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MASTERARBEIT / MASTER'S THESIS

Titel der Masterarbeit / Title of the Master's Thesis

„The butyrate and beta-hydroxybutyrate mediated effects of interventions with pro- and prebiotic, fasting and caloric restriction on depression“

verfasst von / submitted by

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1. Abstract:

This master's thesis examines the effectiveness of pre- and probiotic, fasting and caloric restriction interventions for depression and investigates thereby the role of butyrate and beta-hydroxybutyrate, as those metabolites might play a key role in the properties of the studied interventions. Butyrate and beta-hydroxybutyrate are similar due to their structure and additionally seem to have similar physiological influencing properties. That's why literature was obtained, and a meta-analysis conducted with the three included pre-and probiotic intervention RCTs. A significant increase of butyrate (SMD 0,34; [0,02 – 0,67]) and an improvement of depression scores (SMD 0,15, [-0,35 - 0,70]) through the pre-and probiotic interventions could be shown. Beside butyrate, also SCFA concentration seems to be positively associated with pre- and probiotic administration (SMD 0,55 [0,15 - 0,95]. Despite of the significant SCFA and butyrate concentration changes, no significant correlation between either butyrate and depression nor SCFA and depression could be shown through linear regression models. Nevertheless, the calculated outcomes (b1 = 1,57; p = 0,17) for butyrate and (b1 = 0,75; p = 0,44) for SCFA, suggests a strong, positive connection between butyrate and depression, which's effect might be limited by acetate, propionate, valerate and hexanoat concentrations. With also the heterogeneity measure I^2 being 0% all the variances of the effect size estimates are explainable through SE, which means that all effects can be attributed to the interventions. This strengthens the evidence of the findings and underlines butyrate's key role in the gut-brain-microbiome axis properties on mood, depression, and brain-health. Additionally, three studies were qualitatively analyzed examining fasting as intervention. A possible connection between fasting, beta-hydroxybutyrate and depression was found. Also, depression or mood enhancement seemed to be associated to BHB concentration, which may be explainable through similar biochemical properties of BHB and butyrate. Furthermore, caloric restriction as potential long-term intervention was mentioned as alternative to fasting as well as further needed studies stated.

2. Zusammenfassung

Diese Masterarbeit untersucht die Wirksamkeit von prä- und probiotischen Interventionen, sowie von Fasten- und Kalorienrestriktion bei Depressionen und stellt dabei die Rolle von Butyrat und Beta-Hydroxybutyrat in den Untersuchungsmittelpunkt. Butyrat und Beta-Hydroxybutyrat ähneln sich aufgrund ihrer Struktur und scheinen zudem ähnliche physiologische Eigenschaften zu haben. Deshalb wurde aktuelle Literatur zusammengefasst und der aktuelle wissenschaftliche Stand wiedergegeben. Eine systematische Übersichtsarbeit wurde durchgeführt, wobei drei Observationsstudien, die Fasten als Intervention untersuchten, qualitativ analysiert, und drei randomisierte, kontrollierte Studien mit prä- und probiotischen Interventionen im Rahmen einer Meta-Analyse quantitativ behandelt wurden. Dabei konnte ein signifikanter Anstieg von Butyrat (SMD 0,34; [0,02 - 0,67]) und eine Verbesserung der Depressionswerte (SMD 0,15, [-0,35 - 0,70]) durch die prä- und probiotischen Interventionen nachgewiesen werden. Neben Butyrat scheint auch die SCFA-Konzentration positiv mit der prä- und probiotischen Gabe assoziiert zu sein (SMD 0,55 [0,15 - 0,95]). Trotz der signifikanten Veränderungen der SCFA- und Butyrat Konzentration konnte mit Hilfe von linearen Regressionsmodellen weder ein signifikanter Zusammenhang zwischen Butyrat und Depression noch zwischen SCFA und Depression nachgewiesen werden. Dennoch deuten die berechneten Ergebnisse (b1 = 1,57; p = 0,17) für Butyrat auf eine starke, positive Korrelation zwischen Butyrat und Depression hin. Zusammengefasst konnte somit ein positiver Effekt von prä- und probiotischen Interventionen auf Depressionen nachgewiesen, sowie die Schlüsselrolle von Butyrat in dem Einfluss der Darm – Mikrobiom - Hirn – Achse auf die Stimmung, Depressionen und die Gehirngesundheit unterstrichen werden. Die qualitativen analysierten Studien zeigten einen Zusammenhang zwischen Fasten, Beta-Hydroxybutyrat und Depression. Außerdem scheinen Depression negativ mit der BHB-Konzentration assoziiert zu sein, was durch die ähnlichen biochemischen Eigenschaften von BHB und Butyrat erklärt werden kann. Darüber hinaus wurde die Kalorienrestriktion als potenzielle langfristige Alternative zum Fasten genannt und auf die Notwendigkeit weiterer Studien hingewiesen.

3. Objective

The aim of this master thesis was to investigate the effects of pre- and probiotic, fasting, and caloric restriction interventions on depression modulated by butyrate and beta-hydroxybutyrate. Due to the necessary advances in drug therapy for depression, new knowledge about the properties of BHB and butyrate should be obtained, and the efficacy of the interventions studied, should be clarified. Furthermore, the current scientific knowledge should be summarized and further need for studies pointed out. In addition to the new findings, the pathophysiology of depression, the fundamentals of the gut-microbiome-brain axis, as well as the mechanisms of action of the studied interventions should be presented.

Index:

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List of abbreviations:

BHB	Beta-hydroxybutyrate
ICD-10	International classification of diseases-10
DSM-IV	American diagnostic and statistical manual of mental disorders-IV
COVID-19	Coronavirus disease 2019
WHO	World Health Organization
U.S.	United States
MDD	Major depressive disorder
HPA	Hypothalamic-pituitary-adrenal
CRH	Corticotropin-releasing hormone
ACTH	Adrenocorticotropic hormone
GR	Glucocorticoid receptors
MR	Mineralocorticoid receptor
βHSD1	11-β hydroxysteroid dehydrogenase
LC-NE	Locus Coeruleus - Norepinephrine
5-HT	5-hydroxytryptophan
DA	Dopamine
CNS	Centrale nervous system
MAO	Mono amino oxidase inhibitor
NGF	Nerve growth factor
BDNF	Brain-derived neurotrophic factor
NT-3	Neurotrophin-3
NT-4	Neurotrophin-4
TNF-α	Tumor necrosis factor-α
BBB	Blood-brain-barrier
NF-κB	Nuclear factor-κB
SCFAs	Short-chain fatty acids
GLP-1	Glucagon-like peptide-1
GABA	Gamma-aminobutyric acid
QS	Quorum sensing

AI	Autoinducer molecules
GPCRs	G-protein-coupled receptors
MCTs	H+-dependent monocarboxylate transporters
SMCTs	Sodium-dependent monocarboxylate transporters
HADC	Histone deacetylase
LPS	Lipopolysaccharide
I-FABP	Fatty acid-binding protein
B-GOS	Bimuno®-galactooligosaccharide
FOS	Fructooligosaccharide
GOS	Galactooligosaccharide
scFOS	Short-chain fructooligosaccharide
acetyl-CoA	Acetyl-coenzyme A
AcAc	Acetoacetate
HGMCS2	3-hydroxy-3-methylglutaryl CoA synthase 2
HMGCL	3- hydroxy-3-methylglutaryl CoA lyase
Foxo3	Forkhead box O3
MD	Mean difference
SMD	Standardized mean difference
SD	Standard deviation
SEMs	Standard error of the means
TG	Triglycerides

4. Introduction

Hippocrates stated 2500 years ago that humans are constantly threatened by disturbing forces, disbalancing our interior equilibrium. He thereby developed the concept of *Visa medicatric naturare* and meant with that, the intrinsic, natural forces, maintaining and restoring homeostasis and compensating those endogenous, disbalancing influences (1). A century ago, the famous biologist Sir John Arthur Thomson interpreted nature in the context of the concept *Visa mediatric naturare* differently. He shared the view, that homeostasis can be maintained due to the internal forces that occur, but considered nature in general to be a central part of it and was convinced that it provides the body with the necessary energy to maintain equilibrium, considering mindful interaction with the animate and inanimate parts of nature in the external environment to be a prerequisite (2).

> *"What then do I mean tonight by the healing power of nature? I mean to refer to the way in which Nature ministers to our minds, all more or less diseased by the rush and racket of civilization, and helps to steady and enrich our lives. My first point is that there are deeply-rooted, old established, far- reaching relations between Man and Nature which we cannot ignore without loss... there would be less "psychopathology of everyday life" if we kept up our acquaintance... we have put ourselves beyond a very potent vis medicatrix if we cease to be able to wonder at the at the grandeur of the star-strewn sky, the mystery of the mountains, the sea eternally new, the way of the eagle in the air, the meanest flower that blows, the look in a dog's eye."*(3)

Recently it was found, that looking at nature images compared to city images can brighten mood and improve mental health, underlining Sir John Arthur Thomson thesis, and connecting already the perception of nature with mental health. Those results were complemented by further research, showing a negative correlation between naturalness close to home and the incidence of negative health effects of stress, as well as a correlation between increased incidence of stress and distance of residence from green spaces. Through the evident literature results, the connection to nature seems to be a cause of health and wellbeing, suggesting the globally occurring alienation from nature, through the growing cities, the intrusion into ecosystems, and the technological advancements, to be a key cause in the rising prevalence of especially mental disorders (2).

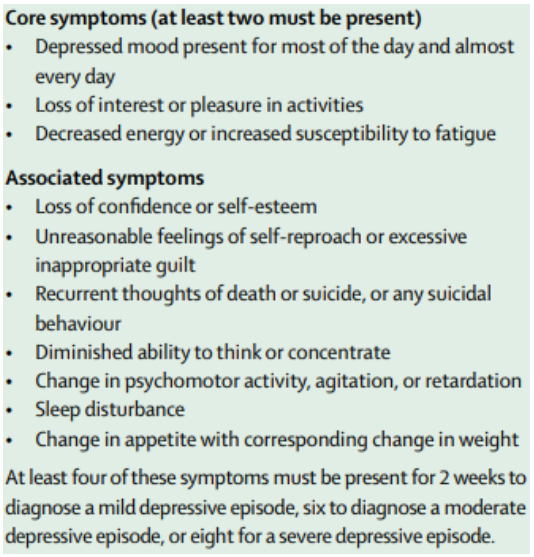
Re-empowering people to develop a stronger connection to nature and to live consciously is difficult to achieve, since the social development determined by efficiency and dynamics would have to be reversed. It therefore seems more promising to adopt Hippocrates' *Visa mediatric naturare* definition, referring to the intrinsic, endogenous mechanisms that constantly provide homeostasis and balance. These could be collectively strengthened if the mechanisms underlying endogenous resilience were better understood and ways to strengthen them effectively were profoundly known.

Butyrate and beta-hydroxybutyrate (BHB), two molecules that modulate intrinsic health-promoting pathways, are of interest in this master's thesis, and the possibility that these molecules may be involved in more cost-effective preventive or curative therapies with fewer side effects establishes the tremendous importance of scientific progress regarding these pathways. However, before presenting the results of the systematic review and meta-analysis and discussing the potential of BHB and butyrate for mental health, it is necessary to explain the basics of the pathophysiology of depression, the gut-microbiome-brain axis, and ketosis, and to summarize the already established scientific knowledge regarding butyrate and BHB.

5. Depression, gut microbiota, and the modulating properties of SCFAs and butyrate

5.1. Depression

Depression is known, as one of the most severe mental illnesses with a global prevalence of 264 million cases in 2020 (4). Depression is defined as the simultaneous occurrence of specific symptoms, identifiable through different scales, including for example the international classification of diseases-10 (ICD-10) and the american diagnostic and statistical manual of mental disorders-IV (DSM-IV) (5). Through to the high prevalence which's increase was further accelerated through the COVID-19 pandemic, as well as the high rates of recurrences, WHO ranked depression as the third leading course of disability worldwide in 2008, projecting it would be the leading course in 2030 (6,7). This is also reflected in the economic burden of depression, which in the U.S. alone increased with a spike of about 48% in the last decade, through which the yearly economic damage recently exceeded 325 billion dollar per year. The pathophysiology of depression is diverse and difficult. For a better understanding it must be mentioned that Major Depression Disorder has many phenotypic presentations (6). Phillip W. Gold distinguishes between melancholic and atypical depression, whereby melancholic depression is defined as the chronic feeling of worthlessness and hopelessness which manifests in pathological hyperarousal and anxiety, in contrast to atypical depression, which is characterized by a combination of disconnectedness and emptiness. Patients suffering from melancholic depression usually try to prevent feelings of pleasantness or proudness on achievements. Also, physiological alterations occur, like the activation of the stress system, as well as the suppression of the reproductive and growth hormone axis. While patients with melancholic depression seem


Core symptoms (at least two must be present)

- Depressed mood present for most of the day and almost every day
- Loss of interest or pleasure in activities
- Decreased energy or increased susceptibility to fatigue

Associated symptoms

- Loss of confidence or self-esteem
- Unreasonable feelings of self-reproach or excessive inappropriate guilt
- Recurrent thoughts of death or suicide, or any suicidal behaviour
- Diminished ability to think or concentrate
- Change in psychomotor activity, agitation, or retardation
- Sleep disturbance
- Change in appetite with corresponding change in weight

At least four of these symptoms must be present for 2 weeks to diagnose a mild depressive episode, six to diagnose a moderate depressive episode, or eight for a severe depressive episode.

Figure 1: Criteria according to ICD-10 for categorizing as depressive episode

to have access to their negative feelings and memories, patients with atypical depression seem to be isolated from themselves. Data suggests, that 1/3 of patients with depression cannot be clearly assigned to one of those variants, while the remaining 2/3 can be equally divided into the two subtypes (6). Fundamentally, depression as well as its types seem to be inheritable, with data also showing a 2-fold higher MDD prevalence in woman than in men, suggesting different underlaying biological and biochemical alterations in the pathology of depression (7). Different studies provided the insight, that MDD is a family disorder, with genetic predispositions seem to explain about 30% of the variance in the development of MDD (8). Previous twin and adoption studies supported this thesis with a reported heritability rate of 31-42%. In this context, different gene loci were found to be potentially associated with MDD risk, mainly influencing synaptic structures and neural transmission, however even those findings did not explain the underlying biochemical mechanics of MDD (9). Consequently, researcher tried to find out which alterations mediate the lasting 70% of the variance of MDD pathophysiology since years, whereby different neurobiological systems have been shown to be promising regarding their potential of explaining MDD emergence.

But before biochemical mechanisms can be discussed in more detail, it hast to be emphasized that physiology disturbances and genetic conditions not only influence the etiology of mental disorders, but depression also impacts different physiological parameters, like disrupts the homeostasis of bone and arterial health and leading to premature osteoporosis and coronary diseases. This underlines the importance of further scientific progress in the understanding of the pathophysiology of depression and the need for new therapeutical methods (6).

5.1.1. Stress system

Stress needs to be viewed as the status of acute danger, to understand its influencing properties properly. In the context of fear, mechanisms are triggered to protect from harm, mainly through inducing simplicity and speed, leading to reproduction of well-rehearsed behavioral and cognitive responses. Those mechanisms also occur during chronic stress or depression, but while during acute crisis the mesolimbic dopaminergic reward system is stimulated to help maintain morale, the reward system is permanently downregulated by stress mediators during chronic stress or depression, resulting in relative anhedonia.

