients. BMC Pediatr. 2019;19(1):84.

215. Sun SS, Chumlea WC, Heymsfield SB, Lukaski HC, Schoeller D, Friedl K, Kuczmarski RJ, Flegal KM, Johnson CL, Hubbard VS. Development of bioelectrical impedance analysis prediction equations for body composition with the use of a multicomponent model for use in epidemiologic surveys. Am J Clin Nutr. 2003;77(2):331-40.

216. Lim S, Lee K, Woo HY, Park H, Kwon MJ. Evaluation of Cobas 8000 Analyzer Series Module e801 Analytical Performance. Ann Clin Lab Sci. 2019;49(3):372-9.

217. Barboza T, Beaufrere H. Comparison of a Point-of-care Cholesterol Meter With a Reference Laboratory Analyzer in Companion Psittaciformes. J Avian Med Surg. 2019;33(1):7-14.

218. Choi HYJ. Flash Continuous Glucose Monitoring (FreeStyle Libre 14-Day System) for Self-Management of Diabetes Mellitus. Am Fam Physician. 2021;103(11):688-90.

219. Stuetz W, Weber D, Dolle ME, Jansen E, Grubeck-Loebenstein B, Fiegl S, Toussaint O, Bernhardt J, Gonos ES, Franceschi C, Sikora E, Moreno-Villanueva M, Breusing N, Grune T, Burkle A. Plasma Carotenoids, Tocopherols, and Retinol in the Age-Stratified (35-74 Years) General Population: A Cross-Sectional Study in Six European Countries. Nutrients. 2016;8(10).

220. Weber D, Stuetz W, Bernhard W, Franz A, Raith M, Grune T, Breusing N. Oxidative stress markers and micronutrients in maternal and cord blood in relation to neonatal outcome. Eur J Clin Nutr. 2014;68(2):215-22.

221. Weber D, Kneschke N, Grimm S, Bergheim I, Breusing N, Grune T. Rapid and sensitive determination of protein-nitrotyrosine by ELISA: Application to human plasma. Free Radic Res. 2012;46(3):276-85.

222. Kochlik B, Stuetz W, Peres K, Feart C, Tegner J, Rodriguez-Manas L, Grune T, Weber D. Associations of Plasma 3-Methylhistidine with Frailty Status in French Cohorts of the FRAILOMIC Initiative. J Clin Med. 2019;8(7).

223. van den Ouweland JMW, Beijers AM, Demacker PNM, van Daal H. Measurement of 25-OH-vitamin D in human serum using liquid chromatography tandem-mass spectrometry with comparison to radioimmunoassay and automated immunoassay. J Chromatogr B. 2010;878(15-16):1163-8.

224. Reeg S, Jung T, Castro JP, Davies KJA, Henze A, Grune T. The molecular chaperone Hsp70 promotes the proteolytic removal of oxidatively damaged proteins by the proteasome. Free Radic Biol Med. 2016;99:153-66.

225. Levent Akyüz AW, Florian Butke, Park Su-Jin, Anja Kuckuck, Hans-Dieter Volk, Gerald Grütz. Validation of novel multiplex technologies. Advances in Precision Medicine. 2017;Vol 2, Issue 1.

226. Krupp LB, LaRocca NG, Muir-Nash J, Steinberg AD. The fatigue severity scale. Application to patients with multiple sclerosis and systemic lupus erythematosus. Archives of neurology. 1989;46(10):1121-3.

227. Kaynak H, Altintas A, Kaynak D, Uyanik O, Saip S, Agaoglu J, Onder G, Siva A. Fatigue and sleep disturbance in multiple sclerosis. Eur J Neurol. 2006;13(12):1333-9.

228. Learmonth YC, Dlugonski D, Pilutti LA, Sandroff BM, Klaren R, Motl RW. Psychometric properties of the Fatigue Severity Scale and the Modified Fatigue Impact Scale. Journal of the neurological sciences. 2013;331(1-2):102-7.

229. Aaron T. Beck RAS, and Gregory K. Brown. Beck Depression Inventory - 2nd Edition (BDI-II)1996.

230. Wintjen L, Petermann F. Beck-Depressions-Inventar Revision (BDI-II). Z Psychiatr Psych Ps. 2010;58(3):243-5.

231. Sacco R, Santangelo G, Stamenova S, Bisecco A, Bonavita S, Lavorgna L, Trojano L, D'Ambrosio A, Tedeschi G, Gallo A. Psychometric properties and validity of Beck Depression Inventory II in multiple sclerosis. Eur J Neurol. 2016;23(4):744-50.

232. Kounali D, Button KS, Lewis G, Gilbody S, Kessler D, Araya R, Duffy L, Lanham P, Peters TJ, Wiles N, Lewis G. How much change is enough? Evidence from a longitudinal study on depression in UK primary care. Psychol Med. 2020:1-8.

233. Button KS, Kounali D, Thomas L, Wiles NJ, Peters TJ, Welton NJ, Ades AE, Lewis G. Minimal clinically important difference on the Beck Depression Inventory--II according to the patient's perspective. Psychol Med. 2015;45(15):3269-79.

234. Smith A. Symbol Digit Modalities Test (Revised). Western Psychological Services; 1982.

235. Strober L, DeLuca J, Benedict RH, Jacobs A, Cohen JA, Chiaravalloti N, Hudson LD, Rudick RA, LaRocca NG, Multiple Sclerosis Outcome Assessments C. Symbol Digit Modalities Test: A valid clinical trial endpoint for measuring cognition in multiple sclerosis. Mult Scler. 2019;25(13):1781-90.

236. Benedict RHB, DeLuca J, Phillips G, LaRocca N, Hudson LD, Rudick R, Assessm MSO. Validity of the Symbol Digit Modalities Test as a cognition performance outcome measure for multiple sclerosis. Mult Scler J. 2017;23(5):721-33.

237. Parmenter BA, Weinstock-Guttman B, Garg N, Munschauer F, Benedict RHB. Screening for cognitive impairment in multiple sclerosis using the Symbol Digit Modalities Test. Mult Scler J. 2007;13(1):52-7.

