

**EFFICACY OF A PROBIOTIC SUPPLEMENT AS AN INTERVENTION FOR THE
SYMPTOMS OF DEPRESSION: A DOUBLE-BLIND, RANDOMISED, PLACEBO-
CONTROLLED TRIAL, OPEN LABEL EXTENSION AND 6 MONTH FOLLOW-UP**

A thesis submitted in partial fulfilment of the requirements for the degree of

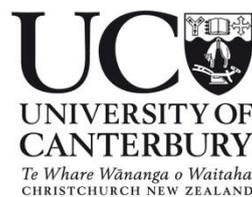
Doctor of Philosophy in Psychology

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Abstract

This thesis presents the first randomised controlled trial (RCT) to investigate whether supplemented probiotic bacteria—“live microorganisms that, when administered in adequate amounts, confer a health benefit on the host” (Sanders, 2008)—affect mood and other psychological outcomes in people presenting with low mood. Seventy-nine participants with at least moderate symptoms of depression were randomised in a double-blind manner to receive either a probiotic preparation containing *Lactobacillus helveticus* and *Bifidobacterium longum* or a matched placebo for eight weeks. The RCT phase was followed by an open label extension in which all participants were offered the active study product for a further 8 weeks. Participants were followed up at 6 months post-study. Based on the existing evidence from gut-brain axis research, and on models linking depression with inflammation, immune activation, low vitamin D levels, and the gut microbiota (outlined in Chapters 1 and 2), it was hypothesised that: the overall sample would have elevated levels of inflammatory biomarkers and low levels of vitamin D at baseline, and that this would be associated with scores on psychological and irritable bowel syndrome (IBS) outcome measures; that group differences (active treatment versus placebo) would be observed in scores on psychological outcome measures after eight weeks of probiotic intervention; that group differences would also be observed in blood levels of proinflammatory cytokines, hsCRP, vitamin D and BDNF, and scores on a measure of gut function/IBS, and that levels of these variables may predict or impact on treatment response; and that group differences would be observed on outcome measures at the point of the 6-month follow-up between those who continued to take the probiotic and those who discontinued probiotic use. In total, 58 of the 77 participants who provided baseline blood samples (75%) had at least one marker of inflammation elevated outside the normal reference range at baseline. Baseline vitamin D was approaching the deficient level, displayed a seasonal pattern, and was associated with severity on one measure of cognition. No significant differences were found between the active treatment and placebo groups on any psychological outcome measure, the measure of gut function or in the level of any blood-based biomarker in the randomised phase.

Baseline vitamin D level was found to moderate treatment effect on several outcome measures. The results of the open label extension supported the lack of efficacy observed in the randomised phase, and also allowed for the comparison of efficacy over intervention periods of varying durations. The results of the follow-up at 6 months post-trial indicated that, while mean scores on psychological outcome measures remained lower than baseline, there was regression on some outcome measures after the study. When the participants who replied to the 6 month follow-up questionnaire were divided into groups based on their self-reported dominant treatment since the trial (probiotics/nutrition, standard treatment or no treatment) there was no difference in mood or other psychological outcomes among the groups at 6 months. The current trial found no evidence that this probiotic formulation is effective in treating the symptoms of depression or IBS, or in moderating the levels of inflammatory and other biomarkers in a sample recruited with moderate depression. This finding does not support the theory proposed in several narrative reviews which suggests probiotics as a possible intervention for depression and other mental health outcomes, but is supported by the systematic review of human probiotics studies presented in Chapter 3 which found overall limited evidence of probiotic efficacy for psychological outcomes. Future studies in the area should attempt to further broaden this field, in particular by recruiting samples with mild and/or non-chronic depression for interventional studies, or by approaching probiotics as a preventative or adjuvant treatment strategy for depression.

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List of Abbreviations

5-HT: 5-hydroxytryptamine (serotonin)
5-HTP: 5-hydroxytryptophan
ACC: Anterior cingulate cortex
ACT: Acceptance and commitment therapy
ANS: Autonomic nervous system
ATP: Adenosine triphosphate
ATQ: Automatic Thoughts Questionnaire
BDNF: Brain derived neurotrophic factor
CBT: Cognitive behavioural therapy
CNS: Central nervous system
CRP: C-reactive protein / hsCRP: High sensitivity CRP
DAS: Dysfunctional Attitude Scale
DASS: Depression, Anxiety and Stress Scale
DSM: Diagnostic and Statistical Manual of Mental Disorders
ENS: Enteric nervous system
GAF: Global Assessment of Functioning
GBA: Gut-brain axis
GI: Gastrointestinal
HMP: Human Microbiome Project
HPA axis: Hypothalamic-pituitary-adrenal axis
IBS: Irritable bowel syndrome
IBS-SSS: Irritable Bowel Syndrome Severity Scoring Scale
ICD: International Statistical Classification of Diseases and Related Health Problems
iCGI: Improved Clinical Global Impressions Scale
Ig: Immunoglobulin (e.g., IgA: immunoglobulin A)
IL: Interleukin (e.g., IL-1 β , IL-6)
LPS: Lipopolysaccharide
MADRS: Montgomery-Åsberg Depression Rating Scale
MAOI: Monoamine oxidase inhibitor
MCT: Metacognitive therapy
MBCT: Mindfulness-based cognitive therapy
IPT: Interpersonal therapy
MDD: Major depressive disorder
MDE: Major depressive episode
O&NS: Oxidative and nitrosative stress
OL phase: Open label phase
PFC: Prefrontal cortex
POMS: Profile of Mood States
PUFAs: Polyunsaturated fatty acids
QIDS: Quick Inventory of Depressive Symptomatology
RCT phase: Randomised controlled trial phase
RNS: Reactive nitrogen species
ROS: Reactive oxygen species
SCFA: Short-chain fatty acid
SFB: Segmented filamentous bacteria
SNRI: Serotonin-norepinephrine reuptake inhibitor
SPF: Specific pathogen-free
SSRI: Selective serotonin reuptake inhibitor
TCA: Tricyclic antidepressant
TNF- α : Tumor necrosis factor alpha

Chapter 1: Major Depressive Disorder

This thesis presents a randomised controlled trial (RCT) investigating the effect of supplemented probiotic bacteria—“live microorganisms that, when administered in adequate amounts, confer a health benefit on the host” (Sanders, 2008)—on mood and other psychological outcomes in people with moderate symptoms of depression. This chapter will provide a detailed outline of major depressive disorder (MDD), as the symptoms of this disorder are being studied in the main RCT. The diagnostic criteria, prevalence, course and functional consequences of MDD described in detail. Patterns of comorbidity between MDD and other psychiatric disorders, and between MDD and systemic (medical) disorders, will be discussed, with relation to possible shared underlying biological mechanisms including inflammation and immune activation. The plausible aetiological mechanisms which may underpin depression will then be discussed, with a primary focus on the possible role of inflammation and immune activation in the development and maintenance of MDD, as these underlying factors support the possible mechanisms of action of probiotics on depressive symptoms. Current standard treatments and alternative treatments for depression will then be reviewed.

1.1 Defined

Major depressive disorder (MDD), also known as clinical depression or unipolar depression, is a mood disorder characterised by low mood and/or a loss of interest in activities and the surroundings. A diagnosis requires a distinct change in mood—usually persistent sadness or irritability—as well as several psychophysiological changes, such as disturbances in sleep or appetite, slowed speech or movement, and suicidal ideation. MDD is associated with high mortality: up to 15% of individuals with severe MDD die by suicide (American Psychiatric Association, 2000). The criteria from the two main diagnostic tools used in psychiatry—the Diagnostic and Statistical Manual of Mental Disorders (DSM) and the International Statistical Classification of Diseases and Related

Health Problems (ICD)—are outlined and compared below. The text revision of the fourth edition of the DSM (DSM-IV-TR) was the latest version available at the beginning of this research; therefore, the criteria in this originally available version will be compared to the latest version (DSM-5) which was released during the course of the present research. The 10th revision of the ICD (ICD-10) was the version available at the time of this research.

1.1.1 DSM-IV-TR criteria. MDD is classed as an Axis I (Clinical) Disorder in the DSM. The diagnostic criteria from DSM-IV-TR state that, to meet criteria for a major depressive episode, at least five of the symptoms from criterion A (Table 1) must have been present during the same two week period, and that these symptoms should represent a change from previous functioning. At least one of the symptoms from this section must be either (1) depressed mood or (2) loss of interest or pleasure.

The other criteria specify that (B) the symptoms must not meet criteria for a mixed episode, (C) the symptoms should cause clinically significant distress or impairment in social, occupational, or other important areas of functioning, (D) the symptoms must not be due to the direct physiological effects of a substance (e.g., a drug of abuse, a medication) or a general medical condition (e.g., hypothyroidism) and (E) the symptoms must not be better accounted for by bereavement (i.e., after the loss of a loved one, the symptoms persist for longer than 2 months or are characterized by marked functional impairment, morbid preoccupation with worthlessness, suicidal ideation, psychotic symptoms, or psychomotor retardation).

Further, the episode will be classed as a single major depressive episode if (A) there is a presence of a single major depressive episode only, (B) the major depressive episode is not better accounted for by schizoaffective disorder and is not superimposed on schizophrenia, schizophreniform disorder, delusional disorder, or psychotic disorder not otherwise specified and (C) there has never been a manic episode, a mixed episode, or a hypomanic episode. The patient will only be diagnosed as having major depressive disorder if they have had two or more major depressive episodes, each separated by at least 2 months in which criteria are not met for a major

depressive episode. Episodes of substance-induced mood disorder or of mood disorder due to a general medical condition do not count towards a diagnosis of MDD.

Table 1. Symptoms from criterion A of the DSM-IV criteria for a Major Depressive Episode

Criterion A	
1	Depressed mood for most of the day, nearly every day, as indicated either by subjective report (e.g., feels sad or empty) or observations made by others (e.g., appears tearful)
2	Markedly diminished interest or pleasure in all, or almost all, activities most of the day, nearly every day (as indicated either by subjective account or observation made by others)
3	Significant weight loss when not dieting or weight gain (e.g., a change of more than 5% body weight in a month), or decrease or increase in appetite nearly every day
4	Insomnia or hypersomnia nearly every day
5	Psychomotor agitation or retardation nearly every day
6	Fatigue or loss of energy nearly every day
7	Feelings of worthlessness or excessive or inappropriate guilt (which may be delusional) nearly every day (not merely self-reproach or guilt about being sick)
8	Diminished ability to think or concentrate, or indecisiveness, nearly every day (either by subjective account or as observed by others)
9	Recurrent thoughts of death (not just fear of dying), recurrent suicidal ideation, or a suicide attempt or specific plan for committing suicide

1.1.1.1 Episode specifiers. There are specifiers in DSM-IV-TR which indicate either the severity of the current major depressive episode or the level of remission if full criteria are no longer met. If criteria are met for a major depressive episode, the episode can be classified as mild, moderate, severe without psychotic features, or severe with psychotic features. Severity is attributed based on the number of criteria symptoms present, the severity of the symptoms, and the degree of functional disability and distress. Mild episodes are characterised by the presence of five or six symptoms, and either mild disability or normal functioning but which requires substantial or unusual effort. A severe episode without psychotic features is characterised by most, if not all, of the symptoms outlined in the criteria, and obvious, observable impairment in functioning or disability, such as the inability to work or care for children. Moderate episodes have a severity that lies between mild and severe. A severe episode with psychotic features refers to the presence of hallucinations or delusions during the episode. These are most commonly mood-congruent psychotic features, which can include delusions of guilt or deserved punishment, and transient

auditory hallucinations, such as voices that berate the person for short-comings or sins. Mood-incongruent psychotic features are less common.

If full criteria are no longer met for a major depressive episode, the specifiers indicate whether the most recent episode is in partial or full remission. Full remission requires a period of at least two months in which there are no significant symptoms of depression. There are two possible ways that an episode can be classified as being in partial remission: firstly, the episode is considered to be in partial remission if some symptoms of a major depressive episode are still present, but full criteria are no longer met; or secondly, if there are no longer significant depressive symptoms, but the two month period required for full remission has not been reached.

Other specifiers which can be applied to the most recent major depressive episode include the “chronic” specifier, which can be applied if full criteria have been met continuously for at least two years. The “with catatonic features” specifier can be applied to the current episode if the episode is characterised by marked psychomotor disturbance, which may include motoric immobility or excessive motor activity, mutism, echolalia and echopraxia. Finally, the “with melancholic features” specifier can be applied to the current or most recent episode if that episode is characterised by a loss of pleasure in all activities, or a lack of reactivity to usually pleasurable stimuli. The individual’s mood does not improve, even temporarily, when something good happens. There must be a complete absence of the capacity for pleasure, not merely a reduction. Additionally, at least three of the following symptoms must be present: a distinct quality of the depressed mood, mood that is regularly worse in the morning, early morning awakening, psychomotor retardation or agitation, anorexia or significant weight loss, or excessive and inappropriate guilt.

1.1.1.2 Subtypes and course specifiers. There are various subtypes of depression, many of which depend upon the onset and course of the expression of the disorder. For example, postnatal depression is a type of depression experienced by women after giving birth which leads to significant impairment in functioning. Seasonal affective disorder (SAD) is a specifier of mood

disorder that refers to a seasonal pattern of depressive symptoms with an onset during autumn or winter and resolving in spring. Other subtypes and diagnoses have been identified, including those which stem from the episode specifiers outlined above; e.g., melancholic depression, catatonic depression, psychotic major depression. Atypical depression is characterised by mood reactivity and positivity, significant weight gain or increased appetite, and a sensation of heaviness in the limbs (leaden paralysis). Depressive Disorder Not Otherwise Specified includes diagnoses such as recurrent brief depression (similar to MDD but much shorter in duration, with episodes occurring frequently and lasting less than two weeks) and minor depressive disorder (depression that does not meet full criteria for MDD, but where at least two symptoms are present for at least two weeks). Dysthymia is not a specifier of depression but a related condition, where the symptoms of depression are present in a milder form but for a longer period.

1.1.2 Changes to criteria in DSM-5. The fifth edition of the DSM was released at the American Psychiatric Association's Annual Meeting in May 2013. For this edition, the core symptoms applied to the diagnosis of MDD and the mandatory two week minimum duration are identical to DSM-IV. Criterion A for a major depressive episode in DSM-5 is identical to that of DSM-IV. The requirement for clinically significant distress or impairment in social, occupational or other important areas of life is also still present, although this is now listed as criterion B rather than criterion C (American Psychiatric Association, 2013a).

Some minor notes have been added to the core criteria for MDD, mostly related to the expression of the disorder in children and adolescents; distinctions which were noted in the "Diagnostic Features" section in DSM-IV-TR but which were not highlighted in relation to the core criterion. Firstly, a note has been added to the first symptom from criterion A ("Depressed mood for most of the day, nearly every day, as indicated either by subjective report (e.g., feels sad, empty, hopeless) or observations made by others (e.g., appears tearful)") which posits that, in children and adolescents, this can be expressed as irritable mood. Secondly, under "Significant weight loss when

not dieting or weight gain (e.g., a change of more than 5% body weight in a month), or decrease or increase in appetite nearly every day”, it is noted that in children, the failure to make expected weight gain should be considered.

Finally, the bereavement exclusion from DSM-IV-TR (i.e., the exclusion of depressive symptoms lasting less than 2 months after the death of a loved one) has been omitted from DSM-5. The American Psychiatric Association (2013b) summarise the reasons for this omission, which include, firstly, to remove the implication that bereavement typically lasts only two months, when the duration is more commonly recognised to be one to two years. Secondly, bereavement is recognised as a severe psychological stressor that can trigger a major depressive episode in a vulnerable individual, generally beginning soon after the loss. Thirdly, bereavement-related major depression is most likely to occur in individuals with past personal and family histories of major depressive episodes. It is genetically influenced and is associated with similar personality characteristics, patterns of comorbidity, and risks of chronicity and/or recurrence as non-bereavement-related major depressive episodes. Finally, the depressive symptoms associated with bereavement-related depression respond to the same psychosocial and medication treatments as non-bereavement-related depression. In the DSM-5 criteria for major depressive disorder, a detailed footnote has replaced the more simplistic bereavement exclusion in DSM-IV-TR, which aims to aid clinicians in making the distinction between the symptoms characteristic of bereavement and those of a major depressive episode.

More attention is given in DSM-5 to suicidality, as it represents a significant concern in psychiatry (American Psychiatric Association, 2013b). Guidance is given on assessment of suicidal thinking, plans, and the presence of other risk factors.

1.1.2.1 Episode specifiers. In addition to the specifiers in DSM-IV-TR, two new specifiers have been added in DSM-5. Firstly, a new specifier has been added to account for the presence of mixed manic and depressive symptoms in a unipolar major depressive episode (“with mixed

features”). Secondly, the new “with anxious distress” specifier allows the clinician to rate the severity of anxious distress in patients with depressive disorders.

1.1.3 ICD-10 criteria. The ICD-10 criteria for MDD are similar to those of the DSM. In addition to the criteria present in the DSM, ICD-10 states that self-esteem and self-confidence are almost always reduced. It also specifies that the lowered mood varies little from day to day, and that it is unresponsive to external circumstances (World Health Organization, 2010). The changes in sleep, appetite, libido, psychomotor speed and levels of interest or pleasure which commonly occur in depression are termed “somatic” symptoms in ICD-10.

ICD-10 also specifies parameters by which the severity of a depressive episode can be defined. A mild depressive episode is defined as one in which two or three symptoms are present, and the patient is “usually distressed by these but will probably be able to continue with most activities”; a moderate depressive episode is where four or more symptoms are usually present, and the patient is “likely to have great difficulty in continuing with ordinary activities”; and a severe depressive episode (without psychotic symptoms) is described as an episode of depression in which “several...symptoms are marked and distressing, typically loss of self-esteem and ideas of worthlessness or guilt. Suicidal thoughts and acts are common and a number of "somatic" symptoms are usually present” (p.140).

ICD-10 defines “recurrent depressive disorder” as a disorder characterised by repeated depressive episodes, without any history of independent episodes of mood elevation and increased energy (mania). It is stipulated that there may be brief episodes of mild mood elevation and overactivity (hypomania) immediately after a depressive episode, which may sometimes be precipitated by the use of antidepressant drugs (World Health Organization, 2010).

1.2 Prevalence

The 12-month prevalence rate of MDD in New Zealand is estimated at around 6%, with higher 12-month prevalence rates in women (7.1%, versus 4.2% in men) (J. E. Wells et al., 2006). The 12-month prevalence rates are five-fold higher for younger people (8.7% in 16-24 year olds) than for older people (1.7% in people 65 years and over) (J. E. Wells et al., 2006). See Figure 1 for the 12-month prevalence rates across different age groups in New Zealand.

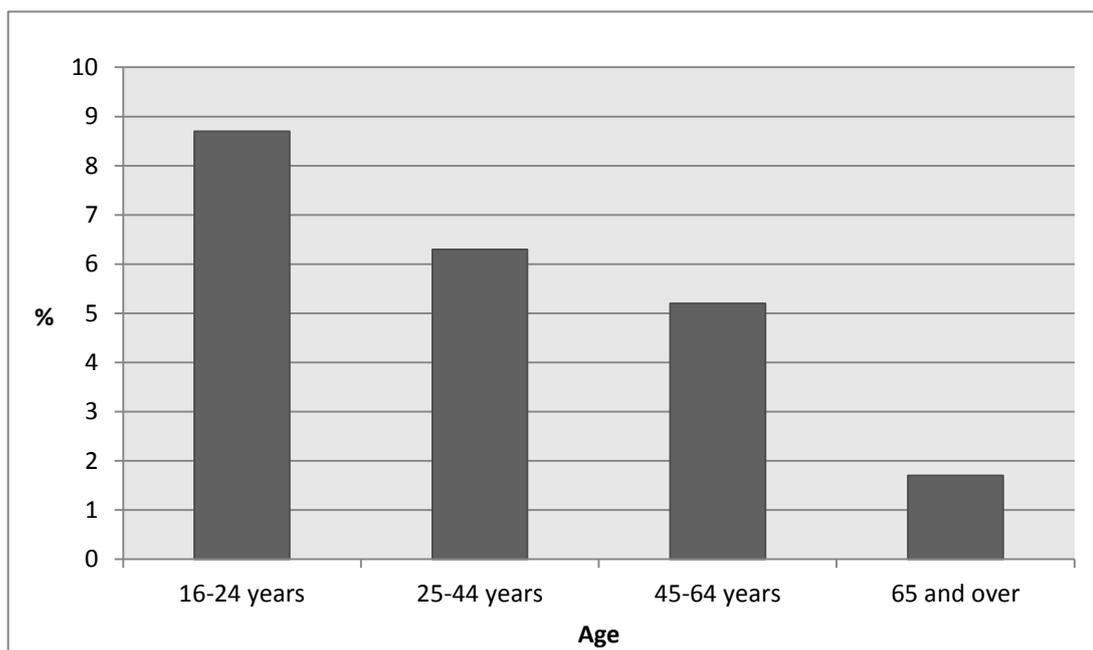


Figure 1. 12-month prevalence rates among different age groups in New Zealand, data from J. E. Wells et al. (2006).

MDD is one of the leading causes of disability worldwide, but the prevalence of MDD varies between countries, with higher rates found in high-income countries compared with low- to middle-income countries: averaged lifetime prevalence 14.6% and 11.1% respectively (Bromet et al., 2011). The 2010 Global Burden of Disease Study identified depressive disorders as a leading cause of burden, with an overall burden of 3.8% of global Disability Adjusted Life Years (DALY) (Ferrari et al., 2013). By the year 2020, depression is projected to be the second leading cause of burden by DALY for all ages (World Health Organization, 2001).

1.3 Course

MDD may first appear at any age, but the likelihood of developing the disorder increases dramatically with the onset of puberty (American Psychiatric Association, 2013a). The National Comorbidity Survey Replication found that the median age of onset for mood disorders was 30 years (Kessler et al., 2005); however, this study was only conducted in adults (18 years and older), and so does not take onset during childhood and adolescence into account. In their longitudinal study on children aged 9-13 years at intake (assessed until age 16), Costello, Mustillo, Erkanli, Keeler, and Angold (2003) found that the three-month prevalence of depressive disorders was 2.2%, this being higher in girls (2.8%) than boys (1.6%). In this study, the highest three-month prevalence rates of depressive disorders were among those who were 15-16 years old (3.1-3.7%).

The course of MDD is highly variable, with some individuals rarely, if ever, experiencing full remission, and others having long periods of remission between distinct episodes. In DSM-5, it is stated that recovery typically begins within three months of onset, for two in every five people with MDD, and within one year for four in five people. It is also stated that the risk of recurrence becomes progressively lower over time as the duration of remission increases (American Psychiatric Association, 2013a).

It has been known for some time that the continued presence of residual depressive symptoms during recovery is associated with faster and more frequent relapse (Judd et al., 1998; Paykel et al., 1995). However, in their 12 year, prospective, naturalistic follow-up trial, Judd et al. (2000) found that the presence of residual subthreshold symptoms, after resolution of the first lifetime major depressive episode, was associated with a more severe, relapsing and chronic future course, which has implications for therapy duration for first episodes of depression. Kovacs (1996) found that the rate of recurrence is similar among children as it is among adults, but that young people convert from unipolar to bipolar depression more frequently than adults do. The author suggests that this may be because the early onset form of the disorder is a particularly serious form

of the disease. Martin et al. (2004) found, however, that age is an effect modifier on the risk of antidepressant-associated manic conversion, with the highest conversion risk among children aged 10-14 years, suggesting antidepressant use as a possible alternative factor underlying the increased rates of conversion from unipolar to bipolar depression in young people.

1.4 Functional Consequences

MDD is typically accompanied by significantly impaired functioning, which may occur either globally, or in specific domains such as occupational or interpersonal functioning. Impairment may be anywhere from very mild, having little effect on daily functioning, to almost complete incapacity: depressed individuals in a severely impaired state of functioning may be unable to attend to basic self-care needs, or be mute or catatonic. Occupational functioning appears to be frequently affected by MDD: Alonso et al. (2004) found that depressed workers lost three to four times more work days than people without any 12-month mental disorder. DSM-5 states that many of the functional consequences of MDD develop from individual symptoms (American Psychiatric Association, 2013a). However, Lam et al. (2013) state that, in contrast to the comprehensive literature detailing the importance of occupational functioning in MDD, there is comparatively little empirical evidence evaluating the impact of specific depressive symptoms on occupational functioning. The authors suggest fatigue as a symptom which has high potential in terms of contributing to functional impairment, but they state that this symptom has received relatively little attention due to the current focus on emotional and cognitive domains in MDD (Lam et al., 2013).

1.5 Comorbidity

1.5.1 Comorbidity with other psychiatric disorders. According to DSM-5, major depressive disorder frequently co-occurs with substance-related disorders, panic disorder, obsessive compulsive disorder, eating disorders, and borderline personality disorder. The National Comorbidity Survey Replication (conducted in the USA; Kessler et al., 2003), found that over 70% of respondents with lifetime MDD also met criteria for at least one other psychiatric disorder, including

around 60% with a comorbid anxiety disorder, and 24% with a substance use disorder. Similarly, the Vantaa Depression Study (Finland; Melartin et al., 2002) found that 79% of patients with MDD suffered from at least one or more comorbid psychiatric disorders, and the rate of comorbid anxiety disorder was found to be 59%. The rates co-occurrence of anxiety disorders with MDD appear to be robust: in a sample of 479 outpatients with DSM-IV unipolar MDD, Zimmerman, Chelminski, and McDermut (2002) found that the rate of any comorbid anxiety disorder was 56.8%. Total comorbidity of MDD with any other Axis I disorder was found to be 64.1%, with 36.7% of patients having two or more comorbid Axis I disorders (Zimmerman et al., 2002). Watson and Stasik (2014) conclude that “most individuals with major depression also meet criteria for comorbid anxiety disorder and vice versa” (p.47).