Key role in the regulation of stress plays the hypothalamic-pituitary-adrenal (HPA) axis. Environmental or endogenous stressors activated the corticotropin-releasing hormone (CRH) system, which modulates the HPA axis through glucocorticoid hormone release in the adrenal glands. More specifically, HPA axis is modulated through CRH secretion into the hypophyseal portal system inducing pituitary release of the adrenocorticotropic hormone (ACTH), witch consequently increases glucocorticoids secretion from the adrenal cortex. Glucocorticoids signal through at least to receptors named to glucocorticoid receptors (GR) and mineralocorticoid receptor (MR). By binding to GR or MR, glucocorticoids mediate physiological processes through their transcriptional influencing properties, affecting a large arsenal of genes through specific alterations regarding gene expression. MR regulates basal circadian and ultradian rhythms and therefore mediates time dependent HPA axis activity. Nevertheless, the MR properties seem to differ between tissues, with glucocorticoid response was shown to be amplified in the brain through 11-β hydroxysteroid dehydrogenase (βHSD1) reductase activities, while in the kidney, high levels of βHSD1 inactivated cortisol. In the brain, the increased glucocorticoid activity activates GR, which mediates stress response, through the mobilization of energy stores (liver, fat and muscle) and the upregulation of inflammation through the expression promoting properties (1,10). Importantly, glucocorticoids also function as feedback inhibitors of ACTH production in the pituitary corticotropes and CRH production in the hypothalamus. In MDD patients, HPA axis is overactive during stressful events, leading to hypercortisolemia and decreased rhythmicity, whereby insufficient inhibition of the glucocorticoid receptor regulatory feedback mechanism, is one key mechanism of HPA axis alterations in the pathophysiology of depression, with cortisol levels being linked to depression severity. Although, connection between depression and hypercortisolemia were shown in different trails, glucocorticoid receptor antagonists, as HPA axis regulating treatment, were not able to alleviate symptoms of depression (9). Especially childhood traumata in depressed subjects seems to be strongly associated to altered stress hormone secretion, possibly mediated through epigenetic regulation of glucocorticoid receptors. Those modulations might connect early life stress to HPA axis alterations and increased MDD risk (8).

Beside HPA axis modulation, the CRH system is responsible for the Locus Coeruleus (LC) - Norepinephrine (NE) (LC-NE) system activation, which functions as an alarm

system of the brain, with activation generally leads to as intense arousal and inhibition of the neurovegetative functions. Thus, CRH is reciprocally activated, mediated by.
NE's potential to inhibit key functions of the prefrontal cortex. This leads to an increased resistance to the extinction of negatively charged emotional memories and therefore represents another relevant pathway in the pathophysiology of depression(1,11).

5.1.2. Neurotransmitter

Also connected to the CRH system is the catecholamine hypothesis, which suggests, that MDD is caused by a deficiency of NE, 5-hydroxytryptophan (5-HT), and dopamine (DA) at critical synapses of the centrale nervous system (CNS). This hypothesis was reasoned through the assumption, that reduction of NE, 5-HT, and DA through reserpine consumption induces major depression, while mono amino oxidase inhibitors (MAOs) and NE uptake inhibitors seem to exert antidepressant effects. Although further research found reduced NE levels in MDD populations, symptoms like decreased flexibility of mood as well as insomnia, anxiety and melancholic depression are shown to be associated with NE excess. This supports the thesis unequivocally, that the key depression underlaying factors is stress, leads in general to increased activity of all stress components, manifesting not only in decreased neurotransmitter concentration, but also in NE, 5-HT, and DA excess (1,12).

5.1.3. Neuroplasticity

The neural system consists of neurons, that constantly form and modulate (strengthen and weaken) connections to appropriately process the stimuli they receive. By neuroplasticity, the adaptability of the neural system to external and internal stimuli, as well as the ability to respond properly to future perceptions is meant. Neuronal plasticity is responsible, not only for processing but also for the development of actions and the storage of behavioral knowledge, whereby neurogenesis and the development of dendritic spines being the key tools. Abnormalities in signaling or neuronal production are always present in neuropsychiatric or neurodegenerative diseases. Again, there is evidence for a link between stress and depression, as stress appears to be associated with the volume of certain brain areas, and a correlation between decreased hippocampal volume and severity of depression was found in postmortem studies of depressed patients. Furthermore,

changes in gray matter density as well as decreased neurogenesis was found in MDD subjects. These results could be complemented by animal studies, showing similar changes as well as decreased neuronal plasticity and decreased expression of proteins due to chronic stress and depression, evidently suggesting neurogenesis to be involved in the pathophysiology of depression (13–15).

5.1.4. Neurotrophic factors

The observed decreased volume of certain brain areas is closely related to neurotrophic factors, as these are associated with underlying cell atrophy and have been found in decreased concentration in limbic brain regions after stress and/or depression. Neurotrophins primarily refer to nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and neurotrophin-4 (NT-4), all of which influence neuronal network formation and plasticity. BDNF is the most studied neurotrophic factor in this regard as it has been found, that serum BDNF levels are reduced in patients with MDD. That the levels could be normalized by treatment with antidepressants provided the initial spark for the interpretation of BDNF as a biomarker for depression. Moreover, stress and depression in rodent models were able to decrease BDNF levels in the hippocampus and PFC, whereas treatment with antidepressants increased BDNF expression in the hippocampus and PFC. This gave rise to the neurotrophic hypothesis, which links the occurrence of depression to decreased neurotrophic support, thereby

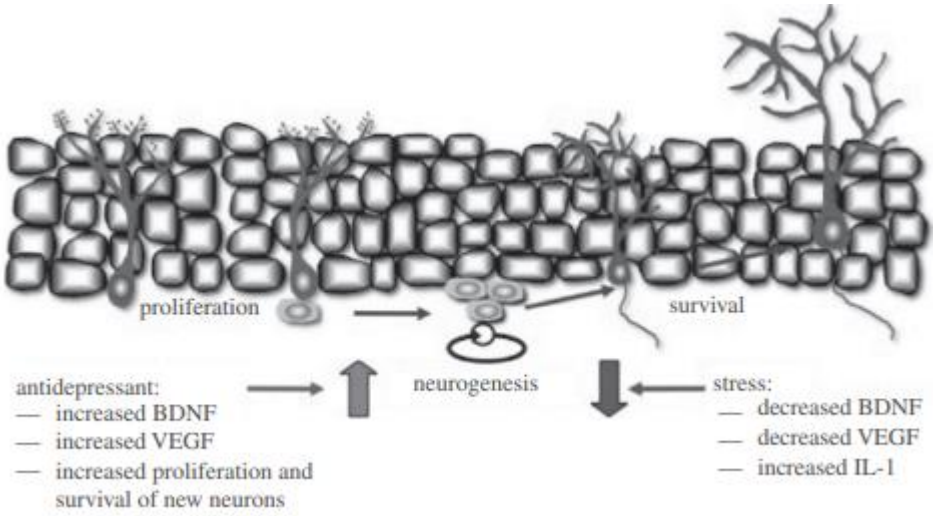


Figure 2: Effects of BDNF on neurogenesis as well as connections between stress and antidepressants therapy on BDNF levels (17)

explaining glial loss, decreased neurogenesis in the hippocampus, and neuronal atrophy. But while trails consistently suggested BDNF's relevant role in modulating the effects of antidepressant therapies, much less evidence supports the thesis, that BDNF depletion causes depression or mood disorders.

The BDNF gene is located at chromosome 11p13 with 11 exons and 9 functional promoters, which are tissue- and brain region-specific. Methylation, as epigenetic mechanisms with expression reducing properties is of increased scientific interest, with evidence already suggests a connection between increased BDNF gene methylation levels, reduced BDNF serum concentration levels and MDD. This effect is especially associated with methylation of CpG sites in exon I, with further findings also reported a significantly increased methylation of the 217 CpG site in BDNF exon IX in MDD patients compared to healthy controls. Beside methylation, also microRNAs seem to be

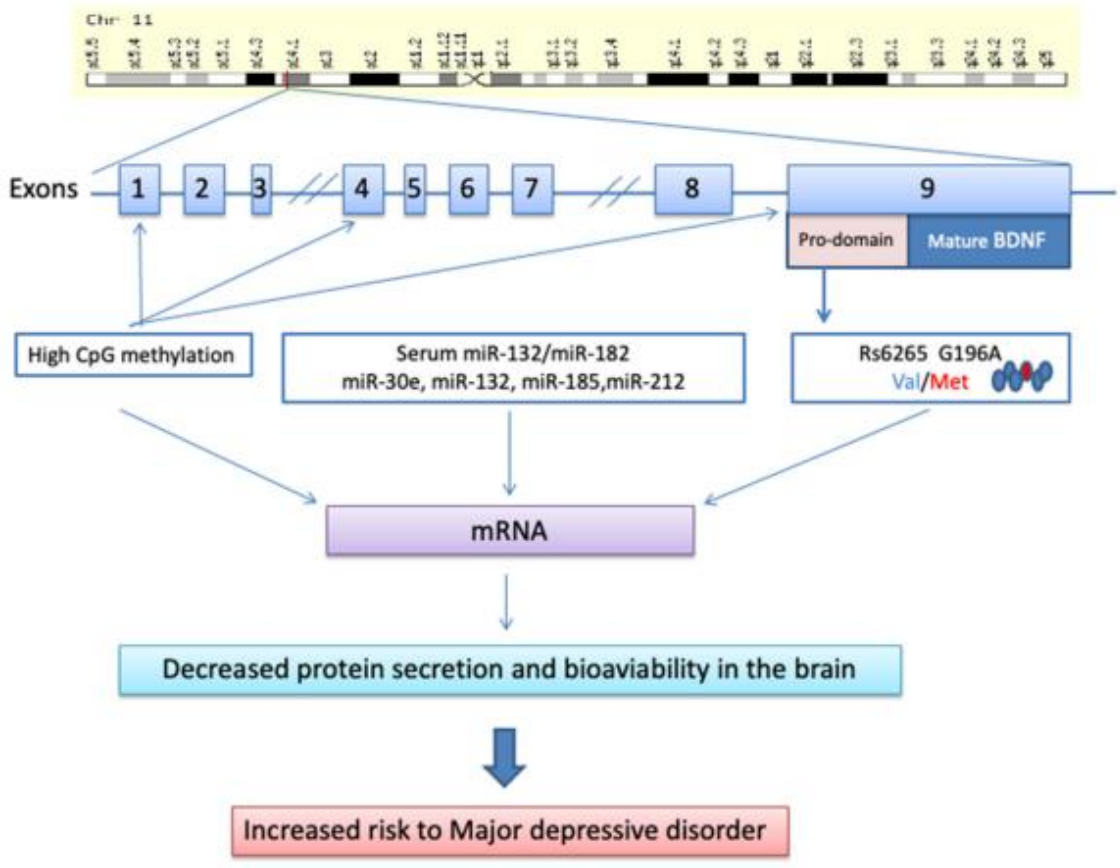


Figure 3: BDNF expression, as well as epigenetic mechanisms modulation transcriptional and translational process (15)

an important epigenetic mechanism, generally influencing expression and translation, with findings reported and inverse relationship between serum BDNF levels and miR-132/miR-182 levels, as well as increased miR-30e, miR-132, miR-185, and miR-212 levels in MDD patients, compared to healthy controls.

After transcription and translation, BDNF is synthesized in the brain, mainly in the hippocampus and hypothalamus where it is released as mature BNDF after the first produced pro-BDNF is cleaved to mature BNDF and pro-peptide. An imbalance between pro-BDNF and mature BNDF is believed to be responsible for neuronal degeneration as well as behavioral impairments. Further research needs to be done, to understand BDNFs influencing properties better, as well as to understand more precisely, which epigenetic mechanisms influence transcription and translation (13–17).

5.1.5. Inflammation

Beside already discussed alterations in MDD subjects, also increased proinflammatory molecules, like cytokines and their receptors as well as chemokines were demonstrated in MDD patients (9). This was underlined through the findings of Syed et al., showing, that untreated depression patients have increased inflammatory markers, with increased anti-inflammatory cytokines after antidepressants therapy, whereas non-responders to antidepressant therapy showed constantly increased proinflammatory cytokines. Those findings further suggested an imbalance between pro- and anti-inflammatory molecules to be involved in the pathophysiology of depression (9,18). This might be also connected to the HPA axis, due to the fact, that increased glucocorticoid concentration and sympathetic signaling results in increased inflammation. This is caused through mobilization of immune cells from the bone marrow, lymph node, and spleen, with activation of monocytes and macrophages results in enhanced secretion of proinflammatory mediators like tumor necrosis factor-α (TNF-α), interleukin-1β, and interleukin-6. Mice models showed the potential of increasing blood-brain-barrier (BBB) permeability through the expression of special tight junctions because of high inflammatory cytokine concentrations, which consequently leads to increased BBB passaging of those inflammatory cytokines.

Another mechanism of action is the mediated by the gut microbiota. Reduction of Lactobacillus strain quantity in rodents resulted in increased concentration of

inflammatory cytokines as well as increased microglial activation in the hippocampus, which is mediated through nuclear factor-κB (NF-κB) activation. Further findings of reduced inflammation and improved depression in chronically stressed mice after Lactobacillus treatment underlined the connection between gut microbiota and depression (19). To understand the huge influencing properties of the gut microbiota more properly, the gut microbiota will be presented in the following part, whereby the current scientific status is explained, as well as possible influencing pathways, relevant for depression, emphasized.

5.2. Gut microbiota

All vertebrates have communities of microorganisms living on the body surface (20). The number of bacteria living in the human body is at least ten times greater than the number of human cells, which led Joshua Lederberg to describe these microorganisms with the term "microbiome", meaning the ecological community of commensal, symbiotic, and pathogenic microorganisms (20). The huge number of bacteria, the diversity in which they occur, and the presence not only in all higher animals but also of a large number of plants stimulated a rethinking of organisms in general and led to newer concepts that conceived the organism as holobionts (21). This summarized the idea, that a more precise way of looking at multicellular organisms is through taking the inhabiting microorganisms into account and regarding the holobiont (host plus symbionts) with the hologenome (host genome plus microbiome) as a biological entity (22). Already before the holobiont concept was published, huge tributes of the influencing properties of microorganisms on humans were found in the analysis of the finished human genome project, where it was consequently argued, that the huge biological progress could only be completed, if the synergistic activities of the microbes and humans are sufficiently understood. Through the central role of microorganism in modern human understanding, different scientific projects were proposed since the 2000s and delivered new insights about the human-microbiota interactions. With increased knowledge it was suggested that human microbiota interactions could be subdivided into the four main colonization sides, oral, gut, vagina, and skin with the majority of the 100 trillion microbes inhabiting the human gastrointestinal tract. The microbial density increases from the proximal to the distal gut with 10^1 microbial cells per gram of content in the stomach, 10^{13} microbial cells

per gram in the duodenum, 10^{14} microbial cells per gram in the jejunum, 10^{17} microbial cells per gram the ileum and up to 10^{112} microbial cells per gram the colon (23,24).

The human microbiota consists out of different types of microbes including bacteria, archaea, eukarya, viruses and parasites, with favorable environmental conditions in the gut for bacteria. The human bowel inhabiting bacteria is almost exclusively divisible in Firmicutes, Bacteroidetes, Actinobacteria, Fusobacteria, Proteobacteria, Verrucomicrobia and Cyanobacteria with Firmicutes and Bacteroidetes contribute to more than 90% of the total population. This can be further segregated in the predominant Bacteroidetes straines Bacteroides and Prevotella, as well as the Firmicutes straines Clostridium, Eubacterium and Ruminococcus. Beside the taxonomic differences, the human microbiome can be further distinguished into the three different enterotypes with enterotype 1 having proteolytic and saccharolytic activities and is involved in the pantothenate, riboflavin, ascorbate and biotin synthesis, while enterotype 2 is a mucin glycoprotein degrader and involved in the thiamin and folat synthesis. Enterotype 3 is characterized through the mucin degrading activities as well as membrane transportations of sugars. While enterotype 1 is dominated by Bacteroides, Prevotella is the main strain in enterotype 2, while the Ruminococcus strain dominates the enterotype 3 (21,23). The combined genome of the bacteria inhabiting the human intestine is with more than five million genes outnumbering the hosts expressible genetic storage by far, underlining to significant influencing properties as well as the biochemical variability of the gut microbiota. Therefore, some consider the gut microbiota as an additional organ, which is also reasoned because of the huge metabolic capacity, comparable to the liver. The great scientific interest in the intestinal microbiota results not only from the numerical dimensions already mentioned, but also from the dynamic habitat intestine in which it unfolds its effects. Lifestyle, nutrition, as well as age and hygiene change the intestine environment and through that the microbial composition (21).

