Does diet matter? The use of polyunsaturated fatty acids (PUFAs) and other dietary supplements in inflammation-associated depression.

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Abstract

An increasingly pertinent issue in psychiatry in recent years is that of the limitations of conventional antidepressants, which are not effective in a large number of patients with major depressive disorder (MDD). Coupled with emerging hypotheses about the role of inflammation in depression, it would appear that it is time to look to alternative treatments for these symptoms.

This review will examine an emerging area in psychiatry, that of dietary supplements and the diet in general to treat depressive symptoms, and inflammation in depression. In particular, polyunsaturated fatty acids (PUFAs), probiotics and folic acid, are three supplements that demonstrate the ability to target inflammation and other underlying systems in depression. While there is a definite need for more research in all these supplements to determine true efficacy, dosage and target populations, they can be used as mono- or adjunctive therapies to good effect, and show superior safety profiles when compared with more traditional alternatives.

Keywords

Diet • Inflammation • Depression • PUFAs • Probiotics • Folic Acid

1. Introduction

While conventional antidepressants have been one of the most notable psychiatric developments since the mid twentieth century, the increase in limitations reported among depressed populations cannot be ignored. Worryingly, figures suggest that only a modest proportion (almost a third) of patients ever achieve remission with monoamine antidepressant therapy alone (Rush et al., 2006). As a consequence, a number of novel therapeutic approaches have been considered in order to ameliorate depressive symptoms, including changes in lifestyle and diet. Developing treatments targeted towards the heterogeneity of depression is not only required for improved quality of life among individuals with Major Depressive Disorder (MDD) but remains an important aspect of translating more recent findings, produced from the *brain-mind-body trichotomy*, into clinical practice (Pariante, 2015). Diet, polyunsaturated fatty acids (PUFAs) and other dietary supplements in particular, have been shown to address this multifaceted origin of depression by targeting multiple biological systems, including the inflammatory system, which has recently been recognized as crucially involved in this disorder.

Since MDD is a multifactorial disorder, several mechanisms are likely to underlie its aetiology. Thus, a number of hypotheses have been put forward in an attempt to elucidate its

origin. The inflammatory hypothesis initially titled the macrophage theory of depression (Smith, 1991), or more recently, the malaise or cytokine theory of depression, has increasing relevance for MDD (Maes et al., 2009, Miller et al., 2009, Dantzer et al., 2008, Miller and Raison, 2015, Zunszain et al., 2011). In short, the bidirectional language between the immune system and the central nervous system (CNS) is thought to be responsible for depressogenic changes caused by an upsurge of pro-inflammatory signalling molecules in certain areas of the brain (Baumeister et al., 2014, Zunszain et al., 2011).

According to a widely accepted model, pro-inflammatory cytokines released as a result of stress, or as a direct consequence of immune activation, can cause disruption to monoamine metabolism, neuroendocrine function, synaptic plasticity, glutamate signalling and neurogenesis (Dantzer et al., 2008, Haroon et al., 2012, Schiepers et al., 2005). Moreover, direct evidence in favour of a causal relationship has been demonstrated by the administration of pro-inflammatory cytokines such as, interleukin (IL)-1β, IL-6, tumour necrosis factor alpha (TNF-α), and interferon alpha (IFN-α) (Capuron and Miller, 2004). In particular, these findings have resulted in an increase in depressive-like symptoms, such as low mood, anxiety, fatigue, anhedonia, cognitive dysfunction and disturbed sleep (Dantzer, 2001, Dantzer et al., 2008).