238. DeLuca J, Schippling S, Montalban X, Kappos L, Cree BAC, Comi G, Arnold DL, Hartung HP, Sheffield JK, Liu H, Silva D, Cohen JA. Effect of Ozanimod on Symbol Digit Modalities Test Performance in Relapsing MS. Mult Scler Relat Disord. 2021;48:102673.

239. Benedict RH, Cohan S, Lynch SG, Riester K, Wang P, Castro-Borrero W, Elkins J, Sabatella G. Improved cognitive outcomes in patients with relapsing-remitting multiple sclerosis treated with daclizumab beta: Results from the DECIDE study. Mult Scler. 2018;24(6):795-804.

240. Planes S, Villier C, Mallaret M. The nocebo effect of drugs. Pharmacol Res Perspect. 2016;4(2):e00208.

241. Goday A, Bellido D, Sajoux I, Crujeiras AB, Burguera B, Garcia-Luna PP, Oleaga A, Moreno B, Casanueva FF. Short-term safety, tolerability and efficacy of a very low-calorie-ketogenic diet interventional weight loss program versus hypocaloric diet in patients with type 2 diabetes mellitus. Nutr Diabetes. 2016;6(9):e230.

242. Stocker R, Reber E, Aeberhard C, Bally L, Schutz P, Stanga Z. [Fasting - Effects on the Human Body and Psyche]. Praxis (Bern 1994). 2019;108(9):593-7.

243. Alva S, Castorino K, Cho H, Ou J. Feasibility of Continuous Ketone Monitoring in Subcutaneous Tissue Using a Ketone Sensor. J Diabetes Sci Technol. 2021;15(4):768-74.

244. Livne-Margolin M, Tokatly Latzer I, Pinhas-Hamiel O, Harari G, Achiron A. Bodyweight Measures and Lifestyle Habits in Individuals with Multiple Sclerosis and Moderate to Severe Disability. J Clin Med. 2021;10(10).

245. Heilbronn LK, de Jonge L, Frisard MI, DeLany JP, Larson-Meyer DE, Rood J, Nguyen T, Martin CK, Volaufova J, Most MM, Greenway FL, Smith SR, Deutsch WA, Williamson DA, Ravussin E, Pennington CT. Effect of 6-month calorie restriction on biomarkers of longevity, metabolic adaptation, and oxidative stress in overweight individuals: a randomized controlled trial. JAMA. 2006;295(13):1539-48.

246. Chen CN, Lin SY, Liao YH, Li ZJ, Wong AM. Late-onset caloric restriction alters skeletal muscle metabolism by modulating pyruvate metabolism. Am J Physiol Endocrinol Metab. 2015;308(11):E942-9.

247. Keenan S, Cooke MB, Belski R. The Effects of Intermittent Fasting Combined with Resistance Training on Lean Body Mass: A Systematic Review of Human Studies. Nutrients. 2020;12(8).

248. Klement RJ, Champ CE, Kammerer U, Koebrunner PS, Krage K, Schafer G, Weigel M, Sweeney RA. Impact of a ketogenic diet intervention during radiotherapy on body composition: III-final results of the KETOCOMP study for breast cancer patients. Breast Cancer Res. 2020;22(1):94.

249. Formica CA, Cosman F, Nieves J, Herbert J, Lindsay R. Reduced bone mass and fat-free mass in women with multiple sclerosis: effects of ambulatory status and glucocorticoid Use. Calcif Tissue Int. 1997;61(2):129-33.

250. Murali N, Browne RW, Fellows Maxwell K, Bodziak ML, Jakimovski D, Hagemeier J, Bergsland N, Weinstock-Guttman B, Zivadinov R, Ramanathan M. Cholesterol and neurodegeneration: longitudinal changes in serum cholesterol biomarkers are associated with new lesions and gray matter atrophy in multiple sclerosis over 5 years of follow-up. Eur J Neurol. 2020;27(1):188-e4.

251. Stampanoni Bassi M, Iezzi E, Buttari F, Gilio L, Simonelli I, Carbone F, Micillo T, De Rosa V, Sica F, Furlan R, Finardi A, Fantozzi R, Storto M, Bellantonio P, Pirollo P, Di Lemme S, Musella A, Mandolesi G, Centonze D, Matarese G. Obesity worsens central inflammation and disability in multiple sclerosis. Mult Scler. 2020;26(10):1237-46.

252. Bueno NB, de Melo IS, de Oliveira SL, da Rocha Ataide T. Very-low-carbohydrate ketogenic diet v. low-fat diet for long-term weight loss: a meta-analysis of randomised controlled trials. Br J Nutr. 2013;110(7):1178-87.

253. Brown BG, Stukovsky KH, Zhao XQ. Simultaneous low-density lipoprotein-C lowering and high-density lipoprotein-C elevation for optimum cardiovascular disease prevention with various drug classes, and their combinations: a meta-analysis of 23 randomized lipid trials. Curr Opin Lipidol. 2006;17(6):631-6.

254. Xie XF, Huang XH, Shen AZ, Li J, Sun YH. Association between circulating leptin levels and multiple sclerosis: a systematic review and meta-analysis. Postgrad Med J. 2018;94(1111):278-83.

255. Bistrom M, Hultdin J, Andersen O, Alonso-Magdalena L, Jons D, Gunnarsson M, Vrethem M, Sundstrom P. Leptin levels are associated with multiple sclerosis risk. Mult Scler. 2021;27(1):19-27.

256. Keyhanian K, Saxena S, Gombolay G, Healy BC, Misra M, Chitnis T. Adipokines are associated with pediatric multiple sclerosis risk and course. Mult Scler Relat Disord. 2019;36:101384.

257. Frisullo G, Mirabella M, Angelucci F, Caggiula M, Morosetti R, Sancricca C, Patanella AK, Nociti V, Iorio R, Bianco A, Tomassini V, Pozzilli C, Tonali PA, Matarese G, Batocchi AP. The effect of disease activity on leptin, leptin receptor and suppressor of cytokine signalling-3 expression in relapsing-remitting multiple sclerosis. J Neuroimmunol. 2007;192(1-2):174-83.

258. Signoriello E, Lus G, Polito R, Casertano S, Scudiero O, Coletta M, Monaco ML, Rossi F, Nigro E, Daniele A. Adiponectin profile at baseline is correlated to progression and severity of multiple sclerosis. European Journal of Neurology. 2019;26(2):348-55.