The patterns of psychiatric comorbidity in people with MDD appear to be fairly consistent across age groups. In a non-systematic review, Kovacs (1996) found the rates of comorbidity of MDD with any other psychiatric disorder in children and youths to be similar to (or only slightly higher than) rates in adults. In this review, it was found that approximately 60-90% of adults with MDD, and 80-95% of children and youths with MDD, had any comorbid Axis I or Axis II psychiatric disorder (Kovacs, 1996). In an epidemiological sample of young people (aged 11-21) in New Zealand around the same time, Newman et al. (1996) found a statistically significant odds ratio of 5.7 for having both a mood disorder and an anxiety disorder, showing that anxiety disorders are frequently comorbid with MDD in young people as well as adults.

1.5.2 Comorbidity with physical disorders. MDD frequently co-occurs with a multitude of medical illnesses and disorders. There is high comorbidity (20-50%) of MDD with neurodegenerative and other brain disorders, including stroke, Parkinson’s Disease and multiple sclerosis (Kanner, 2005). For example, in a systematic review of studies in various settings, Reijnders, Ehrt, Weber, Aarsland, and Leentjens (2008) found that around 50% of patients with Parkinson’s Disease have a comorbid mood disorder (major depression, minor depression or dysthymia).

Concomitant MDD in patients with brain disorders can affect quality of life, is associated with greater use of healthcare systems and more medical hospitalisations, and can lead to greater functional decline (Maes, Kubera, Obuchowiczwa, Goehler, & Brzeszcz, 2011).

There is evidence of high comorbidity of MDD with many systemic disorders, including diabetes (Anderson, Freedland, Clouse, & Lustman, 2001), coronary artery disease (Rudisch & Nemeroff, 2003), chronic obstructive pulmonary disease (Mikkelsen, Middelboe, Pisinger, & Stage, 2004), rheumatoid arthritis (Bruce, 2008), irritable bowel syndrome (IBS; Fond et al., 2014), and inflammatory bowel disease (Graff, Walker, & Bernstein, 2009). Prevalence of comorbid depression in these disorders is very high: for example depressive symptoms may occur, with at least mild severity, in up to 42% of patients with rheumatoid arthritis, and in up to 50% of patients with chronic obstructive pulmonary artery disease (Bruce, 2008; Mikkelsen et al., 2004). The presence of comorbid depression in these disorders affects quality of life and can lead to greater impairment in functioning. For many of these disorders, comorbid MDD is associated with an increase in mortality and poorer long-term outcomes (Maes, Kubera, et al., 2011).

It has been suggested that shared risk factors such as smoking, poor diet or obesity, may be the reason for the high comorbidity of depression with some of the above-mentioned medical disorders. However, the only common attribute of *all* of these brain and other medical disorders is the presence of immune activation and/or induction of inflammatory, oxidative and nitrosative stress (O&NS) pathways (Maes, Kubera, et al., 2011). There is now evidence that such activation may underpin the pathophysiology of MDD, and this proposed shared underlying pathophysiology may explain the pattern of high incidence of comorbid MDD in these medical disorders.

1.6 Aetiology

The aetiology of depression has been debated for centuries. Most recently, MDD has come to be seen as a very heterogeneous disorder, without one specific cause. The most commonly described psychological factors which may contribute to depression will be described below,

including learned helplessness, cognitions and stress. The biological factors which may contribute to the aetiology of depression will then be discussed, including disruption of the endocrine system, genetic factors, brain structure and monoamine neurotransmitters. The monoamine hypothesis of depression will be outlined and critically discussed in light of the newer research on other biological factors which likely contribute to the development and maintenance of depression, including cell-mediated immune activation, induction of inflammatory and O&NS pathways, and mitochondrial function (Maes, Kubera, et al., 2011). Each of these biological factors will then be discussed in further detail, with a primary focus on inflammation and cell-mediated immune activation, as these factors underwrite the rationale for testing probiotics as an intervention for depression.

1.6.1 Learned helplessness. The learned helplessness model of depression was developed after animal studies showed that rats have an emotional reaction to electric shocks outside of their control which mimics human depression (e.g., Overmier & Seligman, 1967). The rats in these studies were shown to function reasonably well if they could do something (e.g., press a lever) to avoid the shocks; however, if the rats learned that they had no control over the shocks they became helpless, manifesting the animal equivalent of depression. The central theory of the model is that a belief in independence between responding and reinforcement is central to the aetiology of reactive depression.

W. R. Miller and Seligman (1975) demonstrated similar impairment in naturally occurring depression and laboratory-induced learned helplessness in humans. They recruited both depressed and non-depressed subjects, and exposed participants from each group to three conditions – escapable noise, inescapable noise, or no noise. They then tested all participants on a series of patterned anagrams, and found that depressed participants subjected to no noise—who were poorer at solving anagrams than the non-depressed group subjected to no noise—showed similar levels of deficits in solving the anagrams as non-depressed subjects exposed to inescapable noise. Inescapable noise did not increase the deficit of the depressed group. This study lends support to

the learned helplessness model of depression by suggesting parallel effects of depression and helplessness.

Several reformulations of the model have occurred over time, attempting to address issues within the model (e.g., Abramson, Metalsky, & Alloy, 1989; Abramson, Seligman, & Teasdale, 1978). Abramson et al. (1989) reformulated the model and named the new version the hopelessness theory of depression. This version focuses less on negative attributional styles than the previous model (Abramson et al., 1978), and highlights the development of a sense of hopelessness as critical in the development of depression. This appears to fit well with current thinking on fundamental differences between anxiety and depression: both depressed and anxious individuals feel helpless and believe that they lack control, but crucially, only in depression do they give up and develop a sense of hopelessness about ever regaining control (Alloy et al., 2006).

1.6.2 Cognitions. Beck's (1963, 1967) cognitive theory of depression emphasises that cognitive structures are critical components in the development, maintenance and recurrence of depression. "Schemas" are central to Beck's cognitive theory of depression. Beck defines schemas as stored bodies of knowledge that affect the encoding, comprehension and retrieval of information, through guiding attention, expectancies, interpretations and memory searches. There is significant interpersonal variation in the content and organisation of schemas dependent on past experiences. Once depressogenic schemas are activated, they provide access to a complex system of negative themes and cognitions. This in turn leads to the onset of a pattern of negative self-referent information processing characterised by systematic errors in thinking, which can lead an individual to develop a "negative cognitive triad". Beck's Negative Cognitive Triad is defined as negative thoughts about the self, the world, and the future. A central component of Beck's theory is that the presence of a negative cognitive triad causes depression, as well as deficits in motivational, behavioural and physiological functioning.

Beck's theory has been empirically supported by a large amount of research, although there are some conflicting findings. For example, a study by Abela and D'Alessandro (2002) on college admissions showed that students' negative views about their future was a strong mediator of the interaction between dysfunctional attitudes and an increase in depressed mood after a negative admissions outcome. However, the study did not find that the relationship was mediated by the students' view of the self, as was hypothesised, suggesting that Beck's theory of depression may not be comprehensive. Beck's theory is a diathesis-stress model of depression. The stress-diathesis model states that diathetic individuals—individuals who have a predisposition to psychological disorders—respond with abnormal or pathological reactions to physiological stimuli or the ordinary conditions of life that are borne by the majority of individuals without injury (Zuckerman, 1999). Without stressful events, individuals who possess depressogenic schemas are no more likely to become depressed than those who do not.

1.6.3 Stress. Psychosocial stressors are associated with the onset (Kendler, Karkowski, & Prescott, 1999), severity (Hammen, Davila, Brown, Ellicott, & Gitlin, 1992) and duration of episodes of MDD (Kendler, Walters, & Kessler, 1997). It has been estimated that around 80% of cases of MDD are preceded by major life events, and stressors are 2.5 times more likely in depressed patients than controls (Hammen, 2005). However, it is possible that stress, including early life stress in the form of childhood adversity, may impact on the risk of developing depression through interacting with a genetic vulnerability to depression (see section 1.6.5 Genetics). Another possible mechanism by which stress may contribute to the onset and maintenance of depression is through its effects on hypothalamic-pituitary-adrenal (HPA) axis function and the endocrine system, discussed below.

1.6.4 The endocrine system. It has been hypothesised that the HPA axis—a brain circuit that begins in the hypothalamus and runs through the pituitary gland, which regulates the endocrine system (Pariante & Lightman, 2008)—may be overactive in depression (Thase, 2009). This overactivity may lead to excess cortisol—a hormone which has been shown to be elevated during

periods of psychological stress (e.g., Kirschbaum et al., 1995)—which may in turn lead to alterations in HPA axis functioning, including resistance to negative feedback (Young, Lopez, Murphy-Weinberg, Watson, & Akil, 2000).

Sachar et al. (1973) found that depressed patients had elevated plasma cortisol concentrations over a 24 hour period, and an altered circadian pattern of cortisol secretion. It has been estimated that around 50% of depressed patients have elevated baseline levels of cortisol (Young et al., 2000), although this rate depends on the population sampled (Maes, Calabrese, & Meltzer, 1994). Depressed patients with high resting plasma cortisol still show a substantial cortisol response to stressors in addition to the elevated baseline levels (van Praag, 2004). One recent finding from animal studies suggests that long-term overproduction of stress hormones may impact on the ability of the organism to form new neurons (neurogenesis), leading to the hypothesis that suppression of neurogenesis in the hippocampus may underlie the connection between high levels of cortisol and depression (Glasper, Schoenfeld, & Gould, 2012).

1.6.5 Brain structure and function. In addition to the hippocampus, the structure and function of several other brain regions have been implicated in the aetiology of depression. However, in many cases, whether differences in brain structure and function precede depression (and may therefore contribute to its development), or are a consequence of being depressed, is still debated. A recent meta-analysis of voxel-based morphometry studies—a neuroimaging analysis technique that uses statistical parametric mapping to map the volume of the whole brain, allowing for regionally unbiased interrogation of brain tissue composition—found that, overall, grey matter in a confined cluster of the anterior cingulate cortex (ACC) was significantly reduced in patients with MDD versus healthy matched controls. They also found grey matter reductions in the dorsolateral and dorsomedial prefrontal cortex (PFC), with reductions in grey matter volumes in the dorsomedial PFC being associated with multiple episodes of depression. Reduction in grey matter volume in the rostral ACC was the most consistent finding overall. The authors state that these grey matter

abnormalities suggest that chronic MDD has a robust effect on brain structure which may be central to the pathophysiology of MDD. Drevets, Price, and Furey (2008), however, posit that the spontaneous and perseverative nature of MDD symptoms, and the responsiveness of these symptoms to antidepressant drugs, suggests that abnormal brain processes may underlie the aetiology of the condition. The authors state that the findings of a variety of neurophysiological, neuropathological, and neurochemical abnormalities in MDD, within the neural systems that modulate emotional behaviour, are consistent with this expectation. However, the authors concede that none of these abnormalities has shown sufficient sensitivity and specificity to prove a useful diagnostic test for MDD (Drevets et al., 2008).

1.6.6 Genetics. A meta-analysis of twin studies—studies comparing concordance rates for major depression in monozygotic and dizygotic twins—suggests a genetic heritability of around 37% for MDD (Sullivan, Neale, & Kendler, 2000). The authors conclude that major depression is a familial disorder, and that this familiarity mostly or entirely results from genetic influences. However, the authors note that environmental influences are also aetiologically significant, and that MDD probably does not result from either genetic or environmental influences alone, but more likely from both. In a large study on 2,974 complete twin pairs, Kendler, Gardner, Neale, and Prescott (2001) confirmed that the genetic heritability of depression was greater in women than in men. The authors conclude that genetic factors play a larger role in the aetiology of MDD in women than in men, but this difference was only observed when using broader, but not narrower, definitions of illness (from DSM-III-R). The authors note that this difference may confound the findings of genetic association studies, as the impact of some genetic factors on risk for MDD will differ in men and women.

Genetic polymorphisms (natural genetic variations) in the promoter region of the serotonin transporter (5-HTTLPR) have been widely studied in relation to depression. In their meta-analysis of studies evaluating associations between genetic polymorphisms and MDD, Lotrich and Pollock (2004) found a small but significant effect of the 5-HTTLPR genotype for MDD (odds ratio. 1.16),

reflecting that individuals who carried the short allele were at elevated risk of depression. However, they drew attention to the size of the effect, and noted that the heterogeneity between studies and other confounders should be taken into account when interpreting their findings. It is also important to note that short allele carrier status elevates the risk of depression in the context of environmental adversity, and that this gene-environment interaction has been observed in a number of studies (e.g., Caspi et al., 2003; Eley et al., 2004).

Su et al. (2009) did not find an association of 5-HTTLPR polymorphisms and depression in their study on 360 male twins, but they did find that a genetic vulnerability involving other single-nucleotide polymorphisms (SNPs) in other areas of the human serotonin transporter gene (SLC6A4) was significantly associated with both increased depressive symptoms and elevated plasma IL-6 levels. This suggests that the genetic vulnerability to depression may also be linked with increased inflammatory activation, which is discussed below as a plausible aetiological factor in the development of depression.

1.6.7 Monoamines. For many years, a deficiency in monoamine neurotransmitters, particularly serotonin (5-hydroxytryptamine; 5-HT), was the prevailing hypothesis in depression. This theory arose from observations that reserpine (a drug that depletes the brain of monoamine neurotransmitters) inadvertently caused symptoms of depression (Gardner & Boles, 2011). Based on this finding, and studies on the mechanism of action of tricyclic antidepressants, a causal relationship was suggested between disturbances in monoamine metabolism and depression (Coppin, 1967). Even at this time, however, Coppin acknowledged that monoamines were probably not the sole mechanism of disease in depression. He stated:

“...we must face the very real possibility that we are far from the primary disturbance in depression. The changes may all be secondary to other abnormalities which have not been taken into account at all, and we may be in the position of investigators of pernicious anaemia before anything was known about vitamin B₁₂.” (p.1237)

There is abundant evidence that the serotonergic system is tightly coupled with depression onset and neuroprogression in the disorder (Maes & Meltzer, 1995). One mechanism by which 5-HT could influence neuroprogression in depression is that 5-HT acts as a neurotrophic factor, promoting neurogenesis and brain derived neurotrophic factor (BDNF) expression (Moylan, Maes, Wray, & Berk, 2012); therefore, lowered metabolism of 5-HT could contribute to reduced neurogenesis, and consequent neuroprogression. However, the idea that the pathogenesis of depression is predominantly a function of monoamines alone is contravened by more recent research (outlined below) which highlights the apparent roles of mitochondrial dysfunction, inflammation, immune activation and O&NS in the pathogenesis of depression. Gardner and Boles (2011) propose that monoamine pathways are intricately linked with inflammatory and mitochondrial processes, allowing for a role for serotonin in the newer aetiological models of depression. Cellular energy (produced by mitochondria) is necessary for the production of serotonin (Kasahara et al., 2006), and neuroinflammation leads to a decrease in tryptophan (Maes et al., 2009) which may ultimately result in reduced serotonin in the brain. Maes (2011) proposes that the disturbances in 5-HT seen in depression are a consequence of cell-mediated immune activation, via disturbances in plasma L-tryptophan production induced by indoleamine 2,3-dioxygenase (an immunomodulatory enzyme produced by activated macrophages and some other immune cells). Autoimmune responses may also interfere with 5-HT neurotransmission, which may explain, in part, serotonin hypo-responsivity in MDD (Maes & Meltzer, 1995).

Since the advent of the monoamine hypothesis of MDD, further exploratory research has uncovered many other biological factors which appear to contribute to the aetiology of depression. Although the serotonergic system still has a role to play in the new explanatory models of the aetiology of depression, it seems likely that the monoamine pathway is interconnected with the biological processes of inflammation, immune activation and mitochondrial function, outlined in more detail below, and that these factors are important targets for future research, and possibly for new treatments for MDD.

1.6.8 Inflammation. The possible role of inflammation in the pathophysiology of depression is not only evidenced by the high prevalence of depression in chronic inflammatory illness (as outlined in section 1.5.2), but also by many studies which have found high levels of biomarkers associated with the inflammation response in depressed people. First reported over two decades ago by Maes et al. (1990), the activation of the inflammatory response in depression has been well documented since then. Although both positive and negative results on this question have been found in individual studies, Dowlati et al. (2010) found, by meta-analysis, significantly higher concentrations of the proinflammatory cytokines tumor necrosis factor-alpha (TNF- α) and interleukin (IL)-6 in people with depression, which supports the notion that depression is accompanied by activation of the inflammatory response system. Although the majority of studies have compared inflammatory biomarkers in depressed versus non-depressed people, some studies have reported positive correlations between proinflammatory cytokines and depressive symptom severity (e.g., Alesci et al., 2005; Suarez, Krishnan, & Lewis, 2003). However, Raison, Capuron, and Miller (2006) draw attention to the fact that some positive studies, which have found an association between depression and elevated inflammatory markers, have failed to find any correlation between depression severity and the levels of inflammatory biomarkers (e.g., Schlatter, Ortuno, & Cervera-Enguix, 2004).

Proinflammatory cytokines themselves can induce sickness behaviours that mimic the symptoms of depression: for example, Reichenberg et al. (2001) report that 20 healthy male volunteers given injections of an endotoxin that stimulated the release of circulating proinflammatory cytokines (without inducing physical sickness symptoms) developed the classical behavioural and cognitive features of depression, including depressed mood, impaired memory and anxiety. Interestingly, antidepressants—particularly selective serotonin reuptake inhibitors (SSRIs), the most commonly prescribed form of antidepressant—exert significant immunoregulatory effects, by either decreasing the production of proinflammatory cytokines and/or increasing the production of anti-inflammatory cytokines (Maes et al., 1999). Hannestad, DellaGioia, and Bloch (2011) found by

meta-analysis that, overall, antidepressants reduced serum levels of IL-1 β . When results were stratified by antidepressant class, IL-6 and TNF- α were found to be significantly reduced by SSRIs, but that this was not associated with improvement in depressive symptoms.

However, determining causality is infinitely more complex than showing an association: the question of whether depression precedes inflammation, or inflammation precedes depression, has been debated for many years, with the results of different studies providing support for both sides of the debate. For example, Pasco et al. (2010) suggest that the presence of high levels of inflammatory activation appears to increase the risk of *de novo* depression. They found, in a population of women with no prior history of depression, that each standard deviation increase in levels of high sensitivity C-reactive protein (hsCRP) resulted in a 44% increase in the hazard ratio for *de novo* depression. After controlling for weight, smoking status and other possible confounders, the authors identified hsCRP levels as an independent marker for risk of major depression in women. Replication of this study in other populations, and with other markers of inflammation, is warranted. Conversely, Copeland, Shanahan, Worthman, Angold, and Costello (2012) tested bi-directional longitudinal associations between CRP and depression, and found no association of prior high CRP levels with later episodes of depression, but found that the cumulative effect of multiple episodes of depression predicted later increases in CRP levels, even when controlling for other factors likely to influence inflammation. Hannestad et al. (2011) report that their data, from a meta-analysis evaluating the effect of antidepressants on proinflammatory cytokines, are consistent with the possibility that proinflammatory cytokines contribute to depressive symptoms, but argue against the notion that resolution of a depressive episode is associated with normalisation of proinflammatory cytokine levels.

There are also studies which suggest that the relationship between depression and inflammation is more complex than simply causal in one direction or the other. Danese et al. (2008) suggest that the conflicting findings on the link between inflammation and depression could be a

signal that a third factor is involved, or that the link is only present in certain subgroups of the depressed population. Based on evidence that childhood adversity predicts adult inflammation (Danese, Pariante, Caspi, Taylor, & Poulton, 2007), the authors suggest that childhood adversity could be that third factor. This is exactly what their study found: depression was associated with high levels of CRP, but this association was attenuated to the point of no longer being significant when childhood adversity was taken into account. G. E. Miller and Cole (2012) found similar results in their population of 147 female adolescents who were at high risk of developing depression, either because of family history or cognitive vulnerability. The authors found that inflammation (as measured by levels of CRP and IL-6) was predictive of later depression only in participants who had a history of childhood adversity. These results suggest that the relationship between inflammation and depression is more complex than previously thought, and that activation of the inflammatory response system could be a mediator of environmental risk factors for depression.

1.6.8.1 Causes of inflammation. Berk et al. (2013), in their formative review on the subject—“*So depression is an inflammatory disease, but where does the inflammation come from?*”—attempt to uncover the source of the inflammatory activation that is a possible mediator of known environmental risk factors in depression. They uncover a range of factors that seem to increase the risk of depression, and also appear to be associated with systemic inflammation. These factors include, among others, stress, diet and obesity. The authors also note the likely importance of vitamin D and the composition of the gut microbiota in the inflammation-depression relationship. The research outlining the possible contribution of these factors to inflammation and depression is outlined below.

1.6.8.1.1 Stress, inflammation and depression. Stress and trauma are particularly well researched, both in terms of links with later risk of developing depression and their impact on immune and inflammatory pathways. In animals, psychosocial stressors have been shown to increase levels of CNS and systemic proinflammatory cytokines. For example, Möller et al. (2013)

demonstrated that social isolation rearing in rats increased proinflammatory versus anti-inflammatory cytokine balance. Similar evidence—that the proinflammatory cytokine network may be stimulated by psychosocial stressors—has been found in humans. For example, Maes et al. (1998) showed that psychological stress (in the form of an academic exam) induced increased production of many proinflammatory cytokines including TNF- α , IL-1RA and IL-6. In addition, where stress is shown to induce decreases in the production of anti-inflammatory compounds such as IL-10 and clara cell protein (CC)16, the response to psychosocial stressors is exaggerated, leading to a more pronounced increase in stress-related anxiety (C. Song et al., 1999). Berk et al. (2013) propose that psychosocial stressors and acute psychotrauma may trigger mood disorders (through the induction of these inflammatory responses) in vulnerable people, for example those with an increased inflammatory burden (e.g., systemic inflammatory disorders). This theory could explain the links between childhood adversity, inflammation and depression mentioned above, and also the high levels of comorbidity with systemic inflammatory disorders.

1.6.8.1.2 Diet, inflammation and depression. The second factor that Berk et al. (2013) suggest as having a possible influence on inflammation and depression is diet. The shifting of the global dietary pattern from one high in fibre, omega-3 fatty acids and nutrient-dense foods to one higher in saturated fats and sugar, and the possible impact of this on mental health, has been the subject of much research. In recent years, there have been many studies reporting inverse associations between diet quality and incidence of depression both in cross-sectional (e.g., Jacka et al., 2010) and prospective studies (e.g., Sánchez-Villegas et al., 2009). Inflammation is a mechanism of action that has been proposed to explain these consistent relationships: it has been suggested that diet quality impacts upon immune functioning and levels of systemic inflammation, which may predispose people to depression (Berk et al., 2013). Associations between dietary pattern and inflammation have been found in population studies. For example Lopez-Garcia et al. (2004) found, in their healthy population sample of 732 registered nurses, that a healthy (or “prudent”) dietary pattern—characterised by high intake of fruit, vegetables, legumes, fish, poultry, and whole grains—

was inversely associated with plasma concentrations of CRP. A less healthy, “Western” dietary pattern—characterised by higher intake of red and processed meats, sugary products, French fries and refined grains—was associated with higher levels of CRP and IL-6. These associations were still present after adjusting for many possible confounding factors; adjusting for BMI meant that the IL-6 result was no longer significant, but all findings remained stable after adjusting for all other possible confounding variables. The associations between dietary patterns and inflammation suggest a possible mechanism by which the association between diet and depression may be explained. It is possible that these effects take place through changes made by diet to the gut microbiota, which will be discussed in more detail in Chapter 2.

1.6.8.1.3 Obesity, inflammation and depression. It is also interesting to note that obesity, a common consequence of poor diet, is an inflammatory state: cytokines have been found in abundance in fat cells and have been associated with all manifestations of obesity, particularly abdominal obesity (de Heredia, Gómez-Martínez, & Marcos, 2012). Obesity and depression are robustly associated (de Wit et al., 2010), with prospective studies finding both that depression predisposes obesity, and vice versa (Faith et al., 2011). The independent positive association of obesity with inflammatory cytokines (adjusted for age and other potential confounding factors) provides a likely explanation for the high levels of comorbid depression in obesity.

1.6.8.1.4 Gut bacteria, inflammation and depression. Another possible cause of increased inflammatory activation is the increase in immune responses against elements produced by some bacteria in the intestines, such as the endotoxin lipopolysaccharide (LPS; part of the bacterial wall of gram-negative bacteria). Increased plasma levels of immunoglobulin A and/or M (IgA/IgM; blood-based antibodies), directed against a number of gram-negative bacteria which are normally present in the gut, have recently been demonstrated in clinical depression (e.g., Maes, Kubera, Leunis, & Berk, 2012). Berk et al. (2013) propose that the presence of increased plasma immunoglobins could be indicative of an immune response against LPS. Maes et al. (2013) suggest bacterial translocation,

or leaky gut, as a plausible causal mechanism driving the increased immune and inflammatory activation seen in depression. The links between immune activation and depression are discussed below. The gut and the intestinal microbiota, and their possible impact on mental health, will be discussed in more detail in Chapter 2.

1.6.8.1.5 Vitamin D, inflammation and depression. Despite some conflicting evidence, low levels of serum vitamin D have been found by meta-analysis to be associated with an increased risk of depression, although this relationship has not been proven to be causal (Anglin, Samaan, Walter, & McDonald, 2013). There are many possible causes of vitamin D deficiency, including intestinal fat malabsorption and reduced exposure to sunlight due to season and/or latitude (Holick, 2007; Lo, Paris, Clemens, Nolan, & Holick, 1985), and low vitamin D has been associated with a number of adverse health outcomes including osteoporosis and cancer (Nowson et al., 2012). Vitamin D exerts modulatory effects on immune function, including the modulation of immune responses to infections (Battersby, Kampmann, & Burl, 2012) and also plays a role in regulatory T (T_{REG}) cell and dendritic cell development and function (Griffin, Xing, & Kumar, 2003). Given the association between depression and elevation in inflammatory activation, the long accepted relationship between depression and vitamin D appears to be supported by the modulatory effects of vitamin D on immune and inflammatory pathways. Ly, Litonjua, Gold, and Celedón (2011) suggest that, because of this role of vitamin D in the development and function of immune cells, it is possible that the vitamin D status of the host could have an effect on the relationship between the gut microbiota and the immune system and inflammatory responses.