Given findings in support of the *inflammatory hypothesis*, associations between inflammatory status and treatment response have opened up the possibility of producing reliable inflammatory biomarkers to improve upon treatments for this heterogeneous disorder (Eller et al., 2008, O'Brien et al., 2007). Emerging is the notion that attenuating inflammatory-mediated processes provides relief from depressive symptomology (Miller and Raison, 2015, Stirling et al., 2005, Muller, 2013, Fond et al., 2014, Loftis et al., 2010). Similarly, work conducted in our laboratory has found that higher baseline gene expression of IL-1β and TNF-α predicts antidepressant resistance, thus these findings could have translational value for those who do not respond to traditional antidepressants (Cattaneo et al., 2013). While the use of anti-inflammatory strategies in the context of depression has obtained most attention, a putative anti-inflammatory and antidepressant effect could be delivered also by dietary supplements that regulate the immune system. Is there any evidence that dietary supplements with anti-inflammatory action can be beneficial for MDD, through inflammatory modulation?

Upon answering this question we will review the findings for different classes of dietary supplements throughout this chapter, with a view to obtain a greater understanding of the pharmacological concepts underlying the mechanisms of these agents, their efficacy and, finally, their relevance to MDD.

2. Dietary supplements in depression

Epidemiological studies have consistently demonstrated that diet plays a huge part in overall wellbeing, both mental and physical. It has also been shown in recent years that the gastrointestinal tract (GI) and the brain are closely related systems, with the GI system able to improve memory and learning, reduce anxiety, and regulate stress levels (Magnano et al., 2012). This bi-directional relationship between the GI tract and CNS has been referred to as the 'brain-gut-axis' (Diaz Heijtz et al., 2011). The high co-morbidity between stress-related psychiatric disorders, such as anxiety and depression, and gastric disorders, such as irritable bowel syndrome (IBS) and inflammatory bowel disease (IBD), further strengthens this association (Magnano et al., 2012). Patients with MDD can also display altered GI function, increased oxidative stress, and abnormal nutritional status with deficits in multiple micronutrients, which demonstrates that nutrition must be considered in treatment strategies by clinicians managing these patients (Logan and Katzman, 2005).

Dietary supplements in general demonstrate a good safety profile, with minor adverse effects, especially when compared to other antidepressants and anti-inflammatory pharmacological alternatives. Hence they could be recommended as a safer treatment strategy, when indicated. Supplements can work in tandem with conventional antidepressants to help lessen their more unwanted side effects. For example, some antidepressants may increase blood pressure, and PUFAs may help to counteract these symptoms as they have been suggested to have anti-hypertensive effects (Cicero et al., 2009). Moreover, SSRIs have been associated with changes in bone metabolism, with an increased risk of hip fracture in females and loss of bone mineral density (Diem et al., 2007, Haney et al., 2007), whereas folic acid has been proposed as a supplement to help to decrease fractures and so could be considered in tandem with a more traditional approach (Swart et al., 2013). However, the most important use of dietary supplements is by a synergistic action in improving the antidepressant effects of other compounds, mainly through an anti-inflammatory action.

At present, the majority of clinical research related to inflammation in depression has been conducted on PUFAs, and so, this topic will encompass the largest portion of this review. However, consideration will also be given to probiotics and folic acid, which may prove, in coming years, to be as valuable.

2.1 Polyunsaturated fatty acids (PUFAs)

Polyunsaturated fatty acids (PUFAs) are omega-3 (n-3) fatty acids derived entirely from dietary sources (Burr and Burr, 1929), and include eicosapentaenoic (EPA) and docosahexaenoic acid (DHA) (McNamara, 2015). Their main function is to act as an essential component of membranes, maintaining their integrity and fluidity, and also to give rise to eicosanoids, which affect inflammation (Das, 2006). Eicosanoids from n-3 fatty acids reduce the synthesis of arachidonic acid (AA)-derived eicosanoids that are pro-inflammatory, and it is the balance between these fatty acids which maintains homeostasis in the immune system (as discussed in Fond et al., 2014). Indeed, consumption of n-6 PUFAs increases the production of pro-inflammatory cytokines, such as IL-1, TNF- α, and IL-6, and consumption of n-3 reduces the activity of these n-6 acids, therefore reducing the production of these cytokines (Calder, 2009, Calder, 2010). Moreover, inflammatory processes in the central immune system are partly mediated by microglial activation, and n-3 acids inhibit constitutive and lipopolysaccharide (LPS) -induced microglial activation, pro-inflammatory cytokine production and cyclooxygenase (COX) expression (McNamara, 2015). The therapeutic use of n-3 acids for inflammatory conditions has long been known, and EPA can be used therapeutically in hypertension, diabetes, psoriasis, eczema, coronary heart disease, atherosclerosis and cancer (Das, 2006).