259. Signoriello E, Mallardo M, Nigro E, Polito R, Casertano S, Di Pietro A, Coletta M, Monaco ML, Rossi F, Lus G, Daniele A. Adiponectin in Cerebrospinal Fluid from Patients Affected by Multiple

Sclerosis Is Correlated with the Progression and Severity of Disease (Jan, 10.1007/s12035-021-02287-z, 2021). Mol Neurobiol. 2021;58(6):2671-.

260. Musabak U, Demirkaya S, Genc G, Ilikci RS, Odabasi Z. Serum adiponectin, TNF-alpha, IL-12p70, and IL-13 levels in multiple sclerosis and the effects of different therapy regimens. Neuroimmunomodulation. 2011;18(1):57-66.

261. Guerrero-Garcia JJ, Carrera-Quintanar L, Lopez-Roa RI, Marquez-Aguirre AL, Rojas-Mayorquin AE, Ortuno-Sahagun D. Multiple Sclerosis and Obesity: Possible Roles of Adipokines. Mediators Inflamm. 2016;2016:4036232.

262. Li S, Shin HJ, Ding EL, van Dam RM. Adiponectin levels and risk of type 2 diabetes: a systematic review and meta-analysis. JAMA. 2009;302(2):179-88.

263. Grassmann S, Wirsching J, Eichelmann F, Aleksandrova K. Association Between Peripheral Adipokines and Inflammation Markers: A Systematic Review and Meta-Analysis. Obesity. 2017;25(10):1776-85.

264. Ruiz-Arguelles A, Mendez-Huerta MA, Lozano CD, Ruiz-Arguelles GJ. Metabolomic profile of insulin resistance in patients with multiple sclerosis is associated to the severity of the disease. Mult Scler Relat Disord. 2018;25:316-21.

265. Tavazzi B, Batocchi AP, Amorini AM, Nociti V, D'Urso S, Longo S, Gullotta S, Picardi M, Lazzarino G. Serum metabolic profile in multiple sclerosis patients. Mult Scler Int. 2011;2011:167156.

266. Kallaur AP, Reiche EM, Oliveira SR, Simao AN, Pereira WL, Alfieri DF, Flauzino T, Proenca CM, Lozovoy MA, Kaimen-Maciel DR, Maes M. Genetic, Immune-Inflammatory, and Oxidative Stress Biomarkers as Predictors for Disability and Disease Progression in Multiple Sclerosis. Mol Neurobiol. 2017;54(1):31-44.

267. Flauzino T, Simao ANC, Pereira WLDJ, Alfieri DF, Oliveira SR, Kallaur AP, Lozovoy MAB, Kaimen-Maciel DR, Maes M, Reiche EMV. Disability in multiple sclerosis is associated with age and inflammatory, metabolic and oxidative/nitrosative stress biomarkers: results of multivariate and machine learning procedures. Metabolic brain disease. 2019;34(5):1401-13.

268. Mayo I, Arribas J, Villoslada P, DoForno RA, Rodriguez-Vilarino S, Montalban X, de Sagarra MR, Castano JG. The proteasome is a major autoantigen in multiple sclerosis. Brain. 2002;125:2658-67.

269. Thuy-Tien H, Haugen M, Aarseth J, Storstein A, Vedeler CA. Proteasome antibodies in patients with cancer or multiple sclerosis. Scand J Immunol. 2008;67(4):400-3.

270. Zou K, Rouskin S, Dervishi K, McCormick MA, Sasikumar A, Deng CH, Chen ZB, Kaeberlein M, Brem RB, Polymenis M, Kennedy BK, Weissman JS, Zheng JS, Ouyang Q, Li H. Life span extension by glucose restriction is abrogated by methionine supplementation: Cross-talk between glucose and methionine and implication of methionine as a key regulator of life span. Sci Adv. 2020;6(32).

271. Bonelli MA, Desenzani S, Cavallini G, Donati A, Romani AA, Bergamini E, Borghetti AF. Low-level caloric restriction rescues proteasome activity and Hsc70 level in liver of aged rats. Biogerontology. 2008;9(1):1-10.

272. Puri R, Jain N, Ganesh S. Increased glucose concentration results in reduced proteasomal activity and the formation of glycogen positive aggresomal structures. FEBS J. 2011;278(19):3688-98.

273. Sies H, Stahl W. Vitamins E and C, beta-carotene, and other carotenoids as antioxidants. Am J Clin Nutr. 1995;62(6 Suppl):1315S-21S.

274. Couillard C, Lemieux S, Vohl MC, Couture P, Lamarche B. Carotenoids as biomarkers of fruit and vegetable intake in men and women. Br J Nutr. 2016;116(7):1206-15.

275. Ames BN. Prolonging healthy aging: Longevity vitamins and proteins. Proc Natl Acad Sci U S A. 2018;115(43):10836-44.

276. Kenig S, Petelin A, Poklar Vatovec T, Mohorko N, Jenko-Praznikar Z. Assessment of micronutrients in a 12-wk ketogenic diet in obese adults. Nutrition (Burbank, Los Angeles County, Calif). 2019;67-68:110522.

277. Prudencio MB, de Lima PA, Murakami DK, Sampaio LPB, Damasceno NRT. Micronutrient supplementation needs more attention in patients with refractory epilepsy under ketogenic diet treatment. Nutrition (Burbank, Los Angeles County, Calif). 2021;86:111158.

278. Holecek M. Histidine in Health and Disease: Metabolism, Physiological Importance, and Use as a Supplement. Nutrients. 2020;12(3).

279. Kochlik B, Gerbracht C, Grune T, Weber D. The Influence of Dietary Habits and Meat Consumption on Plasma 3-Methylhistidine-A Potential Marker for Muscle Protein Turnover. Mol Nutr Food Res. 2018;62(9):e1701062.

280. Morris G, Maes M. Myalgic encephalomyelitis/chronic fatigue syndrome and encephalomyelitis disseminata/multiple sclerosis show remarkable levels of similarity in phenomenology and neuroimmune characteristics. BMC medicine. 2013;11.

281. Armstrong CW, McGregor NR, Butt HL, Gooley PR. Metabolism in chronic fatigue syndrome. Adv Clin Chem. 2014;66:121-72.