1.6.8.2 Summary: inflammation and depression. There is evidence of increased inflammatory activation in people with depression, compared with healthy controls (Dowlati et al., 2010). Many factors which have been previously linked with depression, including stress, diet, obesity and low vitamin D levels are also associated with an increase in inflammatory activation (Berk et al., 2013). It appears that the link between inflammation and depression is not as

straightforward as merely causal, in one direction or the other, and is probably a mediator of, or mediated by, one or more other factors. However, it is also important to note that the increase in markers of inflammation and cell-mediated immune activation are not seen in all patients with depression; therefore it is important to continue to consider other factors in the pathogenesis of depression. The associations between increased inflammatory activation and the gut microbiome will be discussed in more detail in Chapter 2.

1.6.9 Immune activation. Although it is likely that depression is an inflammatory disease (Berk et al., 2013), it is probable that cell-mediated immune activation is also a key component of depression. Cell-mediated immunity is the part of the immune system that does not involve antibodies, and that involves cellular interactions between T cells (or T lymphocytes) and monocytes (both types of white blood cell). In the above-mentioned meta-analysis of cytokines in major depression—in which Dowlati et al. (2010) found overall higher levels of IL-6 and TNF- α in depressed patients than in controls indicating inflammatory activation—no significant differences were found in cytokines which are more likely indicators of cell-mediated immune activation, such as T helper cell (T_H)-1 like cytokines (e.g., IL-2 and interferon (IFN) γ) and T_H-2 like cytokines (e.g., IL-10). Maes (2011) states that this result has been interpreted to mean that depression is an inflammatory disorder and that no cell-mediated immune response is present in depression; however, Maes (2011) argues that the markers used in the meta-analysis by Dowlati et al. (2010) to detect T cell activation—namely IL-2 and IFN γ —are not particularly sensitive markers of T cell activation. These are T lymphocytic markers that are frequently not even measurable in blood plasma: Maes suggests serum IL-2 receptor (IL-2R) levels as a more adequate assay for T cell activation. IL-2 receptors are acquired by T lymphocytes during the cell-mediated immune response, and may be released into the serum by activated T cells. Serum levels of IL-2R have been shown to correlate well with the degree of T cell activation, and thus cell-mediated immune response (Caruso, Candore, Cigna, Colucci, & Modica, 1993). Maes (2011) argues that there is considerable evidence of elevation of serum IL-2R in

depressed patients relative to normal controls (e.g., Maes et al., 1990), and that this evidence was not included in the Dowlati et al. (2010) review.

1.6.10 Oxidative and nitrosative stress. Evidence now shows that major depression is accompanied by increased oxidative and nitrosative stress (O&NS; Moylan et al., 2012). Inflammatory processes can increase production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) in the body, but even normal metabolic processes generate these potentially harmful molecules. The body has defence systems which usually counterbalance the potentially damaging effects of ROS and RNS, such as the release of antioxidants and antioxidant enzymes, but O&NS pathways become activated when these systems are unbalanced, either by an increase in ROS and RNS, or when antioxidant defences are compromised. ROS and RNS molecules can damage fatty acids, proteins, DNA and mitochondria. These molecules may also initiate autoimmune responses through altering the structure of endogenous fatty acids and proteins, rendering them immunogenic, thereby inducing Ig-mediated autoimmune responses (Moylan et al., 2012). Oxidative stress can contribute to apoptosis (programmed cell death) and neurodegenerative diseases such as Alzheimer's disease (López, Tormo, De Blas, Llinares, & Alom, 2013).

Maes, Mihaylova, Kubera, Leunis, and Geffard (2011) found that MDD is associated with both lowered antioxidant levels and reduced antioxidant enzyme activity. The authors found that autoimmune responses to O&NS (directed against functionally active neo-epitopes, which would usually not be detected by the immune system but which become immunogenic because of damage caused by O&NS) were significantly higher in depressed patients than in controls. They propose that this autoimmune response plays a role in the pathophysiology of depression, and may mediate the cellular dysfunctions that contribute to neuroprogression in the disorder.

1.6.11 Mitochondrial function. There is increasing evidence that dysfunction of the mitochondria—sometimes described as “cellular power plants”—may be a crucial factor in the pathogenesis of mental disorders, including depression. Mitochondria are membrane-bound

organelles found in all human cells, which supply a large part of the cell's energy in the form of adenosine triphosphate (ATP). Mitochondria produce the majority of ATP using the products of the tricarboxylic acid cycle, also known as the Krebs cycle . Briefly, carbohydrates, fats and proteins are broken down, and acetyl coenzyme-A is produced, which is sequentially oxidized in the Krebs cycle. This produces high energy molecules which can then donate their molecules to the electron transport chain (another process which is being studied in relation to mental disorders). These electrons are transferred through a sequence of chemical reactions which create a chemical environment suitable for the generation of ATP. Many coenzymes are needed for the Krebs cycle and the electron transport chain to function correctly, and many of these coenzymes are synthesised from dietary nutrients (Kaplan, Rucklidge, Romijn, & Dolph, 2015).

Dysfunctional mitochondrial energy production is associated with depression (e.g., Karabatsiakos et al., 2014). In addition to demonstrating that patients with MDD had significantly impaired mitochondrial functioning in comparison to controls, Karabatsiakos et al. (2014) found that mitochondrial respiration was significantly negatively correlated with the severity of depressive symptoms. It is possible that mitochondrial dysfunction contributes to the pathogenesis of MDD by influencing neurogenesis (Voloboueva & Giffard, 2011). Interestingly, some antidepressants have been shown to upregulate mitochondrial energy generation (Scaini et al., 2011). In mice, stress has been found to limit mitochondrial energy generation and damage mitochondrial structure (Gong, Chai, Ding, Sun, & Hu, 2011), which provides another plausible biological explanation for the association between stress and depression. Mitochondrial dysfunction also leads to apoptosis, which primes the immune system with antigens leading to an inflammatory response (Gardner & Boles, 2011).

It is possible that mitochondrial dysfunction may play a central role in the pathophysiology of depression. Although no definitive conclusion can yet be drawn as to whether this is the case, many trials suggest this possibility. Just as some characteristic depressive symptoms may be

explained by proinflammatory states and sickness behaviour, these symptoms (e.g., lack of motivation, working memory deficits) can also be explained by changes in energy metabolism and ATP availability. Similarities in cognitive impairments seen in major depression and mitochondrial disorders suggest common underlying factors (Gardner & Boles, 2011). Gardner and Boles (2011)—in their seminal paper “*Beyond the serotonin hypothesis*”—demonstrate that mitochondrial energy metabolism, inflammation, and monoamines are interrelated in many complex ways.

1.6.12 Aetiology summary. There are many possible aetiological factors which may contribute to the development of MDD. Earlier psychological models including learned helplessness and Beck’s cognitive triad are still used today for the development of diagnostic tools and psychotherapies for MDD. Stress is recognised to play a major role in the development of depression, and there is evidence that this may be linked with other mechanisms discussed, such as inflammation and genetics. Although genetic heritability studies show that depression is highly heritable, the links between individual genetic polymorphisms and MDD show only small effect sizes. It appears that gene-environment interactions are important in the development of MDD. Although it is clear that the serotonergic system is disrupted in MDD, it appears that this is not the sole biological process involved in the pathogenesis of depression. Inflammation and cell-mediated immune activation both impact on the serotonergic system, and have been linked with the development of depression, although there are other factors, for example childhood adversity, which seem to be involved in the relationship. Mitochondrial function and O&NS also seem to play a role in the pathogenesis of depression, particularly in the neuroprogression of the disorder. However, it is important to note that these organic processes alone are probably not a full explanation of the aetiology of MDD, particularly given that the elevation in markers of inflammation and cell-mediated immune activation are not seen in all patients with depression. Chapter 2 will outline the associations between the gut microbiome and increased immune/inflammatory activation, which provide support for testing probiotics as an intervention for depression.

1.7 Treatment

Currently, the first line of treatment for depression is pharmacological in nature. The mechanisms of action of the various antidepressant drugs available will be discussed below, and the efficacy and safety of these treatments will be discussed. The various forms of psychotherapy, another current standard treatment for MDD, will then be outlined. Following this, some alternative treatments for MDD will be discussed.

1.7.1 Pharmacological treatment. The most commonly used classes of antidepressant drugs are monoaminergic antidepressants such as the earlier tricyclic antidepressants (TCAs) and monoamine oxidase inhibitors (MAOIs), as well as newer, more targeted pharmacological treatments such as SSRIs and serotonin-norepinephrine reuptake inhibitors (SNRIs). Although other drug types may be used in the treatment of depression (e.g., low dose antipsychotics), monoaminergic antidepressants are the most commonly prescribed and so will be the main focus of this section.

1.7.1.1 Mechanisms of action. Consistent with the monoamine hypothesis of depression, monoaminergic antidepressant drugs primarily act on levels of monoamine neurotransmitters, such as serotonin and norepinephrine, in the brain (Feighner, 1999). TCAs work by blocking the reuptake of both norepinephrine and 5-HT receptors (Stahl, 1998)—inhibiting the reuptake of the neurotransmitter into the presynaptic cell, thereby leaving an increased level in the synaptic cleft which may bind to the postsynaptic receptor—although they also act on a number of other receptor sites including histamine, acetylcholine, and epinephrine which may contribute to their significant side effect profile (Feighner, 1999). MAOIs inhibit the activity of monoamine oxidase enzymes, which catalyse the breakdown of the monoamine neurotransmitters, consequently preventing the breakdown and increasing the availability of norepinephrine, dopamine and 5-HT (Stahl, 1998).

SSRIs are believed to increase extracellular levels of the monoamine neurotransmitter serotonin, which is usually explained simply as “selective inhibition of the serotonin transporter”

(Stahl, 1998; p.4). However, Stahl (1998) asserts that, when an SSRI is administered, it blocks serotonin reuptake which does indeed lead to a sudden increase in serotonin, but not in the area of the axon terminals where it is needed to exert therapeutic action. The author proposes that, when SSRIs are administered chronically, the sustained increase of serotonin leads to the desensitisation of the serotonin autoreceptors, which in turn disinhibits neuronal impulse flow and leads to more serotonin being released from the axon terminal. This process may explain why SSRIs do not have a rapid onset of therapeutic actions (Stahl, 1998). SNRIs similarly block the reuptake of serotonin, but also inhibit the reuptake of norepinephrine at higher doses, and in some cases may have an effect on dopamine reuptake at very high doses (Stahl, 1998).

However, as mentioned previously, antidepressants—particularly SSRIs—also exert significant immunoregulatory effects, either by decreasing the production of proinflammatory cytokines and/or increasing the production of anti-inflammatory cytokines (Berk et al., 2013; Hannestad et al., 2011). One study on the SSRI escitalopram found that non-response to treatment with the antidepressant was associated with persistently elevated levels of proinflammatory cytokines (Eller, Vasar, Shlik, & Maron, 2008). This anti-inflammatory action provides another possible mechanism by which antidepressant drugs—although originally targeted at monoamines—may exert their effect on depressive symptomatology, and suggests the possibility of inflammation targeted treatments for depression. This idea is discussed in more detail below. In addition, hippocampal neurogenesis—the production of new neurons within the brain of an adult—appears to mediate the behavioural efficacy of antidepressant medications (Santarelli et al., 2003), indicating effects on neurogenesis as another possible mechanism of action by which antidepressants may exert their effects on depressive symptoms.

1.7.1.2 Efficacy. A considerable number (>40%) of depressed patients do not respond satisfactorily to monoaminergic antidepressants (Maes et al., 2009). Meta-analyses of antidepressant drug efficacy based on data from published trials have shown that the benefits of

antidepressants compared with a placebo are statistically significant, but do not meet the recommended level for clinical significance, which is defined as an effect size (Cohen's *d*) of 0.5 or above, or a drug/placebo difference of 3 points on the Hamilton Depression Rating Scale (HDRS) (National Institute for Clinical Excellence, 2004). Analyses which also include unpublished clinical trials fall well below the recommended level for clinical effectiveness (National Institute for Clinical Excellence, 2004). Kirsch et al. (2008) suggest that mean improvement scores may be obscuring differences between subgroups of patients, particularly in varying severity of depression at baseline. Their meta-analysis, which included both published and unpublished data, found that, as they had predicted, antidepressant efficacy increased as a function of baseline severity of depression, with severely depressed patients experiencing the most benefit compared with placebo (Kirsch et al., 2008). However, the benefits for severely depressed patients were still small, and it is possible that the benefits observed were a consequence of the decreased responsiveness of severely depressed individuals to placebos, rather than an increased responsiveness to medication. Fournier et al. (2010) also found, by patient-level meta-analysis of published research, that Cohen's *d* effect sizes for response to antidepressant medications, in those with HDRS scores below 23, were less than 0.2 (small). The authors of this meta-analysis found that the threshold for clinical significance was met when intake HDRS scores were around 25 or greater, confirming the larger difference between responses to medication and placebo in more severely depressed patients found by Kirsch et al. (2008).

The STAR*D trial (Sequenced Treatment Alternatives to Relieve Depression) was designed to test which treatments are most effective following intolerance or nonremission to initial treatment with an SSRI (citalopram) (Warden, Rush, Trivedi, Fava, & Wisniewski, 2007). They found an initial response rate of 47% to citalopram, with 28% of the total sample achieving remission (based on the Hamilton Rating Scale of Depression; HRSD). After a medication switch to sertraline (another SSRI), bupropion-SR (a nonserotonin active agent) or venlafaxine-XR (an extended release SNRI), approximately 25% of those who had experienced intolerance or nonremission to citalopram

achieved remission, with no difference between the various drug types. When cognitive behavioural therapy (CBT) was added to medication treatment, around one third of patients achieved remission. In the final two levels of their study, the nonresponders from previous levels were prescribed a combination of medications, including lithium at level 3, and an MAOI at level 4. During levels 3 and 4, remission rates were described as “modest” and “remarkably low” respectively. After a 1-year naturalistic follow-up, the investigators found that participants who were in remission at follow-up were around 30% less likely to relapse than those who had not achieved remission, despite possibly having responded to treatment (Warden et al., 2007). The authors conclude that their study highlights the prevalence of treatment-resistant depression, and the importance of achieving remission with acute treatment in preventing relapse.

1.7.1.3 Safety and tolerability. Since 2004, the Food and Drug Administration in the USA has required that all antidepressants carry a black box warning which clearly states that antidepressants carry an increased risk of suicidal thinking, feeling and behaviour in children and adolescents (Friedman & Leon, 2007). In 2007, this black box warning was expanded to include information about an increased risk of suicidal symptoms in young adults aged 18-24. The decision to include young adults in the black box warning was made after a large meta-analysis (on 99,839 participants in total) found that, when the active antidepressant treatment was compared to a placebo (so that the general effect of depression on suicidality was randomized out), the risk of suicidal behaviour or ideation was elevated for patients 18 to 24 years of age (Friedman & Leon, 2007). Although the result for this age group was not significant, the black box warning was expanded to include this age group because the threshold for threat to safety is generally lower than that for efficacy, and the trend towards an association between antidepressant use and suicidality across age groups was convincing (Friedman & Leon, 2007).

A large New Zealand study of 1,829 adult antidepressant users found that 83% of participants self-reported that antidepressants had reduced their depression, but that the number of

adverse events experienced by the group while taking antidepressants was very high—higher than previously reported. For example, 62% of participants experienced sexual difficulties and 60% experienced feeling emotionally numb (Figure 2; Read, Cartwright, & Gibson, 2014). Although it is often argued that the adverse events experienced by those taking antidepressants are caused by the depression, rather than the drug, Read et al. (2014) asked participants about both their level of depression and occurrence of adverse events prior to taking any antidepressants, and found no significant relationship between level of depression and total adverse events scores prior to taking antidepressants. They propose that, if adverse events are caused by depression, rather than by antidepressant drugs, the correlation between the two should exist prior to taking antidepressants, and that the lack of a significant correlation appears to suggest that the adverse events were caused by the antidepressants, not by the depression.

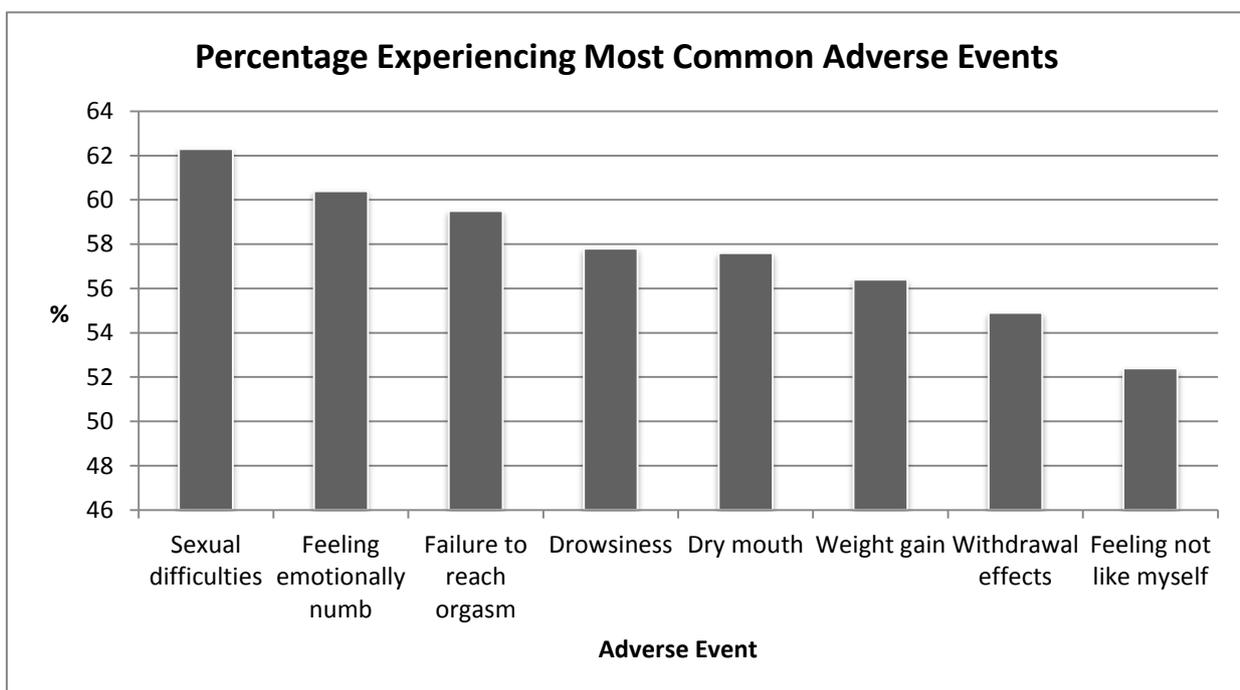


Figure 2. Percentage of participants experiencing the most common (reported by >50%) adverse events while using antidepressants. Data from Read, Cartwright, & Gibson (2014).

1.7.1.4 Pharmacological treatment summary. The most commonly prescribed type of pharmacological therapy for MDD is monoaminergic antidepressants. These drugs appear to reduce the symptoms of depression in many people, with rates of around 50% for response and 30% for remission for individual drugs in 8 week trials (Warden et al., 2007). A significant proportion of patients do not respond to individual drugs, but the STAR*D trial shows that switching and augmenting drugs can improve the rates of response and remission. However, in the final two levels of their study, the remission rates were very low, indicating that switching and combining drugs has little effect on remission after several drugs have been tried. Response rates appear to be higher among those with more severe depression, although it has been argued that this effect is observed because of the reduced placebo response among severely depressed individuals (Kirsch et al., 2008). Antidepressant drugs appear to have a significant side effect profile and the National Institute for Health and Care Excellence (2004) guidelines indicate that antidepressants should not be used for the initial treatment of mild depression, because the risk-benefit ratio is poor. It is possible that antidepressants are acting on depressive symptomatology not only through affecting monoamine neurotransmitter levels, but also through exerting immunological effects and promoting hippocampal neurogenesis.

1.7.2 Psychotherapy. The second current standard treatment for depression is psychotherapy. The most researched of these is CBT. Most recently, “third-wave” psychotherapies such as metacognitive therapy (MCT), acceptance and commitment therapy (ACT) and mindfulness-based cognitive therapy (MBCT) have been developed. The efficacy of these therapies is discussed below.

1.7.2.1 Cognitive behavioural therapy. CBT is an approach to psychotherapy that addresses dysfunctional emotions, and maladaptive cognitive processes and behaviours, using structured, systematic, goal-oriented procedures. CBT has been proven to be effective for depression: despite some negative trials, Butler, Chapman, Forman, and Beck (2006) found, by a

review of 16 meta-analyses, that CBT had large effect sizes for both adult and adolescent unipolar depression when compared with no-treatment, waitlist or placebo controls ($d=0.95$), and that CBT was somewhat superior to antidepressants in the treatment of adult depression ($d=0.38$). However, CBT trials are criticised because double-blind studies are impossible. Despite the empirical support for CBT, access is still limited for many; the New Zealand Mental Health Commission (2007) found that access to CBT and other evidence-based psychological therapies is very limited, and extremely variable in both in terms of cost and regional availability.

1.7.2.2 Metacognitive therapy. Metacognition has been defined as “cognition applied to cognition” (A. Wells, 2009, p.1). MCT is a form of psychotherapy based on a specific theory proposed by A. Wells and Matthews (1994), which is that people become trapped in emotional disturbance because their metacognitions cause a pattern of responding to inner experiences, called Cognitive-Attentional Syndrome, that strengthens negative ideas and maintains emotion. MCT is grounded in this model, and aims to modify Cognitive-Attentional Syndrome and the psychological factors giving rise to it by reducing rumination, increasing flexible control over thinking, and promoting metacognitive awareness (A. Wells, 2009). Negative and positive metacognitive beliefs have been found to be positively associated with state and trait depression (Papageorgiou & Wells, 2001), but the evidence base for MCT as a treatment for depression appears to be lacking large randomised trials. One small multiple baseline trial of MCT in recurrent and persistent depression found that treatment was associated with large and clinically significant improvements in depressive symptoms and ruminations which were maintained at follow-up (A. Wells et al., 2009). However, this study only included four patients, which severely limits the generalisability of these results. A. Wells et al. (2012) treated 12 patients with treatment-resistant depression with up to 8 sessions of MCT and found that large improvements occurred during treatment and were maintained at follow-up. Larger and controlled studies are warranted to assess the impact of MCT on depressive symptoms and relapse rates.

1.7.2.3 Other third-wave psychotherapies. Third-wave therapies such as ACT and MBCT are based on cognitive behavioural therapy, but are particularly sensitive to the context and functions of psychological phenomena (Hayes, Masuda, Bissett, Luoma, & Guerrero, 2004). For example, in MBCT, little emphasis is placed on changing the content of thoughts (as in CBT), but rather emphasis is placed on changing the awareness of and relationship to thoughts (Segal, Teasdale, & Williams, 2004). MBCT is a clinical intervention program designed to reduce relapse or recurrence of MDD by means of systematic training in mindfulness meditation combined with cognitive-behavioural methods. Piet and Hougaard (2011) found by meta-analysis that MBCT significantly reduced the risk of relapse in MDD, with a risk ratio of 0.66 for MBCT compared with treatment as usual or placebo controls.

ACT works on a similar premise to MBCT. Hayes, Luoma, Bond, Masuda, and Lillis (2006) describe ACT as a psychological intervention based on modern behavioural psychology and relational frame theory, which aims to create psychological flexibility through the application of mindfulness and acceptance processes, as well as commitment to behavioural change. Powers, Zum Vörde Sive Vörding, and Emmelkamp (2009) found by meta-analysis that ACT was more efficacious than control conditions with a small to moderate effect (Hedges' $g=0.42$) but that it did not outperform current treatments ($g=0.18$). In an editorial review of the empirical efficacy of ACT, Pull (2009) suggests that currently available data on ACT is promising, and suggests that ACT may work through different processes than CBT. Pull (2009) also states that more well controlled studies are necessary before it can be known whether ACT is generally more effective than other treatments for depression.

1.7.2.4 Interpersonal therapy. Interpersonal therapy (IPT) is a time-limited form of psychotherapy, which focuses on the social and interpersonal triggers that may contribute to depression, developed by Klerman, Weissman, Rounseville, and Chevron (1984). The most recent study of IPT in depression found that IPT performed as well as cognitive therapy, and that both

therapies exceeded the response of the waitlist controls ($d=0.37$) (Lemmens et al., 2015). The study also found that these improvements were maintained up to one year.

1.7.2.5 Other therapies. Psychodynamic psychotherapy is a form of psychotherapy loosely based on Freud's psychoanalysis but more widely practiced, and with an interpersonal and social focus. Short psychodynamic supportive psychotherapy has been found, by meta-analysis, to be as effective as medication for mild to moderate depression (Maat et al., 2008); however, this meta-analysis only included three studies, so more evidence is warranted. Counselling is a term used to describe a non-directive approach to psychotherapy, in which patients are able to talk freely to a trained counsellor. Counselling tends to be more accessible and less costly than other psychotherapies, and may take a variety of approaches including psychodynamic, humanistic and behavioural.

1.7.2.6 Safety and adverse effects. Although many forms of psychotherapy appear to be efficacious for depression, there seems to be a disparity in how antidepressant drugs and psychotherapies are evaluated in terms of cost-benefit ratio. Adverse event monitoring is mandatory in drug trials, whilst psychotherapy trials are generally weighted towards evaluating benefit, rather than measuring possible risk (Berk & Parker, 2009). However, there is evidence of harm arising from psychotherapy. For example, adverse outcomes may occur when psychotherapy is provided for a condition for which it is ineffective or inappropriate, or to the exclusion of other efficacious treatments, thereby exposing the patient to a prolonged period of ongoing symptoms. There are also documented cases of harm caused by inappropriate therapist behaviour, including the well-documented case of Anna O, the first patient of the cathartic method, psychoanalysis and dynamic psychiatry, who was likely exposed to the creation of pseudo-memories and the unintentional amplification of her already present dissociations because of inappropriate type, timing and intensity of interventions delivered (Weissberg, 1993). However, there are also psychotherapies which have been shown to be harmful even when delivered correctly and for the condition for which they were

designed. For example, Werch and Owen (2002) systematically reviewed preventative interventions for substance use in young people and identified 17 studies with documented adverse effects, including increased substance use. Berk and Parker (2009) state that, given the documented occurrence of adverse events in psychotherapy, regulation should be imposed requiring that, in order to be considered 'evidence-based', all treatments must not only prove clinical efficacy but must also assess and take into account any possible adverse effects.