Epidemiological studies

In the last century, the Western diet has shifted away from n-3 rich foods, such as fish and flax seed, and is now high in n-6 foods, such as soy and corn oils, with ratios as high as 17:1 reported in favour of n-6 foods, instead of 1:1 in the case of animals and prehistoric humans (Heinrichs, 2010). This large dietary change may partly contribute to the concomitant rise of inflammatory diseases in modern society, such as cardiovascular disease (CVD), and depression (Simopoulos, 2008).

Epidemiological evidence demonstrates that dietary fish consumption (a major source of n-3) reduces the risk of MDD, Seasonal Affective Disorder (SAD), bipolar disorder and post-partum depression (McNamara, 2015). In an analysis by Hibbeln (1998), a strong negative correlation between worldwide fish consumption and rates of MDD was reported from a cross-national depression database. Specifically, this has been demonstrated in the Mediterranean diet, where there is a decreased prevalence and incidence of depression, and the consumption of oily fish is higher (Rienks et al., 2013). This finding has also been reproduced in parts of Asia, like Japan and Korea, where the diet is higher in n-3 rich seafood. For example, a study by Yoshikawa et al. (2015) found that, in a relatively small sample of 500 Japanese participants, increased fish consumption was associated with resilience to depression. Another small study by Park et al. (2012), with Korean participants and controls,

demonstrated that the increased consumption of seafood and elevated erythrocyte levels of n-3 were associated with a decreased risk of depression.

Contradictorily, some studies have shown more mixed results, with no significant evidence that n-3 consumption reduces risk of depression/depressive symptoms. For example, a longitudinal study with more than 50,000 female participants between the ages of 50-77, and free from depressive symptoms at baseline, did not show a protective effect of n-3, obtained from a fish diet, on depression risk (Lucas et al., 2011). The same was also found in a study with almost 30,000 men in Finland, between the ages of 50-69 years, where no association was found between dietary intake of n-3 from fish and depressed mood, major depressive episodes or suicide (Hakkarainen et al., 2004). These epidemiological studies are limited however, as they do not allow for causality or for certainty on n-3 intake from diet, as this is mainly taken from self-reported measures.

A role of endogenous PUFAs levels in the pathogenesis of depression has also been supported by a few studies that have specifically measured them in the blood of depressed patients. For example, a recent study by Chang et al. (2015) has looked at the levels of n-3 in patients with CVD, and discovered that in sufferers with moderate depression (Hamilton Rating Scale for Depression (HAM-D) score more than 19) there were lower levels of DHA, n-3, and n-6:n-3 ratio than the non-depression group. This finding is mirrored in the general MDD population where lower concentrations of EPA and DHA are shown, with higher ratios of n-6 to n-3 acids when compared with healthy controls (Sontrop and Campbell, 2006, Deacon et al., 2015). A cross-sectional study correlating depressive symptoms with peripheral serum fatty acids and oxidative stress markers in females found that the depression scores were negatively correlated with concentration of n-3 acids, and positively correlated with IL-6 (Tsuboi et al., 2014). Two within-subjects studies found that lower baseline erythrocytes DHA levels and lower DHA plasma levels were correlated with an increased risk of developing depression during IFN-α treatment (a pro-inflammatory cytokine) (Lotrich et al., 2013). A study looking at enzymes which metabolise PUFAs (Phospholipase A2 (PLA2) and cyclooxygenase 2 (COX2)) also found interesting results (Su et al., 2010). In a sample of patients with hepatitis C, the effects of seven single nucleotide polymorphisms in COX2 and PLA2 genes on the development of depression during IFN-α treatment were examined. A subsample of the patients was assessed for the erythrocyte levels of DHA, EPA and AA. It was found that genetic variations in COX2 were associated with lower DHA levels, and PLA2 variations were associated with lower EPA levels. Both increased the risk of IFN-α-induced depression, with the PLA2 Banl GG polymorphism associated with more somatic depressive symptoms. Considering this evidence, it is not surprising that many studies have