282. Zou H, Wang D, Ren H, Cai K, Chen P, Fang C, Shi Z, Zhang P, Wang J, Yang H, Zhong H. Effect of Caloric Restriction on BMI, Gut Microbiota, and Blood Amino Acid Levels in Non-Obese Adults. Nutrients. 2020;12(3).

283. Cassidy AA, Blier PU, Le Francois NR, Dionne P, Morin PJ, Lamarre SG. Effects of fasting and refeeding on protein and glucose metabolism in Arctic charr. Comp Biochem Physiol A Mol Integr Physiol. 2018;226:66-74.

284. Aquilani R, Costa A, Maestri R, Cotta Ramusino M, Pierobon A, Dossena M, Solerte SB, Condino AM, Torlaschi V, Bini P, Boselli M, Ceroni M, Buonocore D, Boschi F, Bruni M, Verri M. Mini Nutritional Assessment May Identify a Dual Pattern of Perturbed Plasma Amino Acids in Patients with Alzheimer's Disease: A Window to Metabolic and Physical Rehabilitation? Nutrients. 2020;12(6).

285. Powers SK, Smuder AJ, Criswell DS. Mechanistic Links Between Oxidative Stress and Disuse Muscle Atrophy. Antioxid Redox Sign. 2011;15(9):2519-28.

286. Ubhi BK, Riley JH, Shaw PA, Lomas DA, Tal-Singer R, MacNee W, Griffin JL, Connor SC. Metabolic profiling detects biomarkers of protein degradation in COPD patients. Eur Respir J. 2012;40(2):345-55.

287. Junttila IS. Tuning the Cytokine Responses: An Update on Interleukin (IL)-4 and IL-13 Receptor Complexes. Front Immunol. 2018;9:888.

288. Gallo E, Katzman S, Villarino AV. IL-13-producing Th1 and Th17 cells characterize adaptive responses to both self and foreign antigens. Eur J Immunol. 2012;42(9):2322-8.

289. Moser T, Akgun K, Proschmann U, Sellner J, Ziemssen T. The role of TH17 cells in multiple sclerosis: Therapeutic implications. Autoimmun Rev. 2020;19(10):102647.

290. Karo-Atar D, Bitton A, Benhar I, Munitz A. Therapeutic Targeting of the Interleukin-4/Interleukin-13 Signaling Pathway: In Allergy and Beyond. BioDrugs. 2018;32(3):201-20.

291. Martinez-Reyes CP, Gomez-Arauz AY, Torres-Castro I, Manjarrez-Reyna AN, Palomera LF, Olivos-Garcia A, Mendoza-Tenorio E, Sanchez-Medina GA, Islas-Andrade S, Melendez-Mier G, Escobedo G. Serum Levels of Interleukin-13 Increase in Subjects with Insulin Resistance but Do Not Correlate with Markers of Low-Grade Systemic Inflammation. J Diabetes Res. 2018;2018:7209872.

292. Ghezzi L, Cantoni C, Cignarella F, Bollman B, Cross AH, Salter A, Galimberti D, Cella M, Piccio L. T cells producing GM-CSF and IL-13 are enriched in the cerebrospinal fluid of relapsing MS patients. Mult Scler. 2020;26(10):1172-86.

293. Rossi S, Mancino R, Bergami A, Mori F, Castelli M, De Chiara V, Studer V, Mataluni G, Sancesario G, Parisi V, Kusayanagi H, Bernardi G, Nucci C, Bernardini S, Martino G, Furlan R, Centonze D. Potential role of IL-13 in neuroprotection and cortical excitability regulation in multiple sclerosis. Mult Scler. 2011;17(11):1301-12.

294. Shin WH, Lee DY, Park KW, Kim SU, Yang MS, Joe EH, Jin BK. Microglia expressing interleukin-13 undergo cell death and contribute to neuronal survival in vivo. Glia. 2004;46(2):142-52.

295. Sinha S, Kaler LJ, Proctor TM, Teuscher C, Vandenbark AA, Offner H. IL-13-mediated gender difference in susceptibility to autoimmune encephalomyelitis. J Immunol. 2008;180(4):2679-85.

296. Tao J, Chen H, Wang YJ, Qiu JX, Meng QQ, Zou RJ, Li L, Huang JG, Zhao ZK, Huang YL, Zhang HF, Zheng JM. Ketogenic Diet Suppressed T-Regulatory Cells and Promoted Cardiac Fibrosis via Reducing Mitochondria-Associated Membranes and Inhibiting Mitochondrial Function. Oxid Med Cell Longev. 2021;2021:5512322.

297. Castaldo G, Pagano I, Grimaldi M, Marino C, Molettieri P, Santoro A, Stillitano I, Romano R, Montoro P, D'Ursi AM, Rastrelli L. Effect of Very-Low-Calorie Ketogenic Diet on Psoriasis Patients: A Nuclear Magnetic Resonance-Based Metabolomic Study. J Proteome Res. 2021;20(3):1509-21.

298. Tahani S, Dehghani L, Jahanbani-Ardakani H, Shaygannejad V, Fazli A, Hamidavi A, Eskandari N. Elevated serum level of IL-4 in neuromyelitis optica and multiple sclerosis patients. J Immunoassay Immunochem. 2019;40(5):555-63.

299. Lee LF, Axtell R, Tu GH, Logronio K, Dilley J, Yu J, Rickert M, Han B, Evering W, Walker MG, Shi J, de Jong BA, Killestein J, Polman CH, Steinman L, Lin JC. IL-7 promotes T(H)1 development and serum IL-7 predicts clinical response to interferon-beta in multiple sclerosis. Sci Transl Med. 2011;3(93):93ra68.

300. Esposito K, Marfella R, Ciotola M, Di Palo C, Giugliano F, Giugliano G, D'Armiento M, D'Andrea F, Giugliano D. Effect of a mediterranean-style diet on endothelial dysfunction and markers of vascular inflammation in the metabolic syndrome: a randomized trial. JAMA. 2004;292(12):1440-6.

301. Cervo MMC, Scott D, Seibel MJ, Cumming RG, Naganathan V, Blyth FM, Le Couteur DG, Handelsman DJ, Ribeiro RV, Waite LM, Shivappa N, Hebert JR, Hirani V. Proinflammatory Diet Increases Circulating Inflammatory Biomarkers and Falls Risk in Community-Dwelling Older Men. J Nutr. 2020;150(2):373-81.