1.7.2.7 Psychotherapy summary. Some forms of psychotherapy—particularly CBT—appear to be as effective as (or somewhat more effective than) antidepressant medication in treating depression. However, evidence for the efficacy of some other forms of psychotherapy appears to be quite limited. CBT, IPT and some third-wave therapies appear to be the most well supported, and comparative trials and meta-analyses appear to show that these forms of psychotherapy are equally effective as one another. MBCT may be most effective for relapse prevention. More trials—preferably large, controlled trials—on MCT are needed to confirm its viability and generalisability as a treatment for MDD. Access to psychological therapies can be limited, and cost is also a factor which may limit the availability of these services for some people. The possible adverse effects of psychotherapy appear to be often overlooked in the literature and should be routinely assessed in psychotherapy trials.

1.7.3 Alternative treatments. There are many treatments for depression which lie outside of the current standard treatments. Some examples of these alternative treatments are discussed below, but these do not encompass all available alternatives.

1.7.3.1 Light therapy. Therapeutic light, in the form of artificial light that mimics real sunlight, is well established as a treatment for depression with a seasonal pattern, or SAD. Golden et al. (2005) found bright light therapy to be more effective than a control condition (white light) in treating SAD, with a large effect ($d=0.84$). The same meta-analysis also found that bright light therapy was effective for nonseasonal depression with a moderate effect ($d=0.53$). Similarly, Even,

Schröder, Friedman, and Rouillon (2008) state that the results of their systematic review suggest that bright light therapy is an excellent candidate for inclusion into the therapeutic portfolio available for the treatment of nonseasonal MDD, as an adjuvant to antidepressant medication.

1.7.3.2 Depression-targeted commercially available supplements. Several commercially available supplements are marketed for depressive symptoms. These include St John's Wort, 5-hydroxytryptophan (5-HTP), tryptophan and S-adenosyl methionine (SAMe) among others. St John's Wort (*Hypericum perforatum*) is a flowering plant which has been developed into a medicinal herb with potential antidepressant and anti-inflammatory properties (Klemow et al., 2011). A meta-analysis of 37 double-blind randomised controlled trials found that, while large placebo-controlled trials in patients with MDD found only minor effects of St John's Wort over placebo (response rate ratio (RR)=1.15), trials comparing St John's Wort with antidepressant medications suggest that they have similar beneficial effects (RR=1.01).

Several amino acids have been developed as dietary supplements marketed for use in depression, including 5-HTP—a naturally occurring amino acid and chemical precursor to the neurotransmitter serotonin—and tryptophan, a chemical precursor to 5-HTP (Shaw, Turner, & Del Mar, 2002). A 2002 meta-analysis by the Cochrane Collaboration which included studies using either 5-HTP or tryptophan, found that 5-HTP and tryptophan were more effective overall than placebo in treating depression (Peto (pooled) odds ratio=4.10), but that the side effects cited included dizziness, nausea and diarrhoea. The authors state that the studies included were few and small, so large controlled trials are necessary to determine the efficacy and relative safety of 5-HTP and tryptophan for depression (Shaw et al., 2002).

S-adenosyl-methionine (SAMe) is a naturally occurring molecule available commercially as a nutritional supplement purported to have antidepressant properties. SAMe is the major donor of methyl groups required in the synthesis of neuronal messengers and membranes (Baldessarini, 1987). The Cochrane Collaboration released a protocol for a systematic review of the evidence for

SAMe in treating depression (Galizia et al., 2014) but this review is not available as yet. The most recent comprehensive (but not systematic) review in the area appears to be from 2009. This review found that, although preliminary evidence suggests that SAMe may be a useful adjunctive therapy in MDD, controlled studies are needed to confirm or refute these preliminary findings (Papakostas, 2009).

1.7.3.3 Micronutrients. Micronutrients (vitamins and minerals) have shown promise in treating low mood, in both single nutrient and broad-spectrum forms. As discussed above, low vitamin D levels have been associated with low mood, but the association between vitamin D and depression has not yet been demonstrated to be causal (Anglin et al., 2013). Randomised controlled trials of vitamin D for the prevention and treatment of depression are needed. Some trials have attempted to address this question; for example, Jorde, Sneve, Figenschau, Svartberg, and Waterloo (2008) report that, in overweight and obese patients, supplementation for one year with high doses of vitamin D appeared to ameliorate the mood symptoms associated with low serum vitamin D levels, which the authors interpreted as indication of a causal relationship. Li et al. (2013) systematically reviewed the literature of randomised controlled trials (RCTs) investigating the efficacy of vitamin D in depression. The review identified six RCTs with inconsistent results and an overall low quality of evidence, with the finding that there is insufficient evidence to support the efficacy of vitamin D supplementation in depressive symptoms.

Rucklidge and Kaplan (2013) systematically reviewed the literature on broad-spectrum nutrients (defined by them as a formula containing at least four vitamins and minerals) and found that there were no trials which recruited patients with MDD. However, there are trials which show an effect of multinutrient supplementation on mood in nonclinical samples; for example, Benton, Haller, and Fordy (1995) tested a multinutrient formula with 10 times the recommended daily dose of nine vitamins versus placebo in a double-blind trial in a healthy population sample over one year, and found improvements in mood as rated on the Profile of Mood States (POMS), particularly in

those who showed improved riboflavin (vitamin B2) and pyridoxine (vitamin B6) status in blood samples. There are also some trials showing improvements in mood in patients with bipolar disorder using a formula called EMPowerplus: these include open label trials (e.g., Kaplan et al., 2001), case studies with reversals (Kaplan, Crawford, Gardner, & Farrelly, 2002), and database analyses (Gately & Kaplan, 2009; Rucklidge, Gately, & Kaplan, 2010), so the lack of RCT data in patients with mood disorders is a limitation of this supplement. However, one randomised controlled trial, using this supplement and assessing mood outcomes in a sample of adults with ADHD, found positive effects on mood compared to placebo in those with moderate to severe depression at baseline, as rated on the Montgomery-Åsberg Depression Rating Scale (MADRS; Rucklidge, Frampton, Gorman, & Boggis, 2014). These positive results warrant further research into the effect of this supplement on mood in samples with mood disorders.

1.7.3.4 Omega-3 polyunsaturated fatty acids (PUFAs). Omega-3 PUFAs have been found, by meta-analysis, to have significant antidepressant efficacy (Lin & Su, 2007). However, the authors note that publication bias and significant heterogeneity in trials may have positively skewed the results, and conclude that further research, in the form of large-scale, well-controlled trials is necessary. Long chain omega-3 fatty acids have anti-inflammatory effects, which provides a possible mechanism of action for omega-3 PUFAs on depressive symptoms (Wall, Ross, Fitzgerald, & Stanton, 2010).

1.7.3.5 Whole diet interventions. Given the strong association between diet and depression (Jacka et al., 2010), it appears logical to implement and test whole diet interventions for depression. The PREDIMED study is a multicentre randomized trial implementing two different Mediterranean diets (one supplemented with nuts and one supplemented with extra virgin olive oil) versus a low fat diet in people at high risk for cardiovascular disease. In one paper resulting from the PREDIMED trial, (Sánchez-Villegas et al. (2013)) found that a Mediterranean diet supplemented with nuts resulted in a decreased risk for new episodes of depression after 3 years, but that this result

was not significant, likely due to a lack of statistical power. However, in participants with type 2 diabetes the effect did reach statistical significance, indicating that a Mediterranean diet supplemented with nuts may have protective effects against depression in people with type 2 diabetes. The researchers also found that, among patients with depression at baseline, significantly higher BDNF levels were found in those assigned to the Mediterranean diet with nuts than those assigned to the low fat control diet (Sánchez-Villegas et al., 2011). Inflammation inhibits the production and secretion of BDNF (A. H. Miller, Maletic, & Raison, 2009), so this result appears to provide support for the inhibition of inflammatory processes as a plausible mechanism of action for the effect of diet on depression. More trials in this area are warranted.

1.7.3.6 Inflammation targeted therapy. Cholesterol-lowering medications such as statins have been shown to have anti-inflammatory properties (e.g., Kwak, Mulhaupt, Veillard, Pelli, & Mach, 2001). However, the results of some prospective, case-control and longitudinal studies suggest aggression (Olson et al., 2008) and an increased risk of suicide (Zureik, Courbon, & Ducimetiere, 1996) as possible side effects of cholesterol-lowering treatment. Conversely, other, randomised trials appear to have shown a reduced risk for depression with statin use, independent of serum cholesterol levels (e.g., Otte, Zhao, & Whooley, 2012). O'Neil et al. (2012) conducted a meta-analysis of the impact of statins on psychological wellbeing in patients both with and without hypercholesterolemia. Although this meta-analysis of seven RCTs did not find any statistically significant difference in psychological wellbeing between participants receiving statins or placebo, when sensitivity analyses were conducted separating depression and mood outcomes, statins were associated with statistically significant improvements in mood scores. The authors state that their findings refute the negative effects of statins on mood, and provide limited support for mood-related benefits of statins. Further studies of statins in patients with MDD would be valuable.

Raison et al. (2013) recently attempted to test the cytokine-hypothesis of depression (outlined above) by treating 60 patients with at least moderately treatment-resistant depression

with infusions of infliximab, a TNF- α antagonist, or a placebo. Although infliximab was not more effective at treating treatment-resistant depression than a placebo, this proof-of-concept study showed that, in those with high CRP levels at baseline (>5mg/L) there were 62% responders in the infliximab group versus 33% in the placebo group. This suggests that treatment with a TNF- α antagonist does not have generalized efficacy in treating treatment-resistant depression, but may improve depressive symptoms in patients with high baseline inflammatory markers. A larger randomised controlled trial in patients with high levels of inflammatory markers at baseline is warranted.

1.7.3.7 Oxidative stress targeted therapy. Berk et al. (2008) investigated the efficacy of N-acetyl cysteine (NAC), adjunctive to usual medication, for depressive symptoms in bipolar disorder, in a double-blind RCT. NAC is a precursor to glutathione, an important antioxidant which has been shown to be perturbed in major psychiatric disorders including depression (Ozcan, Gulec, Ozerol, Polat, & Akyol, 2004). The trial found significant improvement in the MADRS scores of the active treatment group versus placebo group with no significant between-group difference in adverse events, indicating that NAC appears to be a safe and effective augmentation strategy for depressive symptoms in bipolar disorder. The authors state that the benefits observed in their trial indicate that disturbances in oxidative neurochemistry may play a role in bipolar disorder. Replication of this trial is warranted, as are trials using this supplement in patients with major depressive disorder.

1.7.3.8 Probiotics. Logan and Katzman (2005) suggested that probiotics could be an adjuvant treatment for depression, but this is not the first time that this idea has been suggested. In an early effectiveness study, Phillips (1910) gave *Lactobacillus* bacteria to patients with melancholia and reported positive findings. The notion of using probiotics to treat depression will be discussed in more detail in Chapter 3.

1.7.4 Treatment summary. The current standard treatments for depression are pharmacological and psychotherapeutic in nature. While these treatments are efficacious in treating many people with depression, a significant proportion of people do not respond satisfactorily to the current standard treatments. This suggests that the current standard treatments may be failing to address important aetiological factors in depression. In attempting to broaden the treatment options available for depression, alternative treatments for depression should be considered, as well as preventative measures using diet and other lifestyle factors. Treatments that attempt to address the increased immune and inflammatory activation that is emerging as an important underlying factor in depression may be particularly valuable.

1.8 Chapter Summary

Major depressive disorder (MDD), also known as clinical depression or unipolar depression, is a serious, debilitating mood disorder which is highly prevalent in developed countries (Bromet et al., 2011). Rates of comorbidity between MDD and other mental disorders (particularly anxiety) are high, and MDD is also highly comorbid with many physical disorders. It has been suggested that MDD and non-communicable physical disorders such as cardiovascular disease may have a shared underlying pathophysiology: increased immune activation and/or induction of inflammatory, oxidative and nitrosative stress (O&NS) pathways (Maes, Kubera, et al., 2011). There are many other possible aetiological factors which may contribute to the development of MDD including stress, genes, gene-environment interactions, the serotonergic system and mitochondrial function. The current standard treatments for depression are pharmacological and psychotherapeutic in nature; however, it is possible that these treatments may be failing to address important aetiological factors in depression. Alternative directions should be considered for future interventional research. Treatments that attempt to address increased immune and inflammatory activation may be particularly valuable to the field, given the emerging evidence that portrays these factors as important in depression.

Chapter 2: The Gut

This chapter will focus on the human gut and its relation to brain function and behaviour.

The mechanisms and functions of the human gut microbiome (the complex of microorganisms in the digestive tract) will be described, as this concept is central to any discussion involving probiotics.

Given the relationship between increased immune/inflammatory activation and depression outlined in Chapter 1, the associations between microbial balance and the functioning of the immune system described in the present chapter contribute to the rationale for testing probiotics in depression.

Following this, the gut-brain axis—a bidirectional system of communication between the gut and the brain—will be described. The evidence outlined in support of the gut-brain axis, its organisation and functions provides the biological basis for the notion that probiotics may affect brain function and behaviour.

2.1 The Human Gut Microbiome

The human gut microbiome (also known as the gut flora, microflora and microbiota) is an ecological community of 10^{13} to 10^{14} microorganisms in the human gastrointestinal (GI) tract—a number ten times greater than the total number of human cells in the body. Although there are various other types of organisms which inhabit the human gut (including fungi and protozoa), bacteria make up the majority of organisms in the colon and their functions are the most studied; therefore, the bacterial inhabitants of the gut will be the main focus of this section. The microbiome is an integral part of the system of bidirectional communication between the gut and the brain termed the “gut-brain axis”, which is discussed in detail below (see Section 2.2).

2.1.1 Initial colonisation. The colonisation of an infant human’s gut is thought to begin during the natural birth process, when the baby ingests some of the mother’s vaginal and intestinal microorganisms (Round & Mazmanian, 2009). Babies born by caesarean section may come into contact with some of the mothers’ microorganisms, but this is less likely than if the baby is born vaginally: the initial colonisation of babies born by caesarean section is likely to be from

microorganisms in the air or on skin (Schwiertz et al., 2003). The intestinal microbiome of vaginally born infants takes around one month to become well established, while the primary gut flora in infants born by caesarean section may be disturbed for up to six months after birth because of the initial disruption to the normal colonisation process (Grönlund, Lehtonen, Eerola, & Kero, 1999).

After birth, colonisation of the gut with the mother's microorganisms is continued through touch, kissing and breastfeeding. Breastfeeding appears to be particularly important in the colonisation of the gut, and bottle-fed infants may be more susceptible to overgrowth of potentially harmful bacteria. For example, Penders et al. (2005) found that counts of *Colostrum (C.) difficile* and *Escherichia (E.) coli* (both potentially pathogenic bacterial strains) were significantly higher in formula-fed infants in their study, compared with breastfed infants. These differences in initial colonisation may impact on susceptibility to immune-mediated diseases later in life (discussed in more detail below). It takes several years for a stable microbiome to be established in humans. It is possible that factors such as weaning, the introduction of solid foods, diet and early use of antibiotics could have a lasting impact on the composition of the microbiome, as suggested by data from animal studies (e.g., Cho et al., 2012; Turnbaugh et al., 2009), though these findings need to be replicated in humans.

2.1.2 Composition. The bacteria present in the microbiome are predominantly anaerobic, and the number of different bacterial species in the human gut microbiome is likely to reach into the thousands (Geuking, Köller, Rupp, & McCoy, 2014). Most of the bacteria in the gut reside in the lower intestine: there are very few species of bacteria in the upper intestinal tract, which is thought to be because the luminal medium contains acid and bile which kills most ingested organisms (Guarner & Malagelada, 2003). The composition of the microbiome varies greatly from person to person, but it is thought that the composition *within* a person remains relatively stable over time. It is, however, known that certain factors such as diet, medication use and changes in lifestyle can affect the composition of the microbiome over time (O'Hara & Shanahan, 2006).

Despite comprising a relatively large variety of bacterial species, most of the bacteria in the human gut belong to just eight of the 55 bacterial phyla described so far (Geuking et al., 2014), suggesting that the formation of the intestinal microbiome is not a random event but is more likely to be an established evolutionary process. Most of the bacteria in the gut also belong to relatively few genera, including *Bacteroids*, *Clostridium*, *Fusobacterium* and *Bifidobacterium* (Guarner & Malagelada, 2003). Species from the *Bacteroids* genus are particularly prevalent and account for approximately 30% of bacteria in the lower intestine (Salysers, 1984). Some of the bacterial strains present in the human gut are potentially pathogenic, and can cause infection or other adverse effects under certain circumstances, including if the integrity of the intestinal barrier is damaged or breached (Guarner & Malagelada, 2003).

Measuring the composition of the microbiome with any degree of accuracy is challenging, as over 50% of intestinal bacteria cannot be cultured (Macpherson & Harris, 2004). However, advances in gene sequencing technology have allowed for unculturable species of bacteria to be quantified, through sequencing the nucleotide sequence of 16S ribosomal RNA (a ribonucleic acid present in bacterial chromosomes) (e.g., Weisburg, Barns, Pelletier, & Lane, 1991). In 2007, the National Institute of Health (USA) launched the Human Microbiome Project (HMP) which aims to gain a better understanding of the complex interactions between humans and their resident microorganisms, in a logical conceptual and experimental extension of the Human Genome Project (Turnbaugh et al., 2007). Using these new culture-independent techniques across a multitude of different experiments and projects, the HMP aims to identify the microbial communities at several sites on the human body, and to investigate the role of these microorganisms in human health and disease.

2.1.2.1 Enterotypes. Arumugam et al. (2011) argue that intestinal microbiome composition is generally stratified, not continuous, with robust clusters of bacterial genera (enterotypes) which are not dependent on geography, nutritional intake, age or BMI. Using metagenomes of individuals from several different countries and continents, the authors identified three distinct enterotypes

identifiable by the variation in the levels of one of three genera: *Bacteriodes*, *Prevotella* and *Ruminococcus*. The authors propose that the existence of the enterotypes identified provides evidence that there are a limited number of well-balanced host-microbial symbiotic (mutualistic) states. The authors also posit that the discovery of enterotypes may allow for the categorisation of groups that will respond differently to diet or drug intake in future. However, this was a small study in only 39 individuals, and the research did not identify the factors which cause the clustering. The authors also state that there may be subgroups within these enterotypes, but that this would only be detectable with substantially more samples. Replication of this finding is necessary, as well as further research to refine the enterotypes and identify the causes of the clustering.

2.1.3 Functions. The relationship between host and microbiome is a good example of mutualism: two organisms of different species existing in a relationship in which each individual benefits from the activity of the other. The bacteria in the microbiome benefit from a stable habitat within the GI tract, and an almost constant supply of energy from our food, while humans benefit from the many useful functions of the microbiome, including energy released from otherwise indigestible food sources and the crowding out of potentially pathogenic organisms. Other useful functions served by the microbiome include protecting the intestinal barrier defence system, extracting essential nutrients from food, and in some cases synthesizing those nutrients (Carpenter, 2012). The microbiome also contributes to the development and maintenance of normal immune function (Olszak et al., 2012). Some of the main functions of the human gut microbiome are discussed below.

2.1.3.1 Immunity. The GI tract is the main site in the human body where the host's immune system interacts with microorganisms. Over the course of millions of years of co-evolution, the proper development and functioning of the human immune system has come to depend on the presence of these microorganisms (Round & Mazmanian, 2009). Initial intestinal microbial colonisation impacts many different immune cells present in the small and large intestine, and

results in a range of immune adaptations within the host that promote immune homeostasis (Geuking et al., 2014). Innate immune maturation has been shown to be heavily dependent on the presence of gut microorganisms, with germ-free mice—animals delivered directly into a sterile environment so that they are devoid of any microorganisms in or on their bodies—exhibiting a complete lack of some antimicrobial peptides (Cash, Whitham, Behrendt, & Hooper, 2006). Intestinal colonisation has also been shown to induce the secretion of IgA, the most abundant human antibody, and the IgA repertoire has been shown to adapt to changes in the composition of the microbiome (Geuking et al., 2014). The microorganisms of the microbiome can also directly impact levels of inflammation: *Bacteroids fragilis* supports anti-inflammatory responses by activating T_{REG} cells which release IL-10, an anti-inflammatory cytokine (Scher & Abramson, 2011), while the presence of some segmented filamentous bacteria (SFB) have been shown to have the opposite effect, inducing the secretion of proinflammatory IL-17 through activating *lamina propria* T_H17 cells in the small intestine (Ivanov et al., 2009). Immune mediators may also feed back and directly impact the composition of the gut microbiota (Geuking et al., 2014).

There is developing evidence for a functional link between microbiota composition and susceptibility to systemic immune disorders including rheumatoid arthritis (Scher & Abramson, 2011) and Type 1 diabetes (Mathis & Benoist, 2012), and allergies (Hanski et al., 2012). For example, Wu et al. (2010) showed that introducing a single species of gut-residing SFB bacteria into germ-free animals resulted very quickly in arthritis, through the reinstatement of T_H17 cells and the consequent production of antibodies. These results show that a single commensal microbe can drive autoimmune disease; however, it is important to note that these results are not generalizable to humans as yet, and the presence of other species of bacteria may have unknown effects on this relationship.

2.1.3.2 Short-chain fatty acid production. Humans lack the enzymes required to break down and digest many forms of carbohydrate. The same is true of other animals which have a

mutualistic relationship with the microorganisms of their gut flora: Wostmann, Larkin, Moriarty, and Bruckner-Kardoss (1983) demonstrated that germ-free rats excreted 87% more calories with their faeces than normal rats. The bacteria in the human microbiome can break down many carbohydrates by fermentation, releasing otherwise inaccessible energy and creating short-chain fatty acids (SCFAs) in the process. SCFAs are a subgroup of organic fatty acids with one to six carbon atoms. SCFAs suppress inflammation, as well as being important in gut function through modulating intestinal epithelial cells (Aw & Fukuda, 2014; Cook & Sellin, 1998). SCFAs also exert multiple beneficial effects on human energy metabolism. Firstly, they have been shown to regulate the balance between fatty acid oxidation and lipolysis in the body: fatty acid oxidation is activated whilst *de novo* synthesis and lipolysis are inhibited. The net result is a reduction in the concentration of free fatty acids in blood plasma and a decrease in body weight (den Besten et al., 2013). Additionally, SCFAs have been shown to regulate cholesterol metabolism in humans (Kondo, Kishi, Fushimi, Ugajin, & Kaga, 2009) and may also play a role in glucose metabolism, though the available data is sparse and mainly from animal studies, and points to multiple possible mechanisms (den Besten et al., 2013).

2.1.3.3 Nutrient absorption and synthesis. The microorganisms of the colon play an important part in the absorption of calcium, magnesium and iron (Miyazawa, Iwabuchi, & Yoshida, 1996). Absorption is improved by carbohydrate fermentation and production of SCFAs (Guarner & Malagelada, 2003). The microorganisms in the microbiome also play a role in the synthesis of certain vitamins, particularly vitamins B and K (Cummings & Macfarlane, 1997).

2.1.3.4 Suppression of pathogenic microbial growth. The commensal microorganisms in the human gut play an important role in suppressing the growth of potentially pathogenic species, through the process of competitive exclusion, wherein the commensal bacteria of the microbiome prevent overgrowth of potentially harmful bacterial species such as *Colostrum (C.) difficile* through competing for nutrition and attachment sites to the epithelium of the colon (Guarner & Malagelada,

2003). The production of SCFAs in the colon, through the process of fermentation, also serves to lower the pH in the colon, which in turn helps to prevent the proliferation of potentially pathogenic bacteria.

2.1.4 Dysbiosis. Although not a standardised medical term, “dysbiosis” is the name given in the literature to a microbial imbalance in the gut microbiome which produces harmful effects in the host. Gut dysbiosis has been implicated in the pathogenesis of many disorders, from gut disorders such as inflammatory bowel disease (Marteau, 2010) and colitis (Mazmanian, 2008), to chronic fatigue syndrome (Lakhan & Kirchgessner, 2010). In conditions of dysbiosis, certain strains of gram-negative bacteria appear to proliferate, which may affect intestinal permeability and levels of inflammation (Tamboli, Neut, Desreumaux, & Colombel, 2004).

2.1.4.1 Causes. There are many factors which can influence the composition of the microbiome, and so possibly cause dysbiosis, including the use of broad-spectrum antibiotics, a poor diet, stress, and the modern environment being too clean (the hygiene, or “Old Friends”, hypothesis) (Hawrelak & Myers, 2004; Rook, Raison, & Lowry, 2012).

2.1.4.1.1 Antibiotics. Although antibiotics are undoubtedly one of the most important medical discoveries of our time, antibiotic overuse—particularly of broad-spectrum antibiotics—has been implicated in dysbiosis (Blaser, 2011). Broad-spectrum antibiotics act indiscriminately against both gram-positive and gram-negative bacteria, and so are likely to have the most detrimental effect on microbiome composition. There is also evidence that the shifts in microbial composition caused by antibiotics may lead to long-term physiological changes. For example, Trasande et al. (2012) demonstrated that antibiotic exposure during the first 6 months of life was longitudinally associated with consistent increases in body mass later in childhood. The authors stated that, although these effects were modest at an individual level, given the prevalence of both antibiotic exposure in infancy and childhood obesity, their results could have substantial significance at the population level. Given the relatively recent developments in 16S ribosomal RNA analysis techniques, it has

been suggested that we could perhaps use our ever developing understanding of the composition and metagenomics (genetic makeup) of the microbiome to develop new narrow-spectrum antibiotics for more conditions (Cho & Blaser, 2012).