looked at the potential antidepressant effects of PUFAs, either in the natural diet or as experimental supplementation.

Clinical trials of PUFAs

PUFAs have been used successfully in clinical trials looking at improving mood in depression and other psychiatric conditions, and it may be wise to use this more robust evidence when considering their efficacy.

N-3 fatty acids have been shown to be successful when used as an adjunctive therapy (Stoll et al., 1999). This double-blind placebo-controlled trial compared 9.6 g of PUFAs or placebo in addition to usual treatment for four months. The sample receiving PUFAs had a longer remission rate and performed better on the majority of outcome measures when compared with the placebo group. In a study by Nemets et al. (2002) 2 g of EPA were added to antidepressant medication for patients with MDD, all of whom demonstrated baseline scores of 18 or greater on the HAM-D. After 4 weeks, the mean reduction in the HAM-D scores was 12.4 points in the EPA group, compared with 1.6 in the placebo group. These results imply that n-3 may boost the antidepressant effects of traditional medication.

Moreover, meta-analyses which looked at n-3 fatty acid monotherapy trials in patients with MDD have noted a beneficial effect over placebo for the treatment of depressive symptoms (Grosso et al., 2014, Sublette et al., 2011, Lin and Su, 2007). Of note, is the first double-blind placebo-controlled trial using PUFAs as a monotherapy in antenatal depression, which found that significant differences between placebo and n-3 were only seen after 6 weeks, implying that we may need longer trials to note efficacy (Su et al., 2008). Monotherapy has also been used to good effect in a paediatric population. A small pilot study examined 20 children between the ages of 6-12, who had suffered from depression for an average of 3 months. The placebo-controlled trial lasted just over four months. The treatment cohort received 400 mg of EPA and 200 mg of DHA daily, and 7 out of 10 children had a greater than 50% reduction in the Children's Depression Rating Scale (CDRS), when compared to 0 out of 10 children achieving this in the placebo group (Nemets et al., 2006).

However, the success of these supplements does seem to be associated with the level of EPA, rather than DHA (Fond et al., 2014, Grosso et al., 2014, Song et al., 2016). The addition of solely EPA to a conventional antidepressant was first examined by Puri et al. (2001) in a therapeutic case study where it was shown that the dietary supplement had a positive effect on depressive symptoms. This may also explain the heterogeneity in the results of some clinical

trials, which can sometimes be contradictory (Martins, 2009). It has also been noted that the more severe the symptoms and the level of n-3 deficiency at the outset of treatment, the more dramatic the reduction of symptoms over time (Grosso et al., 2014). There is also evidence indicating that females may be more sensitive to n-3, with higher rates of intake predicting lower depressive symptoms (Colangelo et al., 2009, Caslake et al., 2008). The dosage of EPA would also appear to be important, as there has been variance amongst trials when this has been examined (Deacon et al., 2015). However, most studies seem to indicate that the minimum effective dose is approximately 200 to 2,200 mg of EPA/day (Grosso et al., 2014, (Sublette et al., 2011). A final point is one raised by Lesperance et al. (2011), that small significant effects may be overestimated. In their trial they found a marginal statistically significant difference between using 1,050 mg/d of EPA and 150 mg/d of DHA versus placebo, but the clinical benefit was minimal. However, there was a benefit for patients with depression without co-morbid anxiety. The reporting of clinical impact would need to be considered when other studies are reporting their findings. Overall, the data suggests that PUFAs do indeed have an impact on mood, but more research is needed to determine the level of EPA vs. DHA, dosage, treatment duration and what patient population it is most effective in.