302. Azimi G, Ranjbaran F, Arsang-Jang S, Ghafouri-Fard S, Mazdeh M, Sayad A, Taheri M. Upregulation of VEGF-A and correlation between VEGF-A and FLT-1 expressions in Iranian multiple sclerosis patients. Neurol Sci. 2020;41(6):1459-65.

303. Kira J. [Multiple sclerosis]. Rinsho Shinkeigaku. 2005;45(11):912-5.

304. Clark-Raymond A, Halaris A. VEGF and depression: a comprehensive assessment of clinical data. J Psychiatr Res. 2013;47(8):1080-7.

305. Calviello G, Di Nicuolo F, Gragnoli S, Piccioni E, Serini S, Maggiano N, Tringali G, Navarra P, Ranelletti FO, Palozza P. n-3 PUFAs reduce VEGF expression in human colon cancer cells modulating the COX-2/PGE2 induced ERK-1 and -2 and HIF-1alpha induction pathway. Carcinogenesis. 2004;25(12):2303-10.

306. Darwito D, Dharmana E, Riwanto I, Budijitno S, Suwardjo S, Purnomo J, Widodo I, Ghozali A, Aryandono T, Anwar SL. Effects of Omega-3 Supplementation on Ki-67 and VEGF Expression Levels and Clinical Outcomes of Locally Advanced Breast Cancer Patients Treated with Neoadjuvant CAF Chemotherapy: A Randomized Controlled Trial Report. Asian Pac J Cancer Prev. 2019;20(3):911-6.

307. De Groote D, Zangerle PF, Gevaert Y, Fassotte MF, Beguin Y, Noizat-Pirenne F, Pirenne J, Gathy R, Lopez M, Dehart I, Igot D, Baudrihaye M, Delacroix D, Franchimont P. Direct stimulation of cytokines (IL-1 beta, TNF-alpha, IL-6, IL-2, IFN-gamma and GM-CSF) in whole blood. I. Comparison with isolated PBMC stimulation. Cytokine. 1992;4(3):239-48.

308. Emerson SR, Kurti SP, Harms CA, Haub MD, Melgarejo T, Logan C, Rosenkranz SK. Magnitude and Timing of the Postprandial Inflammatory Response to a High-Fat Meal in Healthy Adults: A Systematic Review. Adv Nutr. 2017;8(2):213-25.

309. Kallaur AP, Oliveira SR, Simao ANC, De Almeida ERD, Morimoto HK, Lopes J, Pereira WLDJ, Andrade RM, Pelegrino LM, Borelli SD, Kaimen-Maciel DR, Reiche EMV. Cytokine profile in relapsing-remitting multiple sclerosis patients and the association between progression and activity of the disease. Mol Med Rep. 2013;7(3):1010-20.

310. Mikulkova Z, Praksova P, Stourac P, Bednarik J, Michalek J. Imbalance in T-cell and cytokine profiles in patients with relapsing-remitting multiple sclerosis. Journal of the neurological sciences. 2011;300(1-2):135-41.

311. Tellez N, Rio J, Tintore M, Nos C, Galan I, Montalban X. Fatigue in multiple sclerosis persists over time: a longitudinal study. J Neurol. 2006;253(11):1466-70.

312. Fox RJ, Bacon TE, Chamot E, Salter AR, Cutter GR, Kalina JT, Kister I. Prevalence of multiple sclerosis symptoms across lifespan: data from the NARCOMS Registry. Neurodegener Dis Manag. 2015;5(6 Suppl):3-10.

313. Linnhoff S, Fiene M, Heinze HJ, Zaehle T. Cognitive Fatigue in Multiple Sclerosis: An Objective Approach to Diagnosis and Treatment by Transcranial Electrical Stimulation. Brain Sci. 2019;9(5).

314. Hanken K, Eling P, Hildebrandt H. Is there a cognitive signature for MS-related fatigue? Mult Scler. 2015;21(4):376-81.

315. Roelcke U, Kappos L, Lechner-Scott J, Brunnschweiler H, Huber S, Ammann W, Plohmann A, Dellas S, Maguire RP, Missimer J, Radu EW, Steck A, Leenders KL. Reduced glucose metabolism in the frontal cortex and basal ganglia of multiple sclerosis patients with fatigue: a 18F-fluorodeoxyglucose positron emission tomography study. Neurology. 1997;48(6):1566-71.

316. Costantini LC, Barr LJ, Vogel JL, Henderson ST. Hypometabolism as a therapeutic target in Alzheimer's disease. BMC neuroscience. 2008;9 Suppl 2:S16.

317. Veauthier C, Hasselmann H, Gold SM, Paul F. The Berlin Treatment Algorithm: recommendations for tailored innovative therapeutic strategies for multiple sclerosis-related fatigue. EPMA J. 2016;7:25.

318. Patrick E, Christodoulou C, Krupp LB, New York State MSC. Longitudinal correlates of fatigue in multiple sclerosis. Mult Scler. 2009;15(2):258-61.

319. Chaouachi A, Coutts AJ, Wong del P, Roky R, Mbazaa A, Amri M, Chamari K. Haematological, inflammatory, and immunological responses in elite judo athletes maintaining high training loads during Ramadan. Appl Physiol Nutr Metab. 2009;34(5):907-15.

320. Mitsikostas DD, Doskas T, Gkatzonis S, Fakas N, Maltezou M, Papadopoulos D, Gourgioti R, Mitsias P. A Prospective, Observational, Cohort Study to Assess the Efficacy and Safety of Prolonged-Release Fampridine in Cognition, Fatigue, Depression, and Quality of Life in Multiple Sclerosis Patients: The FAMILY Study. Adv Ther. 2021;38(3):1536-51.

321. Zhang Y, Liu C, Zhao Y, Zhang X, Li B, Cui R. The Effects of Calorie Restriction in Depression and Potential Mechanisms. Curr Neuropharmacol. 2015;13(4):536-42.

322. Manchishi SM, Cui RJ, Zou XH, Cheng ZQ, Li BJ. Effect of caloric restriction on depression. J Cell Mol Med. 2018;22(5):2528-35.

