The Role of Micronutrients in Internalising Symptoms

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Abstract

Recent research suggests that diet quality and mental health are associated. However, the relationship between specific nutritional aspects of diet, such as micronutrient intake, and mental health still remain relatively unconfirmed. This thesis first provides a critical review of theoretical and empirical literature that has examined the role of micronutrients in internalising disorders. Overall, there is epidemiological evidence of an association between specific micronutrient deficiencies, such as zinc and folate, and depressive symptoms. However, evidence from studies examining other micronutrients, such as vitamin C or magnesium, have been tentative. In particular, there is less available evidence supporting an association between micronutrients and anxiety symptoms. In order to further investigate these relationships, a cross-sectional study was performed examining micronutrient intakes and internalising symptoms in undergraduate university students. Results from the study are presented in light of recent epidemiological research and current theoretical models.

Statement of Candidate

I hereby confirm that all material contained in this project is my original authorship and ideas, except where the work of others has been acknowledged or referenced. I also confirm that the work has not been submitted for a higher degree to any other university or institution. The research project was approved by the Macquarie University Human Research Ethics Committee (Approval No. 5201600183).

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Acknowledgements

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Micronutrients and Internalising Disorders: A Review of the Literature

Acronyms

BDNF – Brain-derived Neurotropic Factor

BMI – Body Mass Index

DASS-21 – Depression Anxiety Stress Scale

GAD – Generalised Anxiety Disorder

MDD – Major Depressive Disorder

PHQ-9 – Patient Health Questionnaire

RDI – Recommended Dietary Intake

SAM - S-adenosylmethionine

SES – Socioeconomic Status

SSRI – Selective Serotonin Reuptake Inhibitors

Abstract

Recent prospective research has shown that healthy dietary patterns, such as those consisting of higher intakes of fruit, vegetables, and whole foods, may reduce the risk of developing internalising disorders. However, the biochemical and nutritional pathways involved still remain unclear. This review considers the potential role of micronutrients in attenuating the pathogenesis of internalising disorders. Existing experimental and observational literature that examined a role of zinc, B-group vitamins, vitamin C, and magnesium in depressive and anxiety disorders is critically reviewed. Evidence from observational studies supports an association between several micronutrients and symptoms of internalising disorders. However, at present there is a paucity of research examining the role of micronutrients in anxiety disorders. Increasing research from clinical trials has shown that micronutrient supplementation may assist in reducing depressive symptoms when used as adjunctive therapies. However, more research is needed to confirm the therapeutic role and efficacy of increasing micronutrient intake in internalising disorders. To date, the majority of research has been limited to cross-sectional research. More research is therefore required to further explore the potential clinical utility of micronutrients in assisting symptom remission.

Micronutrients and Internalising Disorders: A Review of the Literature

Introduction

It is estimated that at least 30% of the global human population is deficient in essential micronutrients such as vitamins and minerals (Ramakrishnan, 2002). Adequate nutrition represents an important aetiological component of many physical illnesses. The dietary intake of micronutrients has been shown to prevent serious physical illness, for instance, cardiovascular disease and diabetes (Park & Lee, 2011). To date however, the role of micronutrient deficiency in heightening the risk of developing mental disorders still requires confirmation.

According to epidemiological research, the regular consumption of unhealthy foods, such as junk foods or highly processed and refined foods, is increasing, particularly in young populations (Adair & Popkin, 2005). The diets of young adults, particularly university students living away from home, has been shown to be of poorer nutritional quality compared to those living with their family (Larson et al., 2011; Papadaki et al., 2007).

Micronutrient deficiencies are a common feature of 'Western' diets (i.e. high intakes of processed and refined foods) in comparison to more 'traditional' dietary patterns, such as those consisting of higher consumptions of whole and natural foods (Carrera-Bastos, Fontes, O'Keefe, Lindeberg, & Cordain, 2011). Recent research has shown that healthy dietary patterns are associated with a lower incidence of internalising symptoms (Jacka et al., 2010, 2011a, 2011b, 2013a, 2013b; O'Neil et al., 2014; Sanchez-Villegas et al., 2009; Sanhueza, Ryan, & Foxcroft, 2013). For instance, Jacka et al. (2010) found that Western diets were associated with increased risk of developing depressive disorders, whereas more traditional dietary patterns are associated with a lower incidence of depression in adult participants.

There is evidence to suggest that the dietary intake of specific micronutrients, such as zinc, magnesium, and B-group vitamins, are inversely associated with depressive symptoms (Jacka et al., 2012; Maes et al., 1997; Maserejian et al., 2012; Murakami et al., 2008, 2010; Roozbeh et al., 2011; Stanisławska et al., 2013; Sanchez-Villegas et al., 2009; Yary & Aazami, 2011). However, what specific micronutrient deficiencies are associated with an increased risk of anxiety and depressive disorders respectively still remains relatively unclear.

Several psychobiological theories have been proposed to explain the biochemical abnormalities evident in internalising disorders, such as depression and anxiety. For instance, inflammation and heightened immune function have been identified as psychophysiological features of depressive disorders (Dale et al., 2015; Maes, 1993, 2011; Maes et al., 1992, 1996, 1997; Postolache, 2012; Serafini, 2012). Similarly, dysregulation of neurotransmitters, such as serotonin and GABA, has been observed in both depressive and anxiety disorders (Baumgartner et al., 2012; Guilarte, 1993; Kruiemann et al., 1987). Additionally, specific micronutrients have been shown to be essential for the regulation of immune function (Szewczyk, Kubera, & Nowak, 2011), effective inflammatory processes (Fraker & King, 2004; Szewczyk et al., 2011) , and neurotransmitter synthesis (Georgieff, 2007). Thus it is possible that micronutrients may play a role in the aetiology of mental disorders. Given that most mental disorders begin before adulthood, further exploration of the role of micronutrients in psychopathology, particularly in early life, is important as these may be used to identify potential individuals at risk and to modify risk.

In this review, I aim to critically evaluate the existing observational and experimental literature exploring the role of micronutrients in the development and maintenance of internalising disorders in children, adolescents, and adults. The review firstly provides an overview of psychophysiological models of depressive and anxiety disorders that are relevant to micronutrients. It then includes a comprehensive critique of the existing literature,

including cross-sectional studies and clinical trials involving specific micronutrients and internalising disorders.

<div style="text-align:center">An Overview of Theoretical Models</div>

Micronutrients provide a range of essential biochemical functions, which include supporting neurotransmitter synthesis, hormonal regulation, immune function, and anti-oxidation. Similarly, these processes have been shown to be impaired in patients with depressive (Dale et al., 2015; Maes, 1993, 2011; Maes et al., 1992, 1996, 1997; Postolache, 2012; Serafini, 2012) and anxiety disorders (Baumgartner et al., 2012; Guilarte, 1993; Kruiemann et al., 1987). Figure 1 provides a summary of micronutrient functions that may support the psychophysiological processes of internalising disorders (Figure 1).
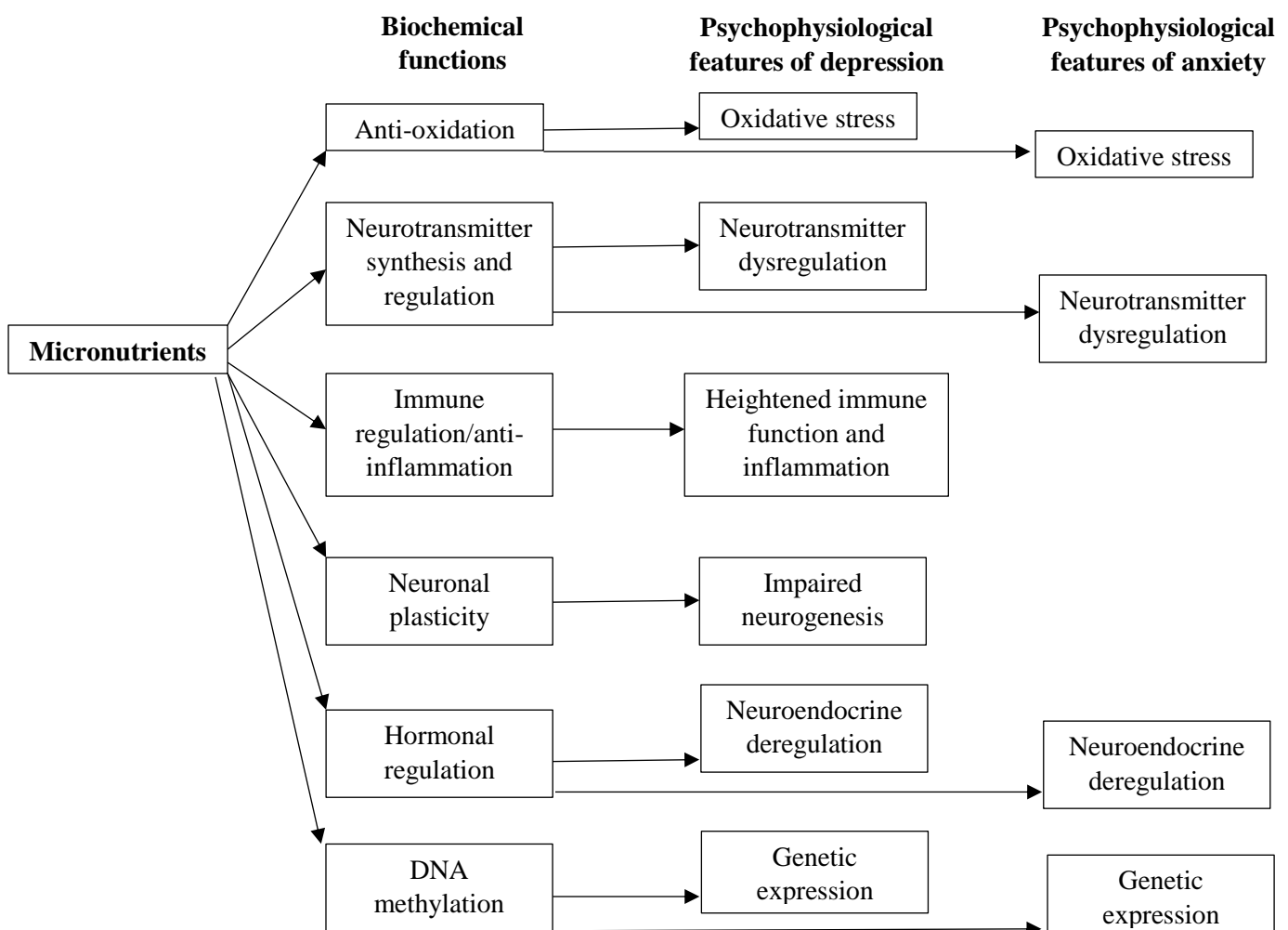
Figure 1. Summary of the biochemical functions of micronutrients and potential therapeutic applications in internalising disorders.

Immunity, inflammation, and the HPA-axis. There is strong evidence to suggest that depressive disorders are associated with heightened immune function, HPA-axis hyperactivity, and inflammation (Maes, 1993, 2011; Maes et al., 1992, 1996, 1997; Miller et al., 2003; Postolache, 2012). Results from animal studies have revealed that rats raised on a high-fat (pro-inflammatory) diet demonstrate more depressive-like behaviours compared to those that received a balanced diet (Abildgaard et al., 2011). Additionally, a longitudinal study in humans found that high inflammatory diets significantly predicted the risk of developing a depressive disorder across a 12 month period. Despite support from prospective studies regarding inflammation and depression, the *causal* pathways of these intricate relationships still remains disputed. For example, O'Brien et al. (2006) found that antidepressant therapy reduced inflammatory markers, but did not simultaneously alter depressive symptoms. Such results suggest that depressive symptomatology may be independent of inflammation. Nevertheless, research supporting the causal pathway between inflammation, immune status, and depressive symptoms has not been well established to date.