2.1.4.1.2 Stress. Psychological distress appears to have a significant effect on the composition of the microbiome: this has been shown in animal models prenatally (Bailey, Lubach, & Coe, 2004), in early life (Bailey & Coe, 1999; Berk et al., 2013), and in adulthood (Bailey et al., 2011). These effects can last for a long time: changes to the gut microbiome of rats who had undergone maternal separation for 3 hours per day, for 10 days after birth, were maintained through to adulthood (O'Mahony et al., 2009). In observing these long term effects, together with changes in behaviour, systemic immune responses and HPA axis function, the authors concluded that early life stress induces persistent changes to the gut-brain axis, and that, if also true for humans, these changes could contribute to symptoms of irritable bowel syndrome and psychiatric disorders in adulthood.

This effect of psychological stress on the microbiome has also been demonstrated in humans. Knowles, Nelson, and Palombo (2008) found that students had significantly altered faecal bacterial levels during a high stress (exam) week than during a low stress week. This study warrants replication and further investigation into this effect in humans: there were a number of inconsistencies and confounders in the study, including that cortisol secretions did not rise significantly during the high stress week as was hypothesised, and aspects of the students' diets also changed significantly from baseline to the high stress week.

2.1.4.1.3 The hygiene hypothesis. The hygiene (or "Old Friends") hypothesis is now the prevailing explanation for the increase in asthma and atopic disorders in Western countries (Maslowski & Mackay, 2011). The hypothesis proposes that the hygienic nature of our modern world means that we no longer come into contact with the infectious bacteria we need for our microbiome, and thus our immune system, to fully develop. Rook et al. (2012) state that humans

evolved and adapted alongside microorganisms associated with faeces, animals and mud, and the proper development of our immune systems came to be dependent upon the presence of these microorganisms; however, because of hygiene practices originating during urbanization, humans in the developed world rarely come into contact with the microorganisms required for the development of a healthy microbiome and immune system, leading to an increase in allergic diseases and inflammatory disorders (Rook et al., 2012).

Evidence for the hygiene hypothesis comes mainly from epidemiological studies. For example, epidemiological studies comparing the prevalence of allergic disorders among children growing up in rural versus urban environments (e.g., Braun-Fahrländer et al., 1999) have consistently found that children from rural areas, particularly those who grow up on farms with livestock (von Ehrenstein et al., 2000), have significantly lower levels of allergic disorders than those growing up in urban environments. However, it is possible that factors other than microbial exposure may be influencing the development of allergic disorders. For example, studies in populations of children from low SES urban areas in the USA (e.g., Webber, Carpiniello, Oruwariye, & Appel, 2002), which show that these children have very high rates of asthma, suggest that confounding factors such as the low income, poor living conditions and high cigarette and drug consumption seen in these populations may be contributing to the development of allergic disorders.

2.1.4.1.4 Diet. Diet has substantial effect on the composition of the microbiota. Turnbaugh et al. (2009) demonstrated in humanized mice—mice transplanted with a human faeces to create a representative animal model of the human microbiome—that switching from a low-fat diet high in plant products to a high-fat, high-sugar “Western” diet significantly altered the structure of the microbiome within a single day, and altered microbiome gene expression. It has been suggested that the Western dietary pattern—characterised by a high intake of refined grains, sugar and processed food, and low intake of fresh produce and fibre—is particularly associated with changes in the gut microbiota, and that these changes may drive the increasing incidence of inflammatory disorders in

developed countries (Maslowski & Mackay, 2011). It is possible that this is mainly due to a lack of fibre: de Filippo et al. (2010) found that children from Europe had a significantly different composition of intestinal microbiota to children from rural Africa, with the African children having significantly more *Bacteroidetes*, and the European children having a complete lack of the bacterial genus *Prevotella* and *Xyaniibacter*. The African children had significantly more SCFAs in faecal samples than the European children; the authors hypothesised that these differences were due to the fibre-rich diet of the African children, compared with the lower fibre intake observed in the European children. However, this was a small study, with a sample size of 30 in total: replication in a larger sample size is warranted to increase generalisability.

2.1.4.2 Effects. In conditions of dysbiosis the microbiome is not able to carry out some or all of its normal functions (as outlined above) to the same level as in conditions of symbiosis. Thus, dysbiosis can have various negative repercussions for the host, including the potential for overgrowth of already-present opportunistic microorganisms such as *Clostridium difficile*, a decrease in production of SCFAs, and an increased susceptibility to intestinal pathogens (Bailey et al., 2011). The decrease in SCFA production and the depletion of microorganisms important in nutrient absorption and synthesis may lead to a worsened nutritional status for the host.

2.1.4.2.1 Effects of dysbiosis on immune functioning. Given the importance of the microbiome in the development and maintenance of a healthy immune system, it follows logically that the depletion or absence of bacteria which are important in this process can lead to an underdeveloped immune system or a breakdown in immune functioning. One mechanism—suggested by Maes et al. (2013)—by which dysbiosis may lead to increased immune responses and inflammation is bacterial translocation (or “leaky gut”): the migration of bacteria to areas outside the intestinal tract. This can happen in states of dysbiosis when the integrity of the mucosal barrier is damaged, when the bacteria which normally protect the intestinal barrier defence system are compromised. Mucosal cells in the gut normally form a barrier that is virtually impermeable to fluid

by forming tight junctions, where two cell membranes join together by forming a network of protein strands. This tight junction barrier normally segregates gram-negative bacteria from the host's immune cells. In cases where the mucosal barrier is compromised by the loosening of the tight junctions, gram-negative bacteria may translocate from the gut into various areas outside of the intestine, including, for example, the mesenteric lymph nodes (Berg & Garlington, 1979). Given that, under normal conditions, immune cells are separated from gram-negative bacteria, they are not primed against the LPS contained within the cell wall of the gram-negative bacteria and so become activated on contact with gram-negative bacteria. This activation leads to the induction of inflammatory pathways; for example, the mesenteric lymph nodes may begin producing proinflammatory cytokines on immune-cell activation (Maes et al., 2013). Maes et al. proposed that this process drives gut-derived inflammation and is responsible for the increase in IgA- and IgM-mediated immune responses seen in patients with depression.

2.1.5 The human gut microbiome summary. The human gut microbiome is an ecological community of 10^{13} to 10^{14} microorganisms in the human gastrointestinal (GI) tract. The composition of the microbiome varies greatly from person to person, but it is thought that the composition within a person remains relatively stable over time. However, certain factors, such as diet and changes in lifestyle, are known to affect the composition of the microbiome over time. The microbiome is known to serve many useful functions, including contributing to the development and maintenance of normal immune function, releasing energy from otherwise indigestible food sources, crowding out of potentially pathogenic organisms, protecting the intestinal barrier defence system, extracting essential nutrients from food, and in some cases synthesizing those nutrients. In conditions of dysbiosis—which has numerous possible causes including an overly hygienic environment, a diet low in fibre and the use of broad-spectrum antibiotics—the microbiome may be unable to carry out its usual functions within the body, which may lead to deficits in immune functioning, increased inflammation and worsening nutritional status of the host. It is possible that these underlying processes could be corrected by supplementing probiotic bacteria, as probiotics

may alter the composition of the microbiome (and thus possibly correct dysbiosis), protect the intestinal barrier by competitive exclusion thereby decreasing immune activation in response to bacterial translocation, and limit proinflammatory cytokine production and in so doing reduce levels of systemic inflammation (Logan & Katzman, 2005; Naruszewicz, Johansson, Zapolska-Downar, & Bukowska, 2002). The microbiome is an integral part of the system of bidirectional communication between the gut and the brain termed the “gut-brain axis”, which is discussed in detail below.

2.2 The Gut-Brain Axis

2.2.1 Definition. The GI tract and the central nervous system (CNS) engage in two-way communication: the CNS is informed of the state of the GI tract by afferent neurons, and is able to modulate digestive function through efferent neurons (Dockray, 2008). The brain sends communications, mainly from the hypothalamus and the amygdala, to the viscera through multiple pathways, including two branches of the autonomic nervous system (ANS) and the HPA-axis (Mayer, 2011). This pathway is known as the gut-brain axis (GBA).

Although much research on the GBA has focused on its role in digestive function and satiety (e.g., Konturek, Konturek, Pawlik, & Brzozowski, 2004), it is becoming increasingly recognised that dysfunction of the GBA may have pathophysiological consequences (Cryan & Dinan, 2012). Alterations in gut-brain signalling have been associated with gut inflammation, GI disorders and eating disorders (Mayer, 2011). It has recently become increasingly recognised that the gut microbiome can have marked effects on the GBA, leading to the conceptualisation of “the microbiome-gut-brain axis” (e.g., Cryan & O'Mahony, 2011), although whether the role of the microbiota in the functioning of the GBA is large enough to warrant this change is still debated (Cryan & Dinan, 2012). Due to its apparent importance in many aspects of pathophysiology, the GBA is increasingly becoming viewed as an attractive target for the development of new treatments for many disorders (Mayer, 2011).

2.2.2 The enteric nervous system. In understanding the interaction of the CNS and the digestive system, an important breakthrough came with the discovery of the enteric nervous system (ENS). The ENS is one of the main divisions of the nervous system; the ENS is considered one of the branches of the ANS, despite the fact that it is similar in size and complexity to the brain (Mayer, 2011). The ENS consists of between 200 and 600 million neurons—afferent, motor and interneurons—organised in networks called plexuses, embedded in the lining of the GI system (Furness, 2006). The ENS is normally controlled by the CNS, through parasympathetic and sympathetic nerve fibres (Boron & Boulpaep, 2012); however, unlike other components of the ANS, the ENS can function as a separate and independent nervous system: the intrinsic neuronal circuits can generate independent reflex activity without any signalling from the CNS (Sasselli, Pachnis, & Burns, 2012).

2.2.3 Methods for studying the GBA. Many methods have been employed to study the organisation and function of the GBA. These methods include those which are directed at the study of organ function (e.g., measuring changes in secretions after sham feeding or electrical stimulation or section of nerve trunks), which provide some valuable insight into the working of the GBA, but do not take endocrine and immune mediators into account and thus do not provide a full picture of gut-brain interactions. Other methods include morphological methods (typically used in animals) which have been used to define the cellular basis of the GBA, such as the use of fluorescent compounds taken up by nerve cells and transported along axons which, when coupled with surgical or chemical lesioning of neurons can be used to provide measurable information on the organisation of nerve pathways. There are some methods which are appropriate for studying the human GBA, from earlier studies using evoked potentials (electrical potential recorded using scalp electrodes after stimulation), to newer techniques such as positron emission tomography (PET) and functional magnetic resonance imaging (fMRI). These approaches each have limitations (e.g. spatial or

temporal resolution) and multiple approaches are often combined in order to overcome some of these limitations (Dockray, 2008).

2.2.3 Gut-brain connections. The major nerve pathways between the gut and the brain are the vagal, splanchnic and sacral nerve trunks, all of which contain both afferent and efferent nerve fibres (Dockray, 2008). It is recognised that afferent signals associated with the physiological regulation of digestion are typically conveyed by the vagal pathway, and pain signals are conveyed by the splanchnic pathway, as first described by Hertz (1911). Afferent neurons (also known as sensory or receptor neurons) are generally described as carrying nerve impulses towards the CNS from sensory organs and receptors. The vagus nerve consists predominantly of afferent nerve fibres: it has been estimated that, in rats, between 75-90% of vagal nerve fibres are afferent (Andrews, 1986). Different populations of afferent nerve fibres respond to stimuli (mechanical, thermal or chemical) arising in different areas of the lumen (the mucosa, the muscle layers or the serosa), carrying impulses to the CNS via the gastric branches of the vagus nerve (Andrews, 1986). Efferent neurons (also known as motor or effector neurons) carry nerve impulses away from the CNS to effectors (e.g., muscles or glands). Tracing experiments (e.g., Berthoud, Jedrzejewska, & Powley, 1990) have shown that vagal efferent fibres supply the GI tract as far as the colon, but that the abundance of these fibres is relatively less in the lower regions of the gut.

The ENS and the CNS are connected through multiple nerve pathways, including the vagal, splanchnic and sacral nerve trunks. However, as mentioned previously, communication between the gut and brain is not only through nerve connections: endocrine and immune signals also play a role in gut-brain interactions.

2.2.4 Signalling. Brain-gut interactions have been studied since they were first recognised independently by psychologist William James and Carl Lange, a physiologist, in the late 19th century. The central concept of the James-Lange theory of emotion is that stimuli which induce emotions first induce changes in gut function through the ANS, and that afferent feedback of these

peripheral changes to the brain is essential to the generation of particular emotional feelings (James, 1884). This was challenged by Cannon (1929), who posited that the bodily changes which often accompany strong emotional feelings (“butterflies” in the stomach, fast heartbeat etc.) are by-products of emotional reaction, rather than causes, and that emotional feelings originate in subcortical brain structures. Advances in neuroimaging techniques, enteric neuroscience and intestinal microbiology have led to a huge expansion in our modern understanding of gut-brain signalling, and microbial-gut-brain signalling.

During homeostasis, the brain communicates to the gut through multiple parallel pathways including the parasympathetic and sympathetic branches of the ANS, the HPA axis, and descending monoaminergic pathways (Mayer, 2011). The two key areas of the brain which generate these signals are the hypothalamus and the amygdala, which receive inputs from the medial prefrontal cortex (PFC) network, which in turn receives input from the lateral PFC and orbitofrontal cortex (OFC; Öngür & Price, 2000). The input from these areas provides integrated multisensory representations of complex homeostatic body states such as food intake, gut homeostasis and visceral pain. The effect of sympathetic signalling from the CNS to the GI tract is largely inhibitory, slowing GI transit and secretion (Mayer, 2011). Parasympathetic innervation of the GI tract—via vagal and sacral nerve pathways, stimulating foregut and hindgut structures respectively (Mayer, 2011)—mediates many visceral functions including vaso-vagal motor reflexes, gastric acid secretion and gastric 5-HT (serotonin) release (Stephens & Tache, 1989).

Signalling from the gut to the brain involves endocrine and immune signalling, as well as neuronal afferent signalling. Information about luminal conditions are conducted to the brain via vagal afferents, with mechanical stimuli such as stretch and pressure activating afferent neurons directly, without endocrine or immune input (Mayer, 2011). For other types of stimuli, signalling molecules—such as histamine, serotonin and cytokines—produced by immune cells within the gut epithelium can activate receptors on vagal afferents (Mayer, 2011). Likewise, neuropeptides and

hormones (gut peptides) released from enteroendocrine cells in response to other luminal factors, such as the presence of nutrients, toxins or agents, can act in both an endocrine manner—reaching the area postrema, dorsal vagal complex and hypothalamus in the brain—or in a paracrine manner through receptor activation (Mayer, 2011).

It is likely that top-down engagement of subgroups of parasympathetic and sympathetic neurons mediates emotion-related changes in motor, secretory and immune activity in the GI tract (Welgan, Meshkinpour, & Beeler, 1988). Mayer (2011) suggests that these emotion-related changes may influence feedback to the brain, and could possibly contribute to the typical prolonged duration of many emotional states, which often last for many hours after the initiating event. These prolonged alterations in ANS output to the gut may induce changes in peripheral target cells—such as downregulation of adrenergic receptors on immune cells (Elenkov & Chrousos, 2002) or changes in primary afferent neurons (Khasar et al., 2008)—which may induce chronic changes to gut-brain signalling and possibly result in the remodelling of brain regions which receive this heightened input (Seminowicz et al., 2010). Mayer (2011) states that these lasting changes in gut-brain signalling “may be associated with tonic ANS dysfunction, which is in turn associated with altered emotional states such as anxiety disorders or depression” (p. 445). Although alterations in the functioning of the GBA have been implicated in many chronic disorders, the strongest associations are between alterations in gut-brain signalling and chronic GI disorders such as IBS (e.g., Tillisch & Labus, 2011), and eating disorders, particularly obesity (Berthoud & Morrison, 2008) and *anorexia nervosa* (Kaye, Fudge, & Paulus, 2009).

2.2.4.3 Microbial-gut-brain signalling. As mentioned above, it is becoming increasingly recognised that the microbiome can have marked effects on the GBA, leading to the conceptualisation of “the microbiome-gut-brain axis”. Some evidence supporting the theory of microbial involvement in gut-brain signalling comes from studies in germ-free, defined flora (gnotobiotic), or specific pathogen-free (SPF) mice. Gnotobiotic animals are germ-free animals which

are intentionally inoculated with one or more non-pathogenic microorganisms, all of which are known; SPF animals are demonstrably free of a specific list of pathogens. For example, germ-free mice have been demonstrated to have reduced anxiety behaviour in the elevated plus maze (a well validated animal model of anxiety), spending more time in the open arms of the maze compared with SPF mice (controls; Neufeld, Kang, Bienenstock, & Foster, 2011). This study also found upregulation in BDNF mRNA expression in the hippocampus of the germ-free mice, and increased levels of serum corticosterone levels in the germ-free mice, which may reflect a stress response to experimental conditions. An exaggerated corticosterone response to stress in germ-free animals has previously been demonstrated by Sudo et al. (2004), and was demonstrably reversible by administration of *B. infantis*, suggesting a role for the microbiome in the development of the HPA axis. The results of the study by Neufeld et al. (2011) suggest that the presence or absence of an intestinal microbiome influences behaviour and induces neurochemical changes in animals, and provide direct evidence that microbiota can influence brain and behaviour. It is important to note, however, that while animal studies generate data which is an important initial step in ascertaining whether the enteric microbiome can alter behaviour, these models are not necessarily generalizable to human disease (Cryan & O'Mahony, 2011). Other evidence for the impact of microbial composition on gut-brain signalling comes from studies using probiotics, which will be discussed in detail in Chapter 3.

2.2.5 The gut-brain axis summary. The GBA is a pathway of communication between the gut and the CNS. The GI tract and the central nervous system (CNS) engage in two-way communication through multiple pathways, and signals may be neuronal, endocrine or immune in nature. Dysfunction in gut-brain signalling is associated with chronic GI disorders and eating disorders, and may also be associated with alterations in emotional states. Evidence for microbial-gut-brain signalling comes mainly from studies in germ-free or gnotobiotic animals, although probiotics studies are also providing some evidence for this modulation, and will be reviewed in Chapter 3.

Chapter 3: Probiotics and Mental Health: A Systematic Review

This chapter contains a modified version of the following published article: Romijn, A. R. & Rucklidge, J. J. (2015). Systematic review of evidence to support the theory of psychobiotics. Nutrition Reviews, DOI: 10.1093/nutrit/nuv025. The introductory and concluding sections have been modified to ensure continuity within this thesis.

3.1 Background and Hypotheses

3.1.1 Overview. As described in Chapters 1 and 2, numerous recent reviews have presented compelling evidence for the roles that inflammation, immune regulation and intestinal permeability may play in the pathophysiology of depression (Berk et al., 2013; Maes, 2011). Other reviews have suggested that the intestinal microbiota may also play a role in the pathophysiology of other psychiatric disorders such as schizophrenia, and psychological states such as stress, via the modulation of immune and inflammatory signalling, and/or via the gut-brain axis (Cryan & Dinan, 2012; Ezeoke, Mellor, Buckley, & Miller, 2013; Fetissov & Dechelotte, 2011; Forsythe, Sudo, Dinan, Taylor, & Bienenstock, 2010; Haroon, Raison, & Miller, 2012; Maes, Kubera, et al., 2011; Mayer, 2011). As a logical continuation to these biological indicators, it has been suggested that probiotic bacteria—“live microorganisms that, when administered in adequate amounts, confer a health benefit on the host” (Sanders, 2008)—may have beneficial effects on the symptoms of mental disorders. This idea appears to be supported by evidence from preclinical trials which demonstrate effects of probiotic supplementation on emotional behaviour in animal models relevant to depression and anxiety (Arseneault-Breard et al., 2012; Bravo et al., 2011; Desbonnet et al., 2010). There have also been trials which show that manipulation of microbial composition (via probiotic supplementation) can induce changes in brain activity in both animal models (Bravo et al., 2011; Desbonnet et al., 2010), and in one well-controlled human trial (Tillisch et al., 2013). In a recent review, Dinan, Stanton, and Cryan (2013) coined the term “psychobiotics”: “a live organism that, when ingested in adequate amounts, produces a health benefit in patients suffering from psychiatric illness”. The preclinical evidence from animal trials for the use of probiotics as psychotropic interventions appears to be robust (as outlined below in section 3.1.3.1). However, the human

research in this area has not previously been systematically reviewed; therefore, systematically reviewing the human research for probiotics as interventions for psychological outcomes will be the focus of this Chapter.

3.1.2 Probiotics. The introduction of the concept of probiotic bacteria is generally attributed to Metchnikoff (1907) who suggested that humans could intentionally modify the gut flora and replace “harmful” bacteria with “useful” microbes. Probiotic bacteria may be supplemented orally in various forms, including fermented foods such as kefir, yoghurts, and freeze-dried powder or encapsulated supplements. In addition to supplementing probiotic bacteria, other means of dietary modulation of the gut microbiome include prebiotics and synbiotics. Prebiotics are food ingredients, such as certain fibres, that cannot be ingested by the human digestive system but are metabolized by enteric microbes which is believed to subsequently stimulate proliferation of select bacterial species (Gibson, Probert, Van Loo, Rastall, & Roberfroid, 2004). Synbiotics are formulated supplements which include a mixture of prebiotics and probiotics (de Preter, Hamer, Windey, & Verbeke, 2011). Since much of the work in this area is focused on the effects of ingestion of probiotic bacteria on health outcomes, supplements that contain viable bacteria will be the focus of this chapter.

3.1.2.1 Probiotic bacterial species. Microbes of the species *Lactobacillus (L.)* and *Bifidobacteria (B.)* are the most commonly researched for their potential health benefits, along with some other lactic-acid-producing bacteria such as *Streptococcus* and even some yeasts such as *Saccharomyces boulardii* (Rauch & Lynch, 2012). Several factors are important in determining the suitability of a bacterial strain as a probiotic, including that bacteria must: maintain stability and retain a suitable number of viable cells during shelf life to confer health benefits; be resistant to physiological stress such as gastric acid; and be non-pathogenic, non-toxic and free of resistance against antibiotics (O'Toole & Cooney, 2008).

3.1.2.2 Effect of supplemented probiotics on microbiome composition. Although the concept of ingesting potentially beneficial bacteria was suggested in the early 20th century, it was not until around a century later that Fuller (1989) suggested that any beneficial effects of probiotics may be mediated by an improvement in intestinal microbiome balance. Some trials (although they are relatively few in number) have attempted to characterise the effect of supplemented probiotic bacteria on the composition of the human gut microbiome. For example, one trial found that the faecal microflora of subjects was altered by administration of *L. rhamnosus* strain DR20, but that the number of detectable bacteria from the DR20 strain was related to the presence or absence of an indigenous *Lactobacilli* population during the control period before the administration of the probiotic (Tannock et al., 2000). The length of time that the strain DR20 presence persisted in faeces after probiotic administration had ceased had high interpersonal variation. This is in agreement with another study by Klingberg and Budde (2006) which suggests that organisms may exhibit extremely variable host-specific persistence behaviour. Some of the possible mechanisms by which exogenously administered probiotic bacteria may alter the composition of the microbiome are through competitive exclusion for binding sites to the gut epithelium, competition for dietary ingredients and production of growth substrates (including vitamins and extracellular polymeric substances such as exopolysaccharides) for other bacteria (O'Toole & Cooney, 2008).

3.1.3 Probiotics for mental health outcomes. As mentioned in Chapter 1, the idea that supplemented probiotic bacteria could impact mental health outcomes is not a new one: in the era of autointoxication—around the late 19th and early 20th centuries, when the digestive tract was viewed as the cause of many illnesses, including mental disorders (Bested, Logan, & Selhub, 2013b)—the idea of supplementing probiotics was extremely preferable to the other invasive treatments of the day, which included (unnecessary) surgical removal of the bowels (Bested, Logan, & Selhub, 2013a). As time, and science, progressed, autointoxication came to be viewed as an unscientific catch-all diagnosis with no real empirical support, and so fell out of favour with the

scientific community. After this controversial history, the notion that probiotics could be used to treat mental disorders lay dormant for almost a century.

The idea of a possible application for probiotics in mental health resurfaced in a paper by Logan and Katzman (2005) which suggested that probiotics could be used as an adjuvant treatment for depression. After the publication of this paper, interest in the area seemed to grow very quickly. Since then, a large number of narrative reviews have been published on the relationship between gut microbes, the brain and behaviour (Carpenter, 2012; Collins, Surette, & Bercik, 2012; Fetissov & Dechelotte, 2011; Forsythe et al., 2010; Foster & McVey Neufeld, 2013; Mayer, 2011; Thakur, Shakya, Husain, Emerald, & Kumar, 2014). Many reviews have also suggested manipulation of gut microbiome composition (usually via ingestion of probiotic bacterial supplements) as an intervention for mental health (Bested et al., 2013b; Cryan & Dinan, 2012; Cryan & O'Mahony, 2011; Dinan & Quigley, 2011; Dinan et al., 2013); however, these reviews appear to rely mainly on evidence from preclinical studies, with only a limited number of human trials.