Potential effects on stress- and inflammation-related mechanisms in depression

Some experimental studies have examined the potential mechanisms by which PUFAs exert an antidepressant effect. The ratio of n-6 to n-3 acids has been demonstrated to influence physiological stress responses in both depressed patients and healthy populations. For example, a study looking at the effects of supplementation with n-3 PUFAs on adrenal activation after an induced stressor (mental arithmetic and Stroop's test) in healthy men found that 3 weeks of fish oil intake resulted in elimination of stress-induced cortisol release and dampened increases in epinephrine (Delarue et al., 2003). This finding suggests that supplementation for individuals with MDD could have great impact in reducing their stress responses.

Most studies mentioned support the idea that n-3 acids hold anti-inflammatory properties and help to relieve depressive symptoms: but is this reduction in depressive symptoms due to a reduction in inflammation? A preclinical study using the olfactory bulbectomised rat model of depression demonstrated that rats which were fed with EPA, rather than the control sham diet, showed significantly decreased behavioural changes in the open field test and improved spatial memory compared with controls, indicating a normalization of behavioural changes induced by stress (increased locomotor and rearing activity, and impaired memory in the Morris water maze). They also showed reduced corticotrophin-releasing factor (CRF)

expression and corticosterone and IL-1β secretion (Song et al., 2009).

Clinical trials have also been conducted, which appear to give good evidence to the theory that PUFAs could be a recommended therapy specifically for treatment of inflammation in depression. In a 2-week, double-blind, placebo-controlled trial comparing EPA, DHA, and placebo in 162 patients receiving IFN-α, it was found that the incident rates of IFN-α induced depression were significantly lower in those treated with EPA, but not DHA, which echoes the findings mentioned earlier in this chapter, although, they both delayed the onset of depression versus placebo (Su et al., 2013). In another double-blind trial, 155 participants with depression were randomized to receive EPA, DHA or placebo, and their baseline biomarker data was also captured. It was found that subjects with high levels of IL-1ra, IL-6, high-sensitivity C-reactive protein (hs-CRP) or leptin, were more likely to respond to EPA and also reported the greatest decrease in HAM-D scores (Rapaport et al., 2016). Other populations with increased baseline inflammation may benefit from PUFAs supplements. This could be true, for example, for old-age subjects, since pro-inflammatory cytokine production is increased after menopause or andropause even without the presence of infection, stress or trauma. In a study examining the ratio of n-6:n-3 and depressive symptoms and pro-inflammatory cytokine synthesis in adults aged 40-86 (mean age of 66.67) it was found that higher levels of pro-inflammatory cytokines were associated with an increase in depressive symptom severity and also higher n-6:n-3 ratios (Kiecolt-Glaser et al., 2007). Other conditions associated with chronic sub-threshold inflammation such as obesity, which is also strongly associated with increased depression risk (Shelton and Miller, 2011), would need to be considered by future research with respect to PUFAs supplementation. This is in the context of the strong evidence showing that supplementary treatment with n-3 can help to balance inflammation and reduce the incidence of mood dysregulation.

In conclusion, the main benefit of PUFAs, and the reason to continue pursuing larger scale randomized controlled trials (RCTs), is their favourable safety profile and lack of adverse reactions, including reactions with concomitant medications. For instance, the most common adverse effect reported for PUFAs is gastrointestinal disturbance (Holub, 2002). Although, the possibility of some supplements containing toxic levels of mercury or vitamin A must be taken into consideration, this risk is negligible in reality. As a result, these supplements would be highly appropriate as a treatment of depression for paediatric populations and also during pregnancy (Su et al., 2008). Moreover, it must be noted that throughout this section the issue of heterogeneity between trials has been mentioned. This issue was discussed further in a meta-analysis conducted by Bloch and Hannestad (2012), where they found significant heterogeneity and publication bias in the thirteen studies they examined. Worryingly,

evidence for the benefits of n-3 was removed when publication bias was adjusted for using the trim-and-fill method. This supports the need for greater attention to the design of RCTs to get a clearer picture of the efficacy of n-3. In this context, the observation that greater efficacy was present when participants showed more severe baseline depression symptoms, should be further examined and explored in future research.