Micronutrients may support imbalanced psychophysiological processes that are associated with depressive disorders. For instance, the dietary intake of specific anti-inflammatory micronutrients, such as zinc, has been associated with a lower incidence of depressive symptoms in community samples (Yary & Aazami, 2011; Jacka et al., 2012), and lower levels of inflammation (Nowak et al., 2003; Szewczyk et al., 2011). As such, it has been theorised that diets high in fundamental nutrients may therefore protect against the onset of depressive disorders, and could even assist in symptom remission by modulating and supporting immune function (Maes et al., 2012; Szewczyk et al., 2011). Yet, more research is

required to confirm whether reducing inflammation and supporting immune function can actually cause a reduction in depressive symptoms.

Heightened inflammation and immune function has also been associated with anxiety symptoms in adults (Pitsavos et al. 2006). However, past research has not established whether pro-inflammation causes increased vulnerability to developing anxiety disorders, or whether it is a symptom of the disorder itself. As such, the causal pathways between immune function and internalising disorders are still disputed in current research.

Neuronal plasticity. Neuronal plasticity refers to the process through which neurons adapt through learning or conditioning (Berlucchi & Buchtel, 2009). This process is fundamental to learning and remembering new information through the modification of neural networks. Additionally, an important component and index of neuronal plasticity is Brain-Derived Neurotropic Factor (BDNF), which functions to support the survival and growth of neurons.

Depressive disorders have been associated with reduced neuronal plasticity (Dale et al., 2015; Serafini, 2012) and BDNF levels (Karege et al., 2002). For example, impaired neurogenesis, or the growth and development of nerves and dendritic abnormalities, has been observed in patients with Major Depressive Disorder (MDD) when compared with healthy control participants (Dale et al., 2015; Serafini, 2012). This pathway has further been supported by research showing that prolonged stress causes increased glutamate release (Musazzi, Racagni, & Popoli, 2011), resulting in neuronal damage (McClelland et al., 2011; Packan & Sapolsky, 1990), and reduced hippocampal volume in patients with depression (Videbach & Ravnkilde, 2015). Additionally, a meta-analysis of twenty studies concluded that BDNF levels are not only altered in MDD, but that antidepressant treatment may increase BDNF and support neuronal plasticity (Brunoni, Russowsky, Lopes, & Fregni, 2008).

Specific micronutrients are essential in supporting neuronal plasticity, particularly during early life when brain structures are still forming (Mattson & Shea, 2003). For instance, Solati et al. (2015) found that zinc monotherapy significantly increased Brain-Derived Neurotropic Factor (BDNF) levels in overweight participants and improved mood. Additionally, in early life, it is well established that prenatal folate deficiency increases the risk of developing neural tube defects (Lucock, 2000). Similarly, Carlson et al. (2009) showed that the regular intake of iron supported neuron development in rodents, and a number of animal trials found that diets high in fat and refined sugar and low in nutrients reduced neuronal plasticity and impaired learning abilities (Molteni, Barnard, Ying, Roberts, & Gómez-Pinilla, 2002). Thus it is plausible to suggest that dietary enhancement of micronutrients may also target this underlying psychophysiological characteristic of depressive disorders through enhancing neuronal plasticity.

Neurotransmitter dysregulation. It is well established that depressive and anxiety disorders are associated with disruptions in neurotransmitter levels, such as serotonin and GABA (Albert, Vahid-Ansari, & Luckhart, 2014; Carlsson, Corrodi, Fuxe, & Hökfelt, 1969; Haase & Brown, 2015; Krishnan & Nestler, 2008; Schildkraut & Kety, 1967). For instance, the dysregulation of GABA and glutamate neurotransmission have been identified in people with MDD (Pehrson & Sanchez, 2015; Sanacora, Treccani, & Popoli, 2012) and Generalised Anxiety Disorder (GAD) (Martin, Ressler, Binder, & Nemeroff, 2010).

Research has shown that one of the major actions of micronutrients is in neurotransmitter synthesis and regulation (Baumgartner et al., 2012; Guilarte, 1993; Kruiemann et al., 1987). Specifically, micronutrients such as B-group vitamins, vitamin C, and zinc, are critical for the formation of monoamines (i.e. serotonin, dopamine, and noradrenaline) through their role as co-factors in enzymatic reactions (Baumgartner et al., 2012). Similarly, Kuriemann et al. (1987) showed that vitamin B6 supplementation increased

GABA levels in humans. As such, adequate micronutrient intake may also support the formation and maintenance of neurotransmitters that have been implicated in internalising disorders. Consequently, further research confirming these relationships is now needed.

Oxidative stress. Free radicals are highly reactive molecules that cause cellular and molecular deterioration. At healthy levels, free radicals play an essential role in cellular signalling and supporting immune function (Ng, Berk, Dean, & Bush, 2008). However, oxidative stress occurs when the body can no longer counter free radical levels and subsequent deterioration caused to the system. The oxidative stress hypothesis posits that neurobiological deficits evident in psychiatric disorders are best explained by high levels of oxidative damage, causing damage and disruptions in biochemical and neurotransmitter levels (Ng et al., 2008). Oxidative stress has been shown to be a key characteristic of not only depressive (Galecki, 2014; Maes, 2008; Maes et al., 2011, 2012) and anxiety disorders (Bouayed, Rammal, & Soulimani, 2009), but also a range of other psychiatric conditions (Ng et al., 2008).

One way free radical damage can be ameliorated is through dietary antioxidants. Several micronutrients have strong antioxidant properties (Evans & Haliwell, 2001). Specifically, vitamins C and E, zinc, magnesium, and selenium function as enzymatic antioxidants in the body (Evans & Haliwell, 2001). Thus it is plausible to suggest that dietary antioxidants have a central role in combating damage caused by oxidative stress to cells and neurons. Though at present these pathways require empirical confirmation.

Gene-Nutrient Interactions - DNA Methylation and Internalising Disorders. One way micronutrients may interact with genes is through epigenetic gene regulation, a mechanistic process by which nutrition and environmental influences interact with the genetic expression of disease (Anderson, Sant, & Dolinoy, 2012). A recent theory has proposed that nutrients interact with genes through DNA methylation, the process used by

individual cells to control the expression of particular genes, or phenotypes. According to the theory, DNA methylation occurs through o*ne carbon metabolism*: a critical methylation process that involves the transfer of methyl groups between molecules (Anderson et al., 2012).
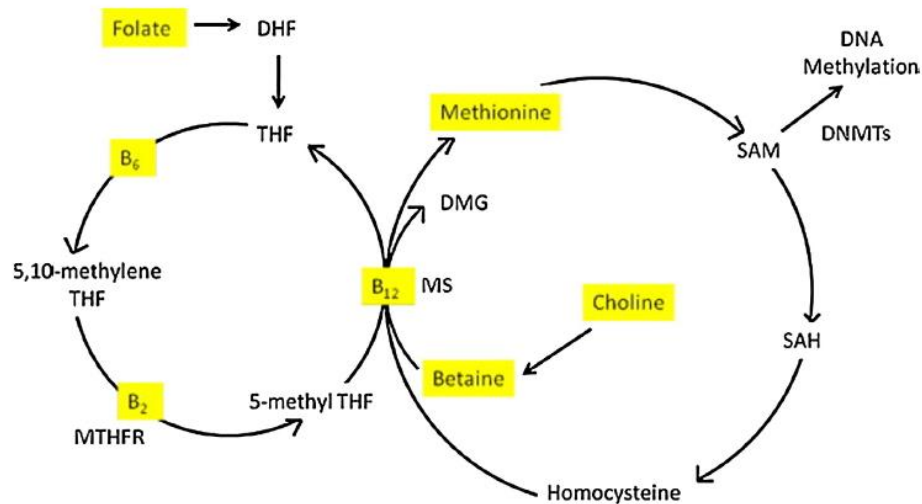

Figure 2. Simplified illustration of the one carbon metabolism pathway. Adapted from Anderson et al. (2012) and Kofnik et al. (2013).

As depicted in Figure 2, the one carbon metabolism pathway relies primarily on the constant availability of specific micronutrients (Kofnik et al., 2013). This pathway is cyclical and regenerates according to the availability of methylfolate (activated folic acid), vitamins B6, B2, B12, as well as choline and betaine (Anderson et al., 2012). The availability of these micronutrients is thus required to produce S-adenosylmethionine (SAM), which is critical for over 40 biochemical reactions in the body. In addition, the one-carbon metabolism process metabolises homocysteine, an amino acid, which is toxic to cells and neurons at elevated levels. Impaired one carbon metabolism, as derived through dietary micronutrient deficiency or impaired metabolism, may therefore lead to reduction in SAM formation, heightened homocysteine levels, and impaired DNA methylation. Therefore, it is theorised that such disruptions to the DNA methylation process lead to psychopathological gene expression (Anderson et al., 2012; Kofnik et al., 2013).

There is preliminary support for the association between disrupted one carbon metabolism and depressive disorders in recent research. For instance, heightened homocysteine levels and lowered SAM serum concentrations have been reported in patients with MDD compared to healthy controls (Bjelland et al., 2003; Bottiglieri, 2005; Bottigileri et al., 2000). Papakostas et al. (2004) also found that lower serum folate levels were associated with a poorer response to an SSRI treatment in patients with MDD. Additionally, Kim et al. (2008) found that lower baseline folate and heightened homocysteine levels were associated with a heightened risk of developing depression over a 2-3 year period. Yet, the relationship between one carbon metabolism and anxiety disorders has not been as strongly supported. In support of this, Bjelland et al. (2003) found heightened homocysteine levels were associated with depressive but not anxiety symptoms in 5948 adult participants. Almeida et al. (2006) also found no associations between homocysteine levels and anxiety symptoms. Although impaired one carbon metabolism may be related to depression, the role of gene-nutrient interactions in other internalising disorders such as anxiety is still unconfirmed at present.

The Relationships between Micronutrients and Internalising Disorders

As shown in Table 1 (Appendix A, p. 46), several lines of research have shown that specific micronutrient intakes and levels, including zinc, B-complex vitamins, magnesium, and more recently, vitamin C, and vitamin E, are associated with internalising symptoms in adults (for a review see Swardfager et al., 2013). Although several other micronutrients have been implicated in neurochemical processes, such as iodine or betaine (Georgieff, 2007), to date research has not examined the role of these micronutrients in internalising disorders. The following section provides a critical review of observational research followed by clinical trials that have examined micronutrients and internalising symptoms.

Zinc

Observational studies. Zinc is an essential mineral that assists in immune and neurological functioning. There is convincing research showing that zinc serum levels and dietary intake are associated with depressive disorders. For example, three cross-sectional studies have shown that dietary intake of zinc is inversely associated with depressive symptoms (Maserejian et al., 2012; Yary & Aazami, 2011; Jacka et al., 2012). Similarly, three studies have shown that zinc deficiency is associated with depressive disorders (Roozbeh et al., 2011; Stanisławska et al., 2013; Maes et al., 1997). A review conducted by Swadfager et al. (2013) examined 17 correlational studies examining serum (blood concentration levels) zinc and depressive symptoms. It was concluded that there was a strong association between serum zinc and depressive symptoms. However, two longitudinal studies have found contrary results (Lehto et al., 2013; Vashum et al., 2014). Lehto et al. (2013), for example, demonstrated that zinc intake at baseline did not increase the risk of developing a depressive disorder at a 20 year follow-up in men. Alternatively, Vashum et al. (2014) examined both men and women and found that a higher intake of zinc was associated with less risk of developing a depressive disorder.

Only one study has established support for the role of zinc in anxiety disorders (Nahar et al., 2010). Nahar et al. (2010) found that serum zinc level was significantly lower among patients with panic disorder compared to healthy controls. However, other research has shown that zinc intake was not associated with symptoms of anxiety (Jacka et al., 2012).