3.1.3.1 Preclinical evidence. Various preclinical studies have been undertaken which demonstrate that probiotic administration affects emotional behaviour in animal models (Arseneault-Breard et al., 2012; Bravo et al., 2011; Desbonnet, Garrett, Clarke, Bienenstock, & Dinan, 2008; Desbonnet et al., 2010). For example one study, by Bravo et al. (2011) treated healthy mice with a probiotic formulation containing *L. rhamnosus* or a placebo, and then subjected them to several anxiety- and depression-related behavioural tests, including the stress-induced hyperthermia (SIH) test, the elevated plus maze (EPM) test and the forced-swim test (FST). Their results suggested that chronic treatment with *L. rhamnosus* had anxiolytic effects, as reductions in anxiety- and depression-related behaviour were observed: on the EPM, animals treated with the probiotic had a significantly larger number of entries to the open arms than controls; on the FST, a significant reduction was observed in time spent immobile in probiotic treated mice versus controls (Bravo et al., 2011). The probiotic treated mice in this study also displayed significantly lower levels of stress-

induced corticosterone compared to the broth-fed controls. This study also found that probiotic administration induced region-dependent alterations in Gamma-Aminobutyric Acid (GABA) Messenger Ribonucleic Acid (mRNA) receptor expression in the brains of the probiotic treated mice: increases in GABA_{B1b} mRNA receptor expression were observed in cortical regions (cingulate and prelimbic), with reductions in various regions including the hippocampus and amygdala, and reductions in GABA_{Aα2} mRNA receptor expression were observed in the prefrontal cortex and amygdala, with increases in the hippocampus, relative to controls. GABA is the main CNS inhibitory neurotransmitter, and alterations in its receptor expression are implicated in the development of anxiety and depression (Cryan & Kaupmann, 2005). Additionally, some mice were vagotomised (a procedure in which the vagus nerve is surgically severed), and these neurochemical and behavioural effects were not found in vagotomised mice, confirming the vagus nerve as a major communication pathway between the gut microbiome and the brain, as outlined in Chapter 2.

3.1.3.2 Possible mechanisms of action. There are many plausible mechanisms of action by which supplemented probiotics could affect CNS function. As previously discussed in the context of animal probiotics research, the vagus nerve is an important pathway in gut-brain communication. However, in another animal study, microbiota-brain interactions have been observed after vagotomy, indicating that vagus-independent mechanisms are also at play (Bercik et al., 2011).

As outlined in Chapter 1 in relation to depression, it has been demonstrated that some mental disorders are associated with increased inflammatory activation (Berk et al., 2013; Dickerson et al., 2014). A possible mechanism by which supplemented probiotics may change emotional states is through altering microbial composition of the gut as described above, which can subsequently limit proinflammatory cytokine production and reduce inflammation, which can have clear effects on gut-brain signalling (Cryan & Dinan, 2012). Direct effects of microbiota on the immune system are another plausible mechanism, given that the immune system is involved in bidirectional communication with the CNS (Dantzer, O'Connor, Freund, Johnson, & Kelley, 2008) and has also

been implicated in depression (Maes, 2011). Improving the composition of the gut microbiota may also lead to increased integrity of the gut wall, which can lower immune activation in response to bacterial translocation (Maes et al., 2012). As discussed in Chapter 2 in the context of the negative effects of dysbiosis, it is also possible that correcting the composition of the gut microbiome through probiotic supplementation could improve the nutritional status of the host, for example through increasing short chain fatty acid production, or improving synthesis and absorption of nutrients (Logan & Katzman, 2005).

In the recent review in which they coin the term psychobiotics, Dinan et al. (2013) express the importance of differentiating between different strains of probiotic bacteria when looking for psychotropic effects. They state that most strains will not have psychobiotic potential, and suggest that the most likely candidate strains of probiotics to affect brain function are those which produce neurochemicals. Prospective psychobiotic strains suggested in this review include certain *Lactobacillus* strains which secrete GABA (Barrett, Ross, O'Toole, Fitzgerald, & Stanton, 2012), *B. infantis* (shown to increase levels of tryptophan, a serotonin precursor; Desbonnet et al., 2008) and *L. acidophilus* (shown to modulate the expression of cannabinoid receptors; Rousseaux et al., 2007).

3.1.4 Importance of the present review. The idea that probiotics can affect the functioning of the CNS and emotional behaviour is an exciting one, and one which could possibly offer a natural alternative to psychotropic medications. While there are a number of narrative reviews in this area, a systematic review has not been conducted to date. Two questions remain: how thoroughly has the theory been tested in controlled human trials, and do the results of these trials support the theory and application of probiotics for psychological outcomes and mental health? Addressing these questions is of the utmost importance. As was asked by Bested et al. (2013b) in a recent narrative review on the topic: are we on the cusp of a major clinical breakthrough, or are we “merely in the quicksand of auto-intoxication II” (p.1)?

3.1.5 Objectives. The primary objective of the current review is to ascertain whether there is evidence of an effect of supplemented probiotics on psychological outcomes and symptoms of psychiatric disorders in humans. To address this question, we will undertake a systematic review of human, double-blind, randomised, placebo-controlled trials which compare probiotic interventions to a matched placebo and use a standardised, validated scale of psychological outcomes or symptoms of psychiatric disorders.

3.2 Methods

3.2.1 Criteria for considering studies for this review. Study selection criteria were defined before searches were completed. Only papers written or available in English and published in full in peer-reviewed journals were included; abstracts, letters, conference reports, and reviews were excluded. A post-hoc decision was made to exclude secondary papers on already included trials.

3.2.1.1 Types of studies. Only double-blind, randomised, placebo-controlled trials were included.

3.2.1.2 Types of participants. Only human trials were included. No restrictions were placed on population health or condition, or sample size.

3.2.1.3 Types of interventions. Only trials investigating the effect of probiotics versus a matched placebo were included. Intervention products were required to provide viable organisms, in keeping with the current accepted definition of probiotics: “live microorganisms that, when administered in adequate amounts, confer a health benefit on the host” (Sanders, 2008). No restrictions were placed on dosage, strain or type of probiotic intervention: supplemented probiotic bacteria, yoghurt formulae with live probiotic bacteria and fermented food with live probiotic bacteria were included. Purely prebiotic formulations were excluded due to the lack of any bacterial

content; however, synbiotic formulations were included. No restriction was placed on duration of intervention period.

3.2.1.4 Types of outcome measures. Only studies which used a standardised, validated measure of psychological outcomes (e.g., stress, mood, anxiety), or symptoms of psychiatric disorders were included. Trials were included regardless of whether the standardised psychological measure was a primary or secondary outcome measure. This will inevitably lead to greater heterogeneity between studies, but was considered necessary due to our prior knowledge that this is a very small area of literature. Health-related quality of life measures were excluded.

3.2.2 Search strategy for identification of studies. Searches were made both electronically (in online databases) and manually (in reference lists of relevant studies), for studies published up to 17th July 2014.

3.2.2.1 Electronic searches. The following databases were searched electronically for studies published up to 17th July 2014: Cochrane Central Register of Controlled Trials, MEDLINE, EMBASE, PubMed, PsycINFO, and PsycARTICLES. Truncation was used to cover several variations of search terms (e.g., probiotic* covers probiotic and probiotics). The following search terms were used: probiotic*, synbiotic, bacteria, bacterial, Lactobacillus, Bifidobacter*, acidophilus, Saccharomyces, Streptococcus AND randomi*, trial* placebo*, controlled study, double-blind AND psych*, depressi*, anxiety, autism, bipolar, ADHD, schizophrenia, eating disorders, anorexia, bulimia, mood, stress. For example, the Cochrane Central Register of Controlled Trials was searched for: (probiotic* OR synbiotic OR bacteria OR bacterial OR Lactobacillus OR Bifidobacter* OR acidophilus OR Saccharomyces OR Streptococcus) AND (randomi* OR trial* OR placebo* OR controlled study OR double-blind) AND (psych* OR depressi* OR anxiety OR autism OR bipolar OR ADHD OR schizophrenia OR eating disorders OR anorexia OR bulimia OR mood OR stress).

The search terms for intervention included the most commonly used terms which describe probiotic interventions, as well as the most commonly used genera and strains in probiotic studies.

The search terms for condition were selected based on the definition of psychobiotics proposed by Dinan et al. (2013): “a live organism that, when ingested in adequate amounts, produces a health benefit in patients suffering from psychiatric illness”. We used terms covering the most common psychiatric illnesses, as well as broadening the search to cover affective states and psychological outcomes in line with the broad nature of the inclusion criteria of this review.

3.2.2.2 Other sources. Reference lists of key reviews and relevant papers were searched manually for studies published up to 17th July 2014.

3.2.3 Selection of studies. Study selection was completed using the PRISMA process (Moher, Liberati, Tetzlaff, & Altman, 2009). One author (ARR) completed the initial screening, using titles, abstracts and key words to exclude citations that were clearly irrelevant. If there was any doubt about inclusion the paper was included for the next stage. After the initial screening, all remaining full text articles were independently screened by both authors (ARR and JJR) to assess their eligibility for inclusion in the review. The reviewers were not blind to authors, journals, results or conclusions of the studies. Any disagreement was resolved by discussion.

3.2.4 Data extraction and management. Data extraction was completed by one author (ARR) using a data extraction form designed prior to the search. Data items extracted from the included studies were: population (including any information on health conditions), sample *N*, number of participants in each group, intervention (including type, dosage, bacterial strain(s), and any method of protecting the bacteria from heat or stomach acid stated in the text—e.g., lyophilisation, encapsulation in acid-resistant capsules), control product, days of intervention period, measures used, and psychological outcomes assessed. If a method of protection of the bacterial organisms was not reported in the text, attempts were made post data extraction to find this information on the website of the manufacturer of the intervention product.

3.2.5 Methodological quality assessment. All studies were scored independently by both authors on the Jadad scale (Jadad et al., 1996)—which assesses the quality of randomisation, the quality of blinding, and reasons for withdrawal/dropouts (0=worst, 5=best)—to assess risk of bias at the study level. Only studies which scored 3 or above (out of a possible 5) on the Jadad scale were included. Any disagreement between reviewers was resolved by consensus.

3.2.6 Data synthesis. Due to the small number of studies, and the wide heterogeneity between studies, we elected to complete a narrative synthesis of results by psychological outcome.

3.2.7 Measures of treatment effect. Where possible, standardised mean difference (SMD; Cohen's *d*) and 95% confidence intervals were calculated to assess the size of treatment effects.

3.2.8 Missing data. Where studies reported only on per protocol data, rather than intent-to-treat, this was made explicit in the narrative synthesis. Where studies did not present sufficient data and/or statistical test results to allow for the calculation of SMD, the authors' interpretation of their results was outlined in the narrative synthesis without SMD data. In all cases, the reasons for this were made explicit in the text, so that these results may be interpreted with caution.

3.3 Description of the Studies

3.3.1 Results of the search. See Figure 3 for the PRISMA flow chart (Moher et al., 2009). In total, 2748 abstracts were retrieved from the electronic database searches, and 15 from study reference lists. After duplicates were removed, 2029 individual abstracts remained. During initial screening, studies that were clearly irrelevant were excluded based on information available in abstracts (n=1986). Full text articles for all included abstracts were searched for, and any articles not published in full, in a peer-reviewed journal, and available in English, were excluded at this point (n=2). All remaining full text articles (n=43) were independently screened by both authors to assess their eligibility for inclusion in the review. Based on the screening process, 10 studies met the full

inclusion criteria. Reasons for exclusion at the full text stage were: did not use probiotic intervention (n=6), did not include at least one standardised, validated measure of psychological outcomes (n=14), not double-blind or not controlled using a placebo (n=11), and secondary paper on already included trial (n=2).

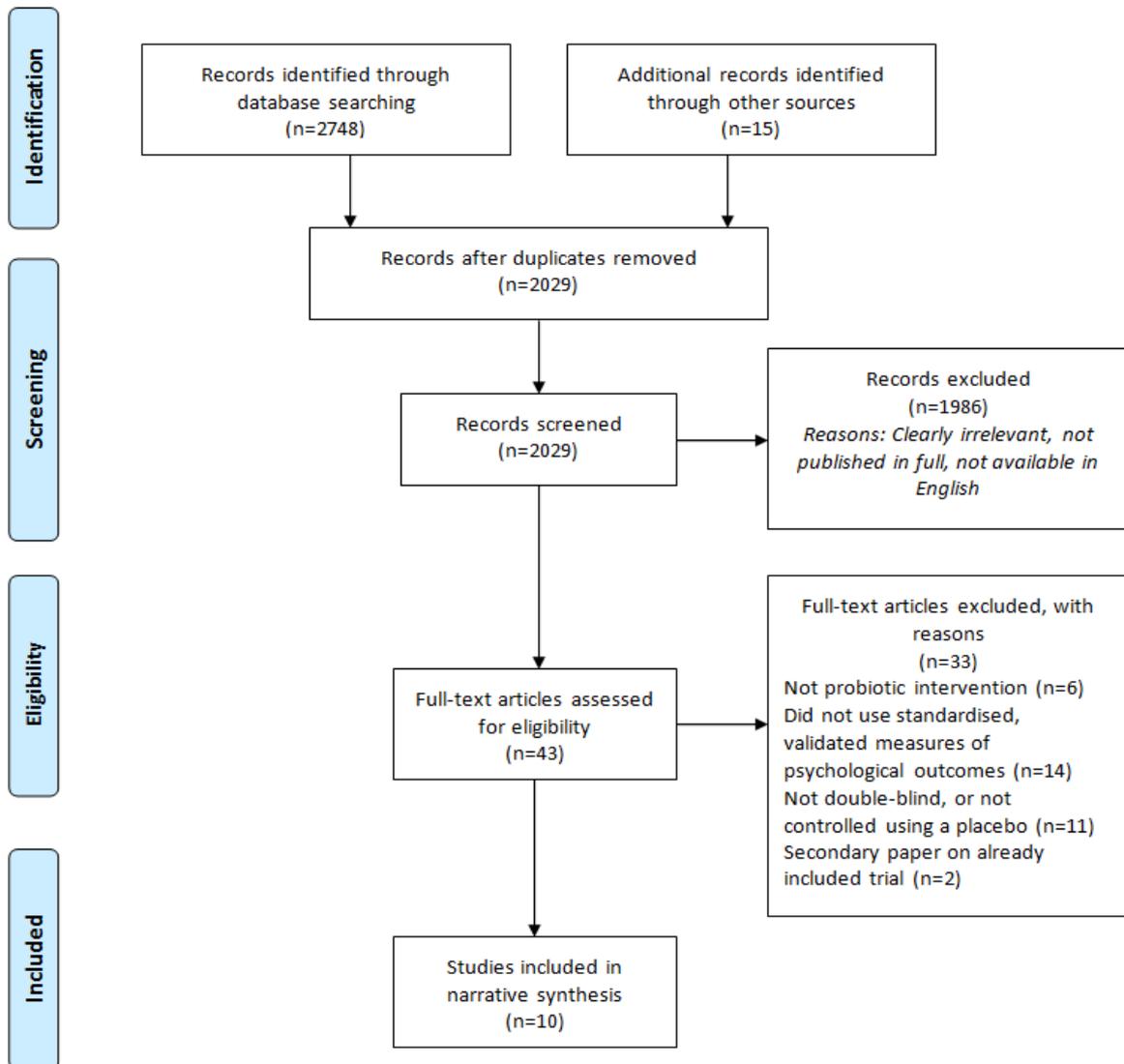


Figure 3. PRISMA flow chart, from Moher D, Liberati A, Tetzlaff J, Altman DG. (2009). Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *Annals of Internal Medicine*, 151(4), 264-269.

3.3.2 Included studies. See Table 2 for characteristics of included trials. All studies in this area were conducted in the last 10 years. Overall, most studies were relatively peripheral to this review with secondary, rather than primary, outcomes being relevant to the review topic.

Table 2. Characteristics of included trials, including population, number of participants (N), number of participants in active treatment and placebo groups, intervention used, duration of intervention period in days, psychological outcomes, relevant measures used and a description of the relevant results.

Author (Year)	Population	N (PF; PL)	Intervention	Control product	Days	Psychological outcomes assessed	Relevant measures used	Results
Benton, Williams, and Brown (2007)	Healthy, general population	124 (PF/PL unknown); PP only	Probiotic formulation: 6.5×10^9 live <i>Lactobacillus casei</i> per day (Yakult, Japan)	Sensorially identical milk product	20	Mood, anxiety	Profile of Mood States	No general effect of probiotics on mood in original sample (SMD not calculable). Further analysis considered an interaction with baseline mood. Subgroup (tertile) with lowest baseline mood showed group difference on the depressed/elated dimension, with the probiotic group showing greater improvement, after probiotic supplementation (SMD not calculable). No effect on composed/anxious or any other subscale of the POMS (SMD not calculable).
Dapoigny et al. (2012)	Patients with irritable bowel syndrome	50 (PF=25; PL=25)	Probiotic formulation: 3 capsules of freeze-dried <i>Lactobacillus casei</i> subsp. <i>rhamnosus</i> with a concentration of at least 2×10^8 CFU per capsule	Identical placebo capsules	28	Mood, anxiety	Hospital Anxiety and Depression Scale	No clinically relevant changes observed from baseline in either group on mood or anxiety scores (SMD not calculable).
Dickerson et al. (2014)	Outpatients with schizophrenia	65 (PF=33, PL=32)	Probiotic formulation: 1×10^9 CFU <i>Lactobacillus rhamnosus</i> strain GG and 1×10^9 CFU <i>Bifidobacterium animalis</i> subsp. <i>Lactis</i> Bb12 in acid resistant capsules (Ferrosan A/S, Søborg, Denmark).	Identical placebo capsules	98	Psychiatric symptoms of schizophrenia	Positive and Negative Symptom Scale	No effect of probiotics over time on the PANSS total score ($d=0.28$, 95% CI [-0.21-0.77]), or on the positive, negative or general scale scores ($d=0.26$, 95% CI [-0.22-0.75]; $d=0.33$, 95% CI [-0.16-0.82], and $d=0.24$, 95% CI [-0.24-0.74] respectively).
Messaoudi et al. (2010)	Healthy, general population	55 (PF=26; PL=29); PP only	Probiotic formulation: 3×10^9 live, freeze-dried <i>Lactobacillus acidophilus</i> and <i>Bifidobacterium longum</i> per day (Institute Rosell-Lallemand, Blagnac, France)	Sensorially identical powder preparation	30	Stress, mood, anxiety, somatisation, psychoticism, interpersonal sensitivity, obsessive-compulsive, coping	Hopkins Symptom Checklist, Hospital Anxiety and Depression Scale, Coping Checklist	Stress: no effect of probiotic on PSS scores over time ($d=0.1$, 95% CI [-0.43-0.63]); no effect of probiotic on UFC levels over time ($d=0.003$, 95% CI [-0.53-0.53]). Anxiety: no effect of probiotics on any anxiety-related subscale of HSCL-90 (d not calculable). Moderate effect of probiotics on HADs-A ($d=.54$; 95% CI [-0.002-1.08]) although not significant. Mood: moderate effect of probiotics HADs global score (mood and anxiety; $d=.63$; 95% CI [0.08-1.17]), but no effect on mood subscale ($d=0.005$, 95% CI [-0.52-0.53]), implying that the effect on the HADs global score may be due to change in anxiety rather than mood. Moderate effect of probiotics on percentage change (baseline to 30 day follow up) on the global severity index of the HSCL-90 ($d=0.62$, 95% CI [0.07-1.16]), particularly due to improvements on three subscales of the HSCL-90: somatisation, depression and anger-hostility. Percentage change on each of these subscales was higher for probiotic treated participants than for controls ($d=0.61$, 95% CI [0.07-1.15]; $d=0.55$, 95% CI [0.008-1.09]; and $d=0.69$, 95% CI [0.14-1.23] respectively. Depression and anger-hostility appear to be associated with mood. No effect on other subscales of HSCL-90, or on coping (d not calculable).

Parracho et al. (2010)	Children with autism	39 (PF=19; PL=20); PP only	Probiotic formulation: <i>Lactobacillus plantarum</i> WCFS1 (4.5x10 ¹⁰ CFU per capsule)	Identical placebo capsules	21	Anxiety, eternalising behaviours	Developmental Behaviour Checklist	No significant difference between treatment group and control on any subscale of the DBC-P (SMD not calculable).
Rao et al. (2009)	Patients with chronic fatigue syndrome	39 (PF=19; PL=17)	Probiotic formulation: 24 x 10 ⁹ CFU <i>Lactobacillus casei subsp. Shirota</i>	Sensorially identical placebo sachet	56	Mood, anxiety	Beck Depression Inventory, Beck Anxiety Inventory	No effect of probiotic on anxiety or mood (SMD not calculable).
Reale et al. (2012)	Healthy male smokers	72 (PF=36; PL=36)	Probiotic formulation: Powder preparation containing 1 x 10 ¹⁰ lyophilised viable <i>Lactobacillus casei subsp. Shirota</i> strain per gram per sachet (four sachets/day).	Sensorially identical powder preparation	21	State and trait anxiety	State-Trait-Anxiety Inventory	No significant effect of probiotic on STAI I (state) or II (trait) scores ($d=0.23$, 95% CI [-0.24-0.69] and $d=0.28$, 95% CI [-0.19-0.74] respectively)
(Simrén et al. (2010))	Patients with irritable bowel syndrome	74 (PF=37; PL=37)	Probiotic formulation: Milk fermented with the yoghurt bacteria <i>Lactobacillus bulgaricus</i> and <i>Streptococcus thermophilus</i> and containing at least 5x10 ⁷ CFU /mL of three different probiotic bacteria, <i>Lactobacillus paracasei, subsp. paracasei</i> F19, <i>Lactobacillus acidophilus</i> La5 and <i>Bifidobacterium lactis</i> Bb12 (Cultura)	Comparable acidified milk product without probiotic bacteria	56	Mood, anxiety	Hospital Anxiety and Depression Scale	No effect of probiotics on HADs-A scores ($d=0$; 95% CI [-0.46-0.46]) or HADs-D scores ($d=0$; 95% CI [-0.46-0.46]).
Vaghef-Mehrabany et al. (2014)	Patients with rheumatoid arthritis	46 (PF=22; PL=24); PP only	Probiotic formulation: capsules containing a minimum of 10 ⁸ CFU of <i>Lactobacillus casei</i> 01	Identical placebo capsules	56	State and trait anxiety	State-Trait-Anxiety Inventory	No effect of probiotic on STAI I (state) or II (trait) scores ($d=0.28$, 95% CI [-0.3-0.86] and $d=0.12$, 95% CI [-0.46-0.7] respectively)
(Whorwell et al. (2006))	Patients with irritable bowel syndrome	362 (PF=90 per group; PL=92)	Probiotic formulation: Freeze-dried <i>Bifidobacterium infantis</i> in varying doses (1x10 ⁶ , 1x10 ⁸ , 1x10 ¹⁰ CFU/ml)	Identical placebo capsules	28	Mood, anxiety	Hospital Anxiety and Depression Scale	No significant change in anxiety or depression scores at any probiotic dose compared with placebo (SMD not calculable).

Abbreviations: PF=probiotic formulation group; PL=placebo group; PP=per protocol; CFU=colony forming units; PANSS=Positive and Negative Symptoms Scale; HSCL-90=Hopkins Symptom Checklist-90; HADs=Hospital Anxiety and Depression Scale (HADs-D=depression subscale; HADs-A=anxiety subscale); DBC-P=Developmental Behaviour Checklist-Parent version; SMD=standardised mean difference (Cohen's *d*).

3.3.3 Participants. The total number of participants in all trials was 928. The number of participants in each trial ranged from 39 to 362, with a median (interquartile range) of 60 (47.5-73.5). As can be seen from the breakdown shown in Figure 4, 50% of studies had samples of between 50 and 99 participants. The eligible trials were conducted on a wide variety of populations (see Figure 5), from healthy volunteers to outpatients with schizophrenia. The most common study population was adults with irritable bowel syndrome (IBS; 30%).

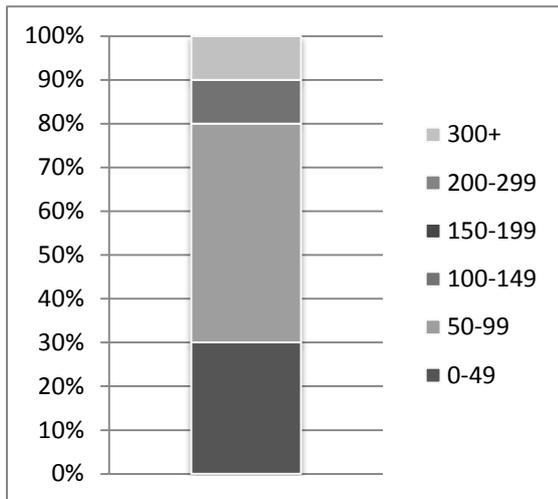


Figure 4. Breakdown of trials by sample *n*.

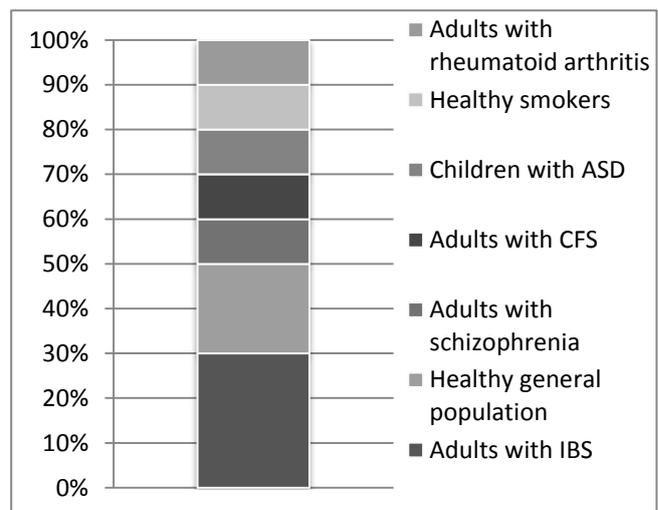


Figure 5. Breakdown of trials by population. *Abbreviations:* ASD, Autistic Spectrum Disorders; CFS, Chronic Fatigue Syndrome; IBS, Irritable Bowel Syndrome.

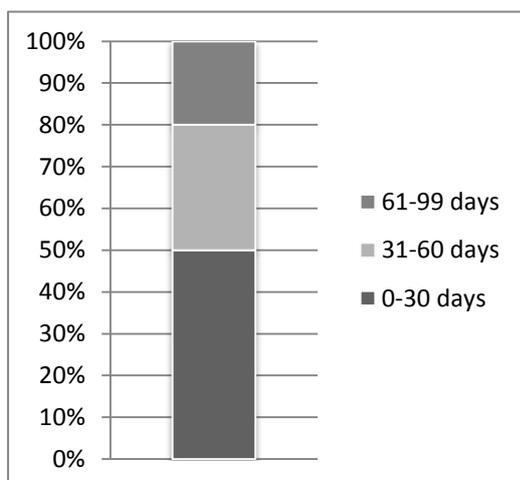


Figure 6. Breakdown of trials by duration of intervention period.

