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Promoting Gut Microbiome Diversity in Anorexic Patients: A Neurobiological Review

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Abstract

Anorexia nervosa (AN), one of the most prevalent eating disorders in the US, is characterized by abnormally low body weight due to an obsessive fear of gaining weight and a distorted perception of one's own body image and weight. This review aims to provide critical insights into the impact of improving patients' gut microbiome diversity, and the use of probiotics, vitamins, and other supplements as potential therapeutic targets for AN. Given the intricate relationship between gut health and psychological well-being, researchers have proposed enhancing gut microbiome diversity through dietary supplements as a potential adjunctive treatment. The proposed thesis asserts that dietary supplements such as vitamins, minerals, and probiotics can be a potential neurobiological treatment method for AN, based on their known ability to modulate gut-microbiome diversity and address alterations in the gut-brain axis that are present in AN. Current treatments, such as cognitive behavioral therapy and family-based therapy, focus primarily on psychological and behavioral aspects. However, they may benefit from a more integrative approach that includes neurobiological treatments targeting the gut-brain axis. This review explores the pathophysiology of AN, the gut-brain axis, and the potential for dietary supplements to modulate gut microbiome composition and improve therapeutic outcomes. Additionally, it discusses the implications of such treatments in combination with existing therapies and explains why further research is needed to establish personalized treatment plans for AN patients based on their unique microbiome profiles.

1. Introduction

Anorexia nervosa (AN) is a psychiatric disorder characterized by restrictive eating behaviors, an intense fear of weight gain, and a distorted body image, leading to significantly low body weight. Patients with AN maintain an abnormally low body weight through restrictive eating behaviors, excessive exercise, and/or other compensatory mechanisms.[1] It is a common and deadly disorder across the world, having the highest mortality of any mental illness.[2] AN patients typically show symptoms of fatigue, low blood pressure, dietary restriction, purging, malnutrition, and excessive exercise. These symptoms can lead to complications like heart failure, kidney problems, and may even alter brain structure. Comorbid psychiatric conditions, including depression and anxiety, are frequently associated with AN and can exacerbate the disorder's severity.[3] Given adolescents' frequent use of social media, their perception of their bodies is constantly being shaped by others on the internet. This pressure causes more people to develop AN, hence pushing research into more treatments for AN. Currently, cognitive behavioral therapy (CBT) is the primary psychotherapeutic approach for AN, focusing on modifying maladaptive thoughts and behaviors related to eating and body image. This helps reduce anxiety surrounding food and promote healthier thought patterns. However, this only helps with the cultural and psychological causes of AN, and is still limited in its effectiveness. Another treatment is the family-based treatment (FBT), where the family of the patient takes over their diet.[3] FBT has shown promise and patients have shown comparable remission rates to those receiving adolescent-focused therapy.[4] While there is no FDA-approved medication, some off-label pharmacotherapies (e.g., olanzapine) are used.[5] This review will look into the relationship between the digestive system and AN to propose a new treatment to combine the mental and physical aspects of AN therapy.

The enteric nervous system (ENS) is a gastrointestinal tract neural network that controls digestive functions, while the vagus nerve serves as a link between the ENS and brain to convey information and

mediate reflexes based on gut conditions, and the microbes present in the gut.[6] Containing the ENS and the vagus nerve, the gut-brain axis (GBA) is a complex network of nerves spanning from the gut to the brain, responsible for monitoring and designing the elaborate interactions between central and autonomic mechanisms regulating the emotional and cognitive parts of the brain along with peripheral intestinal functions such as entero-endocrine signaling, immune activation, intestinal permeability, and enteric reflex.[7] Due to its intricate connection between ENS and the brain, the GBA can play a role in influencing a person's craving for food, feeling of hunger along with food intolerances, digestion, metabolism, and other cognitive functions.[8] Based on previous studies, there is growing evidence that gut microbiota can help in the regulation of the GBA and physiologic homeostasis through the means of vagal transmission, gut hormones, the immune system, and the hypothalamic-pituitary-adrenal axis.8 This allows the microbiome-gut-brain axis to effectively communicate between the central nervous system and the gut.

The gut microbiome is an ecosystem made up of the trillions of microbes that reside in the intestine and the environment surrounding it. These microbes can be categorized into bacteria, viruses, fungi and parasites. Diet and external exposures largely dictate the makeup of the gut microbiome. Maintaining its homeostasis is important as the gut microbiome interacts with different parts of our body such as the digestive, immune, nervous and endocrine system. Bile acids, primarily synthesized in the liver, undergo microbial metabolism in the gut, influencing lipid digestion and signaling pathways.[9] Short chain fatty acids (SCFA) produced by microbes in their metabolism regulates immune response by initiating anti-inflammatory cytokine production. These SCFA are also responsible for releasing neurotransmitters such as serotonin which can affect memory and the learning process. Finally, microbes interact with enteroendocrine cells to release gut hormones and control appetite, nutrition absorption, and digestion.[10] Given how gut microbiomes maintain proper metabolic and neurological functions, the symbiosis of host and microbes is crucial. Such a relationship is regulated through a feedback

system and the improper balance or dysbiosis of the gut microbiome is linked with anxiety, depression, autism-spectrum disorder and eating disorders. The proper balance of a gut microbiome is measured by its diversity, abundance and integrity. Metrics include alpha-diversity, which reflects the abundance of each taxon, and beta-diversity, which reflects differences in species composition. In gut dysbiosis, an increase in originally commensal species can act as pathogenic and the intestinal barrier integrity can be corrupted.[10]

Although traditional treatments such as cognitive behavioral therapy (CBT) and family-based therapy (FBT) offer some relief, they often fall short in addressing the underlying neurobiological disruptions associated with the disorder. Recent studies suggest that alterations in gut microbiome diversity and the gut-brain axis play a crucial role in the mechanisms of AN. This review paper aims to explore the potential of dietary supplements, including vitamins, minerals, and probiotics, as therapeutic agents for AN by restoring gut microbiome diversity and perturbations in the gut-brain axis. By integrating gut microbiome-targeted interventions with traditional psychotherapeutic approaches, a more holistic treatment model for AN may emerge.

This paper will investigate the gut-brain interactions associated with AN, compiling and analyzing scientific literature to identify effective treatment strategies. By examining research on signaling pathways and neurobiological aspects linked to AN, the review aims to highlight how substances affecting these pathways can serve as potential therapeutic targets. The primary objective is to provide insights into the impact of improving gut microbiome diversity and utilizing supplements like probiotics, vitamins, and other additives proposing a comprehensive treatment approach that integrates these with conventional therapies. Given their ability to restore gut-microbiome diversity and modify the gut-brain axis, dietary supplements can be a viable treatment method for AN if combined with traditional approaches, ultimately offering a more holistic approach to managing the disorder.

2. Pathophysiology of Anorexia

2.1 Serotonin Pathway

Serotonin, a monoamine neurotransmitter, is most prominently known for its role in the pathophysiology of many neuropsychiatric disorders. Serotonin receptors, also known as 5-Hydroxytryptamine (5-HT) receptors, are activated by the serotonin neurotransmitter and these receptors are found in central and peripheral nervous systems.[10] These neurotransmitters participate in regulating essential functions in the body such as motor control, cognition, motivation, and the reward system pathway. For example, in the CNS, serotonin plays a role in the regulation of mood and interpersonal perception. Divided into 7 groups of G-protein-coupled receptors, each receptor activates a different intracellular secondary messenger cascade resulting in an inhibitory or excitatory effect. Serotonin is synthesized through a two-step metabolic pathway that involves first hydroxylating tryptophan (TRP) into 5-hydroxytryptophan by tryptophan hydroxylase. The second step involves the decarboxylation of 5-hydroxytryptophan by an aromatic L-acid decarboxylase that finally results in the end-product of 5-HT.[11]

5-HT receptors are responsible for regulating substrates including food consumption, anxiety control, learning, memory, locomotion, as well as prevention of depressive conditions. AN is often observed when changes in connectivity and neurotransmission or dysregulation is present in serotonergic systems.[12] Past studies have shown that animals experience hypophagia or reduced ingestion of food when 5-HT1B and 5-HT2C receptors are stimulated. On the other hand, hyperphagia or the increased ingestion of food, is triggered through the activation of 5-HT1A and 5-HT2B receptors.[13]

As mentioned previously, serotonin is synthesized from TRP, in the two-step metabolic pathway, however, only a fraction of the total TRP available enters this metabolic pathway.[14] Many aspects of AN research focus on this pathway of serotonergic systems since TRP depletion caused by external factors such as stress and inflammation lowers

peripheral and central serotonin levels.14 Some suggest that there are correlations between obsessive or anxious behaviors and serotonin and suggest that due to this correlation, starvation is a common outlet used by patients with AN to reduce emotional discomfort, known as dysphoria.