Clinical trials. Results from clinical trials suggest that zinc supplementation may assist in the reduction of depressive symptoms. Three randomised placebo-controlled trials have demonstrated support for the use of zinc supplementation in enhancing antidepressant efficacy in adult populations (Nowak et al., 2003; Siwek et al., 2009; Ranjbar et al., 2014). Ranjbar et al. (2014) demonstrated that zinc adjunctive supplementation, in addition to antidepressant treatment, significantly reduced depressive symptoms, but no significant effect

on the immune system was identified (measured via interleukin levels). Such results do not support the immune dysregulation hypothesis of depression, whereby promotion of immune regulation would be associated with a reduction in depressive symptomatology. In contrast however, Solati et al. (2015) found that zinc monotherapy significantly increased Brain-Derived Neurotropic Factor (BDNF) levels in overweight participants and improved mood. This would suggest that improvements in mood may be associated with improvements in immune function following zinc supplementation.

Only two trials have examined the effect of zinc supplementation on internalising symptoms in young populations (Digirolamo et al., 2010; Sawada & Yokoi, 2010). Sawada and Yokoi (2010) found that receiving 7mg of zinc daily for 10 weeks significantly reduced depression in young women (mean age 19). However, Digirolamo et al. (2010) found no significant differences between zinc supplementation (5mg/d for one week) and placebo on depression and anxiety ratings of children aged 6-10 years old. It was also observed that increases in serum zinc were inversely associated with depression and anxiety scores (Digirolamo et al., 2010). The effect of zinc supplementation may therefore change according to different developmental periods.

Summary

Despite mixed findings from prospective studies, there is strong overall support for the notion that zinc serum concentrations and intake are concurrently associated with depressive symptoms. Clearly the question of whether zinc deficits can cause depression cannot be determined from current research. As such, it still remains unclear whether zinc has potential anxiolytic (anti-anxiety) and antidepressant effects when used solely as a nutrient monotherapy, although such results have been found in animal trials (Partyka et al., 2011). Additionally, there is evidence supporting the use of zinc in enhancing antidepressant efficacy.

The present state of research regarding zinc and anxiety symptoms is mixed. Further research is needed to determine whether zinc plays an important role in the aetiology of panic disorder and especially whether these findings can generalised to other anxiety disorders.

The available research utilising youth samples suggests that there may be developmentally important biochemical changes in the effects of zinc on internalising psychopathology. In support of this view, a review by Georgieff (2007) concluded that zinc intake was essential for brain development, particularly with regard to mediating brain proteins, biochemistry, and neuronal growth in the formative years. Therefore, future research could examine the role of zinc in attenuating internalising psychopathological vulnerability across different developmental periods.

B-group Vitamins

Observational studies. B-complex vitamins are important for a range of functions including generating energy from carbohydrates, supporting immune function, and neurotransmitter regulation (Higdon & Drake, 2003). As with zinc, there is substantial research supporting an association between the dietary intake of B vitamins and depressive disorders in adults. In particular, dietary intake of vitamins B6, folate, and B12 have been inversely correlated with depressive symptoms in eight studies (Herbison et al., 2012; Sanchez-Villegas et al, 2009, Yary, 2013; Payne et al., 2009; Murakami et al., 2008; Bell et al., 1991; Jacka et al., 2012; Gendall et al., 1999). Alternatively, only one study has found that intakes of vitamins folate, B6, and B12 were not correlated with depressive symptoms in adults (Kamphuis et al., 2008). Conversely, longitudinal research has shown that dietary intakes of B6, folate, and B12 significantly decreased the risk of depression at 2.4 and 12 year follow–ups respectively (Kim et al., 2008; Sharupski et al., 2010). However, this relationship may be moderated by sex. For example, Sanchez-Villegas et al. (2009) found that lower intakes of B12 were associated with depression in women but not men, whereas

folate intake was associated with depression in men only. Similarly, lower folate intakes and increased depressive symptoms have also been found in men but not women (Murakami et al., 2008). Such results have two important implications. First, that the dietary intake of B-group vitamins may ameliorate the risk of developing depressive disorders in adulthood. Second, that the neurobiological features of depression may be different for men and women, particularly with regard to micronutrient absorption.

Only two studies have found similar associations in child and adolescent populations (Murakami et al. 2010; Tsuchimine et al., 2015). In a seminal study, Murakami et al. (2010) examined micronutrient intakes in the diets of 6,517 Japanese adolescents aged 12-15 years old. The study found that B6 and folate intake were significantly associated with depressive symptoms in both boys and girls. However, B2 intake was associated with depression in girls only. In contrast to findings in adult populations, B12 was *not* significantly associated with self-reported depression in boys or girls. Although such research cannot control for the influence of extraneous variables, such as family or peer relationships, these findings suggest that deficiency in B-vitamins may also be associated with the increased risk of developing depressive disorders in children and adolescents.

There is less available support for an association between B-complex vitamins and anxiety disorders in adults. Two studies found that folate levels were significantly associated with anxiety symptoms (Bjelland et al., 2003; Kendrick et al., 2008). In addition, Bjelland also found vitamin B12 was significantly correlated with anxiety levels. Mikawa et al. (2013) found that vitamin B6 was inversely correlated with panic attack frequency. However, in contrast to Bjelland et al. (2003), Mikawa et al. (2013) found that B12 was not significantly associated with symptoms of panic disorder.

Clinical Trials. There is evidence to suggest that folate could improve the efficacy of antidepressant medication (for a review see Taylor et al., 2004). Five clinical trials found that

folate significantly enhanced the reduction of depressive symptoms of patients receiving SSRIs compared to a placebo adjunct (Christensen et al., 2011; Coppen & Bailey, 2000; Papakostas et al., 2012, 2014; Venkatasubramanian et al., 2013). However, these effects appeared to be stronger when the activated form of folate (L-methylfolate) was used (Bedson et al., 2014; Papkostas et al., 2012, 2014). In addition, a longitudinal study found that a B-group vitamins did not increase the efficacy of an antidepressant after 12 weeks, however it improved the treatment effects over a 12-month period (Almeida et al., 2014).

Tentative research suggests that vitamin B monotherapy may be useful in reducing depressive symptoms in adults. In an earlier study, Procter (1991) found that adult participants that received 15mg of folate per day for 6 months demonstrated significantly reduced depressive symptoms. Conversely, five randomised placebo controlled trials found that vitamin B supplementation (including folate, B12, and B6) was not effective at reducing depressive symptoms compared to a placebo when used as a monotherapy (Ford et al., 2008; Hvas et al., 2004; Okereke et al., 2015; Oren et al., 1994; Walker et al., 2010). However, these studies used the non-activated form of B-vitamins, which may not have had the same therapeutic potency as biochemically activated forms.

In adult populations, preliminary research suggests B vitamins may be effective in reducing anxiety symptoms. One study examined the effect of a broad spectrum micronutrient formula, containing vitamins and minerals including B vitamins, on anxiety and stress levels in adults (Carroll et al., 2000). It was found that the formula significantly reduced anxiety levels compared to placebo. Similarly, Souza et al. (2000) found that magnesium and vitamin B6 supplementation significantly reduced pre-menstrual anxiety symptoms. However, these results were not able to further specify which micronutrient (or combination) actually lead to the reported symptom reductions. As such, there is a paucity of research examining the specific role of B-complex vitamins in anxiety disorders.

Summary

There is strong evidence from cross-sectional research supporting associations between several B vitamin deficiencies, particularly folate, and depressive symptoms in both adult and young populations. Although this relationship appears to be moderated by sex, the extent to which sex may play a role in deficiency related psychopathology is unclear at present. However, past findings suggest that there may be sex differences in the neurobiological mechanisms underlying depressive disorders, which may potentially be attributed to endocrine functioning and micronutrient metabolism.

The clinical utility of using B-group vitamins to reduce depressive symptoms is weak at present. However, there is strong evidence to suggest that folate supplementation may be effective at enhancing the efficacy of antidepressant treatments in reducing depressive symptoms in adults. At present there is a paucity of clinical trials examining the efficacy of B-group vitamins in treating paediatric depression. Additionally, the therapeutic utility of using *activated* B vitamins could be further explored in controlled trials.

The association between B vitamins and anxiety symptoms is tentative. Vitamins B6, folate, and B12 have been correlated with generalised-anxiety symptoms, and B6 with panic attack frequency. It is plausible to hypothesise that these differences may be attributed to different neurobiological pathways of panic and other anxiety disorders (Martin et al., 2010). Additionally, poorer diet quality (such as higher intakes of 'junk', processed or refined foods) has been associated with increased risk of developing mental illness in child and adolescent populations (for a review see O'Neil et al., 2014). However, no studies to date have examined the relationship or clinical utility of B-group vitamins and anxiety disorders in childhood or adolescence. As such, future research could further elucidate this relationship through exploring paediatric anxiety symptoms and the dietary intake of B-group vitamins.

Magnesium

Observational studies. There is limited research demonstrating an association between magnesium intake and depression in adults (Stanislawska et al., 2013; Tartleton & Littenberg, 2014; Jacka et al., 2012). Jacka et al. (2012) examined nutrient intakes and internalising symptoms in cross-sectional sample of 1046 women. The study identified a significant inverse association between magnesium intake (as derived through dietary analysis) and depressive symptoms. Similarly, Stanislawska et al. (2013) found magnesium serum levels were inversely associated with depressive symptoms in 171 post-menopausal women. A prospective study also found that low magnesium intake was associated with increased depressive symptoms in a cross-sectional sample of 8894 adults under 65 years of age – but lower levels appeared to protect against depressive risk as people aged (Tartleton & Littenberg, 2014) – indicating potential development differences in micronutrient need. One longitudinal study also found that those who had higher dietary intakes of magnesium were at a significantly decreased risk of receiving a diagnosis of depression (Yary et al., 2015).

There is preliminary research to suggest that magnesium deficiency is associated with anxiety disorders. Only two cross-sectional studies have been performed examining this association (Jacka et al. 2009, 2012). Utilising a sample of 5708 adults, Jacka et al. (2009) found a weak and non-significant relationship between magnesium intake and anxiety in adults. Similarly, Jacka et al. (2012) found no significant associations between magnesium intake and anxiety symptoms in adult women.

Clinical trials. Contrary to observational research, there is some evidence to suggest that magnesium supplementation may be effective at reducing adult internalising symptoms (Barragán-Rodríguez et al., 2008; Souza et al., 2000; Hanus et al., 2004). Barragán-Rodríguez et al. (2008) found that magnesium supplementation significantly reduced depressive symptoms in 23 Type II diabetes sufferers with hypomagnesaemia, a condition of impaired gastrointestinal absorption of magnesium, compared to a receiving a standard anti-

depressant treatment (50mg imipramine). Similarly, Eby and Eby (2006) found 125-300mg of magnesium given daily for one week reduced reported depressive symptoms in three patients. However, the study was limited to case reports and thus no statistical results were reported. In addition, Souza et al. (2000) found that receiving 200mg of magnesium and 50mg of B6 for 1-month significantly reduced pre-menstrual anxiety symptoms in 44 women compared to a placebo. Hanus et al. (2004) also found that magnesium supplementation, when used alongside herbal treatments, significantly reduced anxiety symptoms compared to a placebo. Such promising results require empirical replication and further exploration.

Summary

Evidence from observational studies suggests that magnesium deficiency may be related to depressive symptomatology. To the authors knowledge, only one trial to date has been published, which found magnesium supplementation to significantly reduce depressive symptoms in adults.

Results from observational and experimental literature are mixed regarding the role of magnesium in anxiety disorders. Cross-sectional studies have shown little support for the relationship, and only one clinical trial has shown treatment efficacy for magnesium monotherapy in reducing anxiety symptoms in women only.

There has been no published research examining the relationship between magnesium intakes and internalising disorders in children and adolescents. At present, there is a large gap in the present state of knowledge regarding the role of this micronutrient in developmental psychopathology.