323. Gibson AA, Seimon RV, Lee CM, Ayre J, Franklin J, Markovic TP, Caterson ID, Sainsbury A. Do ketogenic diets really suppress appetite? A systematic review and meta-analysis. Obes Rev. 2015;16(1):64-76.

324. Michalsen A, Schlegel F, Rodenbeck A, Ludtke R, Huether G, Teschler H, Dobos GJ. Effects of short-term modified fasting on sleep patterns and daytime vigilance in non-obese subjects: results of a pilot study. Ann Nutr Metab. 2003;47(5):194-200.

325. Beal CC, Stuifbergen AK, Brown A. Depression in multiple sclerosis: a longitudinal analysis. Arch Psychiatr Nurs. 2007;21(4):181-91.

326. Roar M, Illes Z, Sejbaek T. Practice effect in Symbol Digit Modalities Test in multiple sclerosis patients treated with natalizumab. Mult Scler Relat Disord. 2016;10:116-22.

327. Sheridan LK, Fitzgerald HE, Adams KM, Nigg JT, Martel MM, Puttler LI, Wong MM, Zucker RA. Normative Symbol Digit Modalities Test performance in a community-based sample. Arch Clin Neuropsychol. 2006;21(1):23-8.

328. Henderson ST. Ketone bodies as a therapeutic for Alzheimer's disease. Neurotherapeutics : the journal of the American Society for Experimental NeuroTherapeutics. 2008;5(3):470-80.

329. DM IJ, Postulart D, Lambrechts DA, Majoie MH, de Kinderen RJ, Hendriksen JG, Evers SM, Aldenkamp AP. Cognitive and behavioral impact of the ketogenic diet in children and adolescents with refractory epilepsy: A randomized controlled trial. Epilepsy Behav. 2016;60:153-7.

330. Zhang Y, Kuang Y, Xu K, Harris D, Lee Z, LaManna J, Puchowicz MA. Ketosis proportionately spares glucose utilization in brain. Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism. 2013;33(8):1307-11.

331. Krikorian R, Shidler MD, Dangelo K, Couch SC, Benoit SC, Clegg DJ. Dietary ketosis enhances memory in mild cognitive impairment. Neurobiology of aging. 2012;33(2):425 e19-27.

332. Andaloro A, Russo M, Pastura C, Sessa E, Calatozzo P, Maggio MG, Bramanti P. Is there a correlation between dyslipidemia and cognitive impairment in patients with multiple sclerosis? International Journal of Neuroscience. 2020.

333. Noori H, Gheini MR, Rezaeimanesh N, Saeedi R, Aliabadi HR, Sahraian MA, Moghadasi AN. The correlation between dyslipidemia and cognitive impairment in multiple sclerosis patients. Mult Scler Relat Dis. 2019;36.

334. An Y, Zhang X, Wang Y, Wang Y, Liu W, Wang T, Qin Z, Xiao R. Longitudinal and nonlinear relations of dietary and Serum cholesterol in midlife with cognitive decline: results from EMCOA study. Mol Neurodegener. 2019;14(1):51.

335. Crichton GE, Elias MF, Davey A, Sullivan KJ, Robbins MA. Higher HDL Cholesterol Is Associated with Better Cognitive Function: the Maine-Syracuse Study. J Int Neuropsych Soc. 2014;20(10):961-70.

336. Svensson T, Sawada N, Mimura M, Nozaki S, Shikimoto R, Tsugane S. The association between midlife serum high-density lipoprotein and mild cognitive impairment and dementia after 19 years of follow-up. Transl Psychiat. 2019;9.

337. Yates KF, Sweat V, Yau PL, Turchiano MM, Convit A. Impact of metabolic syndrome on cognition and brain: a selected review of the literature. Arterioscler Thromb Vasc Biol. 2012;32(9):2060-7.

338. Overman MJ, Pendleton N, O'Neil TW, Bartfai G, Casanueva FF, Forti G, Rastrelli G, Giwercman A, Han TS, Huhtaniemi IT, Kula K, Lean MEJ, Punab M, Lee DM, Correa ES, Ahern T, Laurent MR, Verschueren SMP, Antonio L, Gielen E, Rutter MK, Vanderschueren D, Wu FCW, Tournoy J, Grp ES. Glycemia but not the Metabolic Syndrome is Associated with Cognitive Decline: Findings from the European Male Ageing Study. Am J Geriat Psychiat. 2017;25(6):662-71.

339. Jiang J, Zhao L, Lin L, Gui M, Aleteng Q, Wu B, Wang S, Pan B, Ling Y, Gao X. Postprandial Blood Glucose Outweighs Fasting Blood Glucose and HbA1c in screening Coronary Heart Disease. Sci Rep. 2017;7(1):14212.

340. Martinez-Garcia MA, Moncayo S, Insenser M, Montes-Nieto R, Fernandez-Duran E, Alvarez-Blasco F, Luque-Ramirez M, Escobar-Morreale HF. Postprandial inflammatory responses after oral glucose, lipid and protein challenges: Influence of obesity, sex and polycystic ovary syndrome. Clin Nutr. 2020;39(3):876-85.

341. Sandroff BM, Hubbard EA, Pilutti LA, Motl RW. No association between body composition and cognition in ambulatory persons with multiple sclerosis: A brief report. J Rehabil Res Dev. 2015;52(3):301-8.

342. Hernandez-Ledesma AL, Rodriguez-Mendez AJ, Gallardo-Vidal LS, Garcia-Gasca T, Alatorre-Cruz JM, Garcia-Solis P, Lopez Reyes J, Solis-Sainz JC. Lipid profile: causal relationship on cognitive performance in multiple sclerosis? Mol Biol Rep. 2020;47(12):9667-76.

343. Schmidt R, Hayn M, Reinhart B, Roob G, Schmidt H, Schumacher M, Watzinger N, Launer LJ. Plasma antioxidants and cognitive performance in middle-aged and older adults: results of the Austrian Stroke Prevention Study. J Am Geriatr Soc. 1998;46(11):1407-10.

344. Feeney J, O'Leary N, Moran R, O'Halloran AM, Nolan JM, Beatty S, Young IS, Kenny RA. Plasma Lutein and Zeaxanthin Are Associated With Better Cognitive Function Across Multiple Domains in a Large Population-Based Sample of Older Adults: Findings from The Irish Longitudinal Study on Aging. J Gerontol A Biol Sci Med Sci. 2017;72(10):1431-6.