Starvation however, negatively affects serotonergic transmission as it reduces the effectiveness of serotonin in the brain.[14] Not only does this lead to various psychological issues such as anxiety and depression, but also aggravates the course of AN and any course of treatment.

Although these aspects of AN research are of interest and are often of focus, many instances show that experimental data and clinical data are often conflicting in their results of the serotonergic system and how it is affected. Therefore, it is uncertain whether the changes that occur from symptoms of AN are causative or symptomatic.

2.2 Dopamine Pathway

Dopamine is a regulatory hormone within the mammalian nervous system. It is involved with regulating emotions and the motivation and reward system. The mesolimbic dopaminergic system is specifically associated with the food reward system. Food consumption activates the mesolimbic dopaminergic pathway, leading to dopamine release in regions such as the NAcc. This reinforces feeding behavior by associating food intake with pleasurable sensations, thereby motivating future consumption.[15]

Dieting and excessive exercise activate the hypothalamic-pituitary-adrenal (HPA) axis, leading to cortisol release. This chronic stress response influences dopaminergic activity, contributing to heightened reward sensitivity associated with AN behaviors. Sustaining this release of dopamine eventually leads to the symptoms becoming addictive, causing subjects to display the symptoms shown in typical AN patients. Studies show that it becomes more and more rewarding to eat less and exercise more.[16] This reward-driven behavior can become compulsive, reinforcing restrictive eating.

Research has been done to investigate how dopaminergic neurotransmission is specifically affected by gut microbiota change.[17] /Gut microbiota can influence dopamine secretion through the vagus nerve by stimulating the nerve with metabolites, which impacts the dopamine level in the brain. Through many other ways such as the immune system and the hypothalamus-pituitary-adrenal axis, microbiota are tied with dopamine levels.[17] Research shows that changes in these gut microbes target the dopaminergic transmission in the prefrontal cortex, striatum, hippocampus, nucleus accumbens (NAcc), and the amygdala in the brain. These regions are all directly involved with cognitive functions and emotional control, with the NAcc targeting motivation.[18] Meanwhile, intestinal microbe composition also affects the mesocorticolimbic circuit in the brain, which has dopamine as its main neurotransmitter.

Although the precise mechanisms remain under investigation, increasing evidence suggests a significant link between gut microbiota and dopaminergic regulation.[19] Numerous treatments have been tested to see how dopamine may be used to alleviate AN symptoms. Most studies theorize that altering the diet to normal levels would lower or normalize the dopamine levels.[18] Many trials have shown promising true remission in its patients. By using non selective dopamine antagonists, specifically Cis-flupenthixol and olanzapine, AN patients show reduced symptoms. Meanwhile, other studies proposed to increase the dopamine turnover to bring about a normalized dopamine level when eating food.[19] Studies have demonstrated that tyrosine supplementation may help normalize dopamine levels disrupted in AN, though further research is needed in human populations. Fish oil has shown a similar effect, since it can normalize the expression of genes and neurotransmitters in the hippocampus and hypothalamus. More importantly, it increased the dopamine level in the hypothalamus.[19]

2.3 Appetite Regulating Pathway

2.3.1 Orexigenic Pathway

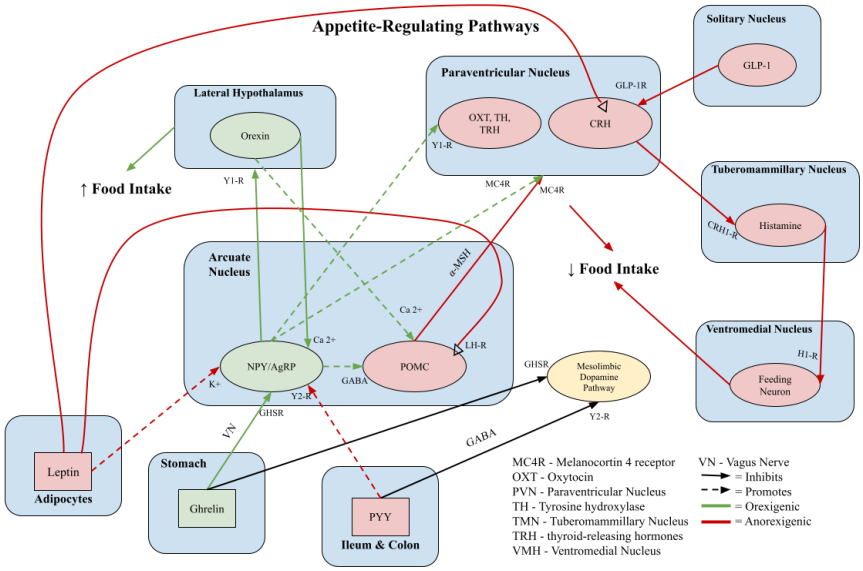

Figure 1. Appetite Regulating Pathways of AN. Describes the orexigenic and anorexigenic signaling pathways that modulates appetite in an inter-dependent fashion. OREXIGENIC: NPY projects to LH and inhibits three anorexigenic neurons in PVN: OXT, TH, and TRH, to increase appetite and fat storage[20]; AgRP acts as a competitive antagonist to α-MSH for the melanocortin 4 receptor (MC4R) in PVN; Orexin-A increases intracellular Ca2+ concentrations to promote NPY expression.21 On the other hand, Orexin decreases intracellular Ca2+ concentrations in POMC-expressing neurons[21]; Ghrelin travels through the vagus nerve to promote NPY/AgRP neurons and inhibits POMC neurons through paracrine signaling[22-23]; ANOREXIGENIC: PYY3-36 depolarized POMC neurons and increased its action potential by 93%.[24] PYY3-36 also stimulates mesolimbic dopamine pathways by acting as a selective Y2 receptor agonist[25]; CRH works through the GLP-1 signaling pathway, which involves histamine and unknown feeding-relating neurons, to reduce food
intake.[26-27] ;Leptin is responsible for lowering NPY secretion by altering its voltage-gated K+ channels and decreasing its excitability.[28] It also upregulates POMC through JAK-STAT3 phosphorylation pathway.[29] In addition, leptin increases CRH levels in the hypothalamus[30];POMC cleavage produces a-MSH which acts on MC4R in PVN to inhibit food intake.

Orexigenic pathways are those that promote food intake and increase appetite. Ingestive behaviors can be categorized into two types: appetite and consummatory. Ingestive behaviors can be divided into two phases: appetitive behaviors, which include food seeking, foraging, and hoarding; and consummatory behaviors, which involve food intake and digestion. For the detailed description of how these appetite-regulating pathways are connected refer to figure 1.

The primary orexigenic pathway involves neuropeptide Y (NPY), a 36-amino acid peptide predominantly expressed in the central nervous system, particularly within the hypothalamic arcuate nucleus (ArcN), where it plays a key role in appetite regulation. The NPY neurons are found in the arcuate nucleus (ArcN). NPY can detect hormones from stomach and fat adipocytes in promotion of food intake and fat tissue storage in adipose tissue cells.

Specifically, NPY neurons in the ArcN project to lateral hypothalamus and feeding-regulating neurons in PVN to enact orexigenic pathways.[31] In the NPY neurons, the orexigenic effects are mainly mediated through the Y1 and Y5 G protein coupled receptors.[32] The Y5 receptor is suggested to contribute more to the consummatory effects of food regulation as the Y5 agonist, increased food intake by 225-800% in foraging hamsters.[33] Although studies remain inconclusive regarding the direct relationship between plasma NPY levels and AN, research indicates that reduced gut microbiome diversity, as observed in antibiotic-treated murine models, correlates with lower NPY levels.[34]

Agouti-related peptide (AgRP), co-expressed with NPY in the arcuate nucleus, serves as a key regulator of energy homeostasis by responding to metabolic signals, such as leptin (satiety) and ghrelin (hunger).[35] AgRP neurons are stimulated by the hunger hormone ghrelin and inhibited by the satiety hormone leptin, thereby modulating feeding behavior. MC4R signaling has been implicated as an inhibitory factor in food intake as administration of MC4R agonists into PVN reduces food intake by 48% four hours post-injection.[36] AgRP neurons also express GABA neurotransmitters which are capable of directly

inhibiting POMC anorexigenic neurons. Due to the nature of AgRP as an indicator of energy homeostasis, AN patients are concluded to have abnormally higher AgRP levels, as studies with acute and weight-restored AN showed that the plasma AgRP levels are elevated, and are correlated with a lower BMI score.[35]

The appetite-regulating pathway is interconnected and involves multiple parts of the brain. In fact, orexin are orexigenic hormones synthesized in the lateral hypothalamus (LH) nucleus and promote NPY-positive neurons while inhibiting POMC-positive neurons in the ArcN. Orexin offers insight into how the physiological and psychological properties of AN are connected.