Vitamin C, Vitamin E, and Iron

Observational studies. Vitamin C acts as strong anti-oxidant and promotes immune function (Higdon & Drake, 2003). Presently, there is evidence to suggest that vitamin C intakes are associated with depressive symptoms. For instance, three studies found significant

inverse correlations between dietary vitamin C intake and depressive symptoms (Oishi, Doi, & Karakami, 2009; Payne et al., 2012; Kinsman & Hoot, 1971). Oishi et al. (2009) reported significant inverse association between vitamin C dietary intake and depressive symptoms in 401 community-dwelling person aged 65-75 years old. Similarly, Payne et al. (2012) found that vitamin C intakes were lower among people (aged over 65) with depression (n = 144) compared to those without depression (n = 134). However, there is a paucity of research examining the relationship between vitamin C and internalising symptoms in child and adolescent populations. To date in the extant literature, only a single case study involving a 5-year old girl, has found improvement in depressive symptoms following one week of vitamin C supplementation (50mg per day) (Cocchi et al., 1980).

Vitamin E also functions as a potent anti-oxidant and assists in the body's immune regulatory function. The findings supporting an association between vitamin E and depression are mixed (Banikazemi et al., 2014; Maes et al., 2000; Owen et al., 2005). For instance, Banikazemi et al. (2014) found that vitamin E intake was inversely related to self-reported depression scores in adults. Additionally, Maes et al. (2000) found vitamin E serum levels were lower in depressed patients compared to healthy controls. Further, a longitudinal study found that lower vitamin E serum levels significantly predicted the 4-year progression of depression, but only in men, even after adjusting for potential confounding variables such as age and educational attainment (Shibata et al., 1999). In contrast, Owen et al. (2005) found lower serum levels of vitamin E in depressed patients. However, these findings were not related to or mediated by meeting the recommended daily intake of vitamin E. No published research has examined these associations in younger populations.

Iron is a fundamental micronutrient that is responsible for maintain blood levels, anti-oxidation, and immune-related DNA functions. There is no strong support of an association between iron and depressive symptoms in adults (Stewart & Hirani, 2012; Hunt & Penland,

1999; Baune, Eckardstein, & Berger, 2006). Only one study has found that iron status was related to depressive symptoms in elderly individuals (Stewart & Hirani, 2012). However, two studies found no association between iron levels or metabolism and depressive symptoms (Baune, Eckardstein, & Berger, 2006; Hunt & Penland, 1999).

One study has found that iron deficient anaemia (IDA) was significantly associated with unipolar depression in children and adolescence (Chen et al., 2013). Chen et al. (2013) investigated 2957 children and adolescents with diagnosed with IDA. It was shown that IDA was significantly correlated with unipolar depression and anxiety disorders. However, the study was not able to control for other aspects of nutritional deficiency, or psychosocial variables that also impact on psychological onset risk. The present state of research regarding this association is therefore novel. Further research is needed to confirm these findings in both adult and youth populations.

Clinical Trials. Only one RCT has examined the effect of vitamin C monotherapy on levels of depression in adults (Mazloom et al., 2003). Mazloom et al. (2003) found significant reductions in depressive levels with vitamin C compared to vitamin E monotherapies and a placebo.

There is evidence to suggest that vitamin C may also enhance the effectiveness of antidepressant medication (Amr et al., 2013; Saharian et al., 2015). One RCT, for instance, found support for vitamin C as an adjunct in enhancing antidepressant efficacy in children (Amr et al., 2013). Amr et al. (2013) randomised 24 children with clinical levels of depression to receive either vitamin C or placebo alongside standard fluoxetine medication for six months. Children that received the vitamin C adjunct reported significantly lower depression symptoms compared to the placebo group. Such results were limited by a relatively small sample size and thus require further replication and confirmation. In contrast however, Saraharian et al. (2015) found no significant differences between vitamin C and

placebo groups when the supplement was used as an adjunctive treatment alongside standard antidepressant medication. As such, the present understanding is mixed in the literature as to whether vitamin C is an effective pharmacological adjunct.

Only one trial has found vitamin C to be an effective monotherapy for treating anxiety symptoms in adults (Mazloom et al., 2013). Mazloom et al. (2013) found that self-reported anxiety levels were significantly lower in a vitamin C supplement group compared to vitamin E and placebo groups in patients with Type II diabetes. Such results have also been shown in child and adolescent populations (De Oliveira et al., 2015). De Oliveira et al. (2015) randomised healthy high school students to receive either 500mg of vitamin C per day or placebo for two weeks. The vitamin C treatment group had significantly greater reductions in self-reported anxiety symptoms compared to the placebo group. These findings suggest that vitamin C may assist in the remediation of anxiety symptoms.

To date, there is no evidence suggesting a therapeutic benefit of vitamin E on internalising symptoms. Only one RCT has examined the effect of vitamin E on symptoms of anxiety (Mazloom et al., 2013) and found no significant differences in anxiety levels between placebo and treatment groups. There has been no other published research to the author's knowledge examining the effect of vitamin E supplementation on depressive symptoms.

Similarly, only one trial has been published examining the specific effect of iron supplementation on anxiety levels (Zhang et al., 2013). Zhang et al. (2013) examined the effect of multiple micronutrient supplementation on rates of anaemia and anxiety respectively in 2730 Chinese children aged 10-12 years old over a 36 week period. It was found that micronutrient supplementation significantly reduced anaemia and anxiety symptoms compared to placebo treatment. However, a prevailing limitation was identifying which particular micronutrients (or combination of) had therapeutic efficacy with regard to anxiety symptoms. Nevertheless, this research does suggest that alleviating micronutrient deficiency

may be associated with reductions in psychopathological symptoms. There have been no reported trials of iron supplementation in adult populations.

Summary

There is tentative research supporting a potential therapeutic role of vitamin C in reducing internalising symptoms. There is less available evidence supporting a role for vitamin E and iron. This may be due to the paucity of actual published clinical trials examining the specific therapeutic effect of micronutrient supplementation. The combined evidence from observational studies supports an association between dietary intakes or serum concentrations of vitamin E and depressive symptomatology. The underlying biochemical causal pathways still remain unclear. Vitamin E or C deficiency may contribute to the development of internalising disorders; alternatively, depression may produce biochemical disruptions to vitamin E serum status (Maes et al., 2000).

<center>**Conclusion and Directions for Future Research**</center>

In conclusion, there is evidence indicating that micronutrients may play a role in the pathogenesis of internalising disorders. However, how micronutrients actually affect the psychophysiological processes involved in disorder expression is largely unknown. This interaction is likely to be highly complex, involving multiple nutrients and biochemical processes.

Despite unclear causal pathways, there is some evidence to support the role of specific micronutrients in the pathophysiology of anxiety and depressive disorders respectively. These views are supported by observational studies linking specific micronutrient deficiencies with these disorders. Current evidence supports the relationships between zinc, B vitamins (specifically B6, folate, and B12) and magnesium, and depression. However, results from clinical trials only support a therapeutic role of zinc and B-group vitamins in treating depression. Further, zinc, vitamin C and folate were found to enhance the effect

pharmacological antidepressant therapies. Additionally, tentative evidence supports an association between zinc, magnesium, and folate and anxiety symptoms. In addition, there is moderate evidence supporting the clinical use of magnesium and vitamin C in the treatment of anxiety. At this stage, only speculation can be made as to the causal biochemical pathways involved between nutrition and internalising psychopathology. However, the overall current evidence suggests that this an important area and requires further investigation. For example, it still remains unclear which particular micronutrient deficiencies are associated with depression and anxiety disorders respectively.

The current available evidence base has the strongest support for a potential therapeutic role of micronutrients in supporting remission from depressive disorders. There is less available evidence to confirm such a role for anxiety disorders. This therefore remains a prevailing gap in the current state of knowledge. Past research has also been limited by a paucity of studies and trials in younger populations. This is surprising, given there is significant evidence to supporting a role of specific micronutrients, such as zinc, iron, essential fatty acids, iodine, selenium, and vitamin A, in early brain development (Georgieff, 2007). Thus further research into this area is needed.

As such, this review proposes several directions for future research. Firstly, the extent to which micronutrients play an important role in the pathogenesis of specific internalising disorders needs further confirmation. For example, are specific micronutrients related to specific symptoms of disorders? Secondly, it is important to confirm the findings from adult research in younger populations. Thirdly, the underlying biochemical pathways between micronutrients and psychopathology need to be established.

The potential implications of micronutrients in assisting with symptom remission are quite large. Understanding such pathways may have population-level implications for preventing the onset of internalising disorders. Future controlled trials involving clinical and

younger populations are required to confirm the potential role of micronutrients in the development of psychopathology across the lifespan.

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Table 1

Summary of Included Studies Examining Micronutrients and Internalising Disorders.

Micronutrient	Disorder	Authors	Sample	Design	Outcome assessment	Results
Zinc	Depression	Maserejian et al. (2012)	Community sample of adults ($n = 3708$)	Observational	CES-D	Lower dietary intakes of zinc were associated with depression in women but not men.
Zinc	Depression	Lehto et al. (2013)	Community sample of adult men ($n = 2317$)	Observational/prospective	Human Population Laboratory Depression Scale	Zinc intake was not associated with an increased risk of depression across a 20 year follow-up in men.
Zinc	Depression	Yary and Aazami (2012)	Postgraduate students ($n = 402$)	Observational	CES-D	A significant an inverse relationship between dietary intake of zinc and depression was found.
Zinc	Depression	Roozbeh et al. (2011)	Adult patients with End-Stage Renal disease ($n = 135$)	Observational	BDI	Lower zinc levels were observed in patients with depression.
Zinc	Depression	Vashum et al. (2014)	Community sample of adult men and women ($n = 9738$)	Observational	CES-D	An inverse association was observed between dietary zinc intake and risk of depression in men and women.
Zinc/Magnesium	Depression	Stanisławska et al. (2013)	Community sample of postmenopausal women ($n = 171$)	Observational	BDI	Those with higher zinc levels had significantly less depressive symptoms.
Zinc	Depression	Maes et al. (1997)	Clinical sample ($n = 31$) vs. community sample ($n = 15$)	Observational	HDRS	Those diagnosed with Major Depressive Disorder had significantly lower

						levels of zinc, and increased immune proteins, compared to healthy participants.
Zinc/Magnesium, Folate	Depression/Dysthymia/Anxiety	Jacka, Maes, Pasco, Williams, and Berk (2012)	Community sample of adult women (*n* = 1494)	Observational	Diagnosis of Major Depression/Dysthymia, or anxiety disorders (SCID-I/NP; GHQ-12)	Intakes of zinc, magnesium and folate were significantly associated with reduced odds ratio for major depression/dysthymia. Magnesium and zinc intakes were also inversely correlated with psychological symptoms.
Zinc	Depression	Nowak et al. (2003)	Clinical sample of patients with Major (unipolar) Depressive disorder (*n* = 20)	Randomised, placebo double-blind controlled trial of zinc (25mg/day vs. placebo) as an antidepressant adjunct for three months	HDRS; BDI	Zinc supplementation significantly enhanced antidepressant efficacy at 6 and 12 weeks for patients with unipolar depression.
Zinc	Depression	Siwek et al. (2009)	Clinical sample of patients with Major Depression (*n* = 60)	Randomised, placebo double-blind controlled trial of zinc (25mg/day vs. placebo) as an antidepressant adjunct for three months	HDRS; BDI; CGI; MADRS	Zinc supplementation augments the efficacy and speed of onset of therapeutic response to antidepressant treatment.
Zinc	Depression	Ranjbar et al. (2014)	Clinical sample of patients with Major Depression (*n* = 44)	Randomised, placebo double-blind controlled trial of zinc (25mg/day vs. placebo) as an antidepressant adjunct for three months	HDRS	Zinc supplementation significantly reduced HDRS compared to placebo ($P < 0.01$ at 12th week).
Zinc	Depression	Solati et al. (2015)	Community sample of adults (*n* = 46)	Randomised, placebo double-blind controlled trial of zinc (30mg/day vs. placebo) as an monotherapy for three months	BDI	Zinc monotherapy significantly improved depressive symptoms compared to placebo.