2.2 Probiotics

Probiotics, which have been coined 'psychobiotics', are defined as a live organism which, when consumed, can have beneficial effects for those suffering from psychiatric disorders (Dinan et al., 2013). The use of probiotics in psychiatric research is still in its infancy, its use as an adjunct therapy in depression being first proposed by Logan and Katzman (2005). Moreover, it has been shown that certain bacteria produce neurochemicals relevant to depressive symptoms; for example, strains of *Lactobacillus* and *Bifidobacterium* secrete gamma-aminobutryic acid (GABA), an inhibitory neurotransmitter, which is implicated in anxiety and depression, and this has been suggested to have an effect on the *brain-gut axis* (Schousboe and Waagepetersen, 2007). Other bacteria have also been shown in mice to affect serotonin plasma levels and to produce dopamine and norepinephrine (Collins and Bercik, 2009, Lyte, 2011). The *brain-gut axis* is connected predominantly via the vagus nerves which perform a direct connection between the brain and gut and transmits all hormonal, neuronal, and bacterial changes from the intestines (Evrensel and Ceylan, 2015).

Clinical studies

The disorder that has received the most treatment with probiotics to date has been IBS, which is well documented to be associated with depression and anxiety. This has been studied with successful results in both the disease-specific and mood symptoms, and also with a parallel reduction in pro-inflammatory cytokines in the blood (Clarke et al., 2009, O'Mahony et al., 2005). In a study with sufferers of chronic fatigue syndrome (CFS) consuming a probiotic, it was found that there was a significant improvement in anxiety (Rao et al., 2009). Two-month supplementation with probiotics also demonstrated improvements in inflammatory markers and oxidative stress in pregnant women and patients with type 2 diabetes mellitus (Asemi et al., 2013, Asemi et al., 2012). This improvement in well-being was also echoed in a study with healthy subjects consuming a probiotic yogurt for a 3-week period (Benton et al., 2007): the third of the sample with the lowest baseline mood reported themselves happy rather than depressed after the study was completed. Moreover, in a study by Mohammadi et al. (2015) consuming a probiotic yogurt or multispecies probiotic supplement for six weeks showed improvements in mental health.

To date, there has been one RCT conducted which examines the effects of probiotics on depression and inflammation. This study by Akkasheh et al. (2016) examined 40 patients with MDD receiving a probiotic supplement (*Lactobacillus acidophilus*, *Lactobacillus casei*, and *Bifidobacterium bifidum*) or placebo for 8 weeks. The authors found significantly decreased Beck Depression Inventory (BDI) scores, oxidative stress and serum hs-CRP concentrations for the participants receiving the probiotic when compared to the placebo group. In addition, while there is a need for more large-scale placebo-controlled trials with probiotics in depression, there have been other studies that have produced some interesting results. For example, a double-blind, placebo controlled randomized parallel group study using healthy human volunteers for 30 days showed that probiotic consumption reduced psychological distress on several scales (Hopkins Symptom Checklist (HSCL-90), the Hospital Anxiety and Depression Scale (HADS), the Perceived Stress Scale (PSS) and the Coping Checklist (CCL), and reduced urinary free cortisol (UFC) levels (Messaoudi et al., 2011).