Food aversion increases the expression of orexin in the LH, resulting in an increase in dopamine release in the accumbens nucleus.[37] Such a connection to the brain reward circuit contributes to aversion to food consumption and encourages anorexic behaviors. Moreover, studies show that AN patients have abnormally high neural responses to both pleasant and aversive food stimuli, suggesting a dysfunction in pathways leading up to the neural reward response.[38] Such evidence demonstrates the need to expand current treatment options for AN to address the physiological issues.

Such interconnectedness is an example of the gut-brain relationship as ghrelin is a 28-amino acid peptide produced in the stomach that promotes NPY/AgRP expression. Acylated ghrelin is orexigenic, while non acylated ghrelin is involved in synthesizing anorexigenic mediators like urocortin in the hypothalamus.[39] Ghrelin heavily relies on the vagus nerve to carry out its signaling functions.[40] Its secretion is up-regulated with negative energy balance such as fasting and AN, and down-regulated with positive energy balance such as feeding and obesity.

Furthermore, ghrelin acts on the reward circuit through the dopaminergic pathway.[41] Paradoxically, ghrelin levels are elevated in

AN patients, likely as a compensatory response to chronic energy deficit, despite persistent appetite suppression.[42]

2.3.2 Anorexigenic Pathways

Anorexigenic pathways are those that reduce food intake, suppress appetite, and are interconnected with regulation of orexigenic pathways. One of the key anorexigenic pathways involves Peptide YY (PYY), a 36-amino acid peptide produced by enteroendocrine L cells in the ileum and colon.[43] Circulating PYY levels increase postprandially, peaking approximately 1–2 hours after feeding. PYY1-36 and PYY3-36, belong to the same family as NPY and consist of five G protein-coupled receptors (Y1, Y2, Y4, Y5, Y6). PYY is able to cross the blood-brain barrier and inhibit NPY neurons through transmembrane diffusion from the circulation.[44] Anorexigenic actions are carried out through the Y2 inhibitory receptor, which is abundantly expressed on NPY neurons in ArcN, since these effects were not observed in Y2 receptor knockout mice.[45] On the other hand, PYY3-36 in ArcN promotes POMC neurons.[45] PYY3-36 also stimulates mesolimbic dopamine pathways by acting as a selective Y2 receptor agonist.[46] This finding offers insights to evidence suggesting the dysfunction of the mesolimbic dopaminergic pathway in AN patients.[47] Although results are inconsistent with regards to the PYY levels in AN patients, increased PYY levels reduces food intake and fasting PYY levels are higher in all AN presentations.[48]

Although its specific anorexigenic action requires further research, Corticotropin-releasing hormone (CRH) acts as a hypothalamic neurotransmitter to reduce appetite.[49] One of the ways CRH exerts its anorexigenic effects is through the glucagon-like peptide-1 (GLP-1) signaling.[50] Another study suggested that NPY mediates the anorexigenic effects of CRH, as impaired activity of CRH neurons led to neuropeptide Y (NPY)-induced hyperphagia.[51] Within the HPA axis, adrenocorticotropic hormone (ACTH), a downstream product of CRH, has been implicated in appetite suppression, as ACTH signaling precedes POMC activation, which exerts anorexigenic effects.[52] CRH levels are elevated in AN patients, potentially as a

compensatory response to cortisol resistance, a phenomenon frequently observed in AN.[53-54]

Mirroring the role of orexigenic ghrelin, Leptin acts as a "satiety hormone" and is responsible for regulating multiple feeding-regulating neurons including NPY, POMC, CRH.[55-58] It is secreted from adipocytes, which are fat cells that store energy and its abundance is a key indicator of available energy due to its role as a peripheral hormone. In AN patients, reduced plasma circulating leptin levels were observed while a significant increase in the NPY inversely correlates with BMI scores.[59] These studies hint at the dysregulation of neuropeptides and results in abnormal eating behaviors observed in AN.

Finally, Proopiomelanocortin (POMC) functions similarly NPY/AgRP and produces anorexigenic effects by producing a-MSH to act on the PVN and decreases food intake. Due to the permeability of ArcN, POMC is heavily regulated by leptin and GABA. Stress has been linked to be a marker for AN, and under such conditions, POMC mRNA expression increases and activation of its neurons results in anorexigenic effects.[59] As POMC neurons can express a long isoform of leptin receptors, leptin can activate the STAT3 pathway and increase the mRNA expression of POMC. Leptin modulates neighboring NPY-GABA neurons by reducing GABAergic inhibition onto POMC neurons, thereby enhancing anorexigenic signaling.[60]

3. Current Treatments for AN

Cognitive behavioral therapy (CBT) is among the most effective treatments for eating disorders, demonstrating relatively low relapse rates and significant symptom reduction. CBT begins by identifying sources of emotional distress that contribute to dysphoria. Using that knowledge, the therapist can understand the behavior and motivation of the patients and develop their problem-solving skills to cope with their situations. Each CBT strategy is tailored specifically between therapist and patient to help the patient eventually be able to practice

self-regulation.[61] For patients with phobia, CBT has shown to have reduced hyperactivity in the insula and anterior cingulate cortex, regions known to have increased activity when processing phobic threats. In patients with OCD, CBT improved the hyperactivation of circuits that are associated with OCD, including the orbitofrontal cortex, anterior cingulate gyrus, and basal ganglia. In anxiety cases, patients had lower glucose uptake after CBT particularly in the right hippocampus, medial prefrontal cortex, and left ventral cingulate cortex, restoring the normal circulation.[62] CBT facilitates cognitive restructuring, leading to neurobiological changes in brain regions involved in reward processing and emotional regulation. Studies report a remission rate of 33% for AN, with 77.3% of adult patients experiencing a significant reduction in disordered eating behaviors after a year of treatment.[63-64]

Family based treatment (FBT) also focuses on restructuring the patient's self-perception and any cognitive distortion on food intake. The major difference is that FBT seeks to include families as a resource to the child's aid, as shown in figure 2 below. In Phase 1 of FBT, parents assume full responsibility for meal planning and supervision, ensuring adequate nutritional intake while reducing conflict surrounding food. Once the patient reaches a medically stable weight and eating-related distress decreases, Phase 2 gradually returns control over eating to the adolescent under parental guidance. Phase three revolves around resolving the still-lingering symptoms and any developmental issues disrupted by the condition, such as identity formation and self-image. The first randomly controlled trial (RCT) of FBT on AN patients showed that FBT was more efficient for 18-year-olds and younger children in preventing relapse and weight restoration. In other RCTs, FBT has been proven to be effective in the long term for weight restoration and maintaining gains even five years after treatment. Studies show that merely 10 sessions of FBT course is just as effective as 20 sessions of individual oriented course, in terms of weight maintenance.[65] At six and twelve months after trials, FBT was shown to be statistically superior to adolescent-focused therapy, differing by over 20 percentage points in remission rates.[66]

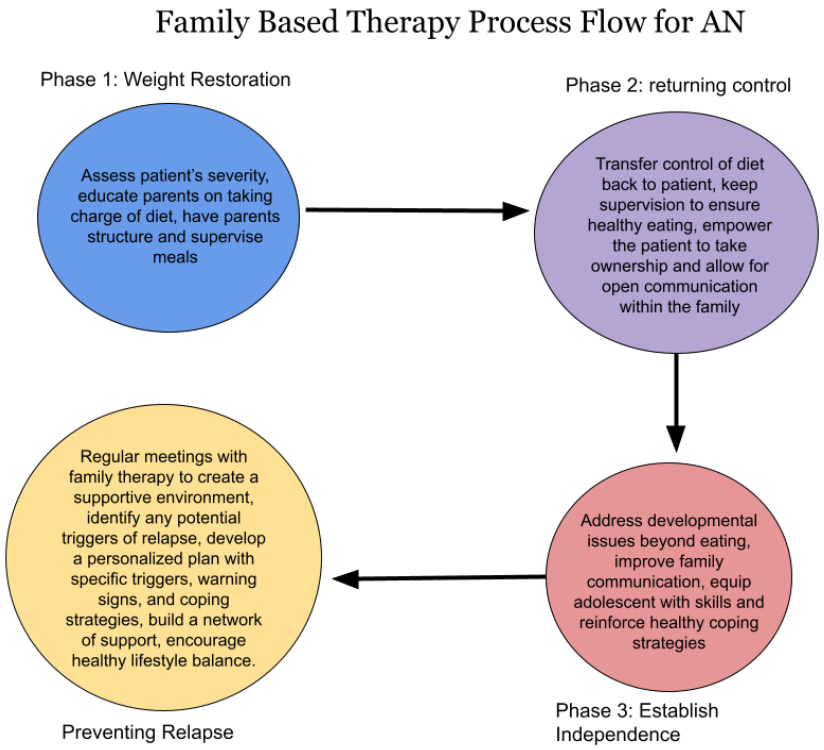

Figure 2. Family Based Therapy Process Flow for AN.