3.3.4 Duration of intervention period. The duration of probiotic intervention period in included trials ranged from 20 days to 98 days. Most commonly, probiotics were administered for less than 30 days (Figure 6). Although there was a wide variation in duration of intervention period in included trials, there were too few eligible studies to see any relationship between duration of intervention period and change on psychological outcome measures.

3.3.5 Protection of the bacteria. Despite the huge number of probiotic-based health products available today, reports have indicated that there may be poor survival of the bacteria in many of these products (Kailasapathy, 2002). Providing these bacteria with protection from heat and stomach acid could be crucial in ensuring their survival through transportation, storage, and through the digestive tract.

Protection status of bacteria was found for six eligible trials (Benton et al., 2007; Dapoigny et al., 2012; Messaoudi et al., 2010; Rao et al., 2009; Reale et al., 2012; Whorwell et al., 2006). Protection methods used included breeding acid-resistant strains (n=2)(Benton et al., 2007; Rao et al., 2009), and lyophilisation (freeze-drying; n=4) (Dapoigny et al., 2012; Messaoudi et al., 2010; Reale et al., 2012; Whorwell et al., 2006). Some of the manufacturers of products for which no protection information could be found had stated in promotional pamphlets that their product had “proven survival of the gastrointestinal tract”; however, for the purposes of this review, this was not considered protected bacteria as no formal protection method was declared. This information is included in Table 2, under the “Intervention” column.

3.3.6 Bacterial strains. The probiotic formulae used in included studies varied widely in composition. The number of strains in each formula ranged from one to five. Seven studies used single strain formulae (Benton et al., 2007; Dapoigny et al., 2012; Parracho et al., 2010; Rao et al., 2009; Reale et al., 2012; Vaghef-Mehrabany et al., 2014; Whorwell et al., 2006). The species of probiotics used in eligible studies included *L. casei*, *L. rhamnosus*, *B. infantis*, *B. lactis*, *B. longum*, *L. acidophilus*, *L. plantarum*, *L. bulgaricus*, *Streptococcus (S.) thermophilus* and *L. paracasei*. Due to the

wide variation in strains used, and the small number of eligible trials, any relationship between probiotic strain and change on psychological outcome measures was not detectable.

3.3.7 Placebo product used. Eight studies used placebo capsules or powder usually containing maltodextrin, which is fully absorbed before the small intestine and so does not affect the gut microbiota (Dapoigny et al., 2012; Dickerson et al., 2014; Messaoudi et al., 2010; Parracho et al., 2010; Rao et al., 2009; Reale et al., 2012; Vaghef-Mehrabany et al., 2014; Whorwell et al., 2006). Two studies used milk products as their placebo (Benton et al., 2007; Simrén et al., 2010). These milk products were heat-treated to kill any bacteria present, and acidified to ensure that they are sensorially comparable to the intervention product; however, it is important to consider the possibility that milk products containing non-living organisms may not be a true placebo, as some reports have suggested that non-living probiotic strains could have an effect on immune responses and inflammation (Adams, 2010).

3.3.8 Methodological quality of included studies. Consensus was reached on the quality assessment in all cases. All included studies met the methodological quality assessment criteria of a score of three or more on the Jadad scale. The Jadad scores of each trial, including results of individual assessment criteria, are presented in Table 3. Jadad scores ranged from 4 to 5. The mean (SD) Jadad score of all trials was 4.3 (0.48).

3.3.8.1 Allocation. Although all included trials were described as randomised, six of the ten eligible studies did not describe the method used to generate the sequence of randomisation (Benton et al., 2007; Dapoigny et al., 2012; Dickerson et al., 2014; Rao et al., 2009; Vaghef-Mehrabany et al., 2014; Whorwell et al., 2006). Due to these omissions in reporting, we are unable to rule out the possibility that the randomisation methods in these six trials may have been inappropriate, which would bias the results of this review.

Table 3. Results of methodological quality assessment of all trials using the Jadad scale. Includes the results of each study on all criterion of the Jadad scale, and the final Jadad score.

Author (Year)	Described as randomised:	Described as double-blind:	Description of withdrawals and dropouts*:	Method of randomisation:	Method of double blinding:	Final Jadad score
	Yes (+1)	Yes (+1)	Yes (+1)	Appropriate (+1)	Appropriate (+1)	Max: 5
	No (0)	No (0)	No (0)	Inappropriate (-1)	Inappropriate (-1)	
				Not described (0)	Not described (0)	
Benton et al. (2007)	Yes	Yes	Yes	Not described	Appropriate	4
Dapoigny et al. (2012)	Yes	Yes	Yes	Not described	Appropriate	4
Dickerson et al. (2014)	Yes	Yes	Yes	Not described	Appropriate	4
Messaoudi et al. (2010)	Yes	Yes	Yes	Appropriate	Appropriate	5
Parracho et al. (2010)	Yes	Yes	Yes	Appropriate	Appropriate	5
Rao et al. (2009)	Yes	Yes	Yes	Not described	Appropriate	4
Reale et al. (2012)	Yes	Yes	No	Appropriate	Appropriate	4
(Simrén et al. (2010))	Yes	Yes	Yes	Appropriate	Appropriate	5
Vaghef-Mehrabany et al. (2014)	Yes	Yes	Yes	Not described	Appropriate	4
(Whorwell et al. (2006))	Yes	Yes	Yes	Not described	Appropriate	4

*Description of withdrawals and dropouts must include the number of withdrawals in each group and the underlying reasons for withdrawal (Jadad et al., 1996).

3.3.8.2 Selective reporting. There were a large number of omissions in reporting of data in eligible trials. Group mean and standard deviation data were not reported for psychological outcome measures in any trial. Four trials (Benton et al., 2007; Messaoudi et al., 2010; Parracho et al., 2010; Vaghef-Mehrabany et al., 2014) only reported on per protocol data, without reporting on intent-to-treat analysis. Six trials (Benton et al., 2007; Dapoigny et al., 2012; Messaoudi et al., 2010; Parracho et al., 2010; Rao et al., 2009; Whorwell et al., 2006) did not report sufficient data, or results of statistical tests, to allow for the calculation of SMD for some or all psychological outcome measures assessed. The majority of results for which SMDs could not be calculated were described in study reports as showing no effect of probiotics on psychological outcomes, with only one trial (Benton et al., 2007) reporting a significant difference between treatment and control group for

which SMD could not be quantified. It is possible that this could negatively bias this review, as interpretation of many of the results showing no effect of probiotics on psychological outcomes relies on the original interpretation of the study authors.

3.4 Results

3.4.1 Effect of probiotics on stress. Of the ten included trials, one study was found which used a measure of stress (Messaoudi et al., 2010). This study investigated the effect of probiotic administration on the psychological impact of everyday life events in 55 healthy adult participants from the general population. The scale used to measure change in perceived stress during the trial was the Perceived Stress Scale (PSS-14). The intervention period was 30 days in duration, during which the probiotic intervention (3×10^9 colony forming units (CFU) of freeze-dried *L. acidophilus* and *B. longum* per day) was compared to an identical maltodextrin-based placebo. This study only reported on per protocol data.

The study found no effect of probiotic on their measure of perceived stress over time ($d=0.1$, 95% CI [-0.43-0.63]). In addition, there was no effect of probiotic on urinary free cortisol levels over time ($d=0.003$, 95% CI [-0.53-0.53]). The results of this study do not provide evidence that probiotics are a useful treatment for stress in a healthy population. A preclinical trial reported on in the same paper showed that the same probiotic reduced conditioned defensive burying behaviour in rats (a rat model of stress/anxiety) significantly more than placebo, which the authors interpreted as favouring the hypothesis that the probiotic formulation has anxiolytic effects. However, it appears that this preclinical evidence does not generalise to this measure of human perceived stress. The authors suggest that the PSS may not be sensitive over a longer treatment period, which may explain the lack of any significant change on this scale during their study.

3.4.2 Effect of probiotics on anxiety. In total, nine of the eligible trials used measures of anxiety (Benton et al., 2007; Dapoigny et al., 2012; Messaoudi et al., 2010; Parracho et al., 2010; Rao et al., 2009; Reale et al., 2012; Simrén et al., 2010; Vaghef-Mehrabany et al., 2014; Whorwell et

al., 2006). Measures used included the Profile of Mood States (POMS; composed/anxious dimension), Hospital Anxiety and Depression Scale: Anxiety subscale (HADS-A), the Hopkins Symptom Checklist-90 (HSCL-90; anxiety, phobic anxiety and paranoid-ideation subscales), Developmental Behaviour Checklist: Primary Carer Version (DBC-P; anxiety problems dimension), the State-Trait Anxiety Inventory (STAI) and the Beck Anxiety Inventory (BAI). Population types in these studies included healthy volunteers (n=2), healthy smokers (n=1), IBS patients (n=3), children with autism (n=1), patients with rheumatoid arthritis (n=1) and patients with chronic fatigue syndrome (CFS; n=1).

Rao et al. (2009) investigated probiotics as an adjuvant treatment for CFS. The study sample was 39 CFS patients, the intervention duration was eight weeks, and anxiety was measured using the BAI. The probiotic formulation used in this study contained 24×10^9 CFU *L. casei subsp. Shirota*. The authors state that a sensorially identical placebo formula was used; however, the ingredients in the placebo formula were not published.

There appears to be some disparity in the reporting of the results of this trial. Although it was reported in the text of this paper that “Overall there was a significant improvement in anxiety among those taking the active *L. casei subsp. Shirota* compared to the placebo (Table 3)” (p.3), it is unclear whether the statistics reported in the referenced table (labelled “Probiotic Treatment Pre vs. Post”, p.4) refer to a pre- to post-treatment change within the probiotic group, or a group difference. We contacted authors on this paper for clarification of their results and were informed that there was a group difference between probiotic and placebo for the BAI. However, when we asked the authors to clarify whether the data reported in their Table 3 refers to this group difference, and to provide mean and standard deviation data for both groups pre- and post-treatment, we did not receive a response within the necessary timeframe. Therefore, while this paper is usually cited as evidence for a group difference between treatment and placebo on the BAI, we are neither able to draw any firm conclusions about the results of this trial, nor calculate any reliable effect size estimates.

Parracho et al. (2010) conducted a 12 week crossover probiotic feeding study in 39 children with autistic spectrum disorders (ASD): three weeks of treatment with probiotics *Lactobacillus plantarum* WCFS1 (4.5×10^{10} CFU) or placebo, followed by a three week washout period, and then a crossover phase in which participants switched to the opposite condition. The authors report that there was no significant difference between groups on the “anxiety problems” dimension of the DBC-P, and standardised mean difference was not quantifiable from the data reported. Median and rank sum data were reported for the DBC-P; however, only per protocol data were evaluated (for the 15 participants for whom a completed DBC-P was available at both baseline and follow-up), and the authors did not report the *n* of active treatment and placebo groups for the participants included in this analysis. No results from statistical tests were reported. This study does not appear to provide supporting evidence for the use of probiotics for anxiety in children with ASD. However, the power of this study was greatly affected by a substantial dropout rate, and the authors speculate that the three week intervention period may not have been long enough to see a notable response in behaviour and emotional responses. It is also debatable whether a crossover design is appropriate for probiotics trials, as the duration of persistence of different strains of supplemented bacteria in the gut after ceasing supplementation has yet to be firmly established, and, as mentioned above, organisms may exhibit extreme variability in persistence behaviour dependent on the host (Klingberg & Budde, 2006).

Benton et al. (2007) studied 132 healthy participants; however, only per protocol data were presented for the 124 participants who finished the trial. Their probiotic drink contained *L. casei* *subsp. Shirota* at a dosage of at least 6.5×10^9 CFU per day, and the intervention period was 20 days in duration. A milk-based placebo without live bacteria was used in this study. The authors report that they found no general effect of taking the probiotic on the composed-anxious dimension of the POMS. Standardised mean difference was not calculable as no data or results from statistical tests were reported for the POMS subscales. It is possible that the lack of any effect may be due to the short intervention period, or a lack of presence of anxiety in participants at baseline. As discussed

above, it is also possible that the control milk was not a true placebo, as it may have contained non-living organisms.

There were four trials which used the HADs-A to measure anxiety (Dapoigny et al., 2012; Messaoudi et al., 2010; Simrén et al., 2010; Whorwell et al., 2006). The study by Messaoudi et al. (2010) described above, investigating the effect of probiotic administration on the psychological impact of everyday life events in 55 healthy adult participants, found a moderate effect of probiotics on the HADs global score ($d=.63$; 95% CI [0.08-1.17]), and a moderate effect of probiotics on HADs-A subscale scores ($d=.54$; 95% CI [-0.002-1.08]) although the latter was not significant. These results suggest that probiotics may have an effect on anxiety levels in a healthy population sample. However, these effect size estimates should be interpreted with caution, as it is possible that the data reported on in this paper is skewed: only median and interquartile range (IQR) data were reported on (rather than mean and standard deviation), which suggests that the data may have a non-normal distribution. This study found no group differences between the treatment and placebo groups on any anxiety-related dimension of the HSCL-90 (anxiety, phobic anxiety and paranoid-ideation); effect size estimates were not quantifiable for these results due to the type of data reported for these subscales (median and IQR only).

The remaining three trials which assessed anxiety using the HADs-A were trials in samples of patients with IBS, with the HADs-A being a secondary outcome measure. Whorwell et al. (2006) conducted a dose-response study in 362 patients with IBS over a 4 week intervention period, comparing three different doses of freeze-dried *B. infantis* (1×10^6 CFU, 1×10^8 CFU or 1×10^{10} CFU) with matched placebo capsules. No group data or results from statistical tests were reported for the HADs, but the authors report that they found no significant group differences at any dose. Dapoigny et al. (2012) compared 6×10^8 CFU encapsulated *L. casei rhamnosus* with a placebo over four weeks. They also reported no group data or results from statistical tests for the HADs, rendering effect size estimates impossible, but the authors stated that they observed no clinically relevant changes on the HADs-A in either group from baseline. Simrén et al. (2010) studied 74 patients with IBS, using a

probiotic fermented milk supplement containing at least 5×10^7 CFU of *L. paracasei*, *L. acidophilus* and *B. lactis*, versus a control milk product without live bacteria, over an intervention period of eight weeks. They did not find an effect of probiotics on HADs-A scores ($d=0$; 95% CI [-0.46-0.46]). They found a larger change in HADs-A scores in the control group than in the treatment group, which may be due, in part, to the fact that the placebo effect is very high in IBS trials (40-45%); however, as discussed previously, it is also possible that the control milk product was not a true placebo, as it may have contained non-living organisms.

Two trials used the STAI to measure anxiety: one trial in healthy smokers (Reale et al., 2012) and one in patients with rheumatoid arthritis (Vaghef-Mehrabany et al., 2014). The trial in 72 healthy smokers used freeze-dried *L. casei subsp. Shirota* powder versus a matched placebo (maize starch powder) for three weeks (Reale et al., 2012). The authors report the dosage as “ 1×10^{10} CFU per gram per sachet” (p. 309), at four sachets per day for the duration of the intervention period; however, the number of grams per sachet is not reported, so determining actual daily dosage is problematic. They found no significant effect of probiotic on STAI I (state) or II (trait) scores ($d=0.23$, 95% CI [-0.24-0.69] and $d=0.28$, 95% CI [-0.19-0.74] respectively). Baseline levels of anxiety among their participants were within the normal range, which may partly explain this lack of effect. Finally, the trial in 60 patients with rheumatoid arthritis by Vaghef-Mehrabany et al. (2014) tested *L. casei* at a minimum dosage of 1×10^8 CFU per day for eight weeks, also found no significant effect of probiotic on STAI I or II scores at the end of their study ($d=0.28$, 95% CI [-0.3-0.86] and $d=0.12$, 95% CI [-0.46-0.7] respectively); however, the participants most frequently reported both state- and trait-anxiety in the “Not at all” or “Low” categories at baseline, indicating relatively low levels of anxiety among the sample.

There are no results which suggest an effect of probiotic strains *L. casei subsp. Shirota*, *L. acidophilus*, or *L. casei rhamnosus* on anxiety. Messaoudi et al. (2010) found a moderate effect of their probiotic formula on the HADs global score in their human population, and although there was a trend towards an effect on the anxiety subscale, this result was not significant. They stated that

their results provide preliminary evidence that *L. acidophilus* and *B. longum* in combination “mitigated psychological distress” (p. 7), and further evidence that the “gut microflora play a role in stress [and] anxiety” (p.7). Although there are many more negative trials than positive trials for anxiety, given the moderate effect found in the Messaoudi et al. (2010) study using *L. acidophilus* and *B. longum*, coupled with their preclinical evidence, it is possible that this is an issue of probiotic strain. Interestingly, the duration of intervention period in the Messaoudi et al. (2010) trial was 30 days, suggesting that this may be a long enough intervention period to see effects of probiotics on anxiety in a healthy population sample. However, the reporting of only per protocol data in this trial is a potential source of bias. It is also important to note that no trials have been conducted which have recruited patients based on the presence of anxiety at baseline; it is only possible to draw from the results of Messaoudi et al. (2010) that these strains of probiotic may have an effect on anxiety in a healthy population. Probiotics trials recruiting participants based on a level of anxiety at baseline, or recruiting participants from clinical populations with anxiety disorders, are necessary before the possible efficacy of probiotics in treating anxiety can be established.

3.4.3 Effect of probiotics on mood. Six probiotic intervention trials were found that used measures of mood (Benton et al., 2007; Dapoigny et al., 2012; Messaoudi et al., 2010; Rao et al., 2009). Scales used to measure mood included the POMS (depressed/ elated subscale), Hospital Anxiety and Depression Scale: Depression subscale (HADS-D), the HSCL-90 (depression and anger-hostility subscales), and the Beck Depression Inventory (BDI). Population types included patients with IBS (n=3), patients with CFS (n=1) and healthy volunteers (n=2). No included trials recruited participants based on a level of low mood at baseline, or patients with any clinical mood disorder.

Two studies on healthy populations used measures of mood (Benton et al., 2007; Messaoudi et al., 2010). The study by Messaoudi et al. (2010) described above, using *L. acidophilus* and *B. longum* in 55 healthy participants for 30 days, found no effect of probiotic treatment on HADS-D scores ($d=0.005$, 95% CI [-0.52-0.53]). As previously stated, a moderate effect was found for

probiotics on the HADs global score ($d=.63$; 95% CI [0.08-1.17]); however, the lack of any effect on the HADs-D subscale, compared with the moderate (though not significant) effect on the HADs-A subscale, suggests that this change was mostly due to group differences in anxiety, rather than mood. This trial also found a moderate effect of probiotics on percentage change (baseline to 30 day follow up) in HSCL-90 global severity index scores ($d=0.62$, 95% CI [0.07-1.16]). This was particularly due to improvements on three subscales of the HSCL-90: somatisation, depression and anger-hostility. Percentage change on each of these subscales was significantly higher for probiotic treated participants than for controls ($d=0.61$, 95% CI [0.07-1.15]; $d=0.55$, 95% CI [0.008-1.09]; and $d=0.69$, 95% CI [0.14-1.23] respectively). Two of these subscales appear to be associated with mood (depression and anger-hostility). Although the moderate effect of probiotic treatment on HSCL-90 subscales associated with mood suggests that probiotics may affect mood outcomes, it is important to note that, as detailed above, effect size estimates may not be reliable due to the probable skewed nature of the distribution of their data. In addition, this trial only reported on per protocol data. They also used a healthy population sample which limits the generalisability of these results to populations recruited for low mood or populations with clinical mood disorders.

Benton et al. (2007) found no effect on mood in their healthy general population sample of 124 participants who were treated with a probiotic supplement containing 6.5×10^9 CFU *L. casei* *subsp. Shirota* for 20 days. However, the authors believed that a generally positive mood among their sample may have masked any response to supplementation. To investigate this possibility the authors added a factor into their analysis dividing the study sample into tertiles by baseline mood (as rated on POMS). This analysis showed a statistically significant interaction on the depressed/ elated subscale of the POMS between treatment group, test session and baseline mood ($F(2, 111)=4.19$, $p<0.02$), reflecting that, in the third of the sample with the lowest mood at baseline, the probiotic group were significantly less depressed after 20 days of probiotic supplementation than controls ($p<0.04$). Effect size estimates could not be extracted from the F -ratio, as the ANOVA compared more than two groups (i.e., degrees of freedom were more than 1). No mean or standard deviation

data were reported. It is also noteworthy that this study only included participants who were self-reportedly healthy, and excluded participants who had current depression. These data suggest that probiotics may be a useful treatment for healthy people with lower than average mood; however, the study does have some limitations relevant to this review. Only per protocol data are reported on, and effect sizes were neither reported nor quantifiable from the data reported. These results should therefore be interpreted with caution.

In the study by Rao et al. (2009) on patients with CFS (as described above), given the apparent disparity between the reporting in the text and the labelling of the table rows, interpretation of the statistics reported is problematic. They state that they found no significant difference between treatment groups and controls on the BDI, but estimates of effect size were not quantifiable as we were unable to ascertain from the authors whether the results reported in the table referred to a between or within group difference.

The three remaining trials were on patients with IBS and used the HADS-D to assess mood (Dapoigny et al., 2012; Simrén et al., 2010; Whorwell et al., 2006). Of these trials, two trials had an intervention period of four weeks (Dapoigny et al., 2012; Whorwell et al., 2006) and one was eight weeks long (Simrén et al., 2010). Probiotic strains used included *B. infantis* (Whorwell et al., 2006), *L. casei rhamnosus* (Dapoigny et al., 2012) and a formulation containing *L. bulgaricus* and *S. thermophilus*, *L. paracasei*, *L. acidophilus* and *B. lactis* (Simrén et al., 2010). Whorwell et al. (2006) in their dose-response study (*B. infantis* 1×10^6 CFU, 1×10^8 CFU or 1×10^{10} CFU) in 362 IBS patients, found no significant group differences on the HADs-D at any dose. As described above, no group data or results from statistical tests were reported for the HADs in this study; therefore, the authors' interpretation is discussed here. Dapoigny et al. (2012) also reported no group data or results from statistical tests for the HADs in their study comparing 6×10^8 CFU *L. casei rhamnosus* with a placebo over four weeks. The authors stated that they observed no clinically relevant changes on the HADs-D in either group from baseline. Finally, Simrén et al. (2010) studied 74 patients with IBS, using a probiotic fermented milk supplement containing 5×10^7 CFU of *L. paracasei*, *L. acidophilus* and *B.*

lactis, versus a control milk product without live bacteria, over an intervention period of eight weeks. They did not find an effect of probiotics on HADs-D scores ($d=0$; 95% CI [-0.46-0.46]). It is worthy of note that, of these three trials, one excluded depressed patients (Dapoigny et al., 2012), one excluded anyone who had suffered from a major psychiatric disorder within the past two years (Whorwell et al., 2006), and one excluded those with any severe psychiatric disorder (Simrén et al., 2010). Mean (SD) HADs global score of treatment and control groups were reported by Dapoigny et al. (2012) and appeared to be within the normal to borderline range (16.3 (6.5) and 6.5 (6.4) respectively). The mean (SD) baseline HADs-D score was reported by Simrén et al. (2010) and was well within the normal range (3.6 (3.1)). These exclusion criteria and baseline mood data suggest that there may not have been enough room for change in mood in these studies to see an effect of probiotics on mood.

While one study (Messaoudi et al., 2010) and one post-hoc analysis (Benton et al., 2007) appear to show some support for the efficacy of probiotics in improving mood, the majority of trials do not show an effect of probiotics on measures of mood. This could possibly be due to the lack of available trials on populations reporting low mood at baseline. The results of the Messaoudi et al. (2010) trial are somewhat conflicting, with no effect of probiotic found on HADs-D scores, but moderate effects found on two dimensions of the HSCL-90 which appear to be related to mood (depression and anger-hostility). The result from Benton et al. (2007) should be interpreted with caution as standardised mean difference could not be quantified from the data reported. In addition, both trials which provide any supporting evidence for an effect of probiotics on mood were conducted in healthy samples, limiting their generalisability to samples with low mood at baseline or clinical mood disorders. Randomised controlled trials in patients with clinical mood disorders, or in participants screened for a level of low mood at baseline, may be useful in providing evidence of an effect of probiotics on mood.

3.4.4 Effect of probiotics on externalising behaviours. Gastrointestinal (GI)

symptoms are very common in people with autism spectrum disorders (ASD; Kidd, 2003) and studies have suggested that ASD may be linked to an imbalance in gut microbiota and a higher incidence of certain *Clostridium* bacteria and clusters (Bolte, 1998; Finegold et al., 2002; Parracho, Bingham, Gibson, & McCartney, 2005; Y. Song, Liu, & Finegold, 2004). These links led to the development of a theory suggesting that addressing the imbalance of bacteria in the gut could be an important step in reducing GI symptoms in people with ASD, and could also lead to significant clinical improvement in behaviour, attention and development, possibly through improving the integrity of the intestinal wall (d'Eufemia et al., 1996). However, there is conflicting evidence: for example, a study by Gondalia et al. (2012) found no clinically significant differences in the composition of the microbiota of their cohort of children with ASD relative to healthy controls. They suggest that the gastrointestinal dysfunction in the ASD population could be caused by a variety of other factors, including elevated anxiety and self-restricted diets.

One study was identified which explored the use of probiotic feeding in a sample of children with ASD (Parracho et al., 2010). As described above, this was a 12 week crossover probiotic feeding study in 39 children with ASD: three weeks of treatment with the probiotic *L. plantarum* WCFS1 (4.5×10^{10} CFU) or placebo, followed by a three week washout period, and then a crossover phase in which participants switched to the opposite condition. Despite the classification of autism as a neurodevelopmental disorder, this study was included because it measured behaviour, which was considered within the scope of the review. The behavioural measure used was the Developmental Behaviour Checklist: Primary Carer Version, which includes the following dimensions: disruptive/antisocial behaviour, self-absorbed, communication disturbances, anxiety (covered above) and social relating, as well as a total behaviour problem score. The authors found no significant group difference on any dimension of the DBC-P. As described above, standardised mean difference was not quantifiable from the data reported in this study.