Although the huge scientific progress of microbial research was made in the last one or two decades, Elie Metchnikoff of the Pasteur Institute in Paris developed the theory, that lactic acid bacteria influence the aging process already more than 100 years ago. Simultaneously, Hubert J. Norman, working at the Camberwell House asylum, and George Porter Phillips, working in the Bethlem Royal Hospital, both in London, investigated about the effect of lactic acid bacteria on depression (25). So, although the

influence of bacteria on human health is of scientific interested in already a century, the pressure of making further progress first increased exponentially, as the dimension of occurrence of microbial inhabitants, as well as their influencing potential became clear.

5.2.1. Gut – microbiota – brain axis

As mentioned earlier, one of the most, if not the most important microbial colonization side is the human gastrointestinal tract especially the colon, not only due the high density of inhabitants, but also because of the influencing properties on health and diseases through the gut-brain-axis. The concept of the gut brain axis was born in the 1980s through the realization, that hormone signaling of the gastrointestinal endocrine system also affects neurons and brain cells (26). The concept was strengthened since then, as well as its key physiological functions on food intake, sleep and immune regulation examined and the bidirectional mode of operation ascertained. Only in the last years the concept was extended due to a shift of the understanding of the gut's physiological properties and is today rather understood as the gut -microbiome-brain axis (26,27).

With the gut-microbiota-brain axis, a concept is meant, which points out the connections among the CNS, the immune system, metabolic activities, the development of various organs and the gut (28). Consequently, gut to CNS signaling has been studied for a short

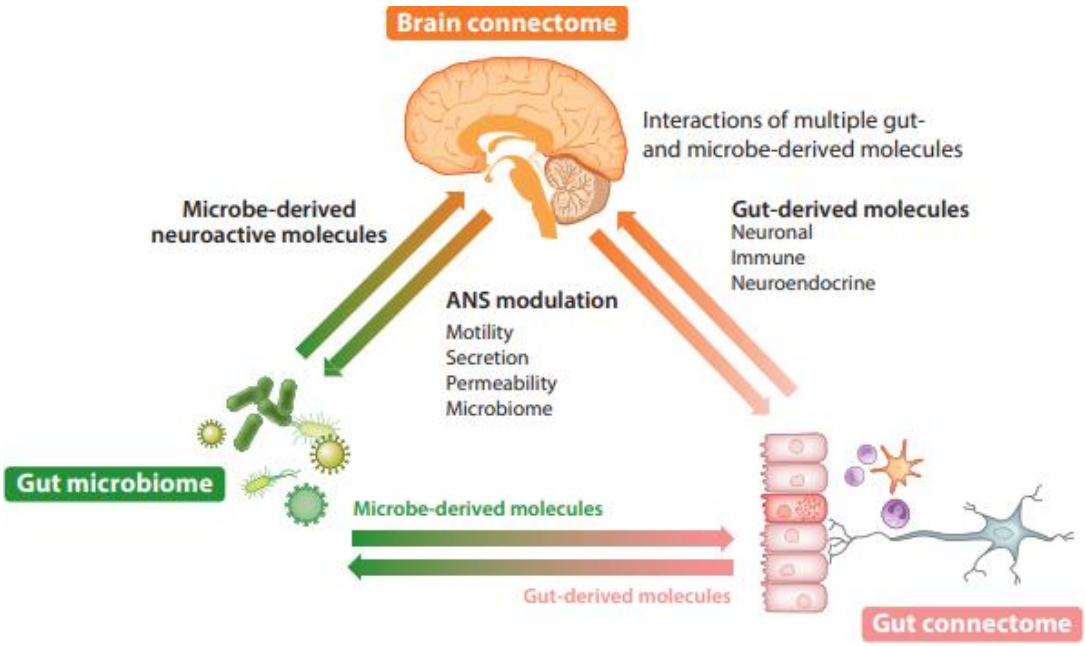


Figure 4: Gut - brain - microbiome - axis (27)

period, with, although several brain disorders could be linked to gut microbiota disruption, no clear evidence could determine whether these alterations in the microbiota are induced by brain signaling or whether brain dysfunction is driven by changes in the gut microbiota (29).

To understand the variety of influenced processes through the gut and the gut-microbiota, the mechanisms of action of inhabiting bacteria and especially the gut – microbiota – brain axis must be analyzed in detail.

5.2.2. Mechanisms of action

The hypothesis of the influencing potential of the gut microbiome in the pathophysiology of depression was underlined through findings of reduced α-diversity as well as total decreased quantity of species in depressed subjects. Gut microbiotas envelop their influencing potential through different pathways, which are connected with, among others, the vagus nerv, the HPA axis and the enteric nervous system. Especially the by the gut microbiota synthesized molecules, like short-chain fatty acids (SCFAs) as well as tryptophan, because of the critical role in modulating essential cytokines release and peptide YY, glucagon-like peptide-1 (GLP-1), 5-HT, melatonin, catecholamines, and gamma-aminobutyric acid (GABA) building, seem to be crucially involved in the modulating properties of the gut microbiota (30).

Starting directly after birth, commensal bacteria are influencing human development through childhood, maturity and aging, which is among others mediated by the bidirectional communication properties of the gut microbiota with the endocrine system. Especially the detection of inter kingdom signaling pointed out a possible way of action. It was first noted that gut bacteria produced and responded to a variety of different hormones and neurotransmitters, like serotonin, norepinephrine and estrogen, before with the detection of quorum sensing (QS), new insights regarding the communication between bacteria and the endocrine system could be gained. With QS, a bacterial cell to cell communication method is meant, in which’s middle the production and detection of autoinducer molecules (AI) stands. AI are hormone-like elements, produced by bacteria and accumulated in bacteria rich environments, through which information about density could be shared, and collectively genetic expression changed. This allows in synchrony acting bacteria tribes to simultaneously change their influence on processes like

bioluminescence, sporulation, antibiotic production, biofilm formation, and virulence factor secretion. Besides that, it was found that, AI seem to have influencing properties at host – cell signal transduction and beside that, modulate the hormone signaling pathway. Furthermore, it must be mentioned that similar processes were detected vice versa with hormones influencing bacterial gene expression and thereby affect for example growth or virulence. These results underscore the ability of the microbiota to act synchronously, validate the holobiont concept and highlight the potential impact on health and disease (31,32).

5.2.2.1. SCFAs and butyrate

Observational studies allowed the conclusion that MDD leads to an increased occurrence of proinflammatory microbial strains, as well as a decreased relative abundance of SCFA producing bacteria (33). SCFAs emerge by fermentation of dietary fiber through bacteria and can be further subdivided into formate, acetate, propionate, butyrate, valerate, isovalerate and hexanoate with especially acetate, propionate and butyrate are usually meant, normally occurring in a ratio of 60:20:20 (29). The short-chain-fatty acids can be transported or diffused before they can bind on epithelial and immune cells though G protein-coupled receptors (GPCRs), with only a minor fraction reaches the systemic circulation, because of their usage as energy substrates. The absorption is mediated by either H+-dependent or sodium-dependent monocarboxylate transporters (MCTs and SMCTs), with MCTs showing tissue specific differences regarding subtypes and expression patterns. SCFAs improve gut health and barrier integrity, with, when considered that increased permeability and translocated bacterial products impact neuronal function, might significantly affect mental health. Furthermore, histone deacetylases (HADC) inhibitory properties through acetylation of lysine residues of nucleosomal histones were found in various cell populations through SCFAs, suggesting another highly influencing pathway (29). Histone hyperacetylation is associated with activation of gene expression, while the removing of acetyl groups from lysine residues, which is mediated by HDACs, leads to transcriptional suppression. So, the HADCs inhibitory properties of butyrate are associated with increased expression through higher acetylation in order of decreased HADC activity (34). Furthermore, the findings, that propionate and butyrate modulate the expression of the enzyme tryptophan 5-hydroxylase

1 underlined the suggested properties of SCFAs on influencing neurochemistry, due to the fact, that tryptophan 5-hydroxylase 1 is involved in serotonin as well as the dopamine, noradrenaline and adrenaline biosynthesis (29).

Although, anti-depressant effects were associated with SCFAs in general, butyrate is of increased interest, because of the promising recent results. It was found that butyrate leads to an identical HADC inhibition pattern on hippocampal neurons, then shown by antidepressants (29,35–37). Furthermore, studies proved the ability of butyrate to cross the BBB, assisted by butyrate-transporting transmembrane proteins, which allows the

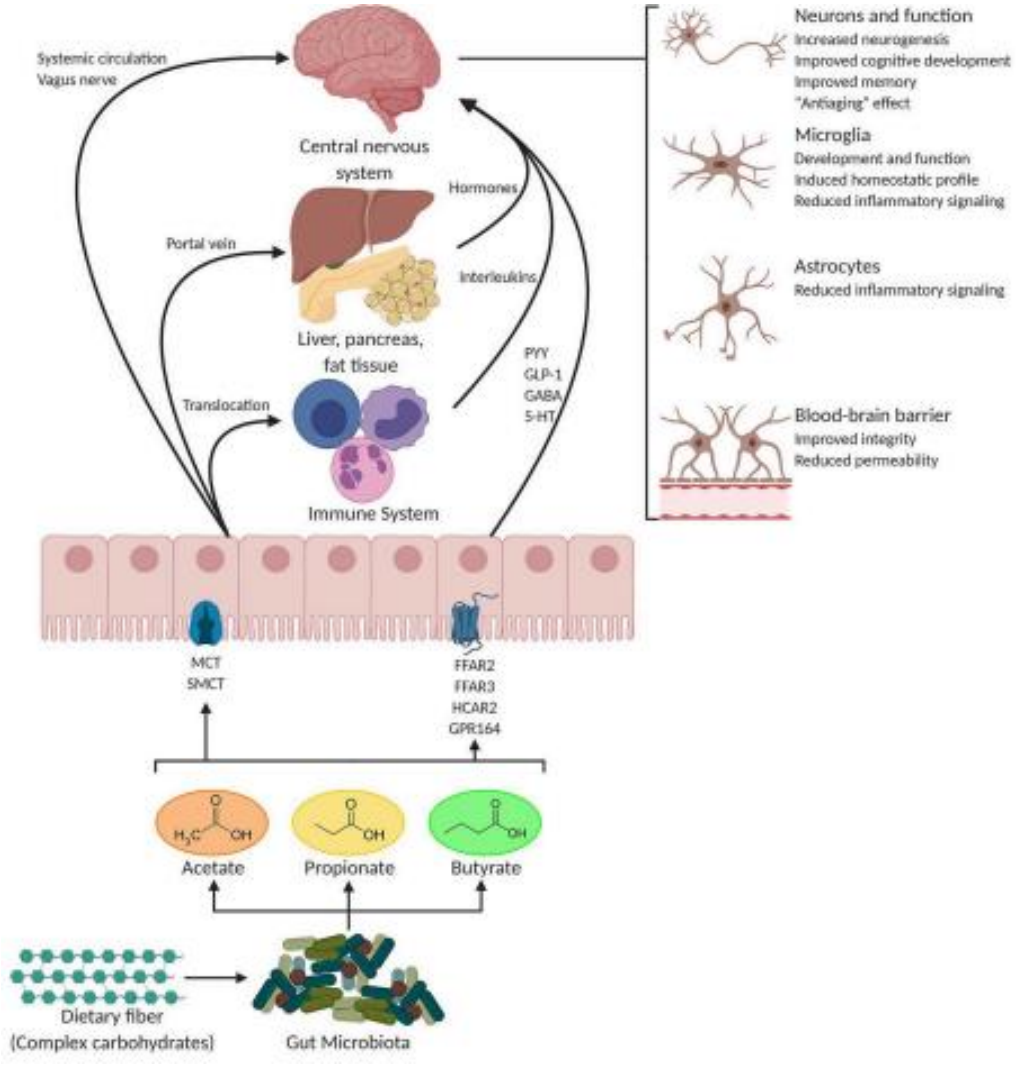


Figure 5: SCFA emerging and enveloping of physiological influencing properties (29)

occurrence of butyrate in the brain and lays the foundation of the influencing potential of neurological processes (37,38).

5.3. Studied interventions

5.3.1. Prebiotics

With prebiotics, non-digestible but fermentable food components are meant, which can be distinguished from fibers, due to the fact, that fibers are metabolized by most colonic microorganisms, while prebiotics are selectively fermented by specific health promoting bacterial species. That is why prebiotics are also defined through their health promoting properties, because of the strain specific growth modulation effects of especially, but not exclusively, lactobacilli and bifidobacteria (39,40)

5.3.2. Probiotics

In contrast to prebiotics, probiotics are live microorganisms with also contribute to health when administered. The FAO defined probiotics as: “live microorganisms, which when administered in adequate amounts, confer a health benefit on the host”. Although live microorganisms are meant, not exclusively bacterial strains but also health promoting yeasts are recognized as probiotics (41).

5.4. Current evidence

5.4.1. Connection between pre- and probiotic administration and depression

As earlier mentioned, the connections between the gut microbiota and mental disorders were of increased scientific interest in the last years. In order of generating better understanding, the gut microbiota composition of MDD subjects were studied and compared to healthy controls, as well as possible differences examined (42). Gut microbiota composition can be distinguished in α and β diversity. While α diversity is the variety of species at a local side, with β diversity, the differentiation among inhabitants of different samples is meant. Studies found inconsistent results regarding α and β diversity, but consistently reported a lower microbial diversity in MDD patients. Interestingly, alterations in the concentration of gastrointestinal permeability markers

seem to be associated with depression. Stevens et al., reported increased levels of zonulin, lipopolysaccharide (LPS) and fatty acid-binding protein (I-FABP) as well as higher prevalence of dysbiosis in MDD patients, while Alvarez-Mon et al. found no differences of zonulin levels but increased LSP and I-FABP concentration in depressive patients compared to healthy controls (43–45).

Pro- and prebiotic administration seems to be the most effective method counteracting dysbiosis und improving gut health. That is why, systematic studies, examining the effects of pre- and probiotic interventions are summarized.

5.4.1.1. Prebiotics and probiotics for depression and anxiety

The in 2020 published systematic review and meta-analysis of Liu et al., summarized 36 trails investigating about the effects of pre- and probiotic administration on depression and anxiety, whereby the included studies can be further distinguished into 7 prebiotic and 29 probiotic trails.

The prebiotic substances used, were Bimuno®-galactooligosaccharide (B-GOS), fructooligosaccharide (FOS), galactooligosaccharide GOS, and short-chain FOS (scFOS) all of which are suggested to have bifidogenic properties, with administration duration ranged from four hours to four weeks. No significant differences were found regarding depression and anxiety outcomes after prebiotic administration compared to control group in all included trails.

The included probiotic trails focused on the effect of lactobacilli administration, either alone or with extension of other strains, must often Bifidobacterium, with two exceptions focusing exclusively on Bifidobacterium longum and Bacillus coagulans. The duration of the included trails ranged from eight days to 45 weeks. Probiotic interventions decreased depression severity across the different studies, with significant effects were only observed in studies lasting more than one month. Interestingly, when analyses were restricted to Lactobacillus-only trials, no significant effect for depression and anxiety could be shown, while Lactobacillus complemented by other bacterial strains as well as other genera alone, reached statistical significance, with stronger depression enhancing effects. This connection was also shown by a model which compared the effect on depression of Lactobacillus-only trails with the remaining probiotic studies. A significant difference, not favoring Lactobacillus-only trails was calculated ($p < 0,01$). Summarized,

this systematic review suggested probiotic administration to be more effective as prebiotic consumption, with probiotic success seemed to be strain specific as well as duration dependent (46).