As aforementioned, FBT has multiple stages and one of its advantages is the gradual improvement the patient will experience as it goes through each phase. Through creating a unity amongst the patient's loved ones, the patient can feel more encouraged to correct their diet compared to CBT.

4. The Relationship Between the Gut Microbiome & Gut Brain Axis

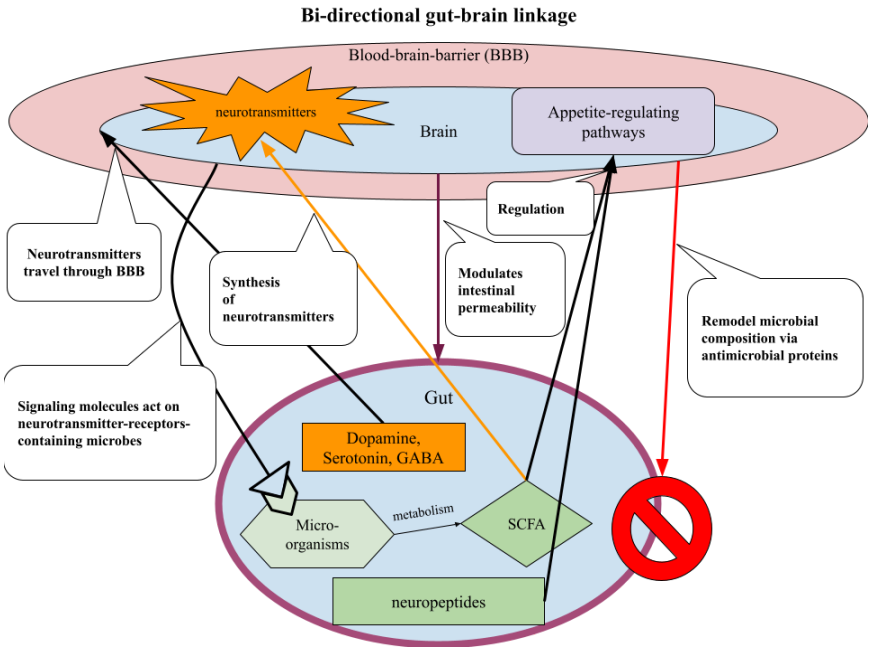

Figure 3. Bi-directional Gut-Brain Linkage. Summary of examples of the bi-directional communications between the gut microbiota and the brain. This linkage suggests the important role gut microbiota play in mental health disorders.

As mentioned previously and depicted in Figure 3, the GBA is a bi-directional linkage between intestinal processes and the central nervous system. The central nervous system mainly communicates through vagus and pelvic nerves to intestinal targets such as the enteric nervous system. The hypothalamic–pituitary–adrenal axis, endocrine pathways and bacterial metabolites via blood circulation are all major connections between the GBA and gut microbiome.[67] The gut microbiome consists of trillions of microbes that reside in the intestine and spans up to 500 species.[68-69] The gut transmits signaling molecules through the enteric nervous system and vagus nerve, modulates immune responses and gut permeability, and maintains barrier integrity.[8,67] The role of gut microbiota in neuropsychiatric disorders was first recognized in hepatic encephalopathy, where oral antibiotic treatment improved neurological symptoms.[8] More recent studies link gut microbiome dysbiosis to AN and other psychiatric conditions.[68] More recently, the gut microbiota dysbiosis, referred to as the imbalance of microbial communities, has been linked closely with

anxiety, depression, autism-spectrum disorder and eating disorders.[8,70] Administration of lactobacillus and bifidobacterium strains can reduce cortisol release, which has been associated with reduced depression, lower pro-inflammatory cytokine levels and a healthy response to stress.[71]

The gut microbiome can communicate with the brain through their bacterial metabolites. Gut microbes produce short chain fatty acids (SCFA) such as butyric acid, propionic acid, and acetic acid during metabolism.[67] SCFAs activate the sympathetic "fight or flight" nervous system, influences memory process, and modulates food intake. For example, butyric acid, the product of sugar fermentation by obligate anaerobic bacteria, is responsible for inhibiting the release of anorexigenic peptide, CRH, levels and influencing appetite.[72] These bacterial metabolites can also signal and regulate the synthesis of neurotransmitters in intestinal epithelial cells that act on the brain through the vagus nerve.[67]

One of the other ways the gut microbiome communicates with the brain is through neurotransmitters. The gut microbiota produces metabolites that include neurotransmitters or their precursors. As shown in figure 3, certain neurotransmitters and precursors produced by gut microbiota can travel through the blood-brain-barrier (BBB) for signaling or further synthesis of neurotransmitters.[67] Dopamine, serotonin and GABA are three neurotransmitters closely correlated to the pathology of AN. As mentioned in earlier sections, hypophagia is present when 5-HT1B and 5-HT2C receptors are stimulated. Staphylococcus in the gut can decarboxylate aromatic amino acid, dihydroxyphenylalanine and 5-hydroxytryptophan into dopamine and serotonin. Dopamine can then be transported via the blood-brain-barrier and reach the mesolimbic dopaminergic system usually associated with food reward. On the other hand, serotonin is mainly synthesized through the metabolites, SCFAs, secreted by bacteria strains that signal enterochromaffin cells to produce tryptophan hydroxylase.[73] In fact, 90-95% of serotonin are mainly present in the epithelial enterochromaffin cells of GI tract.[74] The

serotonergic and 5-HTergic system plays a role in mediating the onset of AN. GABA production is influenced by gut microbiota, with dietary factors such as a ketogenic diet increasing GABAergic activity. As an inhibitory neurotransmitter, GABA plays a role in appetite suppression, which may contribute to AN pathophysiology.[75]

On the other hand, since this gut-brain-axis is a bi-directional linkage, the brain also affects the make-up of gut microbiota. The brain primarily communicates with the gut microbiota through secretion of signaling molecules from neuron, immune, and enterochromaffin cells. These signaling molecules depend on the neurotransmitter receptors present on bacteria. For example, E. coli O157:H7 contains a receptor for host epinephrine and norepinephrine.[76] The brain can also alter microbiota composition through influencing the intestinal permeability. Stress increased colonic paracellular permeability by increasing the production of interferon-g and decreasing the expression of ZO-2 and occludin.[77] The CRH receptors participate in colonic barrier dysfunction under mild stress in adult rats.[78] Stress in the brain also induces the production of an antimicrobial protein, a-defensin, influencing the existing microbiota composition.