345. Ajana S, Weber D, Helmer C, Merle BM, Stuetz W, Dartigues JF, Rougier MB, Korobelnik JF, Grune T, Delcourt C, Feart C. Plasma Concentrations of Lutein and Zeaxanthin, Macular Pigment

Optical Density, and Their Associations With Cognitive Performances Among Older Adults. Invest Ophth Vis Sci. 2018;59(5):1828-35.

346. Christensen K, Gleason CE, Mares JA. Dietary carotenoids and cognitive function among US adults, NHANES 2011-2014. Nutritional neuroscience. 2020;23(7):554-62.

347. Renzi-Hammond LM, Bovier ER, Fletcher LM, Miller LS, Mewborn CM, Lindbergh CA, Baxter JH, Hammond BR. Effects of a Lutein and Zeaxanthin Intervention on Cognitive Function: A Randomized, Double-Masked, Placebo-Controlled Trial of Younger Healthy Adults. Nutrients. 2017;9(11).

348. Lindbergh CA, Renzi-Hammond LM, Hammond BR, Terry DP, Mewborn CM, Puente AN, Miller LS. Lutein and Zeaxanthin Influence Brain Function in Older Adults: A Randomized Controlled Trial. J Int Neuropsychol Soc. 2018;24(1):77-90.

349. Lindbergh CA, Mewborn CM, Hammond BR, Renzi-Hammond LM, Curran-Celentano JM, Miller LS. Relationship of Lutein and Zeaxanthin Levels to Neurocognitive Functioning: An fMRI Study of Older Adults. J Int Neuropsychol Soc. 2017;23(1):11-22.

350. Mewborn CM, Terry DP, Renzi-Hammond LM, Hammond BR, Miller LS. Relation of Retinal and Serum Lutein and Zeaxanthin to White Matter Integrity in Older Adults: A Diffusion Tensor Imaging Study. Arch Clin Neuropsychol. 2018;33(7):861-74.

351. Palacios N, Lee JS, Scott T, Kelly RS, Bhupathiraju SN, Bigornia SJ, Tucker KL. Circulating Plasma Metabolites and Cognitive Function in a Puerto Rican Cohort. J Alzheimers Dis. 2020;76(4):1267-80.

352. Yuan C, Chen H, Wang Y, Schneider JA, Willett WC, Morris MC. Dietary carotenoids related to risk of incident Alzheimer dementia (AD) and brain AD neuropathology: a community-based cohort of older adults. Am J Clin Nutr. 2020.

353. Shrank WH, Patrick AR, Brookhart MA. Healthy user and related biases in observational studies of preventive interventions: a primer for physicians. J Gen Intern Med. 2011;26(5):546-50.

11 Statutory declaration/Eidesstattliche Versicherung

„Ich, Lina Samira Bahr, versichere an Eides statt durch meine eigenhändige Unterschrift, dass ich die vorgelegte Dissertation mit dem Thema: "Effects of fasting and a ketogenic diet on neuropsychiatric outcomes in multiple sclerosis patients – a randomized controlled trial / Auswirkungen von Fasten und ketogener Ernährung auf neuropsychiatrische Symptome bei Multipler Sklerose – eine randomisierte kontrollierte Studie" selbstständig und ohne nicht offengelegte Hilfe Dritter verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel genutzt habe.

Alle Stellen, die wörtlich oder dem Sinne nach auf Publikationen oder Vorträgen anderer Autoren/innen beruhen, sind als solche in korrekter Zitierung kenntlich gemacht. Die Abschnitte zu Methodik (insbesondere praktische Arbeiten, Laborbestimmungen, statistische Aufarbeitung) und Resultaten (insbesondere Abbildungen, Graphiken und Tabellen) werden von mir verantwortet.

Ich versichere ferner, dass ich die in Zusammenarbeit mit anderen Personen generierten Daten, Datenauswertungen und Schlussfolgerungen korrekt gekennzeichnet und meinen eigenen Beitrag sowie die Beiträge anderer Personen korrekt kenntlich gemacht habe (siehe Anteilserklärung). Texte oder Textteile, die gemeinsam mit anderen erstellt oder verwendet wurden, habe ich korrekt kenntlich gemacht.

Meine Anteile an etwaigen Publikationen zu dieser Dissertation entsprechen denen, die in der untenstehenden gemeinsamen Erklärung mit dem/der Erstbetreuer/in, angegeben sind. Für sämtliche im Rahmen der Dissertation entstandenen Publikationen wurden die Richtlinien des ICMJE (International Committee of Medical Journal Editors; www.icmje.og) zur Autorenschaft eingehalten. Ich erkläre ferner, dass ich mich zur Einhaltung der Satzung der Charité – Universitätsmedizin Berlin zur Sicherung Guter Wissenschaftlicher Praxis verpflichte.

Weiterhin versichere ich, dass ich diese Dissertation weder in gleicher noch in ähnlicher Form bereits an einer anderen Fakultät eingereicht habe.

Die Bedeutung dieser eidesstattlichen Versicherung und die strafrechtlichen Folgen einer unwahren eidesstattlichen Versicherung (§§156, 161 des Strafgesetzbuches) sind mir bekannt und bewusst.“

_____ _____

Datum Unterschrift

12 Curriculum Vitae

Mein Lebenslauf wird aus datenschutzrechtlichen Gründen in der elektronischen Version meiner Arbeit nicht veröffentlicht.

Mein Lebenslauf wird aus datenschutzrechtlichen Gründen in der elektronischen Version meiner Arbeit nicht veröffentlicht.

13 Scientific publications 2017-2022

Sperber PS, Brandt AU, Zimmermann HG, **Bahr LS**, Chien C, Rekers S, Mähler A, Böttcher C, Asseyer S, Duchow AS, Bellmann-Strobl J, Ruprecht K, Paul F, Schmitz-Hübsch T. Berlin Registry of Neuroimmunological Entities (BERLimmun): protocol of a prospective observational study. Submitted at BMC Neurology. March 2022.