5.4.1.2. Effect of Probiotics on Depression

Huang et al. investigated about the effects of probiotic administration on depression and therefore conducted a quantitative synthesis of RCTs. Five studies were included, corresponding to inclusion criteria with the duration ranging from 30 days to 20 weeks. Combinations of different bacterial strains were supplemented in every trail exclusively belonging to Lactobacillus and Bifidobacterium strains with just Shinkai, 2013 et al, administering exclusively Lactobacillus pentosus strain b240. All studies reported an improvement of depression through probiotic administration with two of those reaching statistical significance. Consequently, the overall effect was positive, and showed a significant depression score enhancement after probiotic administration [MD (CI): -0,30 (-0,51 – -0,09)]. Interestingly, also different effects were found in the examined subgroups, with bigger effects in MDD patients [MD (CI): -0,73 (-1,37 - -0,09)] compared to healthy individuals [MD (CI): -0,25 (-0,47 - -0,03)]. Especially Lactobacillus acidophilus and Lactobacillus casei seem to be strongly associated with depression ameliorating effects (47).

When analyzing those results, the subgroup specificity (MDD or healthy control) of the probiotic intervention effects raises attention, as this might be connected to the mechanism of action. Furthermore, strain dependence is shown, evidently connecting specific strains with interventional success.

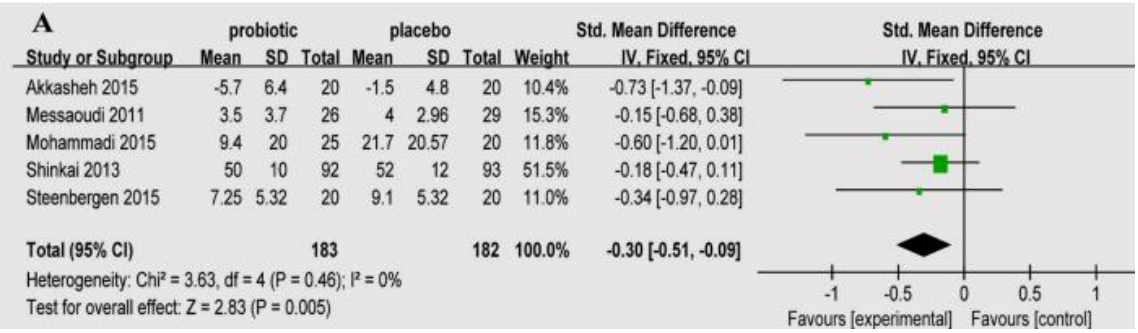


Figure 6: Effects of probiotics on depression (47)

5.4.1.3. Effects of interventions targeting gut microbiota

Hofmeister et al. examined different, the gut microbiota modulating interventions, as well as their effects on depression. Probiotic administration was by far the most frequent intervention, with 44 included trails investigated about the effects of probiotic, 5 about prebiotic and 6 about synbiotic administration on depression.

24 of the probiotic trails used more than one bacterial genus, with Lactobacillus and Bifidobacterium were the most frequent used strains. Included studies could be further subdivided into populations (MDD and healthy subjects), with probiotic intervention seem to influence mood in MDD population even more promising [MDD: Hedges' g 0.78 (0.19 to 1.37); healthy subjects: Hedges' g 0.31 (0.15 to 0.46)].

Prebiotic intervention was defined generally as "compounds in food that induce growth or activity of gut microbiota". Although, no significant effect on depression could be calculated for healthy participants, statistically significant benefits of prebiotic administration were found in MDD patients [MDD: Hedges' g 0.39 (0.04 to 0.73); healthy: Hedges' g 0.13 (–0.23 to 0.48,)].

Included synbiotic intervention trails were just examining healthy populations. Interestingly, synbiotic administration was able to show the most promising effects [healthy: Hedges' g 0.68 (0.36 to 1.00)] (48).

Summarized, again subgroup specific differences were shown, as well as good effects of probiotic and symbiotic interventions found.

5.5. Connection between fasting and CR interventions and depression

Because of the connection between neuroinflammation and depression, whereby current literature suggests neuroinflammation to be the key factor responsible for the alterations underlaying the pathophysiology of depression, interventions with anti-inflammatory properties have been brought into play as possible depression-mitigating therapies (49). Because already conducted trails evidently suggested, that CR and fasting reduce inflammation levels through decreased mRNA expression of proinflammatory cytokines and chemokines, the connection between CR, fasting and depression raised up (50).

This connection might be mainly modulated by beta-hydroxybutyrate (BHB). Since BHB is a ketone body, BHB production is especially stimulated during periods of reduced caloric intake (51). More than 80% of human energy storage is saved as fatty acids in

adipose tissue and is mainly used after muscle and liver glycogen storages are depleted during prolonged fasting periods (52). Fatty acids are thereby transported from different tissues to the liver and are transformed via β-oxidation into acetyl-coenzyme A (acetyl-CoA) before acetoacetate (AcAc) is made. This process is controlled by the mitochondrial enzymes 3-hydroxy-3-methylglutaryl CoA synthase 2 (HGMCS2) and 3- hydroxy-3-methylglutaryl CoA lyase (HMGCL), with is also illustrated in graphic 7 (34,52).

The small polar BHB molecule is well soluble in water and therefore transported into the brain via the blood, where it can be carried across the BBB through monocarboxylic acid transporters, including MCT1 and MCT2, which's expression strongly controls the brain uptake of BHB.

Originally the idea of BHBs modulation properties aroused, because of the chemical similarities to butyrate and could be further underlined by the results of in vitro models,

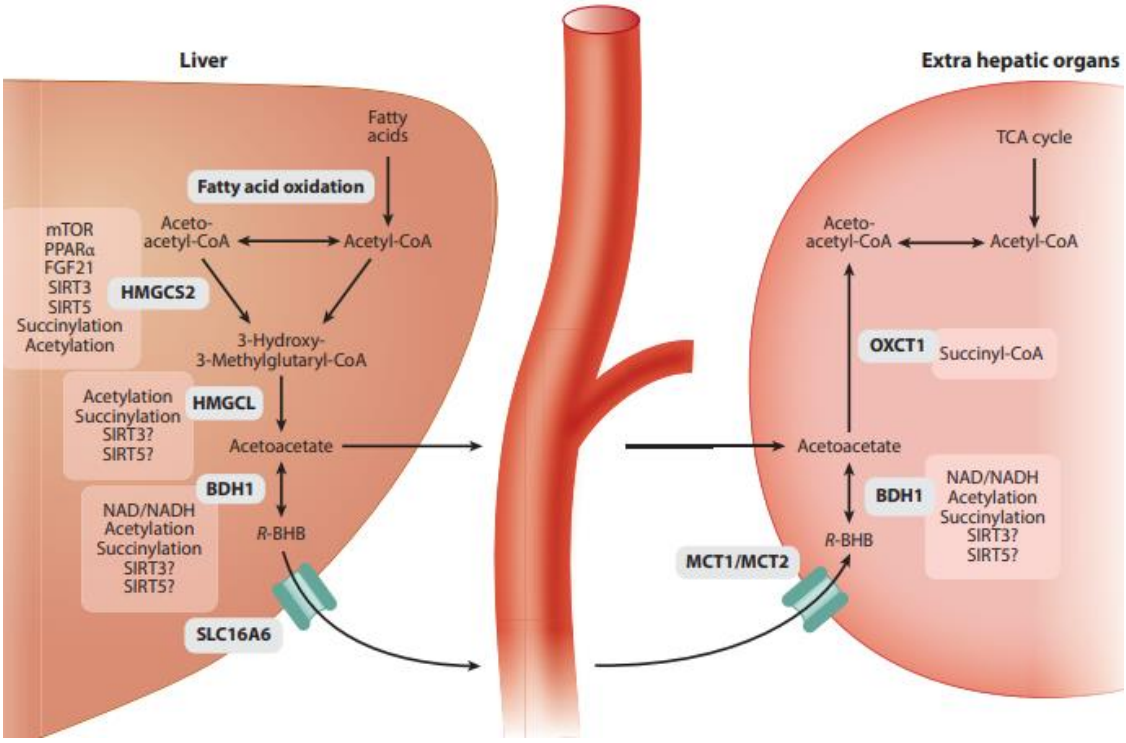


Figure 7: BHB emergence in the liver. HMGCS2 and HMGCL are the key enzymes of this metabolic process. Their expression is strongly influenced by certain molecules enveloping epigenetic alterations influencing translational and transcriptional effects (52)

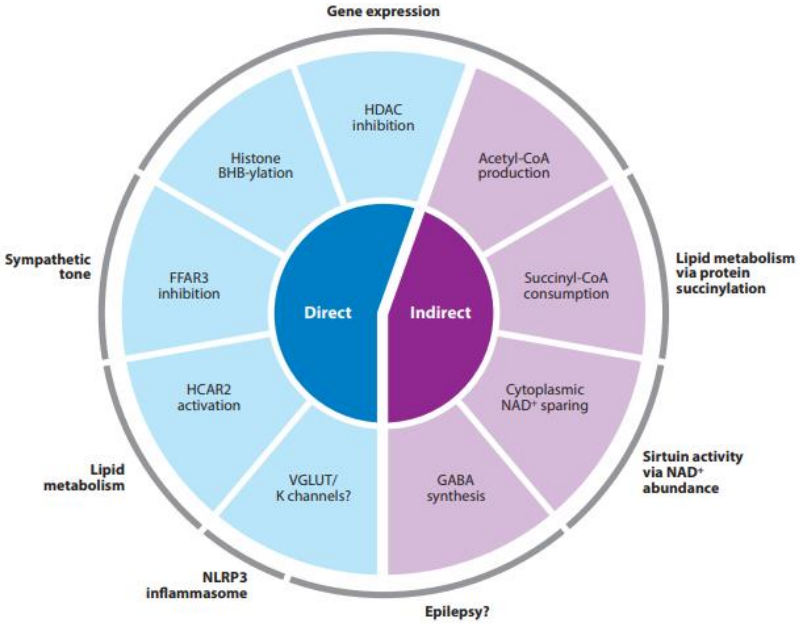


Figure 8: Overview of the influencing properties of BHB (52)

suggesting BHB to have comparable HDAC inhibitory properties than butyrate (53). Especially expression changes of the forkhead box O3 (Foxo3) gene was found to be present in a mice model after BHB infusion, whereby it is thought of HDACs to lead to Foxo3 repression. BHB induced Foxo3 hyperacetylation through HDAC inhibition might mediate health and stress, since Foxo3 is the mammalian ortholog of the stress-responsive transcriptional factor DAF16 which regulates lifespan in worms. Furthermore, it could be shown that HADC inhibition is even present at serum concentrations of 1–2 mM, which is easily reachable through fasting or ketogenic diet (34).

Summarized, evidence suggest looking at BHB mor as a signaling molecule as just an energy substrate, not just because of the HADC inhibitory properties, but also because of effects on autophagy, insulin signaling and inflammation, whereby an overview of possible effects is given in figure 8 (34,52,54).

5.6. Current evidence

In the following part, current systematic reviews and meta-analyses, which investigated about the effects of dietary restriction on depression are summarized.

5.6.1. Fasting Interventions for Stress, Anxiety and Depressive Symptoms

The in 2021 published meta-analysis examined the effects of fasting interventions on depression and therefore included 11 RCTs and observational trails, with a total of 1436 participants. Overall, no effect on anxiety or depression parameters was reached through

fasting and CR. The fasting groups were not found to have lower anxiety or depression levels compared to control groups at the end of fasting. By omitting observational trails and just analyzing RCTs, significance of anxiety [SMD: b = −0.508(−0.988;−0.028)] and depression [SMD: b = −0.281(−0.502;−0.061)] improvements was reached, which also suggests a dilution of potential fasting and CR effects through observational studies. Interventions thereby included time restricted feeding with no caloric reduction, reduction of 300 to 500 kcal/day from their habitual energy intake with additional two days of muslim sunnah fasting per week for 12 weeks, 25% caloric restriction for 104 weeks and a low-calorie diet (800 kcal/J) for 12 weeks.

Interestingly, especially more severe intervention achieved better results, supporting the theses, that only above a certain caloric restriction, desirable biochemical processes occur. In addition, the study design-dependent differences indicate a significant influence of the study design, whereby in principle a better predictive power of RCTs can be assumed in comparison to observational studies (55).

5.6.2. Fasting in mood disorders: neurobiology and effectiveness

No quantitative synthesis, but a summarization of current literature was published in the review of Fond et al., in 2013. One study was also included in the already discussed meta-analysis from Berthelot et al. Mood improvements and depression amelioration were found in all trails, while the duration reached from 8 to 10 days and the interventions were fasting with no or severely limited caloric intake and caloric restriction. Fasting interventions seemed to be effective in healthy and depressed subjects, whereby improving mood in healthy populations and inducing remission in depressed patients. Due to the fact, that just severe fasting interventions were included in the review, no comparison could be conducted between different kinds of caloric restriction and fasting. Nevertheless, because of continuous good effects, more restrictive interventions seem to be significant more effective in improving meant health and wellbeing (56).

6. Systematic Review and Meta-Analysis

6.1. Definition

With systematic review the process of searching and accumulating of all scientific literature to a specific topic is meant, with the aim of analyzing and combining the results.

The quantitative synthesis of included data through usage of statistical tools is thereby called meta-analysis. Consequently, every meta-analysis is part of a systematic review, but not every systematic review contains a meta-analysis. The importance of systematic scientific work arises through the possibility of improving evidence through combining a variety of results and therefore structuring the huge number of published trails (57,58).

6.2. Methods

The objective of this systematic review and meta-analysis was to examine the association between administration of pre-, probiotics, butyrate levels and depression, as well as the connection between fasting or caloric restriction, BHB concentration and depression. Possible similarities and differences between butyrate and BHB are being examined to better understand the biochemical mechanisms of fasting and the health benefits associated with the modulation of the gut microbiota.

Therefore, a systematic search was conducted in Pupmed and Scopus, as well as Google Scholar for potential gray literature. Studies with any study design were included, except for systematic reviews and meta-analyses. Animal studies were also excluded, as were studies that examined mentally ill populations, with overweight or obesity being no exclusion criteria. Furthermore, major exclusion criteria were no analysis of SCFAs and butyrate, ketone bodies and BHB levels as well as administration of additional drugs or substances with a high influencing potential. The advanced search function was used in all of the three databases with the following terms:

((((prebiotic* OR postbiotic* OR probiotic* OR synbiotic*) OR (fasting)) OR ("calori* restriction" OR "energy restriction" OR "dietary restriction")) AND (butyrate OR "beta hydroxybutyrate" OR bhb OR "β hydroxybutyrate" OR "ketone bodies")) AND (depress* OR "affective disorder" OR "bipolar disorder") for the Pupmed search and (prebiotic* OR postbiotic* OR probiotic* OR synbiotic*) OR (fasting) OR ("ca lori* restriction" OR "energy restriction" OR "dietary restriction") AND (butyrate OR "beta hydroxybutyrate" OR bhb OR "β hydroxybutyrate" OR "ketone bodies") AND (depress* OR "affective disorder" OR "bipolar disorder") for Scopus.