5. Targeting Anorexia by Increasing Gut Microbiome Diversity

Anorexia has been correlated with an altered gut microbiome due to the altered expression of orexigenic and anorexigenic peptides which are affected by a subpar gut microbiome shown in previous studies with anorexic mouse models.[79] Studies have shown that alterations in the normal microbial composition, also known as gut dysbiosis, can be linked to AN patients, as well as the development of other eating disorders. In general, AN patients have a lower microbial diversity than healthy controls.[80] During different phases of an anorexic patient's disorder, there are significant shifts in gut microbiome composition.[81] More specifically, alpha diversity drastically decreases during the phase of weight loss.[81]

Morita et al. reported that AN patients exhibit reduced colonization of butyrate-producing bacteria, including *Clostridium coccoides, Clostridium leptum, and Bacteroides fragilis*, which may disrupt appetite-regulating signals.[81] A decrease in butyrate-producing species and an increase in mucin-degrading species are also hallmarks of the gut microbiota in AN patients.[83] In addition, during the phase of weight loss in AN patients, a review on the role of microbiota in the pathogenesis of eating disorders mentions that there is a reduction in Firmicutes and SCFAs in the gut microbiome, while there is an increase in *Bacteroides, Actinobacteria, Enterobacteriaceae, and Methanobrevibacter smithii*.[81] The review, consisting of 16 different studies, concluded that microbial richness increased in anorexic patients after weight regain through stool transplantation. They also concluded that SCFA levels increased and the *Firmicutes/Bacteroides* (F/B) ratio normalized when there was weight gain and renourishment.81 As mentioned in the pathophysiology section, neuropeptide dysregulation is found in AN and the gut microbiota plays a key role in maintaining NPY, AgRP, and PYY levels.[84] An alteration in the gut microbiota affects neurotransmitters which is similar to the case Borgo et al. reported. In the trial, an increase of *Enterobacteriaceae*, an antigen, which produces bacterial peptide caseinolytic protease b, mimics a-MSH and activates anorexigenic neurons.[85]

These studies associating AN and gut-microbiome diversity, show that a restored gut microbiome may be a potential treatment option for eating disorders, and more specifically AN. AN patients' altered gut microbiome conditions have been proven to influence gut permeability and inflammation, appetite changes, weight loss and gain, gastrointestinal symptoms, among other elements that impact the pathogenesis of AN. Understanding the relationship between a patient's unique gut microbiome composition and their psychological condition can also be a key factor of focus for researchers and healthcare providers in order to determine the development of AN.

6. Dietary Supplements as Treatment Method for Anorexia Nervosa

6.1 Probiotics

As mentioned in previous sections, AN patients are often observed with gut dysbiosis, characterized with abnormal levels of pathogenic bacterial species, a disrupted intestinal barrier integrity, and lowered diversity. To study whether such gut dysbiosis contributes to AN symptoms of weight loss and neuropsychiatric disturbances, Fan et al. reported that germ-free mice colonized with AN-derived microbiota resulted in reduced weight gain and increased anxiety-associated activities.[86] Another study by Morisaki et al. conducted a study analyzing the gut microbiota of AN patients using polymerase chain reaction (PCR), and reported that patients exhibited preserving gut dysbiosis during inpatient nutritional therapy, CBT, and supportive psychotherapy, despite improved weight gain and psychological functions.[87] This highlights that gut dysbiosis may not be restored only by weight gain and indicates the importance of targeting the gut microbiota in combination with traditional nutritional intervention therapies. Therefore, probiotics, including commercially available lactobacillus supplements, have been investigated as potential adjunctive treatments for AN, with the goal of restoring gut microbiome balance.

6.1.1 Lactobacillus

Lactobacillus species have been utilized in the clinical setting for improving gastrointestinal health.[88] More recently, it has been linked with modulation of stress as studies of the GBA matures. Administration of lactobacillus bacterial strains can reduce the release of cortisol which leads to reduced depression, lower pro-inflammatory cytokine levels, and a healthy response to stress.[89] Schwarzer et al. recently discovered, Lactobacillus plantarum probiotics supplementation attenuated weight loss and growth impairment in malnutritioned mice, suggesting the possible role of probiotic intervention for AN.[90]

Lactobacillus rhamnosus GG (LGG) is one of the probiotic strains being studied for treating AN due to its protective effect on the gut microbiota.[91] It mostly resides in the colon mucosae and vagina and is key to maintaining genito-urinary health in preventing urinary tract infections.

LGG has been identified as a key probiotic for AN treatment due to its role in maintaining intestinal barrier integrity by upregulating tight junction proteins, such as zonula occludens-1, thereby reducing gut permeability. Moreover, LGG modulates the gut-brain-axis by regulating the 5-HTergic intestinal system, increasing GABA receptors in the brian, increasing serotonin receptor expression and, overall, relieving anxiety-like behaviors.[91] Bai et al. conducted a mouse model study on deoxynivalenol (DON)-induced anorexia, demonstrating that LGG supplementation alleviated anorexic symptoms by reducing intestinal inflammation and modulating appetite-related signaling pathways. Coinciding with information presented in previous sections, DON-induced anorexia mainly releases 5-HT, which acts as a neurotransmitter and leads to anorexia.[92]

In the study's experimental design, antibiotic-treated mice were separated into 4 study groups: control, DON, DON+LGG, LGG and after 4 weeks of treatment, researchers found that LGG prevented DON-induced anorexia by increasing crypt depth of the jejunum and ileum, strengthening the barrier integrity, and promoting nutritional uptake. LGG also remodeled the TLR4-MyD88-NF-κB signaling pathway and since TLR4-MyD88-NF-κB is responsible for lipopolysaccharide (LPS)-induced anorexia, LGG is able to alleviate anorexia mediated by pro-inflammatory cytokines. LGG also maintained butyric acid levels comparable to controls, whereas the DON group had a significant drop. This affirms previous research done that shows AN patients with lower butyric acid levels and demonstrates how LGG promotes food intake as butyric acid inversely correlates with anorexigenic CRH by influencing cortisol receptor in the intestine.[93,72] In addition, LGG's regulation of appetite regulating pathways (NPY, AgRP, PYY) allows for upregulation of food intake and remodels the gut microbiota. More importantly, LGG remodeled

the gut microbiota to ensure long-term benefits as 11/16 metabolites were increased by DON compared with control while they were decreased by LGG + DON. Moreover, Bai et al. conducted fecal microbiota transplantation (FMT) to antibiotic-treated mice and reported that FMT-LGG+DON prevented weight loss and low food intake present in FMT-DON. Further research will be needed to examine its efficacy on humans but its promising results can hopefully present a possible therapy in conjunction with nutritional and behavioral interventions for AN .

Another treatment of interest is *Lactobacillus acidophilus* (L. acidophilus) and has been associated with modulation of weight in multiple clinical studies. L. acidophilus's main function is to produce lactic acid from lactose and has gained popularity for its digestive health benefits with the production of "acidophilus milk" when prevalence for obesity is significant in the US.[94] Although direct studies on L. acidophilus in AN patients are lacking, its ability to restore gut microbiome diversity, regulate inflammatory responses, and improve intestinal permeability suggests its potential as an adjunctive treatment. First of such studies dates back to 1952, in which 124 bottle-fed infants, added with 500,000,000 viable L. acidophilus organisms per quart into daily formula, were compared with 123 healthy controls. The results showed significantly larger weight gain during the first month than did the controls.[95] Another 8-week randomized parallel study of the effects of probiotic drink on LDL-cholesterol and fibrinogen factors revealed that the group intaking yogurt with L. acidophilus showed weight gain.[96] In a more recent study, researchers conducted a genomics study on seven Lactobacillus species associated with weight gain and have been able to classify L. acidophilus and L. reuteri, among others, as weight gain-associated strains.[97] Although other studies indicate L. acidophilus' capability in ameliorating obesity and reduce weight gain, such effects can be explained by L. acidophilus' ability to restore gut microbiome diversity as this same study revealed its reversal of a obesity-induced gut dysbiosis. Overall, L. acidophilus increased alpha-diversity and restored alterations in beta-diversity which is key to the treatment of AN.[98]

Gut dysbiosis is characterized by a disrupted intestinal permeability leading to an outgrowth of Gram-negative LPS producing Proteobacteria and circulating LPS levels.[98] The nutrient deficient environment of AN disrupts the intestinal barrier integrity, as Kleppe et al. reported zonulin family peptides, serum markers for integrity, are significantly lower than healthy controls.[99] A disrupted barrier promotes LPS release resulting in low grade inflammation which exacerbates AN.[100] In fact, LPS plays a crucial role in the pathophysiology of AN as it reduces food intake via a mechanism dependent on myeloid differentiation primary response 88 (MyD88) signaling, an inflammatory signaling pathway, within endothelial cells lining brain capillaries and neurons cells.[101] One of the ways L. acidophilus restores gut microbiome homeostasis is through improving intestinal integrity and lowering gut permeability. Specifically, L. acidophilus strengthens the barrier by increasing the expression of epithelial mucosal proteins, intectin, and the tight junction proteins, Occludin.[98] A strengthened intestinal barrier results in decrease in circulating LPS, as a clinical trial conducted on 71 obese women over the period of 12 weeks reported a decrease in LPS levels by 20.14% after administering L. acidophilus.[102] Although these study subjects were not AN patients, they were all diagnosed with low-grade systemic inflammation caused by a gut barrier permeability dysfunction, which is characteristic of anorexia. In culmination, L. acidophilus' unique ability to restore gut microbiome homeostasis through modulating the intestinal barrier integrity and LPS levels suggests its importance in treating AN.