Potential mechanisms and preclinical studies

As previously mentioned, depression is associated with the presence of biomarkers of inflammation, and in rodent studies it has been shown that stress alters gut-barrier function, with leaked products stimulating Toll-like receptors (TLRs) and resulting in the production of inflammatory cytokines (Ait-Belgnaoui et al., 2012). This has also been demonstrated in rhesus monkeys. Postnatal stress, induced by the separation of the infants from their mothers, altered the microbiota and reduced *Bifidobacterium* and *Lactobacillus* levels (Bailey and Coe, 1999). Dinan et al. (2013) suggested that this might partly explain the pro-inflammatory phenotype, which has been discussed above. The intestinal microbiota also performs an integral role in the immune system; the interaction between the gut mucosa and microbiota balances the production of pro-inflammatory cytokines such as IL-8, IL-1, IL-10 and TGF-β (Neish, 2009). A preclinical study with rats treated with *Bifodobacteria Infantis* for 14 days, and submitted to the forced swim test, found that there were no behavioural effects (Desbonnet et al., 2008). However, there was a significant attenuation of IFN-γ, TNF-α, and IL-6, and an increase in plasma concentrations of tryptophan (a serotonin precursor) and kynurenic acid when compared with controls. Moreover, in another study, rats separated from their mother were placed separately in two groups, and treated with *Bifodobacteria Infantis* or citalopram (Desbonnet et al., 2008, Desbonnet et al., 2010). Plasma cytokine levels, brain monoamine levels, and central and peripheral HPA hormone levels were measured, and depressive behaviours were examined using the forced swimming test. Treatment with the probiotic was found to reverse depression-like behavior, and to decrease peripheral IL-6.

Interestingly, the immunoregulatory action of probiotics is proposed to work in the same manner as conventional antidepressants (Forsythe et al., 2010). For example, traditional antidepressants have been suggested to, in addition to their actions on the serotonergic system, suppress inflammation by increasing production of the cytokine IL-10 (Maes et al., 2005). Probiotics also increase levels of IL-10, but it is unknown if this increase in IL-10 has any effect on mood ((Wall et al., 2010, Smits et al., 2005). Likewise, successful antidepressant treatment affect the HPA-axis in depressed patients, and hyper-response of the HPA-axis found in germ free mice can be reversed with the use of a probiotic, and in these animals it is also found that levels of norepinephrine (NE) and 5-HT are also significantly reduced (Neufeld et al., 2011).

In conclusion, probiotics may prove to be a viable treatment for depression due to their psychoactive and anti-inflammatory properties, changing the gut barrier function and ultimately reducing depressive symptoms. Indeed, Dinan et al. (2013) suggests antibiotics such as minocycline have been shown to have an effect on depressive symptoms and also on inflammation by altering the microbiota. This further strengthens the probability that changes in the gut and inflammation may influence the mood. Other research has also shown that probiotic fortified laboratory chow increases the tissue levels of n-3, which is important given the effects of n-3 demonstrated above (Wall et al., 2010). Probiotics may also favor the formation of folate, the effects of which will be discussed in further detail below (Kanmani et al., 2013). This points to the possibility that dietary supplements could be used in tandem to boost each other's effects. The promising research presented in this section merits further investigation with more full-scale randomized placebo-controlled trials examining the effects of probiotics on inflammation in depression. Various strains of probiotics have been used to date, and it is still unknown which the most effective are. Probiotics also have a good safety profile with few side effects and may work well with other treatments for depression. This would be especially relevant if antibiotics such as minocycline are used more regularly in future for the treatment of inflammation in depression. They may help to balance any effects which minocycline would have on intestinal flora while helping to boost the antidepressant effects.

2.3 Folic Acid

Folic acid, or folate, is a B vitamin, and must be supplied through dietary consumption in order for humans to meet their daily requirements. Foods such as beans and legumes, leafy green vegetables and oranges are good sources. It is necessary for the synthesis and repair of DNA and as a co-factor in enzymatic reactions (Weinstein et al., 2003). It has also been

shown that folic acid deficiencies can be associated with psychiatric conditions such as depression, irritability, altered sleep, and altered cognitive functioning, with these conditions responding to folate therapy (Botez et al., 1979, Manzoor and Runcie, 1976, Gilbody et al., 2007, Gaweesh and Ewies, 2010). Lower folate levels in patients with depression have also been linked to lower rates of treatment response to pharmacological interventions (Fava et al., 1997). This was shown in a study with depressed geriatric patients with low folate levels taking either sertraline or nortriptyline. They found that the higher baseline folate levels predicted greater treatment response on the Profile of Mood States (POMS); a self-report scale (Alpert et al., 2003).