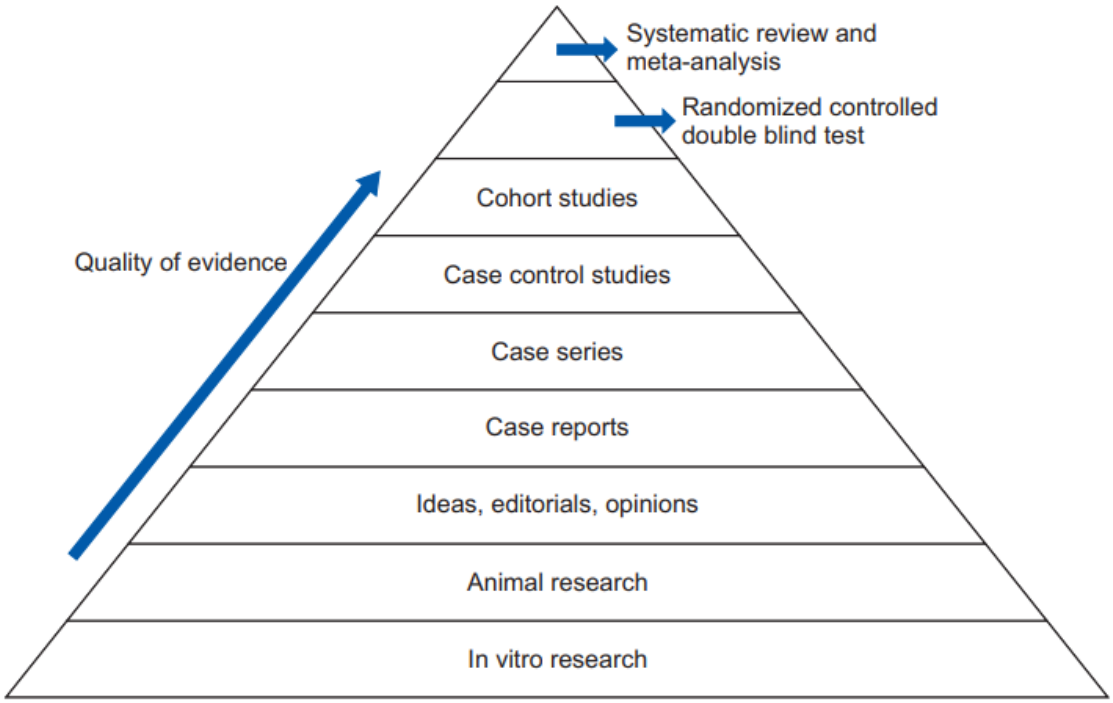


Figure 9: Hierarchy of evidence underlining the opportunity of improving expressiveness through conducting systematic reviews (96)

Additionally, the Publish or Perish software was used to filter out the 500 most fitting search results on Google Scholar, published between 2000 and 2024, for each of the three search terms (1): (prebiotic*|postbiotic*|probiotic*|synbiotic*) (butyrate|"beta hydroxybutyrate"|bhb |"β hydroxybutyrate"|"ketone bodies") (depress*|"affective disorder"|"bipolar disorder"), (2): (fasting) (butyrate|"beta hydroxybutyrate"|bhb |"β hydroxybutyrate"|"ketone bodies") (depress*|"affective disorder"|"bipolar disorder"), (3): ("calori* restriction"|"energy restriction"|"dietary restriction") (butyrate|"beta hydroxybutyrate"|bhb |"β hydroxybutyrate"|"ketone bodies") (depress*|"affective disorder"|"bipolar disorder").

6.3. Findings of the systematic search

122 results were generated using Pupmed, as well as 5017 results gained in Scopus and 1500 results found in Google Scholar. So, 6628 studies were collected during the searching process, of which 1648 existed several times and were deleted using the program Rayyan. 4865 trails could be excluded, because they were not corresponding to inclusion criteria, leaving 116 studies qualified for the full text screening. Finally, 6

studies could be included in the systematic review of which 3 of them were RCTs, examining pro- and prebiotic administration and were used for conducting a meta-analysis (table 1), while 3 observational trails, investigating about the properties of fasting and caloric restriction, were reviewed qualitatively (table 2).

6.4. Meta-Analysis

6.4.1. Data preparation

For the meta-analysis, a fitting effect measure must be chosen. For metric data, as present in the three included trails with SCFA concentration being given either in µmol/l, mg/g or µM and depression measurements as already quantified depression scores, mean difference (MD) and standardized mean difference (SMD) are the common effect measurements. Because of differences regarding measurement loci or depression scores, results could not be compared directly, why SMD seem to be more precise due to its standardizing calculation method. SMDs are calculated by dividing the difference of mean outcome between groups, through the standard deviation (SD) of the outcome among participants.

$$\text{SMD} = \frac{\text{difference in mean outcome between groups}}{\text{standard deviation of outcome among participants}}$$

The SMD calculates the size of the intervention effect compared to the control group, with positive values indicating a favorable intervention effect and negative values favorable results in the control group. In general, SMD values between 0.2-0.5 are considered small, between 0.5-0.8 are considered medium, and greater than 0.8 are considered large. Furthermore, not the outcome measurements were compared, but the changes between baseline and follow-up measurements. This was reasoned because of the small sample sizes and the risk of bias, due to the strongly fluctuating initial values. (59,60).

Study	Country	Population	Age (SD)	Sex (% female)	Study type	Intervention	Control	Duration	Outcomes
Sandberg et al.	Sweden	38 healthy, subjects (52-70y)	63,6 (5,3)	78,9	RCT	Rye-based bread; 75g carbohydrates daily; 5,7g soluble fiber	White wheat bread; 75g carbohydrates daily; 1,2g soluble fiber	3-days	Physiological: Plasma SCFAs, Psychological: Swedish core affect scale,
Nishida et al.	Japan	60 healthy, young adults	25,1 (0,6)	31,6	RCT	Lactobacillus gasseri 1x1010 cfu	placebo	24-weeks	Physiological: Fecal SCFAs Psychological: HADS
Dalile et al.	Belgium	69 healthy, men (20-40y)	26 (4,1)	0	RCT	Extrusion cooked wheat bran; 25g fiber per day	WCC cereal as a placebo; 25g hardly fermentable fiber	4-weeks	Physiological: Fecal SCFAs, Psychological: DASS-21

Table 1: Included trails into the meta-analysis investigating about the effects of pro- and prebiotic interventions on depression

Study	Country	Population	Age (SD)/ BMI (SD)	Sex (% female)	Study type	Intervention	Duration (SD)	Outcomes	Findings
Toledo et al.	Germany	1422 subjects (18-99y)	55,4 (0,4) / 28,2 (0,2)	59,1	Observa-tional study	200-250 kcal per day with 25-35g carbohydrates	Mean 8,2 (0,1)	Physiological: Acetoacetic, Psychological: emotional wellbeing (EWB)	↑ Acetoacetic* ↑ EWB* EWB effects increased with fasting length*
Castro et al.	Spain	20 subjects (18-65y; BMI ≥ 30 kg/m^2	47,2 (10,2) / 35,5 (4,4)	60,0	Observa-tional study	VLKD (from weight loss program); 600-800 kcal/day until targeted weight loss is reached	Depends on weight-loss progress and targeted weight	Physiological: BMI, BHB Psychological: QoL	↑ BHB * ↑ QoL*
Yang et al.	China	13 (28-55y)	39,6 (8,1)/ n.a.	0	Observa-tional study	Water only fasting	10 days	Physiological: Ketone bodies Psychological: POMS, SDS	↑ Ketone bodies* ↓ SDS-score*

*Table 2:Reviewed studies examining the connection between fasting/caloric restriction and depression; significant changes are highlighted with**

6.4.1.1. Impact of rye-based evening meals on cognitive functions, mood and cardiometabolic risk factors

As shown in Table 1, the study by Sandberg et al. titled "Impact on rye-based evening meals on cognitive functions, mood and cardiometabolic risk factors: a randomized controlled study in healthy middle-aged subjects" is included, although this study is conducted with a cross-over design. In cross-over studies, each participant acts as his or her own control, with only the order of intervention and placebo is randomized. Studies with a cross-over design run the risk of a carryover effect, i.e., that the effect of treatment in the first period also affects measurements in the second period (61). This risk was not considered as relevant because of the long washout period of 3 weeks in contrast to the short intervention period of 3 days. Unfortunately, the percentage changes were only reported for a few parameters in the intervention group, so the post-intervention measurements, rather than the comparative values between the pre- and post-intervention measurements were used as the outcome of the intervention. The Plotdigitizer tool was used to calculate fasting post-intervention SCFA measurements and their standard error of the means (SEMs). Due to the absence of highly different baseline measurements in crossover studies, comparison of post-intervention measurements is a valid method anyway. Although the Cochrane Handbook of Systematic Reviews of Interventions does not recommend mixing follow-up values with change values when using SMD as an effect measure, Da Costa et al. found no evidence of systematic differences in studies generated from change and follow-up data (62). This conclusion was inferred after analyzing 21 meta-analyses that examined the association between osteoarthritis and pain scores. Pain scores have a fixed range and therefore tend to be normally distributed and less screwed (63). Because it cannot be assumed that these results also apply to continuous outcome variables, the Cochrane Handbook of Systematic Reviews of Interventions advises against mixing change scores and follow-up data, additionally reasoned because the SD values of change scores tend to be larger (60).

Nevertheless, the use in this case seemed possible and is therefore applied. Furthermore, the SEM values reported must be converted to SD. This is done with the formula: $SD = SEM*\sqrt{n}$ (64).

Study	n (total)	Site (unite)	Follow-up values: intervention group (SD)/control group (SD)			
			Total SCFAs	**Acetate**	**Propionate**	**Butyrate**
Sandberg et al., 2018	38	Plasma (µmol/l)	183,26 (68,79) / 137,91 (64,48)	177,67 (68,86)/ 134,42 (60,23)	3,30 (1,73)/ 3,07 (1,23)	2,08 (1,42)/ 1,52 (0,86)

Table 3: Data SCFAs Sanberg et al.

In addition to SCFA concentrations, mood was assessed using the Swedish Core Affect Scale. This was done using a 100 mm VAS. Here, the participant responded to the questions asked by marking a 100mm long line, showing complete agreement with the question by marking at 100mm, while marking at 0mm indicates absence of agreement. The status between dissatisfied-satisfied, depressed-cheerful, and sad-cheerful was assessed in this way, and the mean values were combined for the valence rating. Higher scores can be interpreted as better psychological outcome. Because SCFA measures were compared in the fasting state after the interventional period, valence scores were also compared in the fasting state after the 3-days of interventional duration. Again, only follow-up values were used to provide a methodologically similar interpretation of the data from this study. Due to the range dependent depression score values, mixing of change and follow up SMD should not account for different findings, as shown in the already mentioned findings of Costa et al. (65).

Study	n (total)	Scale	Follow-up values: intervention group (SD)/control group (SD)
			Valence
Sandberg et al., 2018	38	100 mm VAS	76,9 (14,18) / 70,6 (16,64)

Table 4: Mood assessments Sandberg et al.

6.4.1.2. Health benefits of lactobacillus gasseri cp2305 tablets in young adults exposed to chronic stress

The included study of Nishida et al. is titled "Health Benefits of Lactobacillus gasseri CP2305 Tablets in Young Adults Exposed to Chronic Stress: A Randomized, Double-Blind, Placebo-Controlled Study". The study reported acetate, propionate, n-butyrate, iso-butyrate, n-valerate, and iso-valerate levels at baseline (week 0) and post-intervention (week 24) in mg/g feces, as well as the mean change scores and their SEMs. Since the other included studies only differentiated between the short-chain-fatty acids acetate, butyrate and propionate, with no distinction between the butyrate iso-forms, both the mean and SEMs of the iso- and n-butyrate values must be added together to get total butyrate measurements. Because both measurements were dependent, this was done simply through adding mean changes together, while SEM levels must be converted into SD, using the formula SEM = SD/√n, which can be further transformed into SD = SEM*√n. The SD can then be squared to get the variance which can simply be added together to the final variance of the parameter butyrate, containing iso-butyrate and n-butyrate values (64). To get the SD of the combining mean change, the square root of the final variance must be taken.

Study	n (total)	Site (unite)	Mean change intervention group (SD)/control group (SD)			
			Total SCFAs	**Acetate**	**Propionate**	**Butyrate**
Nishida et al., 2019	60	Feces (mg/g)	-4,10 (18,11) / -10,60 (18,17)	-6,30 (15,03)/-8,00 (15,08)	-0,10 (6,68)/ -0,80 (7,00)	1,30 (7,32)/ -1,00 (7,08)

Table 5: Data SCFAs Nishida et al.

While change values were given for acetate, propionate and total SCFAs, SDs needed to be calculated out of SEMs using the already mentioned formula.

After SCFA values were analyzed, assed mood measurements must be refurbished. Different psychological tests were done in this study, but only the most accurate score,

examining depression, included. Anxiety measurements were of no relevance, because of missing assessments in the other trails of this meta-analysis. Data was present as mean values, with mean change was calculating through subtracting baseline values from follow-up measurements. Baseline and follow-up SDs needed to be calculated out of the given SEM values, before with the following formula, SD of the means of change could be calculated. $SD = \sqrt{SD_b^2 + SD_f^2 - 2 * r * SD_b * SD_\mathrm{f}}$

Thereby, b stands for baseline and f for follow up. Furthermore, the correlation coefficient r must be estimated because no value was given. Due to the already summarized good, reported effects or probiotic intervention, the passively estimated correlation coefficient of 0,5 was taken. The value r = 0,5 was categorized as the smallest correlation coefficient, with which change values, instead of follow-up values can be used. Because the already analyzed meta-analysis also used change score values, the correlation coefficient of ≥ 0,5 seem to be plausible (60,66).

6.4.1.3. Extruded wheat bran consumption increases serum short-chain fatty acids but does not modulate psychobiological functions in healthy men

Study	n (total)	Scale	Mean change scores: intervention group (SD)/control group (SD)
Nishida et al., 2019	60	HADS depression	-1,2 (3,90) / -1,5 (3,99)

Table 6: Mood assessments Nishida et al.

The study of Dalile et al. entitled: "Consumption of Extruded Wheat Bran Increases Serum Short Chain Fatty Acids but Does Not Affect Psychobiological Function in Healthy Men: A Randomized, Placebo-Controlled Trail", was published in 2022. The effects of the intervention were shown by plotting baseline measurements and post-intervention measurements of fecal acetate, propionate, butyrate, and total SCFAs as well as their SDs. Because fecal samples were collected by participants at home, stored in the freezer, and then transported in a frozen state to the study visit, there were many opportunities to influence the outcomes of the measurements, such as improper storage

leading to the decreased concentration of the volatile SCFAs. In addition to fecal SCFA concentrations, serum SCFA levels were measured at baseline and after the intervention period. For this purpose, participants were required to visit the laboratory, where the first measurement was taken in the fasting state and 11 additional tests conducted every 30 minutes, making a total of 12 SCFA measurements. At baseline, both groups ate a standardized, fiber-free breakfast after the first fasting measurement, while participants ate their group-specific meal (placebo or extruded WB) at the follow up laboratory meeting. In the placebo, as well as in the intervention group, baseline or postintervention SCFA concentrations reached a maximum after 2,5 to 4 h, reflecting digestion and transportation duration through the colon. The serum SCFAs were used for the meta-analysis, because of the reduced possibility of bias entry, due to standardized measurements.

Because time-dependent serum SCFA levels were just present in a graphic, Plotdigitizer was used again to find out exact serum acetate, butyrate, and propionate values, as well as their SEs, while total SCFAs were not given. SD was calculated using already mentioned formula $SD = SE*\sqrt{n}$ (62).

The total SCFA values were calculated through adding the values of acetate, propionate, and butyrate together, while the total SCFA SD could be generated by taking the square root of the summarized variances. Furthermore, mean change values were calculated through subtracting the baseline values of the postintervention values. To generate the SDs of the mean change again the formula underneath was used. The correlation coefficient r was again estimated with 0,5, which was again argued through the common

Study	n (total)	Site (unite)	Mean Change: intervention group (SD)/control group (SD)			
			Total SCFAs	**Acetate**	**Propionate**	**Butyrate**
Dalile et al., 2022	69	Serum (µM)	14,94 (12,75) / 7,43 (12,71)	14,34 (12,73) / 7,17 (12,69)	0,40 (0,71) / 0,14 (0,54)	0,20 (0,27) / 0,12 (0,43)

Table 7: Data SCFAs Dalile et al.

use of mean change scores in comparable meta-analysis, which at least requires a correlation of 0,5 (60)

$$SD = \sqrt{SD_b^2 + SD_f^2 - 2 * r * SD_b * SD_\mathrm{f}}$$

Furthermore, assessed mood must be interpreted. DASS score, as an expressive parameter of depression was included. Baseline and follow-up values, as well as SDs, were presented in the study, so that mean changes could be calculated by subtracting baseline from follow-up values. SD of change was calculated through the above-mentioned formula (r = 0,5). Also, all change means were modified, so that positive values represent an improvement of depression, whereby all negative values represent a worsening (67).