Currently, an ongoing phase 2 randomized controlled study conducted by the Medical University of Vienna is examining the effects of daily multistrain probiotics doses including L. acidophilus W22 in 30 adolescent AN patients. This is the first study studying the administration of probiotics on adolescent AN patients and can be conclusive due to a double-blind randomized longitudinal design, the large sample size and the comparison with sex and age-matched healthy controls.

A final bacterial strain of interest is *Lactobacillus reuteri* (L. reuteri) and similar to L. acidophilus, studies have concluded that this strain is associated with weight gain.[97] L. reuteri mainly produces organic acids, ethanol, which aids in inhibiting the colonization of pathogenic microorganisms. Its usual tendency to colonize in the GI tract is the reason it is considered an optimal candidate for probiotic use.[103] With regards to AN, L. reuteri is capable of strengthening the intestinal barrier by preventing microbial translocation from the gut lumen to the tissues, restore microbiome homeostasis through inhibiting pathogenic microorganism colonization, and reduce the production of pro-inflammatory cytokines.[104] A recent 3-year clinical trial in Croatia investigated the effects of L. reuteri DSM17938 on 31 adolescent AN patients with comorbid constipation. After six months, 93% of the L. reuteri group achieved weight normalization, compared to 63% in the placebo group.[105] Constipation is often frequently accompanied with AN weight loss and can further discourage food intake.[106] This study reported that after 3 months, constipation relief was higher in the L. reuteri than in the placebo group (87% vs. 63%), and such relief can pave the way for nutritional recovery interventions. Overall, L. reuteri is a potential safe and effective way of mitigating AN and constipation in adolescents, due to its positive effects on weight normalization, patient compliance, constipation relief, which can lead to more effective nutritional recovery.

6.2 Vitamins

Vitamins are organic compounds that are essential to the human body for organs to perform various essential physiological processes, including metabolism, immune function, and cellular homeostasis. Previous research has shown that there is significant association between vitamins and improving gut microbiome diversity, specifically with Vitamin D, A, and E.[108,115,121] Most of these vitamins need to be consumed through one's diet from sources such as meats, fruits, and vegetables. AN patients are especially at risk of malnutrition can lead to gut complications like having deteriorating intestinal barriers, consequently leading to inflammation and general discomfort from

eating. Vitamin D, A, and E have all been found to assist with preserving gut barrier function and promoting microbiome health.

6.2.1 Vitamin D

Vitamin D is an important nutrient in development and especially in the regulation of the microbiome. With AN patients consuming less food, patients are at high risk of vitamin D deficiency which could create a positive feedback loop of worsening symptoms. Vitamin D influences the microbial composition through vitamin D receptor genes (VDR), which regulates immune responses and microbial homeostasis. Vitamin D regulates the expression of antimicrobial peptides, such as cathelicidins and defensins, which help maintain gut mucosal integrity by preventing dysbiosis and promoting epithelial repair.[108] Through this, Vitamin D controls the health of the gut by protecting against inflammation with the expression of VDR genes.[109] This is shown in one particular study, in which the fecal microbiome of 50 adolescent women were tested after receiving Vitamin D supplements. Results showed an increase in Firmicutes and Bifidobacteria, with a reduction in Bacteroidetes, a marker of inflammatory response. Also, a reduction of VDR gene expression can increase the proteobacteria population, causing an increase in inflammation. This is also supported by a study in which VDR genes in mice were removed and subsequently the subjects showed severe gut inflammation, shown with increased amounts of Bacteroidetes.[110] With adequate supplementation of Vitamin D, the VDR genes are able to prevent the pathogenic microbes from outcompeting the beneficial bacteria, the leading cause of gut dysbiosis.[109]

Vitamin D can help alleviate many other organ complications arising from AN. Vitamin D deficiency is often associated with liver and kidney diseases, and with the inherent malnutrition experienced by AN patients, they are more prone to such diseases. Singh et. al tested the effect of Vitamin D supplementation on subjects and discovered improved liver function along with decreasing the ratio of aspartate aminotransferase (AST)/alanine aminotransferase (ALT). Kidney function also improved as the ratio of serum blood-urea-nitrogen

(BUN)/Creatinine decreased. In the same study, the relative abundance of Bifidobacterium and Akkermansia increased, as did the Firmicutes and healthy Bacteroidetes probiotic phylums, while on the other hand, the Prevotella phylum decreased. In addition, the study also showed that the Vitamin D supplementation led to a statistically significant greater diversity of microbiota.[111] Although more research is still needed, scientists still speculate that AN is related to the dysbiosis of the gut microbiota.[112] If an association between gut dysbiosis and AN is confirmed, Vitamin D supplementation could serve as a potential adjunct therapy for improving gut health and alleviating gastrointestinal symptoms.

An ongoing trial for the past two years aims to further study the effect of Vitamin D on gut microbiota and intestinal barriers, especially in patients with irritable bowel syndrome (IBS). The researchers have currently found that when the concentration of Vitamin D is reduced, symptoms seem to be connected to the concentration. Supplementation of Vitamin D has shown alleviation of IBS symptoms and researchers are investigating the mechanisms of dissecting fecal microbiome samples through 16S rRNA gene sequencing and detecting the expressions of Z0-1, occluding in intestinal mucosa. If Vitamin D can indeed help with alleviating any organ complications arising from AN, then Vitamin D can be a good supplement to start helping alleviate symptoms of AN. Through feeding the patients their proper amount of Vitamin D, they can prevent gut complications arising from AN.

As aforementioned, Vitamin D supplements can be important in restoring the nutritional balance to maintain a healthy digestive system. Vitamin D supplements will aid AN patients who suffer from gastrointestinal issues in immune function and enhance nutrient absorption, a step in the overall recovery process. Through alleviating the general discomfort and the organ complications arising from the disorder, patients can eventually feel comfortable again to eat healthily.

6.2.2 Vitamin A

Vitamin A is an essential nutrient involved in various physiological functions, including the maintenance of gastrointestinal homeostasis.[113] Vitamin A is derived from two primary dietary sources: retinol, found in animal products such as meat and fish, and provitamin A carotenoids, obtained from fruits and vegetables. Not only is Vitamin A essential in homeostasis, but it also maintains a protective effect on the intestinal mucosal barrier and gut mucosal integrity.[114] Patients who suffer from AN tend to not get the required nutrients and supplements they need to maintain their health and gut microbiome. Without the consumption of foods like meat, fish, fruits, and vegetables, anorexic patients drastically lack Vitamin A intake. A research trial conducted by Pattanakitsakul et. al. studied the nature of Vitamin A deficient rats and discovered that additional Vitamin A supplementation protects the intestinal mucosal barrier even after chemotherapy treatment, which is known to drastically affect gut microbiome composition.[115] This protective role of Vitamin A can be useful in implementing and preserving a healthy gut microbiome composition that is required in anorexic patients.

Another study conducted in Chongqing Medical University located in China studied the gut microbiome composition of vitamin A-deficient and vitamin A-sufficient groups amongst patients experiencing diarrhea.[116] They observed that gut microbiota richness was not different between the two groups, but that there was significantly lower microbiome diversity in patients with deficient Vitamin A according to the Shannon and Simpson Index, a traditional method used to measure species diversity.[117] This means that patients with sufficient Vitamin A had ample quantity of gut microbiota species, and in specific the study found that *Escherichia-Shigella* and *Clostridia* phylotypes were found more prominently found in the Vitamin A sufficient group, while the deficient group was largely composed of *Enterococcaceae*, including the enteric pathogen *Enterococcus faecalis*. Enteric pathogens, such as *Enterococcus faecalis*, are typically harmless in the intestinal environment of healthy individuals, but the species' overabundance in the gut can quickly result in infections that spread throughout the

body and even promote tumor formation.[118] In addition, people with underlying health problems, in this instance, patients with AN, with a weakened immune system, are more likely to face additional health issues due to their diet lacking Vitamin A amongst other nutrients. Other studies have also shown that intake of retinol, a dietary supplement derived from Vitamin A and as mentioned previously to be found in meat and fish, can increase or even re-establish ratios between *Proteobacteria* and *Actinobacteria* as well as *Proteobacteria* and *Firmicutes*.[119] This would be useful in anorexic patients since there are significant changes in the quality, quantity, composition of gut microbiota during the phase of weight loss. These studies suggest that Vitamin A may play a role in maintaining gut microbiome balance and mucosal integrity, which could be particularly beneficial for AN patients experiencing intestinal dysbiosis.