Zinc	Depression and Anxiety	Digirolamo et al. (2010)	Community sample of children aged 5-10 years old (n = 674)	Randomised, placebo double-blind controlled trial of zinc (10mg/day vs. placebo) as an monotherapy for six months	CDI; RCMAS; BASC	No significant differences were found in depression or anxiety scores between treatment groups. However increases in serum zinc concentrations were inversely associated with depression and anxiety scores.
Zinc	Depression	Sawada and Yokoi (2010)	Community sample of young women aged between 18-21 years old (n = 30)	Randomised, placebo double-blind controlled trial of zinc (7mg/day + multivitamin vs. multivitamin alone) for 10 weeks	CMI; POMS	Significant reductions in depressive scores were observed in zinc plus multivitamin condition compared multivitamin treatment alone.
B Vitamins	Depression	Sanchez-Villegas et al. (2009)	Community sample (n = 10,094) of adults	Observational/prospective	Formal diagnosis at follow-up	Low folate intake was associated with depression among men. However, a low intake of vitamin B12 was associated with depression among women. No significant associations were found for vitamin B6 intake and depression scores.
B Vitamins	Depression	Yary (2013)	University students (n = 425)	Observational	CES-D	folate intake significantly accounted for depressive symptoms as measured by the CES-D
B Vitamins	Depression	Payne et al. (2008)	Clinical sample of participants with depression (n =	Observational	Duke Depression Evaluation Schedule	Dietary intake of high folate foods was significantly and

						inversely associated with depression.
B Vitamins	Depression	Kim et al. (2008)	Community sample of adults over 65 years of age (*n* = 732)	Observational/Prospective	Geriatric Mental State Schedule	Lower levels of vitamin folate and B12 and higher homocysteine levels at baseline were associated with a higher risk of incident depression at a two-year follow-up. Incident depression was also associated with a decline in vitamin B 12 and an increase in homocysteine levels over the follow-up period
B Vitamins	Depression	Murakami et al. (2008)	Community sample of adults (*n* = 517)	Observational	CES-D	Folate intake was inversely related to depressive symptoms in men but not women.
B Vitamins	Depression	Skarupski et al. (2010)	Community sample of adults (*n* = 3503)	Observational/Prospective	CES-D	Higher intakes of vitamin B6 and B12 were associated with a decreased incident of depression over a 12 year follow-up
B Vitamins	Depression	Kamphuis et al. (2008)	Community sample of men aged 70-90 years old (*n* = 332)	Observational	Zung Self-rating Depression Scale	Dietary intakes of folate, B6, and B12 were **not** related to depressive symptoms
B Vitamins	Depression	Bell et al. (1991)	Clinical sample of geriatric (*n* = 20) and young adults	Observational	HDRS; MSE	Lower levels of B vitamins was associated with

						symptoms of major depression.
B Vitamins	Depression	Murakami et al. (2010)	Adolescent community sample, aged 12-15 years old (*n* = 6517)	Observational	CES-D	Folate and B6 intake was inversely associated with depressive symptoms. Folate intake was inversely associated with depressive symptoms in girls but not in boys. No significant association was found between vitamin B-12 intake and depressive symptoms in either sex.
B Vitamins	Depression	Tsuimine et al. (2015)	Clinical child and adolescent sample of females with depression (*n* = 24) vs. healthy controls (*n* = 26)	Observational	Formal diagnosis of DSM-IV criteria Major Depressive Disorder	Folate serum levels were significantly lower in patients with depression than in healthy controls.
B Vitamins	Depression	Herbison et al. (2012)	Community sample of adolescents (*n* = 709)	Observational/Prospective	Youth Self Report	Dietary intake of vitamin B6 and folate was significantly associated with self-reported internalising behaviours.
B vitamins	Depression	Procter (1991)	Clinical sample of patients with major depression or schizophrenia (*n* = 123) with borderline or folate deficiency	Randomised, placebo double-blind controlled trial (15mg.day of folate for six months vs. placebo)	Not provided	Receiving folate significantly improved depressive symptoms compared to controls.
B Vitamins	Seasonal Affective Disorder	Oren et al. (1994)	Clinical sample of depressed patients (*n* = 27 patients)	Randomised, placebo double-blind controlled trial (4.5mg/day of folate for two weeks vs.	HDRS: SIGH-SAD	Receiving vitamin B12 did not significantly reduce depressive symptoms compared to

						those receiving the placebo treatment.
B Vitamins	Depression	Coppen and Bailey (2000)	Clinical sample of depressed patients taking fluoxetine medication ($n = 127$)	Randomised, placebo double-blind controlled trial (500 mg/day of folate for 10 weeks vs. placebo)	HDRS	Folic acid significantly enhanced the efficacy of fluoexetine treatment in reducing depressive symptoms compared to receiving a placebo.
B vitamins	Depression	Hvas et al. (2004)	Clinical sample of depressed patients with dementia and vitamin B12 deficiency ($n = 140$)	Randomised placebo-controlled trial (1mg/week injection of vitamin B12 vs. placebo for three months)	Major Depression Inventory	Receiving vitamin B12 did not significantly improve depressive symptoms compared to receiving the placebo treatment.
B vitamins	Depression	Walker et al. (2010)	Community sample of adults with elevated levels of psychological distress ($n = 903$)	Randomised controlled trial (400mcg/day folic acid + 100 mcg/day vitamin B12 v. placebo) x (physical activity v. nutrition promotion control) v (mental health literacy v. pain information control)	PHQ-9	Receiving B vitamins did not significantly reduce depressive symptoms at 6 week, 12 week, or 24 month follow-ups.
B vitamins	Depression	Christensen et al. (2011)	Community sample of adults with elevated levels of psychological distress or symptoms of depression aged 60-74 year olds ($n = 900$) taking antidepressant medication	Randomised placebo-controlled trial (400mcg/day of folate + 100mcg-day of vitamin B12 vs. placebo as an adjunctive treatment)	PHQ-9	Taking vitamins folate or B12 did not significantly enhance the efficacy of antidepressant medication compared to a placebo.
B vitamins	Depression	Almeida et al. (2014)	Community sample of adults with DSM-IV-TR	Randomised double-blind placebo-controlled trial (0.5mg/day of vitamins	MADRS	Those that received the B-vitamins adjunct treatment did not show

			Major Depression (*n* = 128)	B12, 2mg/day of folate, and 25mg/day of vitamin B6 as an adjunctive treatment vs. placebo for one year)		significant reductions in depressive symptoms to those receiving the placebo over 12 weeks. However, taking B-vitamins significantly enhanced and sustained the treatment response over one year.
B vitamins	Depression	Okereke et al. (2015)	Community sample of adult women (*n* = 4331) aged 50 years or older	Longitudinal randomised double-blind placebo-controlled trial (1mg/day of vitamins B12, 2.5mg/day of folate, and 50mg/day of vitamin B6 as an adjunctive treatment vs. placebo for seven years)	Self-reported physician/clinician-diagnosed depression or clinically significant depressive symptoms	B vitamin supplementation did not significantly reduce the overall incidence of depression risk in adult women.
B vitamins	Depression	Ford et al. (2008)	Community sample of adult males aged 75 years or older (*n* = 299)	Longitudinal randomised double-blind placebo-controlled trial (400mcg/day of vitamins B12, 2mg/day of folate, and 25mg/day of vitamin B6 monotherapy vs. placebo for two years)	BDI	Receiving B vitamins did not significantly reduce the risk of developing depression or reducing the severity of depressive symptoms.
B vitamins	Depression	Bedson et al. (2014)				
B vitamins	Anxiety/Depression	Kendrick et al. (2008)	Community sample of adult women (*n* = 1264)	Observational/Prospective	GHQ-12	Lower levels of folate were significantly associated with anxiety symptoms at follow-up, even after adjustment for SES and lifestyle factors. No relationship was found with depressive symptoms

Supplement	Disorder	Author	Sample	Study Design	Measure	Findings
B vitamins	Anxiety	Bjelland et al. (2003)	Community sample of adults ($n = 5948$)	Observational	Hospital Anxiety and Depression Scale	Folate and vitamin B 12 levels were significantly associated with anxiety symptoms.
B vitamins/iron	Panic Disorder	Mikawa et al. (2013)	Clinical sample of patients with Panic Disorder ($n = 21$) vs healthy controls ($n = 21$)	Observational	DSM-IV-TR diagnosis of Panic Disorder	Lower serum B6 and iron levels were found in patients diagnosed with Panic Disorder.
B Vitamins	Anxiety	Souza et al. (2000)	Community sample of women suffering from mild premenstrual symptoms ($n = 44$)	Randomised, double-blind, placebo-controlled cross-over trial, consecutive treatments of: 200mg Magnesium, 50mg of vitamin B6, 200mg Mg + 50mg B6, placebo)	Menstrual Health Questionnaire	A small but significant reduction in anxiety symptoms was found in women that received the combination (200mg Mg + 50mg B6) per day compared to other treatments
Magnesium	Depression	Yary et al. (2015)	Community sample of Finnish male adults ($n = 2320$)	Observational/Prospective	Human Population Laboratory Depression Scale	Higher magnesium intake was associated with less risk of receiving a diagnosis of depression at a 20 year follow-up.
Magnesium	Depression	Tartleton and Littenburg (2015)	Community based sample of adults ($n = 8894$)	Observational	PHQ-9	Lower magnesium intake was statistically associated with levels of depression. Low magnesium intake was correlated with depressive symtoms in particpants under 65, but appeared to be protective of those aged over 65.
Magnesium	Depression	Barragán-Rodríguez, Rodriguez-	Clinical sample of elderly Type II diabetes sufferers with newly	Randomised, placebo-controlled trial (50 g of MgCl2 per 1 000 mL of solution vs 50mg of	Geriatric Depression Scale	Magnesium supplementation was as effective in treating symptoms of

		Moran, and Guerroro-Romero (2008)	diagnosed depression ($n = 23$)	Imipramine daily for 12 weeks)		depression in elderly Type II diabetes sufferers as was imipramine.
Magnesium	Anxiety	Jacka et al. (2009)	Community sample of Norwegian adults ($n = 5708$)	Observational	Hospital Anxiety and Depression Scale	A non-significant inverse association was found between magnesium intake and anxiety symptoms.
Magnesium/Zinc/Folate	Depression/Dysthymia/Anxiety	Jacka et al. (2012)	Community sample of adult women ($n = 1494$)	Observational	Diagnosis of Major Depression/Dysthymia, or anxiety disorders (SCID-I/NP; GHQ-12)	Magnesium and zinc intakes were inversely correlated with psychological symptoms. However, no significant association was found between magnesium and having a diagnosis of an anxiety disorder.
Magnesium	Anxiety	Gendle and Ohara (2015)	Sample of university undergraduates ($n = 147$)	Randomised, double-blind, placebo-controlled trial (300mg magnesium per day for five days)	Adult Manifest Anxiety Scale-College	Self-reported pre-exam anxiety did not significantly differ between treatment groups.
Magnesium/B Vitamins	Anxiety	Souza et al. (2000)	Community sample of women suffering from mild premenstrual symptoms ($n = 44$)	Randomised, double-blind, placebo-controlled cross-over trial, consecutive treatments of: 200mg Magnesium, 50mg of vitamin B6, 200mg Mg + 50mg B6, placebo)	Menstrual Health Questionnaire	A small but significant reduction in anxiety symptoms was found in women that received the combination (200mg Mg + 50mg B6) per day compared to other treatments
Vitamin E	Depression	Banikazemi et al. (2014)	Community sample of Iranian adults ($n = 7172$)	Observational	BDI	Dietary intake of vitamin E was significantly associated with depressive scores.