Study	n (total)	Scale	Mean change: intervention group (SD)/control group (SD)
Dalile et al., 2022	69	DASS	0,14 (1,89) / 0,08 (2,77)

Table 8: Mood assessments Dalile et al.

6.4.2. Statistical evaluations

After data preparation, further evaluations were performed using R-Studio. First, SMD was calculated using the metacont function with a random-effects model (e.g., SCFA SMD calculation). The random effects model assumes that because of heterogeneity across studies, e.g., in age, mental health, or diet, the true effect size varies across included studies, whereas the fixed effects model assumes a single true effect size, where differences in measured effect are due to sampling error alone (68): This was done for total SCFAs and the single short-chain-fatty acids butyrate, propionate, and acetate, as well as depression score changes.

e.g., SCFA SMD calculation:

"REscfa <- metacont(ni, scfai, ssdi, nc, scfac, ssdc, sm="SMD",
data=Data_t, comb.fixed=FALSE,
hakn=TRUE)"

Further, the results were merged and illustrated using the forest function. Again, this procedure was also applied on the single SCFAs and depression scores. Hereby, SMD of the included studies, as well as the related confidence intervals (CI) were merged through which an overall effect estimate was calculated as well as the related p-values determined.
e.g., illustration of SCFA SMD saved in object REscfa:

"forest(REscfa, xlim=c(-0.5, 1.5),
studlab=paste(Author),
digits.se = 2,
col.diamond ="blue",
xlab="SCFA concentration changes")"

Furthermore, correlations between the biomarker, which in this case were total SCFAs, butyrate, propionate or acetate and the depression sore changes were calculated through conducting a meta regression model.
e.g., regression model between depression score SMD and butyrate SMD:
"result_B <- rma.mv(Data_t.SMD_D ~ Data_t.SMD_B, V = diag(meta_data$var_D),data = meta_data, random = ~ 1 | Data_t.Study)"

6.4.3. Results

Separate forest plots are presented, evaluating, and illustrating the overall effect of the interventions on butyrate, total SCFAs, propionate, acetate and depression scores (figure 10). Furthermore, effect sizes as well as 95% CI are given. Beside the overall effect, also the heterogeneity is presented in the forest plots. With the heterogeneity measure I^2 being 0% all the variances of the effect size estimates are explainable through SE, which means that all measured effects can be attributed to the interventions. Also, p-values of > 0,05 showing that H_0 is not rejected, and no between study heterogeneity is responsible for SE of the effect size estimates. The estimated effect size of acetate is influenced by heterogeneity of the studies, which accounts for 26% of the variability of the acetate SMD. Since heterogeneity of ≤ 25% is considered low and heterogeneity between 25 and

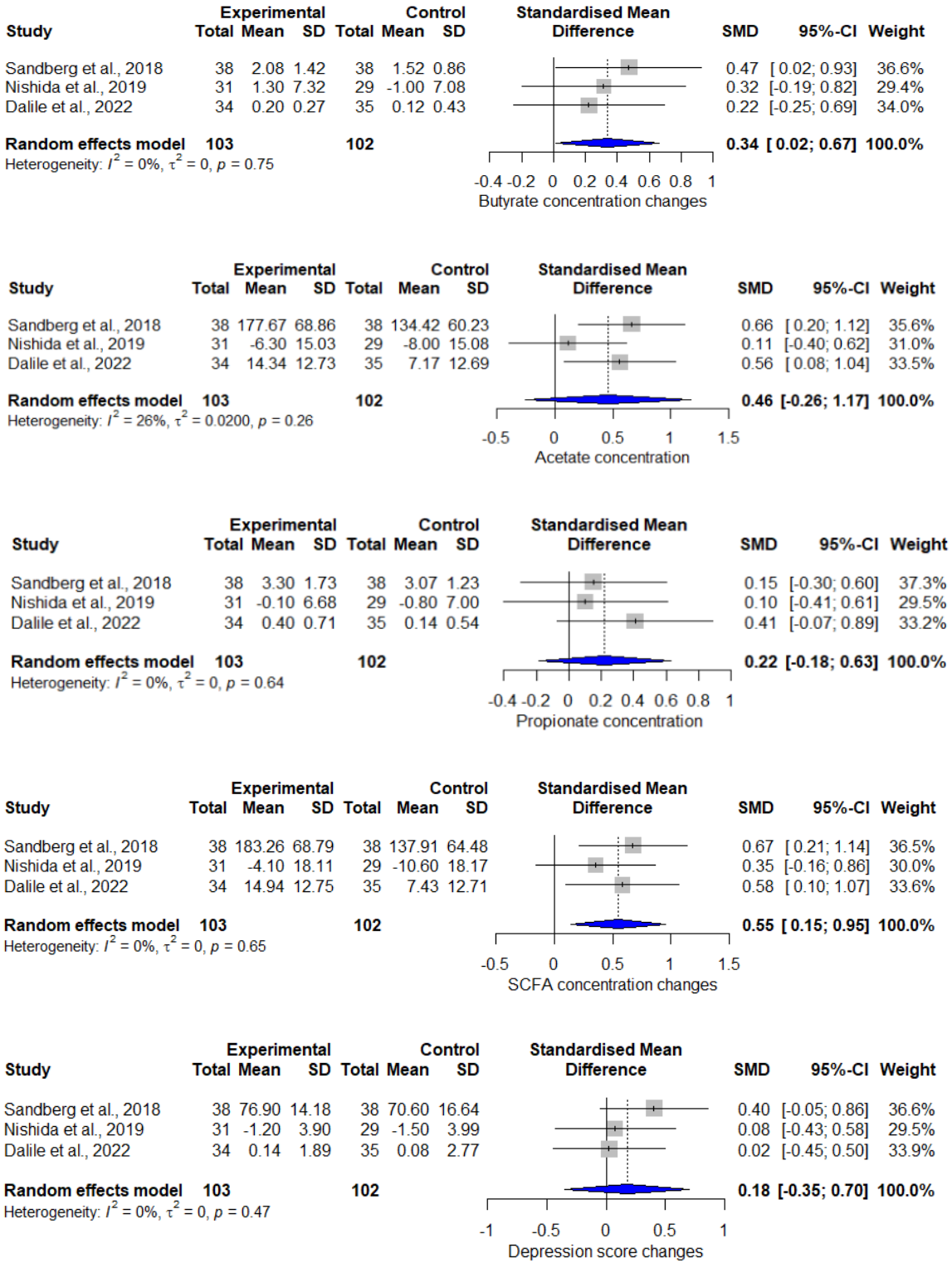


Figure 10: Forest plots, illustrating the SMDs of butyrate, acetate, propionate, total SCFAs and depression score

50 is considered moderate, no studies were excluded in the calculation of the acetate

SMD, although H0 is rejected with a p-value of 0.26 and H1 clearly states that heterogeneity between studies is responsible for the SE of the effect size estimate (69,70). The Intervention led to significant butyrate [SMD (CI) of 0,34 (0,02 - 0,67)] and SCFA [SMD (CI) 0,55 (0,15 - 0,95)] concentration changes, while no significance was found regarding propionate [SMD (CI) of 0.22 (-0.18 - 0.63)] and acetate [SMD (CI) 0.46 (-0.26 - 1.17)] enhancement, whereby acetate seemed to be stronger connected the intervention. The overall SMD of the depression score changes also reached no statistical significance [SMD (CI) 0,18 (-0,35 - 0,7)], but strongly favored the intervention (figure 10).

To assess, a possible connection between the measured metabolites and depression score changes, regression models were conducted, calculating the connection between butyrate, acetate, propionate as well as total SCFAs SMDs and the SMD of the depression score change. No significant relationship could be shown for butyrate (p-value = 0,17), acetate (p-value = 0,45) and SCFAs (p-value = 0,44), but b1 was positive in all three models [(b1 = 1,57; butyrate), (b1 = 0,36; acetate) (b1 = 0,75; SCFA)]. Interestingly, propionate was negatively associated with depression, although no statistical significance was reached [b1 = -0,61 (p-value = 0,57)].

6.5. Qualitative evaluation

Three trails were included, examining the effects of fasting and caloric restriction on depression (table 2).

6.5.1. Included studies

6.5.1.1. Effect of A Very Low-Calorie Ketogenic Diet

The study of Castro et al. from 2018, entitled: "Effect of A Very Low-Calorie Ketogenic Diet on Food and Alcohol Cravings, Physical and Sexual Activity, Sleep Disturbances, and Quality of Life in Obese Patients" examined the effects of a very low-calorie diet in obese individuals. Inclusion criteria were a BMI $\geq$ 30 kg/m^2 and age between 18 and 65 years, while the main exclusion criteria were thyroid changes, diabetes mellitus, and obesity triggered by other endocrine disorders or medications. Twenty subjects were enrolled in the study, which lasted 4 months and was divided into 5 parts. The first three parts were defined as phase with severe reduced caloric intake, with daily energy

consumption consisted of 600-800 kcal/day. The food intake was characterized by low levels of carbohydrates and fat, whereby the first three parts just differed regarding the consumed protein sources, which were expected to provide 0.8 to 1.2 g of protein per kg of optimal body weight. These three levels were maintained until subjects achieved the target weight loss, before a subject-specific low-calorie diet of 800-1500 kcal per day was initiated in levels 4 and 5. Ketosis was measured primarily with BHB, while quality of life was assessed as the variable representing depression (71).

6.5.1.2. Safety, health improvement and well-being during a fasting period

De Toledo et al. examined in 2019, in the study named "Safety, health improvement and well-being during a 4 to 21-day fasting period in an observational study including 1422 subjects" the effects of an 200-250 kcal fasting period. Totally, 1422 subjects were included, and the fasting period divided into 4 different durations, with 5, 10, 15 and 20 fasting days. During fasting, just 3L water, 250 ml fruit juice and 250 ml of vegetable soup were consumed per day, resulting in total daily energy intake of about 200-250 kcal. Acetoacetate concentration was measured representing ketosis. For BHB estimations, the ratio of 1:1 acetoacetate:BHB was used (72). Also, depression was assessed as emotional wellbeing score, whereby subjects were able to answer on specific questions at a scale from 0 to 10 (73).

6.5.1.3. Ten days of complete fasting

Yang et al., investigated 2021 in the study "Ten days of complete fasting affected subjective sensations but not cognitive abilities in healthy adults" about the effects of 10 days water only fasting in 13, 28 to 55 years old, male volunteers. BMI ranged from 18 to 39 kg/m2 with exclusion criteria being history of an eating disorder or any chronic illness, including diabetes mellitus; history of cancer; history of cardiovascular disease; history of metabolic diseases; and tobacco or alcohol dependence. With baseline, fasting, caloric restriction and recovery, 4 phases were introduced, of which especially the fasting intervention was of interested. Ketone bodies were measured, and no further subdivision was made, so it was assumed that the percent change in ketone bodies was identical to the percent change of BHB. Depression was measured with the Self-Assessment Scale

for Depression (SDS), which consists of 20 items, with the corresponding questions were answered on a 4-point scale ranging from 1 ("not at all or occasionally") to 5 ("most of the time or all the time") (74).

6.5.2. Results

De Toledo et al. showed that fasting induced ketosis (p-value < 0,001), as well as significantly enhanced emotional wellbeing can be reached through fasting (p-value < 0,001). Also, the fasting length seemed to be positively connected to emotional wellbeing (p-value = 0,02), but not to ketosis (p-value = 0,81). A plateau of ketone body production seems to be reached after 5 days. Furthermore, weight loss was assessed, and showed a linear correlation to the fasting days, with more fasting days resulting in bigger weight loss (p-value < 0,001). Also, the physiological parameters studied, like HBA1c, triglycerides (TG), HDL, LDL and blood sugar, seem to improve with longer fasting duration.

Castro et al., found a similar plateau effect of ketone body production, but measured BHB levels as the indicator of ketosis. Interventions were individually adapted to the state of ketosis, with the 4-measurement points baseline, maximal ketosis, reduces ketosis and endpoint, whereby QoL increased from measurement point to measurement point. BHB was assesses and piked at timepoint 2, slowly decreasing with increased energy supply. Also, BMI decrease was found at every timepoint, with the timepoint 2 and 3 showed the most BMI reduction. The strongest QoL improvement was measured between timepoint 1 and 2 (0,73; p-value < 0,001), but no significant change was found between the last two measurement points. Due to the fact, the further BMI decrease was measured between those time points, lower BHB levels could be responsible for reduced effect size of QoL change and the failed statistical significance (0,44; p-value = 0,06).

Yang et al., investigated about the effects of water only fasting, where ketone levels increased from baseline: M ± SD = 0.18 ± 0.04 mmol/L to M ±SD =5.62±0.92 mmol/L at day 9 with a plateau was reached after 4-5 days. Those ketone levels decreased rapidly after the fasting duration and reached baseline values and the end of the CR intervention. Assessed psychological parameter decreased at the first days, but interestingly, the trend of decreasing psychological parameters ended at day 3 for depression dejection and day

6 for self-rating anxiety, suggesting that high ketone body concentration may alleviate fasting induced psychological impairments.

6.6. Discussion

An increase of SCFAs, with a significant expression for butyrate through probiotic and prebiotic administration is shown in this meta-analysis. This is supported by other literature examining pro- and prebiotic administrations. Multiple recent studies were able to show increased SCFA and butyrate production after especially probiotic administrations (75–78). The two included trails examining the effects of prebiotic administration used extruded wheat bran and rye-based bread as interventions, which consist mainly of undigestible starches, suitable as reactant for butyrate emerging, as shown through the good effects on butyrate and SCFA production in the included studies, while lactobacillus gasseri was supplemented in the probiotic trail.

Interestingly, the butyrate concentration seems to be strongly subjected to inter-individual variations, also effecting the butyrate concentration raising properties of prebiotic and probiotic administrations (79). Holmes et al. were able to demonstrate differences in microbiota composition of high butyrate producers, compared to low butyrate producers. While especially Firmicutes Lachnospiraceae were thereby associated with butyrate production, Bacteroidetes seem to lack in butyrate emerging properties (80,81). This implies, that butyrate production does not only depend on the composition of the microbiota, but also that the total amount of SCFAs, butyrate, propionate and acetate vary greatly between individuals due to the microbial composition dependency. This also seems to influence the effectiveness of the prebiotic and probiotic interventions. Prebiotics do not significantly alter the gut microbiota but supply substrate for the SCFAs emerging. A good interventional effect requires therefore relevant SCFA producing bacterial strains. In depressed subjects, with findings already showed reduced microbial diversity, a higher prevalence of dysbiotic conditions may lead to reduced capability of transforming those given substrates to SCFAs, while in healthy participants, with a Firmicutes Lachnospiraceae rich environment, the SCFA emerging can be significantly accelerated through prebiotic administration. Exactly the opposite is true for probiotic interventions, where alterations of the gut microbiota can be reached. The expected higher concentrations of SCFAs after probiotic interventions can thereby be attributed to the

higher density of SCFA producing bacterial strains. Because a healthy microbial composition is in balance, just fewer effects of probiotics can be expected in healthy adults, also when focusing on SCFA concentration, due to the already balanced and health-promoting ratio of SCFA producers and non-SCFA producers, while under dysbiotic conditions, significant alterations of the gut microbiota can be reached, with improvements of the bacterial composition and in this course also increased concentrations of SCFA producers.