6.2.3 Vitamin E

A fat soluble antioxidant, Vitamin E functions as a fat-soluble antioxidant that supports plasma membrane repair and maintains intestinal barrier integrity by reducing oxidative stress and inflammation. Vitamin E also helps protect the gut microbiota from pathogenic invasion. There are eight different types of Vitamin E, but the most common form and most present and active in humans is a-tocopherol.[120] Significant Vitamin E uptake shows a positive association with the *Firmicutes* phylum and negative association with *Bacteroidetes*. *Firmicutes* are important gut bacteria for metabolism and *Bacteroidetes*, while sometimes beneficial, overabundance of this species can be damaging for gut barriers as it degrades the mucus barriers of intestines.[121] In addition, a study on lactating women found that those with higher Vitamin E intake were associated with a decrease in *proteobacteria*, known to contain many pathogens and cause inflammation.[119] This supports the idea that Vitamin E deficiency can increase inflammatory response. Due to anorexic patients' lack of nutrient intake, Vitamin E supplementation, along with other vitamins, could drastically improve the state of a patient's gut microbiome and protect them from further health complications that can arise from pathogens and inflammation of the gut.

While research in determining the exact quantities of vitamins to administer in order to obtain the best results for the gut microbiome are lacking, specifically in regards to Vitamin E supplementation, a recent study from 2020 shows that a lower-level consumption of Vitamin E changes the composition of the gut microbiota while also increasing spleen and body weight. The study assigned mice with similar body weight to three varying groups, a control, low-level Vitamin E (0.06 mg per 20 g of body weight), and high-level Vitamin E (0.18 mg per 20 g of body weight). Comparing the body weights of the mice before and after Vitamin E supplementation showed that while all groups increased in weight (including control), the low Vitamin E group had the most weight gain as well as the highest ratio of *Firmicutes* to *Bacteroidetes* in the gut microbiome.[120] Since the ratio of F/B is drastically altered in anorexic patients, implementation of lower quantities of Vitamin E based on a patient's body weight can promote weight gain and renourishment, while also enhancing gut microbiome composition. Meanwhile, another study was conducted where iron and Vitamin E were taken together as supplements. The test group was shown to have a differing microbiome composition with a decrease in *Bacteroidetes* and an increase in *Firmicutes*. There was also an increase in *Roseburia*, a producer of butyrate, a short-chain fatty acid that can help with gut mucosal barrier.

The study concluded that ultimately the combination of iron and Vitamin E benefited microbiome composition by assisting the growth of butyrate-producing bacteria.[122]

6.3 Fatty Acids and Ketone Supplements

Ketones are metabolic intermediates formed as byproducts of fat metabolism. When people starve, their bodies enter ketosis, a state where the brain shifts from using glucose to using ketones for energy. This state can reduce anxiety and provide a sense of reward, which might explain why some people with AN find starvation soothing. A ketogenic diet (KGD) can replicate the state of ketosis without actual starvation.[123] The diet is high in fat from foods like avocados and nuts, moderate in lean protein like fish, eggs, and lean pork/beef, and is very

low in amounts of carbohydrates from leafy greens and non-starchy vegetables.[124-125] It has been used effectively to reduce seizures in epilepsy and is being studied for other neurological conditions. This shift affects various brain pathways and can have significant effects on neurological health, similar to the effects seen in epilepsy treatment. While no published studies have evaluated KGD specifically for AN, preliminary evidence suggests potential benefits in addressing energy deficits and gut microbiome alterations. However, further research is needed to determine its clinical applicability.

6.3.1 Ketone Body Metabolism Overview

Ketone body metabolism involves coordinated processes across multiple organs, including the liver, muscles, and brain, to regulate energy homeostasis. Ketone bodies are vital energy sources for the nervous system, especially when glucose is scarce. In healthy adults, ketone levels fluctuate daily, contributing to about 5% of energy expenditure in the fed state and up to 20% during fasting or starvation.[126] These levels rise significantly during fasting, exercise, neonatal periods, or late pregnancy.[126] Other tissues such as skeletal muscle, heart, and kidneys also metabolize ketone bodies. This turnover includes production from fatty acids in liver hepatocytes and disposal by extrahepatic tissues.[127]

Fatty acids, released from adipose tissue, enter hepatocytes and undergo β-oxidation in the mitochondrial matrix, leading to ketone body production, primarily from acetyl-CoA.[127] Ketogenesis is regulated by hepatic glucose availability, mitochondrial redox state, β-oxidation efficiency, TCA cycle flux, and the hormonal balance between glucagon and insulin, which collectively determine ketone body production.[126] Overall, ketone bodies are crucial energy sources for various processes ranging across all bodily systems and mechanisms and changing levels in a person's body highly affects how energy is utilized and the balance of hormones, and other substances, in the body. Therefore, reinforcing the ketone body prevalence in a patient's body may have a significantly positive impact on reestablishing homeostasis and energy efficiency throughout recovery from AN.

6.3.2 Ketones and Short-Chain Fatty Acids (SCFAs)

Ketone bodies serve as an alternative energy source during periods of low carbohydrate intake, supporting various organs, including the brain and muscles. They help modulate the redox state within cells, potentially reducing oxidative stress and supporting cellular health.[126] They are precursors for lipid synthesis, playing a role in lipid storage and metabolism.[126] Ketone bodies can also act as signaling molecules that influence pathways involved in inflammation, cell growth, and differentiation.[126] The production of ketone bodies in the gut is regulated by the microbiome and dietary factors, suggesting an intricate relationship between ketones and gut health.

Given the severe caloric deficits in AN patients, ketone bodies could provide a critical energy source, helping to restore overall energy balance.[128] AN is often associated with gut dysbiosis which is essentially the imbalance of the microorganisms that reside in the body, especially in the gut or skin.[129] Due to their multifaceted role in the body, ketone bodies could help restore microbial diversity, which is crucial for gut health and nutrient absorption. They may reduce inflammation in the gut, a common issue in AN, thereby promoting a healthier gut environment.[126] The ability of ketone bodies to influence cell proliferation and differentiation in the gut lining could also help maintain or restore gut mucosal integrity, which is essential for proper nutrient absorption.[127] The ambiguous roles of ketone bodies suggest they could be used as part of a comprehensive treatment strategy for AN. Their benefits in energy provision, gut health, and inflammation modulation make them a promising adjunct to traditional treatments like CBT and FBT which address the disease from a more neurological basis without also assessing the physical imbalances caused by AN.

According to other studies, ketone bodies, such as beta-hydroxybutyrate (BHB), significantly impact cerebral fuel metabolism.[128] They exhibit a glucose-sparing effect by substituting for glucose in the brain, particularly noticeable during fasting or ketogenic diets.[128] This metabolic adaptation not only supports brain function but also suggests potential therapeutic benefits for conditions like AN,

where compromised glucose metabolism can occur.[128] Ketone bodies enhance cerebral energy metabolism without altering ATP levels, indicating their efficiency as a neuroprotective substrate.[128] Additionally, ketone bodies possess antioxidant and anti-inflammatory properties, potentially mitigating oxidative stress and neuroinflammation, common in neurodegenerative diseases.[128] These insights highlight the therapeutic potential of elevating ketone bodies in managing disorders characterized by impaired glucose metabolism, including those affecting brain health like AN.

There are a few ongoing clinical trials that are investigating the impact of short-chain fatty acids and ketone supplements on eating behavior and overall nutritional states. One current trial led by Dr. Elske Vrieze in Belgium is specifically testing the psychobiological effects of SCFAs in patients suffering from AN.[130] The study consists of administering SCFAs or a placebo through colon-delivery capsules over six weeks, in addition to standard psychotherapeutic treatment regimens, to evaluate changes in stress responses, eating habits, body mass index (BMI), gut microbiota, gastrointestinal symptoms, and related psychological conditions.[130] These scientists aim to understand how metabolites in the gut microbiota can impact human stress systems and eating behaviors to facilitate potential therapeutic targets for anorexia and other psychiatric disorders.[130] Another ongoing clinical trial being done at UC San Diego is specifically testing the effect of BHB as a nutritional supplement for anorexic and bulimic patients, and how BHB ingestion impacts brain function.[131] Researchers will compare the effects of ketone supplementation versus placebo on brain activity using EEG measurements, with participants taking a ketone drink twice daily for two weeks.[131]

Overall, the growing number of studies surrounding the role of ketone bodies in human health and disease emphasizes the importance of understanding their role as potential therapeutic targets. While there are still limited studies on how ketone bodies directly correlate to AN, there has already been substantial research regarding how it influences other psychiatric and chronic diseases like Alzheimer's, Parkinson's Disease, fatty liver disease, heart failure, among others.[128] Ketone

bodies play vital roles in energy metabolism, redox balance, lipid synthesis, and cellular signaling. For individuals with AN, these properties suggest that ketone bodies might help address some of the condition's critical aspects, such as energy deficiency and gut health. They may serve as crucial mediators of a patient's microbiome. While promising, these potential applications require further research to confirm their efficacy and safety in clinical settings, ensuring that interventions are tailored to the unique needs of AN patients.