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Vitamin E	Depression	Shibata, Kamagi, Watanabe, and Suzuki (1999)	Community sample of 504 elderly adults	Observational/Prospective	Geriatric Depression Score	Vitamin E at baseline significantly predicted the progression of depressive symptoms in men only.
Vitamin E	Depression	Maes et al. (2000)	26 healthy volunteers and 42 major depressed patients	Observational	Structured interview of the DSM-III-R; HDRS	Major depressive symptoms were significantly correlated with serum vitamin E levels.
Vitamin E	Depression	Owen et al. (2005)	Clinical sample of outpatients with major depression ($n = 49$)	Observational	BDI	Serum levels of vitamin E were significantly associated with depressive symptoms.
Vitamin E	Depression	Tiemeier et al. (2002)	Clinical sample of patients with depressive symptoms ($n = 262$) vs. randomly selected controls ($n = 459$) aged over 60 years old	Observational	CES-D; DSM-IV-TR diagnoses	No association was found between serum vitamin E levels and depressive symptoms or having a formal diagnosis.
Vitamin E/Vitamin C	Anxiety/Depression	Mazloom, Ekramzadeh, and Hejazi. (2013)	Clinical sample of diabetic patients ($n = 45$)	Randomised single-blind, placebo-controlled study (400IU/day vitamin E, 1000mg/day vitamin C, and placebo for six weeks)	DASS-21	A significant reduction in anxiety levels was observed in the vitamin C group compared to other groups. No significant reductions in the vitamin E group compared to other groups.
Vitamin C	Anxiety	De Oliveira, De Souza, Motta, and Da-Silva (2015)	Community sample of high school students ($n = 42$)	Randomised, double-blind, placebo controlled trial (500mg/day vitamin C vs. placebo for 14 days)	BAI; Mean heart rate and blood pressure	Students that received the vitamin C supplement showed significantly greater reductions in anxiety levels and mean heart rate compared to

						students that received the placebo treatment.
Vitamin C	Depression	Oishi, Doi, and Karakami. (2009)	Community sample of eldery persons (n = 279)	Observational	CES-D	Vitamin C intakes were significantly associated with depressive symptoms in men but not women.
Vitamin C	Depression	Payne et al. (2012)	Clinical sample of elderly participants (n = 144) vs. healthy controls (n = 134)	Observational	Duke Depression Evaluation Scale	Participants with depression had significantly lower dietary intakes of vitamin C
Vitamin C	Depression	Kinsman and Hoot (1971)	Community sample of prisoners (n = 5)	Observational	Depression scale of the Minnesota Multiphasic Personality Inventory (MMPI)	Scores on the depression subscale became elevated as vitamin C deficiency progressed.
Vitamin C	Depression	Cocchi et al. (1980)	Case study of a five year old girl with chronic hepatitis and depressive symptoms	Case study examining the effect of 50mg.day intravenous vitamin C for one weeks	Clinical observations	Depressive symptoms subsided after one week of treatment.
Iron	Depression	Stewart and Hirani (2012)	Community sample of adults aged over 65 years (n = 1875)	Observational	Geriatric Depression Scale	Depressive symptoms were associated with levels of anaemia in both men and women.
Iron	Depression	Hunt and Penland (1999)	Clinical sample of non-pregnant postmenopausal women aged 20-45 years (n = 384)	Observational	Depression scale of the MMPI	No significant association was found between iron status and depressive symptoms in women.
Iron	Depression/Anxiety	Chen et al. (2013)	Cross-sectional clinical sample of children and adolescence with iron deficient anemia (n = 2957) compared to	Observational	Formal diagnosis	Iron deficiency increased the risk of having a unipolar depressive and anxiety disorder respectively, compared to healthy controls.

			gender matched controls (1:4)			
Iron	Depression	Baune, Eckardstein, and Berger (2006)	Cross-sectional community sample of adults aged over 65 ($n = 374$)	Observational	CES-D	No significant association was found between iron levels and depressive symptoms in men or women.
Iron/B vitamins	Panic Disorder	Mikawa et al. (2013)	Clinical sample of patients with Panic Disorder ($n = 21$) vs healthy controls ($n = 21$)	Observational	DSM-IV-TR diagnosis of Panic Disorder	Lower serum B6 and iron levels were found in patients diagnosed with Panic Disorder.
Iron	Anxiety	Zhang et al. (2013)	Community sample of children age 10-12 years in rural China ($n = 2730$)	Randomised controlled trial (Multivitamin + 5mg iron supplement vs. waitlist)	CMAS	Receiving the multivitamin + iron supplementation significantly reduced both anaemia and anxiety levels compared to controls.

Note. Revised Children's Manifest Anxiety Scale (RCMAS), Behavior Assessment System for Children (BASC), Centre for Epidemiological Studies Depression Scale (CES-D), Beck Depression Inventory (BDI), Beck Anxiety Inventory (BAI), Hamilton Depression Rating Scale (HDRS), Global Health Questionnaire (GHQ-12), Structured Clinical Interview for DSM-IV Axis I Disorders – Non Patient (*SCID-I/NP*), Clinical Global Impressions Scale (CGI), Montgomery-Asperg Depression Scale (MADRS), Profile of Moods Scale (POMS), Mental State Examination (MSE), Structured Interview Guide for the Hamilton Depression Rating Scale – Seasonal Affective Disorder (SIGH-SAD), Patient Health Questionnaire (PHQ-9), Depression, Stress, and Anxiety Scale (DASS-21).

Dietary Intake of B-vitamins, Magnesium, and Zinc and their Associations with Internalising

Symptoms in University Students

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Abstract

Research suggests that the dietary intake of specific micronutrients is associated with anxiety and depressive disorders. However, the exact relationship between micronutrients and internalising symptoms still remains unclear. As such, this cross-sectional study investigated dietary micronutrient intakes and depressive and anxiety symptoms in an undergraduate university cohort ($n = 46$). Participants completed self-report measures via an online questionnaire about their diet history, food intakes and depressive and anxiety symptoms. Partial correlations were calculated to examine the associations between micronutrient intakes and internalising symptoms while controlling for potential covariates (Age, Gender, Physical Activity, Body-Mass Index, and Socioeconomic Status). Results indicate that the dietary intake of vitamins B3, B6, folate and zinc were significantly and inversely associated with symptoms of depression. No significant associations were found between micronutrient intakes and anxiety symptoms. Findings are interpreted in light of previous epidemiological research and current theoretical models.

Dietary Intake of B-vitamins, Magnesium, and Zinc and their Associations with Internalising

Symptoms in University Students

Recent epidemiological research suggests that nutrition may play an important role in

in the maintenance of mental well-being (Jacka & Berk, 2007; Jacka et al., 2009, 2013; Jacka,

Maes, Pasco, Williams, & Berk, 2012). This finding is significant given an estimated 30% of

the global human population is deficient in essential micronutrients (Ramakrishnan, 2002).

Specifically, the diets of young adults, particularly university students living away from

home, has been shown to be of poorer nutritional quality compared to those living with their

family (Larson et al., 2011; Papadaki et al., 2007). Additionally, recent research suggests

approximately 21% of Australian university students experience moderate levels of

psychological distress compared to their non-student peers (Cvetkovski et al. 2012). In

particular, students aged 18-22 years old have been reported to be more vulnerable to

developing mental illness compared to those aged over 25 years, with up to 16% of tertiary

students experiencing clinical levels of depression and anxiety symptoms (Eisenberg et al.,

2007)..

It is theorised that some micronutrients may enact therapeutic benefits by attenuating

the psychophysiological processes associated with internalising disorders. For instance, zinc

and B-group vitamins have been shown to reduce inflammation (Nowak, Szewczyk, & Pilc,

2005), support immune function (Fraker & King, 2004; Szewczyk, Kubera, & Nowak, 2011),

and promote neuronal plasticity (Carlson et al., 2009; Mattson & Shea, 2003). These

processes have been shown to be impaired in patients with depression (Dale et al., 2015;

Maes, 1993, 2011; Maes et al., 1992, 1996, 1997; Postolache, 2012; Serafini, 2012). The

dietary intake of micronutrients is also essential for the synthesis and maintenance of

neurotransmitter levels in the brain, such as serotonin and GABA (Baumgartner et al., 2012;

Guilarte, 1993; Kruiemann et al., 1987). Neurotransmitter imbalance is a well-understood

hypothesis as to the biochemical mechanisms underlying internalising disorders (Carlson, Corrodi, Fuxe, & Hökfelt, 1969; Cowen, 2008; Krishnan & Nestler, 2008; Möhler, 2012; Schildkraut & Kety, 1967). Thus, diets with adequate micronutrient intake may play an important role in the maintenance of mental well-being.

In support of this hypothesis, previous research has shown that diet quality is associated with symptoms of internalising disorders (Bakhtiyari et al., 2013; Jacka et al., 2010a, 2010b, 2011a; Jacka, Ronthon, Taylor, Berk, & Stansfield, 2013; Milaneschi et al., 2011; O'Neil et al., 2013). For example, Jacka et al. (2010a) found that adolescents who consumed healthy diets (e.g., higher fruit and vegetable intake) had fewer depressive symptoms than those that had unhealthy diets (e.g., higher junk food intake). Similarly, Western dietary patterns (e.g., higher intake of processed and refined foods) in adult women have been associated with an increased risk of developing depressive and anxiety disorders, whereas traditional diets, such as those consisting of a high intake of whole grains, fruit, vegetables, and meat are associated with a lower onset risk (Jacka et al., 2010b). Opie, O'Neil, Itsiopoulos, and Jacka (2014) completed a systematic review of randomised controlled trials examining the effect of dietary interventions on internalising disorder symptoms. They found that almost 50% of the trials reported significant treatment effects for symptoms of depression, while only 20% showed significant reductions in anxiety symptoms. Taken together, these findings suggest that improving diet quality may assist in treating internalising symptoms, especially symptoms of depression. However, it still remains largely unconfirmed as to what specific aspects of diet, or what specific micronutrients, are protective against depressive and anxiety disorders.

Strong evidence suggests that the dietary intake of zinc and B-group vitamins, particularly B6, folate, and B12, have been negatively associated with depressive symptoms in large cross-sectional community adult studies (Jacka et al., 2012; Maes et al., 1997;

Maserejian et al., 2012; Murakami et al., 2008, 2010; Roozbeh et al., 2011; Stanisławska et al., 2013; Sanchez-Villegas et al., 2009; Yary & Aazami, 2011). However, evidence supporting the association between other micronutrients and internalising symptoms has been mixed. For instance, some research has shown that magnesium and vitamin C intake may also be related to depressive and anxiety symptoms (Amr et al., 2013; De Oliveira et al., 2015; Kinsman & Hoot, 1971; Oishi et al., 2009; Payne et al., 2012; Mazloom et al., 2003; Jacka et al., 2012; Tarleton & Littenburg, 2015). In contrast, other research has found no association between these variables (Jacka et al., 2009; 2012; Sahraian et al., 2015). Thus, understanding specifically which micronutrients may be directly related to specific internalising symptoms also requires further clarification.

Limited research has examined the association of dietary micronutrients and internalising symptoms in younger populations. Therefore, the aim of this study was to examine the association of dietary micronutrient intakes and internalising symptoms in a sample of young adults. Such knowledge would support previous findings and extend current understanding of these relationships specific to anxiety and depressive symptoms among a younger sample.

Therefore, the purpose of this study was to further examine the relationship between the dietary intake of micronutrients and internalising symptoms in undergraduate university students. Based on current theoretical models and previous empirical findings, it was predicted that dietary intake of zinc, and vitamins B6, folate, and B12 would be inversely correlated with depressive symptoms. Similarly, based on previous research (Amr et al., 2013; De Oliveira et al., 2015; Kinsman & Hoot, 1971; Oishi et al., 2009; Payne et al., 2012; Mazloom et al., 2003; Jacka et al., 2012; Tarleton & Littenburg, 2015), it was predicted that the dietary intake of magnesium and vitamin C would be negatively correlated with anxiety symptoms. The role of additional B-vitamins and internalising symptoms was also explored.