Due to their different mechanisms of action, probiotics seem to exceed more promising results in dysbiotic conditions, which often occur in depressed or sick subjects, while prebiotics might be more promising in healthy individuals. This was also shown in the summarized trails, were Huang reported significantly better results of probiotic interventions in MDD patients, than in healthy individuals (47). All included trails in this meta-analysis were conducted with healthy subjects, with SMD of SCFA increase was 0.63 [0.06; 1.20] prebiotic trails compared to 0.35 [-1.24; 1.94] in the probiotic trail, underlining the hypothesis of stronger SCFA improvements in through prebiotic supplementation in healthy subjects, also emphasized through the reached statistical significance. But not only the population seem to influence the study results significantly, also the study design might play a crucial role, with cross-over design seem to be more plausible, because of the lower weighting of the inter individual fluctuations (79). Interestingly, the included trail of Sandberg et al. was a cross-over RCT and was able to show the biggest SMD regarding SCFAs and butyrate changes as well as statistical significance regarding the increase of butyrate concentration. Due to the fact, that in prebiotic interventions no long-lasting effects are expected, this might be the best study design, but seems to be unsuitable for probiotic interventions, due to the persistent modulating of gut microbiota reasoned high risk of carry-over effect.

Beside study design and population, also kind of prebiotic as well as bacterial strain are relevant factors influencing the interventional effects. Although especially complexes of probiotics are associated with SCFAs increase, enhancement of SCFA concentration was found during Lactobacillus gasseri administration in this meta-analysis, but significance was not reached (81,82).

In conclusion, further studies need to be conducted to better understand the relationships between these influencing parameters and thereby interpret the study results more confidently to finally develop appropriate, efficient microbiota modulating treatments.

Due to the complexity of the cross-linking between gut health, microbial composition, inflammation, brain health, and mental health, not one mechanism, but many different pathways are responsible for the interactions, and mutually influence health and well-being. SCFAs and especially butyrate emerging of the gut microbiota might be one significant mechanism of action, particularly plausible due to the occurrence in the brain and the resulting possibility of influencing cognitive processes.
The key role of butyrate was highlighted in this meta-analysis through the good association with depression, calculated in the regression model. Interestingly, propionate showed a negative correlation with depression, with acetate being positively associated with depression as well, but in a weaker manner. This might be the case, through the limited substrate occurrence, needed for SCFAs emerging, which is reduced by bacterial strains producing propionate and acetate, resulting in a decreases capability of butyrate emerging, which mainly mediates the depression ameliorating effects. This suggests that synbiotic administration is more effective due to bacterial and substrate supplementation and avoidance of limited substrate concentrations strongly influencing the effects of the intervention. Interestingly, Hofmeister et al. was able to underline this thesis in a meta-analysis where also synbiotic administration was included, showing the best effect size regarding depression amelioration.

In the qualitative analysis, a significant improvement of depression through fasting was found in two of the three studies. This observation applies to 1442 of the total 1453 included subjects, while just water ad libidum fasting resulted in a decreased depression score. This suggests that severity of the fast strongly correlates with depression parameters, and 200kcal/day – 800kcal/day fasting correlates positively with depression score enhancement. When analyzing depression amelioration, it has to be taken into account, that even if BHB levels were elevated through the intervention, the effects on depression might not be connected to BHB, since in an overweight or obese population, weight loss modulated by fasting could also be responsible for a higher sense of well-

being. Also in a normal weight cohort, better well-being could be modulated through improvement of physiological parameters (HBA1c, blood glucose, HDL). So, many opportunities exist for bias entry, and have to be take into account, when analyzing effects of fasting on depression in the future.

Ketone body, as well as BHB concentration could be increased significantly through fasting, while no fitting trails were found, examining caloric restriction as intervention. Usually, with CR an energy reduction of about 10 to 25 % is meant, which also led to significant increases of BHB, shown in animal and human trails. Fasting and CR are both methods of energy restriction, associated with weight reducing, lifespan prolonging and cancer fighting properties (83). However, those methods as well as their potential on depression must be distinguished. Since all included trails found a plateau of BHB concentration reached after 5 days of fasting, as well as a fast reduction of BHB levels after the end of the fasting period, no persistent BHB increase seem to be possible through fasting interventions. Because CR is less severe and therefore can be implemented for months and years, continuous high BHB levels are just possible with CR, suggesting CRs ability to generate longer lasting effects. The effect of fasting and CR is strongly limited by the adherence of participants (84). Hunger and cravings could be responsible for the high drop-out rates but tend to decrease after the first days of fasting and caloric restriction. Because of that, CR maybe more practical for long-term administration, since periodic fasting always leads to severe hunger and cravings on the first days, while with CR the demand for food stays tolerable after passing the first days once.

So summarized, fasting and caloric restriction seem to have depression ameliorating properties. The already analyzed systematic trails on fasting interventions on depression underlined this hypothesis but highlighted the needed severity of energy restriction for the interventional success. This might point out the connection between depression enhancement and BHB, due to the fact, that ketone body production is negatively associated with caloric intake.

To provide more evidence, further research needs to be conducted to understand the pathophysiology of depression and the role of butyrate and BHB better. Recent studies suggest that MDD is a neurogenerative disorder due to insufficient levels of neurotrophic factors, particularly BDNF, in certain brain regions. Studies in mice have shown that BHB can activate CREB and nuclear factor κ-B, a DNA transcription, cytokine production, and

cell survival controlling protein complex. Furthermore, BDNF expression in neurons was enhanced through BHB, suggesting HDAC inhibition of gens associated to BDNF as one of the key mechanisms of action, not only balancing reduced BDNF levels of MDD patients, but also improve the immune system and reduce inflammation as further depression improving mechanisms (85). The neuroinflammation reducing properties of BHB may be furthermore mediated through protein kinase B (Akt), which's inhibition abolished BHBs positive influence on microglial polarization, phagocytosis, and inflammation (86). Furthermore, butyrate was shown to increase acetylation on BDNF genes in the prefrontal cortex, through HDAC inhibitory properties, as well as enhance glutathione (GSH) production through increased expression of enzymes, which might be a relevant mechanism of action, through the big antioxidative capacity of GSH and the potential of reducing neurodegenerative risk factors like hydrogen peroxide and lipid peroxide (87,88).

BDNF appears to be involved in each case, but whether there is a difference in the modulation of BDNF levels by butyrate or BHB needs to be investigated, to successfully develop new drugs and to best perform pro- and prebiotic or energy restriction and fasting therapies for disease-specificity.

7. Conclusion

Beside depression, the prevalence of other diseases is increasing as well. Overweight can officially be considered as normal for Europeans, since the last WHO report showed that more than 50% of the Europeans are at least overweight. This development gets even more dramatical, when considering that nearly 30% are considered obese, comparable to 10% in 1980 (89). As evidentially known, with adiposities and depression, the risk of a variety of diseases rapidly increases leading to higher rates of multimorbidity. When taken the demographic developments with into account, which by itself is a huge burden of the health care systems of many western countries, prognosis of future health care gets even more dramatical. Beside needed preventive strategies, also the therapies need to get improved, the prices lowered, and effectiveness increased. A healthy lifestyle with plenty of exercise and a varied and balanced diet represents the framework of a healthier social development, which would lead do decreased prevalence of adiposities and depression as well as its concomitant symptoms. Since the current development rather points in the

opposite direction, the questions need to be asked, how realistic those changes in the sooner future are.

This raises the attention to interventions, which can be easily applied and may exceed some of those effects, reachable through a healthy lifestyle. BHB and butyrate and two key molecules, naturally involved in homeostasis preventing and health promoting pathways. Due to their potential beneficial effects on health and wellbeing with low levels of adverse effects, due to the fact, that they are also synthesized in the body, and occur naturally, strategies of raising BHB and butyrate levels are examined as well as synthesized butyrate and BHB supplementation studied. But before BHB and butyrate can be administered properly, further understanding needs to be gained regarding their modulating pathways, as well as their effectiveness on different diseases.

This systematic review and meta-analysis is the first, summarizing the butyrate and BHB dependent effects of pro-and prebiotic administration as well as fasting and CR regimes on depression and was able to underline to key roles of BHB and butyrate in mediating depression ameliorating effects.

Due to the fact, that promising effects of butyrate and BHB supplementation can also be expected on a variety of other diseases, the need of understanding the pathways of action better and examine effective supplementation strategies must be underlined, as well as the huge potential of therapies, involving those molecules emphasized.

8. Publication

sensors

MDPI

Review

The butyrate and beta-hydroxybutyrate mediated effects of interventions with pro- and prebiotic, fasting and caloric restriction on depression

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Abstract: To examine the through butyrate and beta-hydroxybutyrate modulated effects of pre-and probiotic, fasting and caloric restriction interventions on depression, a systematic search was carried out in order to conduct a systematic review and meta-analysis. Three pre-and probiotic intervention RCTs were included into the meta-analysis. A significant increase of butyrate (SMD 0,34; [0,02 – 0,67]) and an improvement of depression scores (SMD 0,15, [-0,35 - 0,70]) through the pre-and probiotic interventions could be shown. Beside butyrate, also total SCFA concentration seems to be positively associated with pre- and probiotic administration (SMD 0,55 [0,15 - 0,95]. Despite of the significant SCFA and butyrate concentration changes, no significant correlation between either butyrate and depression nor SCFA and depression could be shown through linear regression models. Nevertheless, the calculated outcomes (b1 = 1,57; p = 0,17) for butyrate, suggests a strong, positive connection between butyrate and depression. Additionally, three studies were qualitatively analyzed examining fasting as intervention. A connection between fasting, beta-hydroxybutyrate and depression was found. Also, depression or mood enhancement seemed to be associated to BHB concentration, which may be explainable through similar biochemical properties of BHB and butyrate. Furthermore, caloric restriction as potential long-term intervention was mentioned as alternative to fasting, as well as further needed studies stated.

Keywords: butyrate; beta-hydroxybutyrate; probiotics; prebiotics; fasting; caloric restriction; depression; major depression disorder

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1. Introduction

The scientific interest in the pathology of depression has increased during the last decades and is further increasing because of its rising prevalence (6,7). Currently it is estimated, that 3,8% of the global population experience depression during der lifespan with approximately 280 million cases of depression worldwide (90). The economic burden of depression to the U.S. alone increased with a spike of about 48% in the last decade through which the yearly economic damage recently exceeded 325 billion dollar per year (4). The development of depression underlies multiple factors, with family history and genetic predisposition as well as abuse and stressful events as highly influencing variables (5,90). Beside the disturbing epidemiological development, also pharmacological problems arise within the drug therapy of depression. The efficiency of antidepressants underlies a variability of influences with highly individual variations regarding the response to different medications. Besides that, and in defiance of antidepressant therapy improvements, treatment-resistant types of depression exist as well as problems with delayed onset of antidepressants in treatable depression variants. As a result, the needs of many patients are not being met, especially when considering the high relapse rates that already complicate treatment for patients with depression (91,92). In this context of

therapeutical problems with foltering scientific progress because of the unknown biochemical formation processes of depression, the concept of the gut-microbiota-brain-axis gained scientific interest, with increasing evidence supporting the thesis, that the gut microbiota plays a vital role in regulating brain function and human behavior, and therefore might be involved in the pathophysiology of depression (33,92).

Observational studies allowed the conclusion, that Major Depressive Disorder (MDD) leads to an increased occurrence of proinflammatory microbial strains, as well as a decreased relative abundance of short-chain-fatty-acid (SCFA) producing bacteria (33). SCFAs emerge by fermentation of dietary fiber through bacteria and can be further subdivided into formate, acetate, propionate, butyrate, valerate, isovalerate and hexanoate. The short-chain-fatty acids can be transported or diffused before they can bind on epithelial and immune cells though G protein-coupled receptors. Especially the butyrate production is of increased interest for depression research because butyrate has already shown similar histone deacetylase (HADC) inhibition properties on hippocampal neurons, as antidepressants (35–37). Butyrate seems to have HADC inhibiting properties in general, which leads to increased transcriptional procedure because of the weakened DNA-histone binding complex. Moreover, butyrate can cross the blood-brain-barrier (BBB), assisted by butyrate-transporting transmembrane proteins and therefore may be able to influence neurological processes through its modulating properties (37,38).

Beta-hydroxybutyrate (BHB) has a strong chemical similarity to butyrate but is instead synthesized in the liver as the major ketone body, supplying energy under low glycemic conditions (53). BHB can be transported to the brain via the monocarboxylate transporter MCT1, where BHB is an energy substrate for the neurons, and beside that displays a variety of brain function influencing properties. Furthermore, raised BHB levels through ketogenic diet led to increased social behavior in mice, modulated through expression upregulation of certain, with myelin associated genes. Besides that, also TNFα and Cxcl15 downregulation in the hippocampus was found, which all together suggests BHB's potential on influencing neurobiological processes (93). Because BHB was linked to similar HDAC inhibitory properties than butyrate in in-vitro models, similarities regarding the biochemical influencing potential of BHB and butyrate raised up, which should be further examined through this systematic review and meta-analysis (53).

Since ketone bodies are alternative energy substrates, ketone body production is accelerated especially during periods of reduced caloric intake (51). This systematic review and meta-analysis sought to examine the properties of butyrate and BHB on depression, so fasting and caloric restriction, as well as administration of pro- and prebiotics were the interventions studied.

Fasting is defined as a strongly restricted dietary energy intake, with a duration of at least 48h for reaching the optimal medical fasting state, while caloric restriction (CR) being the reduction of the caloric intake without malnutrition and can be applied in prolonged periods (56,94).

Probiotic and prebiotic administration is connected to SCFA level changes and increased butyrate production. Probiotics are defined as living microorganisms, which contribute to the host gut microbial flora and improve health, while prebiotics are chemical compounds, positively influencing the host's health through their effects on the microbiome (95).

2. Materials and Methods

The systematic search was conducted using PubMed and Scopus, as well as Google Scholar for potential grey literature. The advanced search function was used in every database with the terms being: ((((prebiotic* OR postbiotic* OR probiotic* OR synbiotic*) OR (fasting)) OR ("calori* restriction" OR "energy restriction" OR "dietary restriction")) AND (butyrate OR "beta hydroxybutyrate" OR bhb OR "β hydroxybutyrate" OR "ketone

bodies")) AND (depress* OR "affective disorder" OR" "bipolar disorder") in PupMed and (prebiotic* OR postbiotic* OR probiotic* OR symbiotic*) OR (fasting) OR ("calori* restriction" OR "energy restriction" OR "dietary restriction") AND (butyrate OR "beta hydroxybutyrate" OR bhb OR " β hydroxybutyrate" OR "ketone bodies AND (depress* OR "affective disorder" OR "bipolar disorder") for Scopus. Additionally, the Publish or Perish software, was used to filter out the 500 most fitting search results on Google Scholar, published between 2000 and 2024, for each of the three search terms (1): (prebiotic*|postbiotic*|probiotic*|synbiotic*) (butyrate|"beta hydroxybutyrate"|bhb|"β hydroxybutyrate"|"ketone bodies") (depress*|"affective disorder"|"bipolar disorder"), (2): (fasting) (butyrate|"beta hydroxybutyrate"|bhb|"β hydroxybutyrate"|"ketone bodies") (depress*|"affective disorder"|"bipolar disorder"), (3): ("calori* restriction"|"energy restriction"|"dietary restriction") (butyrate|"beta hydroxybutyrate"|bhb|"β hydroxybutyrate"|"ketone bodies") (depress*|"affective disorder"|"bipolar disorder").

3. Results

122 results were found in PubMed, as well as 5017 results in Scopus and 1500 results in Google Scholar. Altogether, 6628 studies were collected during the searching process, of which 1648 existed several times and were deleted using the program Rayyan. 4980 single studies were left, and one additional study was found during secondary literature analysis, leaving 4981 studies ready for the title and abstract screening process. Thereby, inclusion criteria were primary literature, human trails, no population with diagnosed mental illnesses and no administration of potentially influential controls. 4865 trails could be excluded, because they were not corresponding to inclusion criteria, leaving 116 studies qualified for full text screening. Finally, six studies could be included in the systematic review of which 3 of them were RCTs, examining pro- and prebiotic administration and were used for conducting a meta-analysis (65–67) (Table 1) while 3 observational trails investigating about the properties of fasting and caloric restriction were reviewed qualitatively (71,73,74) (Table 2). For the meta-analysis, the standardized mean difference (SMD) was the effect measure of choice, with change values, meaning the differences between post- and preinterventional measurements, were used.