König RS, Albrich WC, Kahlert CR, **Bahr LS**, Löber U, Vernazza P, Scheibenbogen C, Forslund SK. The Gut Microbiome in Myalgic Encephalomyelitis (ME)/Chronic Fatigue Syndrome (CFS). Front Immunol. 2022 Jan 3;12:628741. doi: 10.3389/fimmu.2021.628741.

Bahr LS, Franz K, Mähler A. Assessing the (anti)-inflammatory potential of diets. Current Opinion in Clinical Nutrition and Metabolic Care. June 2021. doi: 10.1097/MCO.0000000000000772

Bahr LS, Bock M, Bellmann-Strobl J, Franz L, Liebscher D, Kessler CS, Steckhan N, Michalsen A, Paul F, Mähler A. Nutritional Approaches in Multiple Sclerosis – a randomized controlled trial. Trials. 2021**,** 3 (2020) doi:10.1186/s13063-019-3928-9

Bahr LS, Bellmann-Strobl J, Michalsen A. Die ketogene Diät – was sie kann, wie sie wirkt und wie sie gelingt. Zkm. 2018. 10(02): 22-29. doi: 10.1055/a-0584-5311

Invited talks 2018-2021

Bahr LS. Ketogenic diets and expanded uses in neurological disorders. Talk in the clinical neuroscience lab at the university of Ohio. 2021.

Bahr LS. Ernährungswissenschaftliche Ansätze in Multiple Sklerose. Live Talks „Diverse Wissenschaft.“ Museum für Naturkunde. 2020.

Bahr LS. Potential of ketogenic diets in neurological disorders. Neuroforum Südwest. Frankfurt am Main. 2020.

Bahr LS. Ketogenic diets – expanded uses. Global Symposium on ketogenic therapies: Global potential and emerging applications. Würzburg, Germany. 2019. Oral presentation. https://evolution-medizin- gesundheit.org/wp/wp-content/uploads/2019/04/Symposium-2019-Programm.pdf

Bahr LS. What is the ideal diet in Multiple Sclerosis? Neurologische Fachtagung im Zentrum für ambulante Rehabilitation. Berlin. 2018.

Reviewer activities

Journal “Der Nervenarzt“

Journal “trials”

Conference contributions 2017-2019

Bahr LS, Bellmann-Strobl J, Franz L, Michalsen L, Paul F, Mähler A. Long-term effects of a ketogenic diet on oxidative stress in multiple sclerosis patients. Abstract for the 6th Global Symposium on KETOGENIC THERAPIES FOR NEUROLOGICAL DISORDERS, Jeju, Korea. 2018. Poster

Franz K, Otten L, **Bahr LS**, Müller-Werdan U, Norman K. Energy Intake and functional Limitations in Malnourished, geriatric Patients at Hospital Discharge**.** Innovations in Aging 2017; 1(S1):551.

Franz K, Kiselev J, Otten L, **Bahr LS**, Müller-Werdan U, Norman K. Prävalenz von Frailty bei mangelernährten, geriatrischen Patienten bei Krankenhausentlassung. Zeitschrift für Gerontologie und Geriatrie. 2017; Suppl 1:40.

Franz K, Kiselev J, Otten L, **Bahr LS**, Müller-Werdan U, Norman K. Risikofaktoren für eine schwere Mangelernährung bei geriatrischen Patienten bei Mangelernährung. Zeitschrift für Gerontologie und Geriatrie 2017; Suppl 1:40.

Franz K, Otten L, **Bahr LS**, Kiselev J, Müller-Werdan U, Norman K. SUN-P151: Post-Hospital Syndrome: Determinants of Readmission in Malnourished, Geriatric Patients Within 3 Months Of Hospital Discharge. Clinical Nutrition 2017. 36:S110.

Franz K, Otten L, **Bahr LS**, Kiselev J, Müller-Werdan U, Norman K .SUN-P152: Mobility, Functional Status and Fatigue in Malnourished, Geriatric Patients at Hospital Discharge. Clinical Nutrition 2017.

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Abschließend widme ich diese Arbeit allen StudienteilnehmerInnen, ohne dessen Einsatz diese Studie nicht möglich gewesen wäre.

15 Statistical consultation/Statistische Beratung

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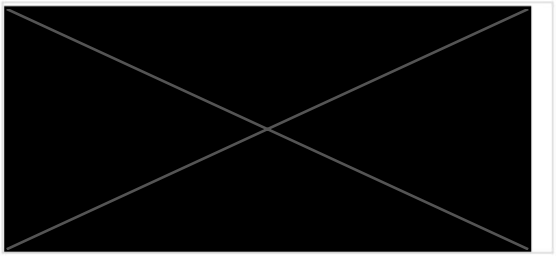


Bescheinigung

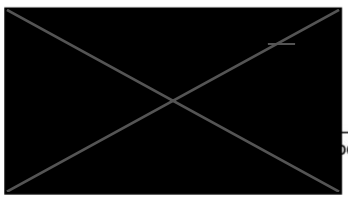
Hiermit bescheinige ich, dass Frau Lina Samira Bahr innerhalb der Service Unit Biometrie des Instituts für Biometrie und klinische Epidemiologie (iBikE) bei mir eine statistische Beratung zu einem Promotionsvorhaben wahrgenommen hat. Folgende Beratungstermine wurden wahrgenommen:

- Termin 1: 19.10.2020, Termin 2: 30.11.2020. Termin 3: 12.01.2021, Termin 4: 22.01.2021, Termin 5: 17.06.2021

Folgende wesentliche Ratschläge hinsichtlich einer sinnvollen Auswertung und Interpretation der Daten wurden während der Beratung erteilt:

- Wahl der Lage-und Streuungsmaße je nach Skalenniveau und Verteilung des Merkmals
- Wahl des statistischen Tests je nach Skalenniveau des Merkmals und verbundenen bzw. unverbundenen Stichproben
- Interpretation der p-Werte als explorativ

Diese Bescheinigung garantiert nicht die richtige Umsetzung der in der Beratung gemachten Vorschläge, die korrekte Durchführung der empfohlenen statistischen Verfahren und die richtige Darstellung und Interpretation der Ergebnisse. Die Verantwortung hierfür obliegt allein dem Promovierenden. Das Institut für Biometrie und klinische Epidemiologie übernimmt hierfür keine Haftung.



Name des Beraters/ der Beraterin: Alice Schneider

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