7. Practical Applications

These findings highlight the potential role of dietary supplements, including vitamins, minerals, and probiotics, in treating AN. Integrating vitamin supplementation, dietary supplements, ketogenic diets with current therapies will overall provide a more comprehensive approach to treating AN. This review paper may also encourage ongoing and future clinical trials to evaluate the effectiveness of these supplements and find ways to develop more personalized treatment plans based on individual gut microbiome profiles. Further research on these substances may enable physicians to assess gut microbiome composition and optimize nutritional interventions tailored to individual patient needs.

7.1 Clinical and Nutritional Rehabilitation Treatment Plans

Patients with AN often suffer from severe nutritional deficiencies and altered gut microbiome, which contribute to both physical and psychological symptoms. Effective nutritional rehabilitation is crucial for the recovery of AN patients. Traditional refeeding programs may benefit from the inclusion of dietary supplements to address specific deficiencies and support gut health. Integrating specific dietary supplements and tailoring them to patient-specific needs introduces a way to provide a more personalized treatment plan for AN patients. Since the makeup of everyone's gut microbiome is unique, understanding the impact of specific substances and how varying amounts of them affects each patient is key to targeting the distinct

ways the disorder is influencing them. Dietitians can also create individualized supplement plans based on the specific needs and deficiencies of each patient. Potential outcomes of individualized supplement-based interventions may include improved gut microbiome diversity, enhanced nutrient absorption, and potential benefits for mental well-being through gut-brain axis modulation. However, these effects require further clinical validation.

7.2 Psychiatric Support Strategies

AN is also often accompanied by psychiatric symptoms such as anxiety, depression, and obsessive-compulsive behaviors, which can be influenced by gut health. Incorporating dietary supplements into psychiatric support strategies can provide a holistic approach to treatment. Psychiatrists and therapists can work with dietitians to ensure that patients receive supplements that support both mental and physical health. This integrated strategy aims to reduce psychiatric symptoms, improve adherence to treatment plans, and increase the likelihood of sustained recovery.

The integration of dietary supplements into treatment strategies for AN offers a promising approach to addressing the complex nutritional and psychiatric needs of patients. By restoring gut-microbiome diversity and supporting the gut-brain axis, these supplements can enhance both physical and mental health outcomes. Continued research and careful implementation will be key to maximizing the benefits of this approach.

8. Limitations/Implications

While dietary supplements hold promise as a potential treatment for AN by restoring gut-microbiome diversity and addressing alterations in the gut-brain axis, significant limitations exist. Addressing these limitations through rigorous research, personalized treatment approaches, and enhanced regulatory oversight is crucial to fully realize the potential benefits of dietary supplements in the treatment of AN.

As of now, there is an extremely limited scope of research regarding incorporating these supplements into AN treatment regimens. Research on dietary supplements in AN treatment is still in its early stages, with most studies being limited by small sample sizes, short follow-up periods, and a lack of randomized controlled trials. The limited scope of resources prevents us from fully examining variability in individual responses and the heterogeneity in patient responses necessitates personalized treatment approaches, complicating the standardization of supplement-based interventions. There is also lack of research on the potential risks and side effects of dietary supplements in relation to AN, how they interact with other AN treatments, and regulatory and quality control issues. Over-supplementation of certain vitamins and minerals can lead to toxicity and therefore correct quantities of supplements to administer needs to be determined. Probiotics may cause gastrointestinal disturbances in some individuals. Additionally, the purity and quality of supplements can vary widely. The interactions between dietary supplements and other treatments are not well-understood, and there is a risk of adverse interactions or diminished efficacy when combined with certain medications or therapeutic modalities. Variability in supplement quality, dosing, and labeling accuracy can also pose challenges for ensuring consistent and safe administration of supplements.

Another limitation to this treatment approach to consider is patients' compliance and acceptance of the treatment regimen. Individuals with AN often exhibit resistance to treatment, including dietary interventions.[132] Fear of weight gain, mistrust of new treatments, and the psychological complexity of the disorder can lead to poor compliance with prescribed supplement regimens.

Ensuring patient compliance and acceptance of dietary supplements requires extensive education, support, and possibly behavioral interventions, which can be resource-intensive.[132] Overall, the limited scope of current research restricts our ability to draw definitive conclusions about the efficacy and safety of dietary supplements for treating AN.

9. Future Directions

While the components in this review show advantages for using dietary supplements as a treatment for improving gut microbiome diversity, there is limited research on its effectiveness in relation to AN specifically. It is important to consider areas of future research that can maximize the potential of dietary supplements and improve the effectiveness of current treatment methods. As mentioned before, utilizing dietary supplements for AN along with conventional therapies (i.e. CBT), can propose a comprehensive treatment approach that offers a holistic approach to managing and treating the disorder. Studying this combination of traditional treatment methods with additional supplementation, scientists can compare the effectiveness of the combined approach versus solely traditional methods.

Future research should focus on comparative studies evaluating the efficacy of various dietary supplements, including probiotics, vitamins, fatty acids, and ketone supplements. Examining their effects both individually and in combination may help identify the most effective strategies for improving gut microbiota diversity in AN patients. In addition, although time consuming, conducting long-term studies would help assess the effects of dietary supplements in improving gut diversity in specifically anorexic patients as well as examine the longer term effect and side effects of using these supplements. These long-term studies can also be closely connected to evaluating the impacts of weight restoration in AN patients while monitoring a patient's psychological well-being.

Personalized medicine, including microbiome-based interventions and genetic screening, may improve treatment outcomes by tailoring dietary supplement regimens to individual AN patients. However, further research is needed to develop standardized implementation strategies. Since each individual's gut microbiome is unique in composition and is developed fully approximately one to two years after birth, these personalized treatment plans targeting specific areas in a patient's gut profile can improve, in this circumstance for example, microbiome imbalances in patients with AN.[133] These personalized

approaches can also take into account genetic predispositions which can be beneficial in tailoring dietary supplement intake and regimens to target specific deficiencies that are present in a particular patient.

10. Conclusion

Patients with AN suffer from both nutritional deficiencies and a disrupted gut microbiome, and traditionally nutritional intervention strategies and behavioral therapies are employed to restore weight gain and improve psychological functions. However, this leaves the gut microbiome unaddressed, as studies increasingly reported the presence of gut dysbiosis after traditional nutritional therapy, CBT, and supportive psychotherapy. Dietary supplements can act as a promising add-on in meeting the unaddressed areas in AN treatment. Probiotic strains from the lactobacillus genus, such as L. acidophilus, L. reuteri, and L. rhamnosus GG, have proven to improve weight gain, relieve constipation (a symptom often preventing AN patients from receiving nutritional interventions), positively modulate the appetite-regulating pathways, and strengthen the intestinal barrier integrity.

While probiotics are the most extensively studied dietary intervention for gut microbiome modulation in AN, further research is needed to determine the specific effects of different probiotic strains in human clinical trials. Vitamin D plays an integral role in microbial composition through its receptor genes, regulation of gut mucosa, and is shown to positively remodel the gut microbial makeup of AN patients. Vitamin A maintains the intestinal barrier integrity and is positively correlated with microbiome diversity and Vitamin E, similar to Vitamin A and D, strengthens barrier integrity, protects against pathogenic microbes, and reduces intestinal inflammation. While some vitamins, such as vitamin D, have shown potential in microbiome modulation, direct clinical evidence supporting their role in AN treatment remains limited, warranting further investigation. Ketone bodies offer an alternative intervention acting as a critical energy source, restoring cerebral fuel metabolism, and in repairing gut mucosal integrity due to its role in influencing cell proliferation in the gut lining. Fatty acids play a role in ketone body production, and

SCFAs modulate appetite-regulating pathways. While there are no published studies on the effects of ketogenic diet for AN, ongoing clinical trials investigating SCFAs and Ketone supplements as potential anorexic treatment are promising.

By combining traditional therapies with dietary supplements, dietitians can tailor-make treatments to address the unique nutritional and gut microbiome deficiencies in each patient. However, more comprehensive and diverse clinical trials are needed to determine the efficacy of both individual dietary supplements and its combination with traditional therapies in the treatment for AN.

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