Several forest plots are presented, evaluating, and illustrating the overall effect of the interventions on butyrate (Figure 1), SCFAs (Figure 2) and depression scores (Figure 3). Furthermore, effect sizes as well as 95% CI are given. Beside the overall effect, also the heterogeneity is presented in the forest plots.

With the heterogeneity measure I^2 being 0% all the variances of the effect size estimates are explainable through SE, which means that all effects can be attributed to the interventions. Also, p-values of > 0,05 showing that H_0 is not rejected, and no between study heterogeneity is responsible for SE of the effect size estimates (69). The Intervention led to significant butyrate and SCFA concentration changes with a SMD [CI] of 0,34 [0,02 - 0,67] for butyrate and 0,55 [0,15 - 0,95] for SCFAs (figure 1; figure 2). The overall SMD of the depression score changes was not significant 0,18 [-0,35 - 0,7], but favored the intervention (figure 3). To assess, a possible connection between butyrate respectively SCFAs and depression score changes, a regression model was calculated. No significant relationship could be shown for butyrate (p-value = 0,17) and SCFAs (p-value = 0,44), but b1 was positive in both models (b1 = 0,75; SCFA) and (b1 = 1,57; butyrate). This not only suggest that butyrate is able to ameliorate depression but also, that increasing propionate, acetate, valerate and hexanoate levels might limit butyrate's depression ameliorating properties.

Of the qualitatively analyzed trails, De Toledo et al. showed that fasting induced ketosis (p-value < 0,001), as well as significantly enhanced emotional wellbeing can be reached through fasting periods (p-value < 0,001). Because acetoacetic levels represented ketosis, the ratio of 1:1 acetoacetate : BHB was used for the BHB estimation (51). Also, the fasting length seemed to be positively connected to emotional wellbeing (p-value = 0,02),

but not to ketosis (p-value = 0,81). A plateau of ketone body production seems to be reached after 5 days. Castro et al., found a similar plateau effect of ketone body production, but measured BHB levels as the indicator of ketosis. Also, quality of life (QoL) was assessed as the variable representing depression. Intervention was individually adapted to the state of ketosis, and the 4 measurement points were baseline, maximal ketosis, reduces ketosis and endpoint. Interestingly, the QoL increased from measurement point to measurement point, but no significant change was found between the last two measurement points. Due to the fact, the further BMI decrease was measured between those time points, lower BHB levels could be responsible for reduced effect size of QoL change and the failed statistical significance, suggesting that beside weight loss, also BHB modulates QoL improvements.

The study of Yang et al. examined the effects of ten-days water ad libidum fasting on depression score changes and ketone body concentration. Psychological parameters all behaved similar and were dropping during the first days of fasting to finally reach baseline values after the interventional duration. After day 5, a plateau seemed to be reached regarding the ketone body concentration, with stable concentration measurements until the end of the fasting intervention. Interestingly, the trend of decreasing psychological ended at day 3 for depression dejection and day 6 for self-rating anxiety, suggesting that high ketone body concentration may alleviate fasting induced psychological impairments

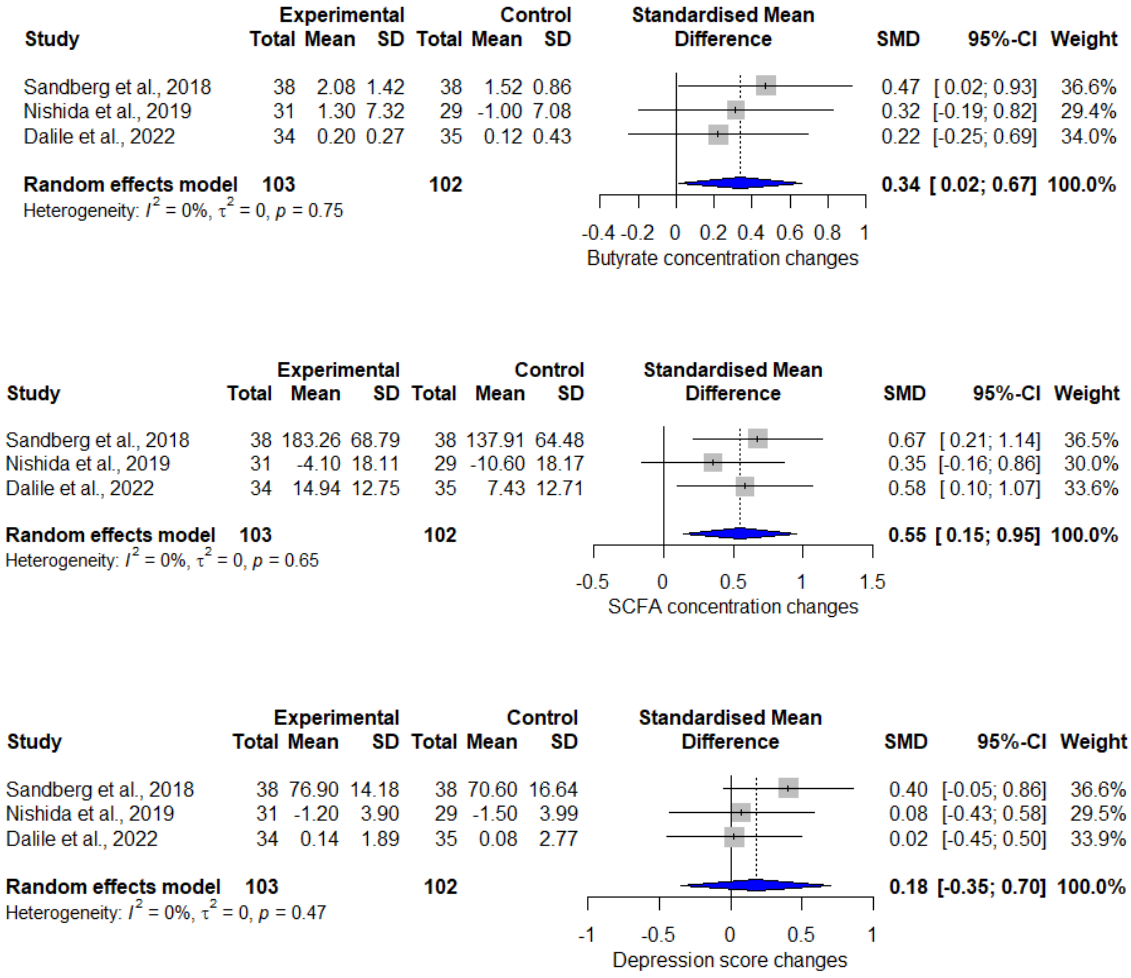

Figure 1. Forest Plots, illustrating the SMDs of butyrate, total SCFAs and depression score changes

Study	Country	Population	Age (SD)	Sex (% female)	Study type	Intervention	Control	Duration	Outcomes
Sandberg et al.	Sweden	38 healthy, subjects (52-70y)	63,6 (5,3)	78,9	RCT	Rye-based bread; 75g carbohydrates daily; 5,7g soluble fiber	White wheat bread; 75g carbohydrates daily; 1,2g soluble fiber	3-days	**Physiological:** Plasma SCFAs, **Psychological:** Swedish core affect scale,
Nishida et al.	Japan	60 healthy, young adults	25,1 (0,6)	31,6	RCT	Lactobacillus gasseri 1x1010 cfu	placebo	24-weeks	**Physiological:** Fecal SCFAs **Psychological:** HADS
Dalile et al.	Belgium	69 healthy, men (20-40y)	26 (4,1)	0	RCT	Extrusion cooked wheat bran; 25g fiber per day	WCC cereal as a placebo; 25g hardly fermentable fiber	4-weeks	**Physiological:** Fecal SCFAs, **Psychological:** DASS-21

Table 1. In the meta-analysis included RCTs, examining pro- and prebiotic interventions on depression

Study	**Country**	**Population**	**Age (SD)/ BMI (SD)**	**Sex (% female)**	**Study type**	**Intervention**	**Duration (SD)**	**Outcomes**	**Findings**
Toledo et al.	Germany	1422 subjects (18-99y)	55,4 (0,4) / 28,2 (0,2)	59,1	Observa-tional study	200-250 kcal per day with 25-35g carbohydrates	Mean 8,2 (0,1)	**Physiological:** Acetoacetic, **Psychological:** emotional wellbeing (EWB)	↑ Acetoacetic* ↑ EWB* EWB effects increased with fasting length*
Castro et al.	Spain	20 subjects (18-65y; BMI ≥ 30 kg/m^2	47,2 (10,2) / 35,5 (4,4)	60,0	Observa-tional study	VLKD (from weight loss program); 600-800 kcal/day until targeted weight loss is reached	Depends on weight-loss progress and targeted weight	**Physiological:** BMI, BHB **Psychological:** QoL	↑ BHB * ↑ QoL*
Yang et al.	China	13 (28-55y)	39,6 (8,1)/ n.a.	0	Observa-tional study	Water only fasting	10 days	**Physiological:** Ketone bodies **Psychological:** POMS, SDS	↑ Ketone bodies* ↓ SDS-score*

Table 2. The in the qualitative review included observational trails, investigating about the effects of fasting interventions on depression; significant improvements were highlighted with *

4. Discussion

A significant increase of SCFAs and especially butyrate through probiotic and prebiotic administration is shown in this meta-analysis. This is supported by other literature examining pro- and prebiotic administrations. Multiple recent studies were able to show, that prebiotics increase SCFAs and butyrate production (75–78). Thereby, butyrate is formed through cleavage of carbohydrates over the intermediate acetyl-CoA and butyryl-CoA (75). The two in the meta-analysis included prebiotic trails used extruded wheat bran and rye-based bread as prebiotic interventions, which consist mainly of undigestible starch, suitable as reactant for butyrate emerging, as shown through the good effects on butyrate and SCFA production in the included studies, while the probiotic intervention trail supplemented lactobacillus gasseri, which was already able to increases SCFA concentration in conducted trails, with Lactobacillus plantarum being the probiotic associated with the best butyrate concentration-increasing properties so far (81,82).

Interestingly, the butyrate concentration seems to be strongly subject to inter-individual variations, also effecting the butyrate concentration raising properties of prebiotic and probiotic administration (79). Holmes et al. were able to demonstrate differences in microbiota composition of high butyrate producers, compared to low butyrate producers, evidently emphasizing the bacterial composition dependency of SCFAs and butyrate emerging, with especially Firmicutes Lachnospiraceae are associated with butyrate production, while Bacteroidetes seem to lack in butyrate emerging properties (81). Those findings furthermore suggest that the effectiveness of pre- and probiotic interventions underlies bacterial composition dependency (80). Since the success of pre- and probiotic intervention depends on the microbial composition, the question arises which conditions favor pre-, and which favor probiotic administration. It is plausible that the effect of probiotics is better under dysbiotic conditions, due to the long-lasting effects of modulating gut bacteria composition, whereas the success of prebiotic administration seems to be more promising when the gut microbiome is well composed. This is due to differences in the mechanisms of increasing butyrate concentration, with probiotics increasing the concentration of butyrate-producing bacteria, while prebiotics provide substrates for the formation of SCFAs and therefore already require strains of butyrate producing.

Due to the fact, that restrictive energy reduction leads to increased ketone body and BHB production, fasting and caloric restriction are the perfect interventions to examine BHBs potential on alleviating depression symptoms and improving mood. Ketone body, as well as BHB concentration could be increased significantly through fasting, while no fitting trails were found, examining caloric restriction as intervention. Usually, with CR an energy reduction of about 10 to 25 % is meant, which also led to significant increases of BHB, shown in animal and human trails. Fasting and CR are both methods of energy restriction, associated with weight reducing, lifespan prolonging and cancer fighting properties (83). However, those methods as well as their potential on depression must be distinguished. Since Toledo et. al. found a plateau of BHB concentration reached after 5 days of fasting, as well as a fast

reduction of BHB levels after the end of the fasting period, no persistent BHB increase seem to be possible through fasting interventions. Because CR is less severe and therefore can be implemented for months and years, continuous high BHB levels are just possible with CR, suggesting CRs ability to generate longer lasting effects. The effects of fasting and CR are strongly limited by adherence of the participants (84). Hunger and cravings could be responsible for the high drop-out rates but tend to decrease after the first days of fasting and caloric restriction. Because of that, CR maybe more practical for long-term administration, since periodic fasting always leads to severe hunger and cravings on the first days, while with CR the demand for food stays tolerable after passing the first days once.

Although, no significant correlation between the depression SMD and butyrate SMD was found in the linear regression model, a clear tendency suggests big potential of butyrate properties to contribute to brain-health and depression amelioration. Interestingly, the correlation between butyrate SMD and depression SMD is stronger than between SCFA SMD and depression SMD, supporting the hypothesis that butyrate properties might be limited through the other SCFAs as well as suggests butyrate to be one of the key mechanisms of the gut-microbiota-brain axis's connection to depression. Interestingly, propionate was negatively associated with depression, although no statistical significance was reached [b1 = -0,61 (p-value = 0,57)], supporting the thesis, that the through butyrate mediated effects are limited by the other SCFAs, especially propionate. This might be the case through limited substrate concentration, whereby increased propionate emerging, also results in reduced butyrate fermentation.

In the qualitative analysis, a significant improvement of depression through fasting was found in two of the three studies. This observation applies to 1442 of the total 1453 included subjects, while just water ad libidum fasting resulted in a decreased depression score. This suggests that the severity of the fast strongly correlates to depression parameters, and 200kcal – 800kcal fasting correlates positively with depression score enhancement. When analyzing depression amelioration, it has to be taken into account, that even if BHB levels were elevated through the intervention, the effects on depression might not be connected to BHB, since in an overweight or obese population, weight loss modulated by fasting could also be responsible for a higher sense of well-being. This must be evaluated in further studies, investigating about the effects of fasting and caloric restriction interventions on depression in healthy, normal weight adults.

As mentioned above, the meta-analysis and the qualitative analysis suggest that butyrate and BHB may be able to improve depression. To provide more evidence, further research needs to be conducted to understand the pathophysiology of depression and the role of butyrate and BHB better. Recent studies suggest that MDD is a neurogenerative disorder due to insufficient levels of neurotrophic factors, particularly BDNF, in certain brain regions. Studies in mice have shown that BHB can activate CREB and nuclear factor κ-B, a DNA transcription, cytokine production, and cell survival controlling protein complex. Furthermore, BDNF expression in neurons was enhanced through BHB, suggesting HDAC inhibition of gens associated to BDNF to be one key mechanism of action, not only balance reduced BDNF levels of MDD patients, but also improve the immune system and reduce

inflammation as further depression improving mechanisms (85). The neuroinflammation reducing properties of BHB may be furthermore mediated through protein kinase B (Akt), which's inhibition abolished BHBs positive influence on microglial polarization, phagocytosis, and inflammation (86). In contrast to BHB, which's transcription regulation properties are rather discussed recently, butyrate was one of the first endogenous substances known to inhibit HADC activity. This led to the connection between butyrate and depression, because especially BDNF genes in the prefrontal cortex led to butyrate-induced increased acetylation (88). Furthermore, butyrate seems to enhance glutathione (GSH) production through increased expression of enzymes, which may be another relevant mechanism of action, through the big antioxidative capacity of GSH and the potential of reducing neurodegenerative risk factors as hydrogen peroxides and lipid peroxides (87).

BDNF appears to be involved in each case, but whether there is a difference in the modulation of BDNF levels by butyrate or BHB needs to be investigated, to successfully develop new drugs and to best perform pro- and prebiotic administration or energy restriction therapies for disease-specificity.

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