It has been proposed that folic acid supplementation may result in an antidepressant-like effect, with a reduction in norepinephrine secretion and increased serotonin activity (Lucock et al., 1995, Botez et al., 1982). This would tie in with folate's importance in the methylation of homocysteine, for its conversion to S-adenosyl-methionine (SAMe), which has been shown to influence serotonin metabolism and have some antidepressant effects (Bottiglieri et al., 1984). However, a meta-analysis looking at RCTs using folic acid as an augmentation to antidepressants found that its use may be effective, but would not suffice as a replacement for conventional antidepressants. They were also unable to assess whether this effectiveness was particularly evident in those with low folate, as the studies had not taken a baseline reading from participants (Taylor et al., 2004). Therefore, endogenous levels of folate would need to be considered in future research.

Folate has also been found to have an impact on the immune system, or rather, a deficiency in folate has been found to be associated with higher levels of pro-inflammatory cytokines. Folate levels are a determinant for plasma homocysteine, and increased levels of homocysteine are considered a marker for folate deficiency (Nagele et al., 2011). High levels of homocysteine make endothelial cells more prone to injury, which in turn leads to inflammation in the blood vessels and this may act as a risk factor for atherogenesis, coronary artery disease and stroke (De Bree et al., 2002). It has also been shown that folic acid treatment for hyperhomocysteinemia reduced the levels of both homocysteine and pro-inflammatory cytokines (Wang et al., 2005). In a study looking at B-vitamin deficiency as a risk factor for vascular disease, it was found that *in vitro* folate deficiency in mouse cells (achieved by growing the mouse monocyte cell line RAW264.7 under folate restriction) increased the release of pro-inflammatory cytokines IL1-β, IL-6, TNF-α and MCP-1 2-to 3-fold (Kolb and Petrie, 2013). This study also showed that nitric oxide (NO) production was attenuated by folate deficiency and, in contrast with other findings; these changes were independent of the concentration of homocysteine. This last study suggests that folate levels

rather than homocysteine are responsible for attenuation of inflammation.

To date, there have been few studies linking the anti-inflammatory effect of folic acid with mood changes in depression. One study by Resler et al. (2008) compared adjunctive therapy of folic acid or placebo with fluoxetine. Patients receiving folate had a significantly lower depression score on the HAM-D after 6 weeks than the placebo group, and also plasma homocysteine was significantly decreased, with a significant negative correlation between homocysteine and folate. Although this only provides indirect evidence, a reduction in homocysteine levels is likely to influence immune function, as discussed above. Finally, it has been shown that folic acid can enhance concentrations of n-3 acids, which again can have an anti-inflammatory action, as discussed earlier in this section (Das, 2008).

In conclusion, while folate has been found to have some effects on inflammation and on depressive symptoms, further research is needed to determine whether these effects on depression could be associated with a reduction in inflammation. It would also be important to measure baseline levels of folate as it has been shown that this may have an effect on the improvement in depressive symptoms.

3. Final remarks and future research

For future consideration, there is great potential to design and implement novel treatments for depressive subtypes. The dietary supplements discussed in this chapter have been shown to work well in conjunction with traditional approaches, and also on their own, but they should also be considered in tandem with one another, as all of these compounds seem to directly or indirectly have an anti-inflammatory action: this symbiosis ought to be considered in future trials. Inflammatory biomarkers offer a gateway towards personalized medicine, and as a result not only will we be better equipped to understand the mechanisms underpinning MDD, but we may also be able to predict response rates for the novel agents described.

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