To test these hypotheses, dietary micronutrient intakes and internalising symptoms were examined using a cross-sectional sample from an undergraduate university cohort.encies

Method

Participants

A volunteer community sample of forty six young adults aged 18-31 years old ($M =$ 23.95, $SD = 5.17$, female = 74%) participated in the study. It was estimated using G*power 3 (Faul, Erdfelder, Lang, & Buchner, 2007) that approximately 64 participants were required to detect a moderate correlation using a power (β) rating of 0.80, indicating the current study may have been slightly underpowered. To be eligible to participate, university students were required to be more than 18 years of age and fluent in English. Students were recruited online via an internal psychology undergraduate recruitment website. As a recognition of their time, participants were offered course credit for their participation. Advertising materials promoted the study as being related to "diet and mental well-being".

Measures

Depression, Stress, and Anxiety Scale (DASS-21; Lovibond & Lovibond, 1995): Depression and anxiety symptoms were measured using the 21-item DASS self-report measure for adults. Participants were asked to rate their response to a series of statements over the past week using a four-point Likert scale (0 = *Did not apply at all* to 3 = *Applied to me very much, most of the time*). Only the depression and anxiety subscales were administered. The *depression* subscale included such items as "I felt down-hearted and blue", while the *anxiety* subscale consisted of items such as, "I was aware of dryness of my mouth". The subscales of the DASS-21 have demonstrated good internal consistency with Cronbach's $\alpha = 0.94$ and 0.87 for depression and anxiety respectively (Antony, Bieling, Cox, Enns, & Swinson, 1998).

Diet History and Food Frequency: Average micronutrient intakes were estimated using a combination of diet recall and semi-quantitative food frequency questions. Three items asked students to report their most common weekly meals for breakfast, lunch, and dinner, including approximate serving size, and frequency of consumption. These questionnaires were developed according to recommended dietary guidelines for estimating average nutrient intakes (Baghurst & Record, 1984). The survey also asked participants to rate the frequency of their consumption and approximate serving size of commonly consumed foods from a variety of food groups including grains, meat, milk products, vegetables and fruit, fermented foods, coffee and tea. The questionnaire included pictures to assist participants in calculating serving sizes as outlined in the Australian Government Dietary Guidelines (Australian Government Department of Health, 2015).

Body Mass Index (BMI): BMI was calculated by asking participants to report their approximate height and weight using two questions. These scores were then calculated by the researcher as weight/height^2 (kg/m^2) in order to determine overall BMI for each participant.

Physical Activity Level: A single item was used to assess how often students engaged in physical activity during a normal week (1 day – 7 days). This question was developed based upon the Australian Government National Physical Health Standards (Australian Government Department of Health, 2015).

Socio-economic Status (SES): Participants' socio-economic status was estimated using five items that have been used in previous research in adolescent populations (Jacka et al., 2011). Participants responded to questions about their parents' employment, education level, and basic family demographics. Student responses were averaged and rated on the Index of Relative Socio-economic Advantage/Disadvantage (IRSAD). A low score as measured by IRSHAD identifies the most disadvantaged (quartile 1), and a high score identifies the most advantaged (quartile 4).

Procedure

        Participants were initially recruited internally through the University's participant pool website. Interested participants were directed to an online information and consent form in addition to the study link. Participants that gave informed consent were then directed to complete a 30 minute online questionnaire. Participation was voluntary. All aspects of the study were approved by the Macquarie University Human Research Ethics Committee.

Data Preparation

        Micronutrient intake. Daily micronutrient intake was calculated as per the recommendations outlined by Baghurt and Record (1984):

$Frequency\ of\ Consumption\ X\ Serving\ Size\ X\ Relevant\ Nutrient\ Content\ per\ 100gm$

        Participant responses from the dietary questionnaire were initially entered into nutrient analysis software, which included the type of food consumed, the approximate serving size, and the frequency of consumption per week (FoodWorks Version 4). FoodWorks contains relevant nutrient content profiles (per 100mg) for almost all Australian foods and food products. Foodworks also produces an estimated average total energy intake per participant, which was used in the current study to estimate energy-adjusted micronutrient intake. The software also calculates average micronutrient intakes based upon the specific food and serving sizes consumed, and the frequency of consumption per day. Finally, dietary micronutrient intakes were energy adjusted into mg or μg per 1000mg/kcal per day according to the formula recommended by Willet et al. (1997). As such, an energy adjusted micronutrient intake profile was created for each participant based upon their responses. This statistical procedure for calculating energy adjusted micronutrient intakes has been used in previous related research (e.g. Murakami et al., 2008; 2010).

Internalising symptoms. Total scores for depressive and anxiety symptoms respectively were calculated through summing relevant subscale item responses according to DASS-21 guidelines.

Data Analysis

Data were analysed using IBM SPSS Statistics Release Version 19.0.0.1 (IBM SPSS Inc., 2010, Chicago, IL) and Microsoft Office Excel (2010). The information entered included the type of food, servings size, and frequency of consumption.

To examine the association between micronutrient intakes and internalising symptoms while controlling for potential covariates (namely, Age, Gender, BMI, Physical Activity, and SES), partial correlations were calculated.

Prior to calculating the correlations, the assumptions of normality, linearity, and homoscedasticity were assessed. Shapiro-Wilk's test indicated several variables were not normally distributed. However, visual inspection of histogram and Q-Q plots confirmed that these departures from normality were only mild, which were deemed acceptable. Additionally, visual inspection of scatterplots of between micronutrients and internalising symptoms confirmed that the relationship between these variables was linear and homoscedastic, indicating equal variances across all variables.

<div align="center">

Results

</div>

Descriptive Statistics

Participant demographics and variable means, including average micronutrient intakes, are presented Table A1. Approximately 35% of participants did not meet the recommended daily intake (RDI) for zinc, 40% did not meet the RDI for vitamin B1, 25% did not meet the RDI for vitamin B3, 30% did not meet the RDI for vitamin B6, and 38% did not meet the RDI for vitamin C and magnesium (Linus Pauling Institute, 2016).

The mean BMI of the sample was 24.45 for males and 22.07 for females. The majority of participants exercised for at least 30 minutes 4-5 times per week ($M = 4.64$, $SD = 1.99$). The DASS-21 provides an assessment of distress or disturbance associated with depression or anxiety. A total of 71% of participants reported distress associated with anxiety symptoms in the Normal range, 13% of participants reported Mild distress, 8% reported Moderate distress, 4% reported their distress in the Severe range, and 4% fell within the Extremely Severe range according to DASS-21 guidelines. Additionally, 71% of participants fell within the Normal range for distress associated with depressive symptoms, 13% fell within the Mild range, 7% reported Moderate distress, 2% reported Severe distress, and 4% reported Extremely Severe distress associated with depressive symptoms.

Table 2 shows the correlations between all variables examined before controlling for covariates. All micronutrients were significantly correlated with each other ($p < .05$), with the exception of vitamins B3, zinc, and magnesium. Similarly, depressive and anxiety symptoms were positively and significantly associated ($p < .001$).

Micronutrient Intake and Internalising Symptoms

Table 3 provides the partial correlations, while controlling for Age, Gender, BMI, SES, and Physical Activity level.

As shown in Table 3, there were significant inverse associations found between the dietary intakes of vitamins B3, B6, folate and zinc and depressive symptoms ($p < 0.05$) after controlling for covariates. The strength of these associations were all considered medium-large according to Cohen's (1988) conventions. Surprisingly, the relationship between vitamin B3 intake and anxiety symptoms was non-significant after controlling for covariates. There was no significant associations found between the dietary intake of any micronutrients and anxiety symptoms after covariates were accounted for.

Table 2. *Pearson Correlation Coefficients between All Variables (n = 46)*

	Anxiety Symptoms	Depressive Symptoms	Vit B1	Vit. B2	Vit. B3	Vit. B6	Folate	Vit. B12	Vit. C	Zinc	Magnesium
Anxiety Symptoms	1										
Depressive Symptoms	.75**	1									
Vit. B1	-.27	-.26	1								
Vit. B2	-.18	-.28	.45*	1							
Vit. B3	-.30*	-.32*	.24	.04	1						
Vit. B6	.05	-.10	.48*	.34*	.33*	1					
Folate	-.17	-.35*	.36*	.12	.22	.13	1				
Vit. B12	.06	-.00	.36*	.35*	.12	.36*	.16	1			
Vit. C	-.12	-.08	.43*	.45*	-.01	.21	.17	.50*	1		
Zinc	.09	-.28	.23	.37*	.36*	.57*	.17	.18	-.09	1	
Magnesium	.00	-.06	.15	.21	-.07	.41*	.16	.01	-.11	.37*	1

Note. Energy-adjusted micronutrient intakes mg/1000kcal * = $p < .05$. ** = $p < .001$

Table 3

Partial Correlations (r) Between Micronutrients and Internalising

Symptoms (DASS) (n = 46).

Micronutrient^	Anxiety Symptoms	Depressive Symptoms
Vitamin B1	-.17	-.15
Vitamin B2	-.23	-.32
Vitamin B3	-.28	-.42*
Vitamin B6	.26	-.43*
Folate	-.28	-.46**
Vitamin B12	.22	-.13
Vitamin C	-.33	.32
Zinc	-.27	-.35*
Magnesium	-.10	-.16

Note. After controlling for Age, BMI, SES, Physical Activity, and Gender.

^ = Energy-adjusted micronutrient intakes mg/1000kcal.

* = $p < 0.05$. ** = $p < 0.01$

Discussion

This study is one of the first cross-sectional analyses of micronutrient intake and specific internalising symptoms in university students. The primary aim of the study was to examine the association between the dietary intake of micronutrients and depressive and anxiety symptoms. Based on previous research, it was predicted that zinc and vitamins B6,

folate, and B12 would be inversely associated with depressive symptoms. Similarly, it was predicted that magnesium and vitamin C would be negatively correlated with anxiety symptoms. The results of this study partially supported these hypotheses. Firstly, the dietary intake of vitamins B3, B6, folate and zinc were found to be significantly and negatively related to depressive symptoms. However, contrary to expectations vitamin B12 was not found to be associated with depressive symptoms. Secondly, zinc, magnesium and folate were not found to be significantly related to anxiety symptoms. Interestingly, no micronutrient intakes were significantly correlated with anxiety symptoms after adjustment for covariates.

B-Group Vitamins

The dietary intake of vitamin B3, B6 and folate were found to be inversely related to depressive symptoms. Such associations have also been reported in previous research with regard to B6 and folate (Gilbody et al., 2007; Merete et al., 2008, 2010, 2012; Kim et al., 2008; Jacka et al., 2012; Payne et al., 2008; Sanchez-Villegas et al., 2009; Yary, 2013). The current results are in contrast to Fulkerson et al. (2004), which found no association between B6 intake and depressive symptoms in US adolescents. However, Fulkerson et al. (2004) reported that all participants met RDI for these micronutrients, whereas 30% of participants did not meet the RDI for vitamin B6. Thus, the current study confirms previous research that vitamin B6 deficiency is inversely related to depressive symptoms.

Unlike the current results, Herbison et al. (2012) did not find a significant association between B3 intake and internalising symptoms, although their identified relationship approached significance ($p = .08$). However, Herbison et al. (2012) measured overall internalising symptoms via the Youth Self-report measure, whereas current study utilised the DASS, which allowed for more specific analysis of internalising symptoms. Thus, the current results also extend previous research through highlighting a possible implication of vitamin

B3 in depressive disorders specifically. The relationship between vitamin B3 deficiency and depression may be attributed to the role of B3 in the L-tryptophan-serotonin biochemical process (Fuchs et al., 1990; Maes et al., 1993). Nicotinamide adenine dinucleotide (NAD), the bioavailable form of niacin, is endogenously converted from L-tryptophan above the formation of serotonin whenever vitmain B3 deficiency is present (Sarris, Schoendorfer, & Kavanagh, 2009). Sarris et al. (2009) thus maintain that serotonin formation could be jeopardised whenever vitamin B3 deficiency is present in the body. Additionally, a central enzyme needed for the L-tryptophan-serotonin pathway relies on the availability of vitamin B6 and vitamin C, both of which were also found to be related to depressive symptoms in the current study. Therefore, it is plausible to suggest that a deficiency in vitamin B3 may lead to serotonergic dysregulation, and subsequently to depressive symptoms, a pathway that needs to be examined in future research.

Similarly, vitamin B6 and folate may enact neurological benefits through neurotransmitter synthesis (Herbison et al., 2012; Lok et al., 2014). Combs (2008) propose that vitamin B6 and folate act as cofactors in enzymatic reactions that synthesise monoamines (serotonin, epinephrine), which have been implicated in depression. In addition, vitamin B6 and folate acts as critical cofactors in the one carbon metabolism process, which has important implications in gene-nutrient interactions. Accordingly, one carbon metabolism relies primarily on the dietary intake of vitamin B6 and folate, and deficiency may lead to impaired DNA methylation, reduced S-adenosyl methionine (SAM), and increased neurotoxins through heightened homocysteine levels (Smythies, 2012). Such neurological dysregulations are therefore theorised to contribute to increased vulnerability to developing depressive disorders (Bottigleri, 2005; Smythies, 2012). However, these underlying theoretical processes require further empirical validation.

Surprisingly, the intake of vitamin B12, an additional cofactor required in the one-carbon metabolism pathway, was not associated with either depression or anxiety symptoms in the current study. This finding was similar to the results of Herbsion et al. (2012), which also found a non-significant association between vitamin B12 intake and overall internalising symptoms in adolescents. However, the current study's results were dissimilar to previous research that has examined the relationship between B12 and depressive symptoms specifically (Kim et al., 2008; Sanchez-Villegas et al., 2009; Skarupksi et al., 2010). This lack of consistency in results may be attributed to the amount of vitamin B12 consumed by the participants, with the majority of participants meeting or exceeding the RDI in the current study. As such, the dietary intake of B12 of the participants may have excluded proper examination of deficiency related symptoms.

Zinc

The finding that zinc and depressive symptoms were inversely related was supported by previous research (Maserejian et al., 2012; Yary & Aazami, 2011; Jacka et al., 2012). Zinc deficiency has also been negatively correlated with depressive symptoms in a number of studies (Roozbeh et al., 2011; Stanislawska et al., 2013; Maes et al., 1997). Zinc is hypothesised to exert antidepressant benefit through acting as an anti-oxidant (Prasad, Bao, Beck, Kukuk, & Sarkar, 2004), neuro-protector (Nowak et al., 2005), as well as supporting neurotransmitter synthesis (Takeda, 2000), and overall immune function (Prasad, 2008). Deficits in these mechanisms have been observed in patients with depressive disorders (Dale et al., 2015; Maes, 1993, 2011; Maes et al., 1992, 1996, 1997; Postolache, 2012; Serafini, 2012). Additionally, Maes et al. (1999) demonstrated that zinc deficiency was associated with reduced or impaired essential fatty acids metabolism, including the substrates of Omega 3, 6 and 9, DHA and EPA. Deficiencies in these essential fatty acids have also been noted in depressive illness (e.g. McNamara et al., 2007).

The lack of significant relationship between zinc and anxiety symptoms was inconsistent with previous research (Digirolamo et al., 2010; Jacka et al., 2012). Although Digirolamo et al. (2010) found no significant differences in anxiety scores between zinc and placebo treated groups, serum zinc levels were found to be negatively related to anxiety symptoms. Inconsistencies in findings may be due to differences in the assessment of micronutrient status (e.g., dietary analysis in comparison to actual serum concentrations). Future research could address this through measuring actual serum levels in addition to dietary analysis.

Vitamin C

Contrary to existing research, the current study found vitamin C was not related to internalising symptoms (De Oliveira et al., 2015; Kinsman & Hoot, 1971; Oishi et al., 2009; Payne et al., 2012). For example, De Oliveira et al. (2015) found that students who took a vitamin C supplement had significantly greater reductions in anxiety levels and mean heart rate compared to those that received the placebo. However, the differences between previous research and the present study's results may be due to the amount of vitamin C required to obtain therapeutic benefit. For example, the mean intake of vitamin C in the present study was 215.5mg, whereas previous RCTs have used dose ranges from 500-1000mg per day. Further, it has been proposed that vitamin C may have therapeutic benefits for both anxiety and depressive disorders through having potent anti-oxidant properties (Mazloom et al., 2013). Consuming higher doses of vitamin C could therefore be required in order to enact anxiolytic effects. Future randomised controlled trials could examine this further through titrating vitamin C doses and measuring subsequent changes in anxiety levels relative to placebo.

Magnesium

There was no significant association found between magnesium intake and depressive or anxiety symptoms. The current results were consistent with the findings of Black et al. (2014), which found no significant association between dietary magnesium intakes and internalising symptoms in 684 adolescents aged 14 and 17 years. However, this finding was inconsistent with the results of Tartleton and Littenburg (2015), who found that magnesium intake was negatively related to depressive symptoms in a large community sample of adults. Additionally, Jacka et al. (2012) found magnesium intake to be negatively associated with psychological symptoms, and with a formal depression diagnosis. This inconsistency may be due to differences in measures used across the various studies. Both Jacka et al., (2012) and Tarleton and Littenburg (2015) utilised global psychological symptom measures, such as the Patient Health Questionnaire (PHQ-9; Tartleton & Littenburg, 2015), or categorical diagnosis, whereas the current study utilised a specific measure of both depressive and anxiety symptoms (DASS-21).

There was no significant association found between magnesium intake and anxiety levels. Such results were supported by the findings of Jacka et al. (2009) and Jacka et al (2012), which similarly found non-significant associations between magnesium intake and anxiety symptoms in adults. This finding was also consistent with a previous clinical trial, which showed that 300mg of magnesium supplementation for 5 days did not reduce pre-exam anxiety levels in university students (Gendle & Ohara, 2015).

Strengths and Limitations

The present study should be interpreted in light of several limitations. First, the study was underpowered due to the small sample size. Subsequently, the ability to identify smaller associations between micronutrients and internalising symptoms may have been limited. Therefore, results of the present study should be interpreted with regard to this limitation, and the present findings require replication using larger samples of young adults. Secondly, the

study relied upon self-report measures, which may be subject to social desirability or memory biases. For instance, it may have been difficult for participants to recall serving sizes accurately. Future studies could ameliorate this through the use of food diaries. Thirdly, although a well-developed food analysis program was utilised, nutrient quantities differ between produce, for example organically grown vs. processed food. In addition, it was assumed that dietary intakes were equivalent to serum concentrations of nutrients. However, research has shown that nutrient intake, as derived through diet, is not equal to serum concentration due to differences in bioavailability of foods and actual intestinal absorption of the nutrients. Thus the accuracy of inferences made about participants' actual serum nutrient levels may be different. As aforementioned, measuring dietary intakes in addition to actual blood samples, would allow for more accurate inferences in future research, in addition to utilising a larger sample. Lastly, given the cross sectional nature of the present study, causal pathways between micronutrient intake and internalising symptoms could not be examined. Although several associations were found between micronutrient intakes and internalising symptoms, the results do not suggest that deficiency causes heightened vulnerability to developing internalising disorders. The directionality of this relationship at present still remains unconfirmed. A strength of the study was the use of a well-established measure of depressive and anxiety symptoms, the DASS-21. Additionally, although the study relied upon participants' recalling their own diet history, the study utilised both dietary recall and semi-quantitative food frequency questions in order to estimate nutrient intake accurately. Both methods have been shown to be effective at estimating actual nutrient intakes (Ortiz-Andrellucchi et al., 2009).

Conclusion

This study extends present understanding of the role of micronutrients in specific internalising symptomatology. Current findings suggest that dietary intake of zinc, and

vitamins B3 and B6 are inversely associated with depressive symptoms, while micronutrient intakes were not significantly associated with anxiety symptoms. Such results have important implications for both understanding and treating the neurobiological mechanisms underlying internalising psychopathology. However, the causal pathway between nutrients and mental health still remains unconfirmed. Alternatively, recent research has shown support for overall diet quality as being an important predictor of internalising disorders instead of individual micronutrients (Jacka et al., 2010, 2013b; O'Neil et al., 2014; Sanchez-Villegas et al., 2009; Sanhueza, Ryan, & Foxcroft, 2013). Given the complex interaction of micronutrient metabolism, it is plausible that overall diet quality is a better predictor of mental health than individual micronutrient deficiencies. As such, the current results require further clarification using designs that can infer specific causality such as randomised controlled trials using micronutrient monotherapies. This is particularly pertinent with regard to anxiety, in which there is a large paucity of clinical trial research. Nevertheless, the current results highlight potentially important relationships between zinc and B-group vitamins, and internalising symptoms.

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Appendix A

Table 1

Means (and standard deviations) of all variables

	Male	Female
Physical Activity Level	5.00 (1.94)	4.27 (2.04)
Body Mass Index	24.45 (3.26)	22.07 (4.22)
Total Energy Intake (kj)	7503.01 (2334.5)	7062.69 (2160.72)
Age	22.36 (4.61)	25.30 (8.61)
SES	1.8 (.29)	1.6 (.40)
DASS – Anxiety Subscale	2.50 (2.31)	3.25 (3.49)
DASS – Depression Subscale	2.62 (3.37)	3.42 (3.01)
B1—thiamine* (mg/1000 kcal)	0.19 (.09)	0.18 (.08)
B2—riboflavin* (mg/1000 kcal)	0.23 (.09)	.0.23 (.06)
B3—niacin (mg/1000 kcal)	4.96 (1.35)	4.51 (1.64)
B6 (mg/1000 kcal)	0.23 (.07)	0.24 (.09)
folate (μg/1000 kcal)	57.19 (21.88)	56.03 (23.70)
B12 (μg/1000 kcal)	0.85 (.76)	0.48 (.40)
Vitamin C	22.77 (36.07)	18.88 (11.98)
Zinc	1.40 (.30)	1.34 (0.39)
Magnesium	46.56 (9.07)	49.96 (12.66)

Appendix B

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MACQUARIE
University
SYDNEY·AUSTRALIA

19 May 2016

Dear Prof Rapee

Reference No: 5201600183

Title: *The Association between Micronutrient Intake and Internalising-Externalising Symptoms in Adolescence*

Thank you for submitting the above application for ethical and scientific review. Your application was considered by the Macquarie University Human Research Ethics Committee (HREC (Medical Sciences)).

I am pleased to advise that ethical and scientific approval has been granted for this project to be conducted at:

- Macquarie University

This research meets the requirements set out in the *National Statement on Ethical Conduct in Human Research* (2007 – Updated May 2015) (the *National Statement*).

Standard Conditions of Approval:

1. Continuing compliance with the requirements of the *National Statement*, which is available at the following website:

http://www.nhmrc.gov.au/book/national-statement-ethical-conduct-human-research

2. This approval is valid for five (5) years, subject to the submission of annual reports. Please submit your reports on the anniversary of the approval for this protocol.

3. All adverse events, including events which might affect the continued ethical and scientific acceptability of the project, must be reported to the HREC within 72 hours.

4. Proposed changes to the protocol and associated documents must be submitted to the Committee for approval before implementation.

It is the responsibility of the Chief investigator to retain a copy of all documentation related to this project and to forward a copy of this approval letter to all personnel listed on the project.

Should you have any queries regarding your project, please contact the Ethics Secretariat on 9850 4194 or by email ethics.secretariat@mq.edu.au

The HREC (Medical Sciences) Terms of Reference and Standard Operating Procedures are available from the Research Office website at:

http://www.research.mq.edu.au/for/researchers/how_to_obtain_ethics_approval/human_research_ethics

The HREC (Medical Sciences) wishes you every success in your research.

Yours sincerely

Professor Tony Eyers
Chair, Macquarie University Human Research Ethics Committee (Medical Sciences)

This HREC is constituted and operates in accordance with the National Health and Medical Research Council's (NHMRC) *National Statement on Ethical Conduct in Human Research (2007)* and the *CPMP/ICH Note for Guidance on Good Clinical Practice.*