These results do not provide evidence of an effect of probiotics on externalising behaviours. However, this study had an extremely high drop-out rate which greatly affected its statistical power, and only per protocol data for 15 participants were reported on for this measure. As outlined above, the crossover design of the study and the short duration of the intervention period in this trial may also have contributed to the lack of any significant findings. More trials in this area are necessary to provide empirical support for the theory that probiotics may affect externalising behaviours in ASD.

3.4.5 Effect of probiotics on the symptoms of schizophrenia. One study was found which used a measure of the symptoms of schizophrenia (Dickerson et al., 2014). The study was conducted on a sample of 65 outpatients with long-term schizophrenia, supplementing *L. rhamnosus* (1×10^9 CFU) and *B. animalis* (1×10^9 CFU) over an intervention period of 14 weeks. The measure used was the Positive and Negative Syndrome Scale (PANSS). This study is the first probiotic study to have recruited a sample from a clinical population with a diagnosed psychiatric condition, which is a significant step in psychobiotic research. The authors proposed the theory that probiotics could be useful for people with schizophrenia through the modulation of the inflammatory response, which has been found to be abnormally activated in many people with schizophrenia (Suvisaari & Mantere, 2013). However, the study did not find any effect of probiotic over time on the PANSS total score ($d=0.28$, 95% CI [-0.21-0.77]), or on the positive, negative or general scale scores ($d=0.26$, 95% CI [-0.22-0.75]; $d=0.33$, 95% CI [-0.16-0.82], and $d=0.24$, 95% CI [-0.24-0.74] respectively). The authors suggest that introducing probiotic compounds earlier in the course of the illness may be more effective.

3.4.6 Effect of probiotics on other relevant psychological dimensions. One trial, by Messaoudi et al. (2010) included the Coping Checklist (CCL), which measures five types of coping strategies when confronting an adverse event: problem solving; avoidance and wishful thinking; seeks social support; positive re-evaluation; and self-blame. The authors found no group difference between the probiotic and placebo groups on any dimension of coping. This trial also

included the HSCL-90, which assesses the following relevant dimensions not previously discussed: psychoticism, interpersonal sensitivity and obsessive-compulsive behaviour. No group differences were found on any of these subscales. Estimates of standardised mean difference were not calculable for these results due to the type of data reported for these subscales (median and IQR only).

Benton et al. (2007) used the POMS in their study on healthy participants, which assessed four psychological dimensions not already discussed: agreeable/hostile, confident/unsure, clearheaded/confused, and energetic/tired. The authors report that they found no general effect of taking the probiotic on any of these dimensions. Standardised mean difference was not calculable as no data or results from between groups tests were reported for the POMS subscales.

3.5 Conclusion

3.5.1 Summary of main results. Trials were found which investigated a wide variety of psychological outcomes including stress, mood, anxiety and externalising behaviours in ASD. One trial was also found which assessed the efficacy of probiotics in treating the symptoms of schizophrenia. Two trials used measures which include other dimensions related to psychological outcomes, including psychoticism, somatisation, interpersonal sensitivity, obsessive-compulsive behaviour, coping (problem solving, avoidance and wishful thinking, seeking social support, positive re-evaluation and self-blame), agreeable/hostile, confident/unsure, clearheaded/confused, and energetic/tired.

Overall, there is very limited evidence for the efficacy of probiotic interventions for psychological outcomes. One trial on a healthy population found a moderate effect of probiotics on global HADs scores (Messaoudi et al., 2010): this appeared to be mostly due to change in anxiety (for which a moderate, though non-significant, effect of probiotics was also found) rather than mood (for which no effect of probiotics was found). The same trial also found a moderate effect of probiotics on percentage change (baseline to 30 day follow up) on the global severity index of the HSCL-90,

particularly due to moderate effects on three subscales of the HSCL-90: somatisation, depression and anger-hostility. Two of these subscales appear to be associated with mood (depression and anger-hostility). In a post-hoc analysis, Benton et al. (2007) found a statistically significant result on the depressed/elated dimension of the POMS, reflecting that the third of their sample with the lowest mood at baseline were significantly less depressed after 20 days of probiotic supplementation than the control group. However, it is important to note that effect size estimates could not be calculated for these results due to omissions in reported data.

All other eligible trials did not provide any evidence of an effect of probiotics on any other psychological outcome or dimension, or psychiatric symptom. The interpretation of one trial was problematic due to apparent disparities between the authors' interpretations of findings in the text of the study report, and the statistics shown in the referenced table (Rao et al., 2009). Overall, there were many more negative trials than positive trials in all areas assessed. However, due to the variability in population, method, sample size, duration of intervention period, bacterial strains used and placebo product used, interpreting the results of these trials cohesively is problematic.

3.5.2 Overall completeness and applicability of evidence. Overall, the evidence base is lacking in completeness. This is a very new area of research, and more trials are needed on clinical populations with psychiatric disorders or participants presenting with psychological difficulties at baseline, before any conclusions can be drawn about whether this field is one which is worth pursuing. The evidence from many of the trials included here is not particularly applicable to the area, particularly due to the wide variability in study population.

3.5.3 Quality of the evidence. All of the trials included in this review were randomised controlled trials. However, omissions in reporting made effect size estimates impossible for some results. Group mean and standard deviation data for pre- and post-treatment scores on outcome measures were not reported in any trial.

3.5.4 Potential biases in the review process. A post-hoc decision was made during the study selection process to exclude secondary papers on included trials. Two papers (Alipour et al., 2014; Messaoudi et al., 2011) were excluded for this reason and, although study outcomes were not considered in this decision, it is possible that any decision made during the study selection process (rather than *a priori*) could bias the results of the review. In addition, missing data meant that standardised mean difference could not be calculated for some studies. For those results for which standardised mean difference could not be calculated, the author's interpretation was reported in this review. This could lead to bias, as authors' interpretations of their results may be considered subjective. However, the majority of results for which SMD could not be calculated were reported as no group difference between probiotic and placebo.

3.5.5 Agreement and disagreement with other studies or reviews. The results of this review are in disagreement with the narrative reviews on the topic, and the preclinical evidence outlined in the introduction, which appear to present a relatively strong case for an effect of probiotics on emotional behaviour in animals and psychological outcomes in humans.

3.5.6 Implications for research. The main implication of this review for research in this field is that more randomised controlled trials are necessary before any assertions can be made about the efficacy of probiotics for any psychological outcomes. Indeed, given the number of publications that extol about the potential for probiotics in treating psychiatric illness, it was actually surprising that there are so few clinical trials on affected populations. Future trials should be conducted in populations with clinical psychiatric disorders, or on participants screened for a level of psychological disturbance (e.g., low mood, elevated anxiety, elevated stress levels), as trials sampling these populations would provide the most reliable evidence of efficacy in the field. The issue of probiotic strain and duration of intervention period should be carefully considered in future research. Any trials which find an effect of probiotics on a psychological outcome should be

replicated using different bacterial strains, in order to test the generalisability of the results to other strains of probiotic bacteria.

3.6 Overall Summary

In Chapter 1, emerging evidence for associations between depression, inflammation and immune activation were discussed (e.g., Berk et al., 2013; Maes, 2011). These underlying nutritional and biological influences on depression, as well as the strong links among gut health, microbiome composition and inflammatory and immune activation described in Chapter 2, support a review by Logan and Katzman (2005) suggesting that probiotics may be a useful adjuvant treatment for depression. As described in Chapter 3, many narrative reviews have since been written in support of this notion (e.g., Bested et al., 2013b; Cryan & Dinan, 2012; Cryan & O'Mahony, 2011; Dinan & Quigley, 2011; Dinan et al., 2013), but these mainly rely on evidence from animal studies, with the human literature being sparse and mainly in healthy, rather than clinical, population samples. As described above, there is a need for well-controlled clinical trials testing the effects of probiotics on psychological outcomes in populations with clinical psychiatric disorders, or on participants screened for a level of psychological disturbance (e.g., low mood, high anxiety, high stress levels) at baseline, in order to provide evidence for a possible role for probiotics in treating mental disorders.

3.7 The Current Study

The following chapters present the first randomised controlled trial to investigate whether supplementation of probiotic bacteria affects mood and other psychological outcomes in people presenting with low mood at baseline. The study will also investigate plausible mechanisms of action through measuring levels of blood-based biomarkers and employing measures of cognitions and gut function.

3.7.1 Aims and hypotheses. The current trial aimed to strengthen and grow the field of research through providing the first randomised controlled trial to investigate whether supplementation of probiotic bacteria affects mood and other psychological outcomes in people

presenting with low mood. Another objective of the trial was to discover whether the presence or severity of IBS symptoms, and blood levels of proinflammatory cytokines, BDNF and vitamin D, would predict or impact treatment response, or whether these variables would be affected by treatment with the probiotics. The study also aimed to determine whether there was increased inflammatory activation or evidence of alterations in other blood-based biomarkers in a sample presenting with low mood at baseline.

Based on the existing evidence from gut-brain axis research, and on models linking depression with inflammation, immune activation, low vitamin D levels, and the state of the gut microbiome, it was hypothesised that:

1. The overall sample would have elevated levels of inflammatory biomarkers and low levels of vitamin D at baseline, which would be associated with scores on psychological and IBS outcome measures;
2. When the active probiotic product was acutely administered for eight weeks to participants with at least moderate symptoms of depression, group differences (active treatment versus placebo) would be observed on measures of depressive symptoms, general functioning and symptoms of anxiety and stress;
3. Group differences (active treatment versus placebo) would also be observed in blood levels of proinflammatory cytokines, hsCRP, vitamin D and BDNF, and scores on a measure of gut function/IBS, and levels of these variables would predict or impact treatment response;
4. Group differences would be observed at the point of the 6-month follow-up between those who continued to take the probiotic and those who discontinued probiotic use;
5. The investigational product would have minimal side effects.

The following two chapters describe the methods and results of the first randomised controlled trial to investigate whether supplementation of probiotic bacteria affects mood and other psychological outcomes in people presenting with low mood.

Chapter 4: Methods

4.1 Design

The first phase of the trial used a double-blind, randomised, placebo-controlled design in which participants were assigned (1:1) to receive either the active probiotic or placebo for eight weeks (the RCT phase). This was followed by an open label extension (OL phase) where participants were offered to take the active study product in an open label manner for a further eight weeks. Participants were then followed-up six months after finishing the trial. The trial (Universal Trial Number: U1111-1141-7832) was prospectively registered (Australian New Zealand Clinical Trials Registry: ACTRN12613000438752). No changes were made to the study design after trial registration was completed. The study received ethical approval from both the Southern Health and Disability Ethics Committee (URA/12/05/013/AM04), and the Human Ethics Committee at the University of Canterbury (HEC 2012/67).

4.2 Participants

Participants were recruited in Canterbury, New Zealand, via self-referrals based on advertisements. Screening was completed via an online system: potential participants were directed to a website (<http://bit.ly/UCnutritionresearch>) where they could access comprehensive information about the study (including the study information sheets, see Appendix 1), the contact details of the researchers, and a link to an online screening survey developed using Qualtrics (<http://www.qualtrics.com>). The survey contained a brief information summary, demographic information questions (see Outcomes section), a section of screening questions (see Entry Criteria section), history of mental illness (e.g., anxiety disorder, major depressive disorder), and two self-report mood measures (see Outcomes section). If a potential participant selected an answer which endorsed one of the exclusion criteria, or did not meet score cut offs for inclusion, they were directed to local resources for mental health care.

4.3 Entry Criteria

In order to be considered eligible for the trial, participants were required to score at least the lowest score in the 'Moderate' range on either of the self-report mood measures used for screening purposes: ≥ 11 on the Quick Inventory of Depressive Symptomatology (QIDS) or ≥ 14 on the depression subscale of the Depression, Anxiety and Stress Scale (DASS). Participants were required to be aged 16 or over at the time of screening, and to have a level of understanding sufficient to be able to complete the study protocol reliably. Participants were required to have been free of any psychiatric medications for at least four weeks prior to the trial. Participants who were currently undergoing regular psychological therapies were allowed to participate and continue with their therapy provided that they had been receiving therapy for 6 months or more. Participants were excluded temporarily if they had taken an oral antibiotic in the 6 weeks prior to starting. If an antibiotic or antidepressant was begun during the course of the trial, that participant was considered to have breached the study protocol. Participants who had suffered illness or had signs of inflammation around the time of screening were required to wait for four days after the illness or inflammation had passed before beginning the study for reasons relating to the blood sampling (see Outcomes section). Participants were excluded if they: had any known neurological disorder involving brain or other central function; had any renal, hepatic, cardiovascular or respiratory diseases; had any serious medical condition for which major medical interventions were anticipated during the duration of the trial; had an allergy to any of the ingredients of the intervention; were pregnant or breastfeeding; or were taking any medication with primarily central nervous system activity or any antidepressant herbal supplements including St John's Wort, 5-HTP and SAME. Any subject judged clinically to be at serious risk for suicide or violence in the opinion of the researchers was also excluded, as was any person whose immune system was known to be weakened (through self-reporting any diagnosis of an autoimmune disorder or long term corticosteroid use).

4.4 Interventions

The test product was a probiotic formula developed by Lallemand Health Solutions, Montreal, Canada, which contains freeze-dried lactic acid bacteria strains *L. helveticus* R0052 (strain number I-1722 in the French National Collection of Cultures of Microorganisms (CNCM), Institut Pasteur, Paris, France) and *B. longum* R0175 (CNCM strain number I-3470) at a dosage of at least three billion colony-forming units (3×10^9 CFU) per 1.5 g sachet. Excipients also contained in the sachet were xylitol, maltodextrin, plum flavour and malic acid. This product was the same probiotic preparation used in the study by Messaoudi et al. (2010) described in Chapter 3. The formulation was selected for the present study, as positive results were found by Messaoudi et al. (2010) in their animal models of anxiety and depression, and also in their clinical human trial on two mood-related subscales of the HSCL-90 and on HADs global scores, so the formulation shows promise as a psychobiotic agent. Bacterial enumeration of the product confirmed that the sachets contained at least 3×10^9 CFU at the beginning and end of the study. The placebo product contained only the excipients, without any bacteria, and was sensorially identical to the active product. Both products were room temperature stable and in the form of orally dispersible powder in plain sachets. The sachets are taken by pouring the contents of the sachet onto the tongue, which allows the pleasant-tasting powder to dissolve in the mouth.

4.5 Outcomes

Primary outcome measures were defined *a priori*, and can be viewed on the Australian New Zealand Clinical Trials Registry (Ref: ACTRN12613000438752).

4.5.1 Primary outcome measures. The following measures were the pre-specified primary outcome measures of the trial:

- *Montgomery-Åsberg Depression Rating Scale (MADRS; Montgomery & Åsberg, 1979)*. The MADRS is a 10-item clinician-rated diagnostic questionnaire designed to measure the severity of depressive episodes in patients with mood disorders based on a personal interview, which was

rated in person at baseline, end of the RCT phase and end of OL. The maximum possible score is 60, and the scale demonstrates good internal consistency ($\alpha=0.85$ to 0.94) and high concurrent validity (Bondolfi et al., 2010; Montgomery & Åsberg, 1979). Severity cut-offs used for the purpose of this study were as described by Müller, Himmerich, Kienzle, and Szegedi (2003): None/Recovered (0-8), Mild (9-17), Moderate (18-34) and Severe (≥ 35). A response was defined as a $\geq 50\%$ reduction in score from baseline and a score of < 8 was taken to indicate remission, as described by Riedel et al. (2010).

- *Improved Clinical Global Impressions scale (iCGI; Kadouri, Corruble, & Falissard, 2007)*. The iCGI comprises two companion one-item clinician-rated measures which provide a brief assessment of the clinical severity and treatment response of the participant. The Severity scale (iCGI-S) is a 7-point scale on which the clinician rates the severity of the participant's illness at the time of rating. The Improvement scale (iCGI-I) is a 13-point scale that requires the clinician to rate how much the patient's illness has improved or worsened compared to a baseline. The iCGI was developed from the original CGI scale (Guy, 1976) specifically for use in depression, and has been shown to have a high level of external validity ($r=0.74$) (Kadouri et al., 2007). This measure was rated in person at baseline, end of RCT phase and end of OL.

- *Quick Inventory of Depressive Symptomatology: 16-item self-rated version (QIDS; Rush et al., 2003)*. The QIDS is a 16-item questionnaire designed to assess the severity of depressive symptoms in all criterion symptom domains designated by the Diagnostic and Statistical Manual of Mental Disorders - 4th edition (DSM-IV; American Psychiatric Association, 2000) to diagnose a major depressive episode. Scores range from 0 to a maximum of 27. The measure has been found to have high internal consistency ($\alpha=0.86$) and high concurrent validity with other self-report mood measures (Trivedi et al., 2004). Severity cut-offs are None (< 6), Mild (6-10), Moderate (11-15), Severe (16-20) and Very Severe (≥ 21). The lowest score in the Moderate range (11) was used as the minimum requirement for inclusion in the study at screening, with no upper limit. The measure

was self-rated by participants at screening, baseline and then fortnightly throughout the trial, as well as at the point of 6 month follow-up.

4.5.2 Secondary outcome measures. The following measures were the pre-specified secondary outcome measures of the trial:

- *Global Assessment of Functioning (GAF; Caldecott-Hazard & Hall, 1995).* The GAF is a clinician-rated measure on which the clinician rates the social, occupational, and psychological functioning of adults using a numeric scale (1 to 100), with a higher score indicative of better overall functioning. The scale was rated in person at baseline, end of the RCT phase and end of OL. The scale has been shown to have high concurrent validity with other psychological assessment tests ($r=0.80$)(Hall, 1995).

- *Depression Anxiety and Stress Scale 42-item (DASS; Lovibond & Lovibond, 1995).* The DASS-42 is a self-report questionnaire designed to assess the current severity of an individual's symptoms relating to depression, anxiety and stress. The DASS was used for screening, with the moderate severity cut-off for the depression subscale (14) used as the minimum requirement for inclusion, and no upper limit. The scale shows good internal consistency ($\alpha=0.92$ to 0.97), and high levels of concurrent validity with relevant psychological outcome measures (Antony, Bieling, Cox, Enns, & Swinson, 1998). This measure was rated at screening, baseline, end of RCT phase and end of OL, as well as at the point of 6 month follow-up.

- *Dysfunctional Attitude Scale (Form A) (DAS-A; Weissman & Beck, 1978).* The DAS-A is a self-report measure designed to assess the intensity of dysfunctional attitudes, which, according to Beck's cognitive theory of depression, reflect the content of relatively stable maladaptive schemas in depression (Beck, 1972). The DAS-A lists 40 attitudes or beliefs that are scored by the participant on a 7-point scale from 1 (totally agree) to 7 (totally disagree) and was rated at baseline and then fortnightly throughout the study, as well as at the point of 6 month follow-up. Ten items are reverse scored; a higher score indicates more dysfunctional attitudes. The DAS-A is reported to have very

good internal consistency ($\alpha=.84$ to $.92$) and excellent concurrent validity, significantly correlating with several measures of depression (Weissman & Beck, 1978).

- *Automatic Thoughts Questionnaire (ATQ; Hollon & Kendall, 1980)*. The ATQ is a 30-item self-report measure designed to assess the frequency of occurrence of automatic negative thoughts, or negative self-statements. The participant is asked to rate the frequency of occurrence of 30 statements of negative thoughts (e.g., “I feel like I’m no good”) on a 5-point scale from 1 (not at all) to 5 (all the time), with a higher score indicating more frequent negative thinking. This measure was rated at baseline and then fortnightly throughout the study, as well as at the point of 6 month follow-up. The ATQ has excellent internal consistency ($\alpha=0.97$) and good concurrent validity, correlating with 2 measures of depression: the Beck Depression Inventory and the MMPI Depression scale (Hollon & Kendall, 1980).

- *Irritable Bowel Syndrome Symptom Severity Scale (IBS-SSS; Francis, Morris, & Whorwell, 1997)*. The IBS-SSS contains five 100-point sliding scales which assess the severity of abdominal pain, the frequency of abdominal pain, the severity of abdominal distention, dissatisfaction with bowel habits, and interference with quality of life. Scores may range from 0 to a theoretical maximum of 500, with a higher score indicating greater symptom severity. The score boundaries are as follows: <75, remission or no IBS symptoms; 75-175, mild IBS symptoms; 175-300, moderate IBS symptoms; >300 severe IBS symptoms. Bijkerk et al. (2003) reviewed the most commonly used IBS measures and concluded that the IBS-SSS shows good psychometric and methodological qualities and is appropriate for use. This measure was rated at baseline, end of the RCT phase and end of OL, as well as at the point of 6 month follow-up.

- *Assessment of blood-based biomarkers*. Blood samples were collected by a trained phlebotomist at the baseline and week 8 appointments (end of the RCT phase) to measure levels of high sensitivity C-reactive protein (hsCRP), interleukin (IL)-1 β , IL-6, tumor necrosis factor-alpha (TNF- α), vitamin D and brain derived neurotrophic factor (BDNF). Testing was completed externally at Canterbury Health Laboratories. HsCRP was measured using latex-enhanced nephelometry. Serum

IL-1 β , IL-6, TNF- α and BDNF were measured using commercially available enzymelinked immunoabsorbent assays (ELISA; R&D Systems). Plasma 25 Hydroxycholecalciferol vitamin D was measured using high-performance liquid chromatography (HPLC)-tandem mass spectrometry. For the hsCRP assays, specimens were collected from participants who had not recently, within the last 4 days, suffered illness, or had overt signs of inflammation. HsCRP and vitamin D testing was completed immediately after specimen collection. For the BDNF, IL-1 β , IL-6 and TNF- α assays, blood was centrifuged and serum aliquoted immediately after specimen collection, and was stored at -80°C until batch testing could be completed.

4.5.3 Monitoring. To monitor adverse events, participants were asked at baseline and on each fortnightly questionnaire to rate whether they had experienced any of a checklist of common side effects associated with taking medications (e.g., headaches, rash, nausea). Participants were also asked to estimate their intake of substances including caffeine, nicotine, alcohol and street drugs in a free answer format every fortnight throughout the study.

4.5.4 Demographic information. At screening, participants were asked for their date of birth, ethnicity, household income (measured on a five-point scale from 1 “less than \$20,000” to 5 “more than \$80,000”), occupation, and highest educational qualification (assessed on a scale from 1 “no school certificate” to 7 “university degree” and an option for “other”). Occupation data (or highest educational qualification and age if unemployed) were used to calculate a score of socio-economic status (SES) based on the New Zealand socio-economic index 2006 (NZSEI-06; Milne et al., 2013).

4.5.5 History of low mood. History of low mood, including total duration of low mood in years, duration of current episode, number of episodes, and any other interventions previously trialled for low mood, were recorded in person at the baseline interview using retrospective self-report.

4.6 Randomisation and Blinding

Randomisation was completed by a research assistant not otherwise involved in the study: four tables of 80 numbers (a mix of ones and twos representing probiotic and placebo) were generated using <http://www.randomization.com>, which provides web-based deterministic randomisation software. The online software used a pseudo-random number generator to randomise the tables (1:1) in blocks of 10. One list was blindly selected and sachets (active probiotic and placebo) were pre-packaged according to the randomisation code. This meant that participants, clinicians and raters remained blind to the allocated study group of each participant until after the database was locked and data analysis was completed.

4.7 Sample Size

Due to the lack of any pilot data, the target sample size of 40 per group was largely selected based on the size of similar studies in the literature. Randomised controlled trials in this area using probiotics and assessing psychological outcomes typically have a sample size of 50 to 75 (e.g., Dickerson et al., 2014; Diop, Guillou, & Durand, 2008; Messaoudi et al., 2010). As mentioned above, Messaoudi et al. (2010) used the same probiotic preparation as the current study and found a moderate effect of probiotics on mood-related subscales of the Hopkins Symptom Checklist in their sample of 55 healthy people from the general population. Recruiting 80 participants in total would be sufficient to show a moderate effect size (>0.60) for the comparison of active treatment and placebo as statistically significant (two-tail $\alpha=0.05$) with 80% power.

4.8 Study Procedure

The study was split into two phases, one randomised, placebo-controlled phase (RCT phase) and one open label (OL) phase. The participants were then followed up six months after completion of the OL phase. The procedure of all phases is described below.

4.8.1 Randomised phase. Eligible participants were contacted by email and offered an appointment at the University of Canterbury. Meetings were conducted by a clinical psychologist, trainee clinical psychologist, or research assistant trained in administering all clinician-rated outcome measures. At the initial appointment, the information sheet was reviewed and informed consent was obtained. During informed consent, participants were asked to minimise their usage of nicotine, alcohol, caffeine and street drugs, and to cease their usage of any type of nutritional or herbal supplement believed to enhance the immune system (e.g. vitamin C, vitamin E, Echinacea), for the duration of the study.

After informed consent was obtained, clinician-rated measures were rated on the basis of a personal interview, during which the researcher also collected information about the participants' history of low mood. Blood samples were collected and the participant completed baseline self-report questionnaires and monitoring information on an online survey at the initial appointment.

Participants were then allocated to the next sequentially numbered bag containing 58 sachets of powder. Although the study products were room temperature stable, participants were instructed to keep the sachets in the refrigerator to avoid possible damage to the bacteria. Participants were asked to take one sachet at the same time each day, preferably with a meal. Participants were provided with a study diary in which they were asked to record any adverse events or missed doses. During the eight week intervention period, participants were monitored via a fortnightly online questionnaire completed from home which included the self-report mood measures outlined above and monitoring and compliance questions.

Participants returned for the final assessment meeting at the end of the eight week intervention period and were asked to bring any unused sachets with them. The same procedure was followed during this meeting as during the initial meeting: the clinician-rated measures were rated, blood samples were taken, and participants completed end of study self-report questionnaires and monitoring questions on a computer-based survey at the appointment.

4.8.2 Open label phase. At the completion of the eight week RCT phase, all participants were offered the active study product for a further eight weeks in an open label manner, to ensure that all participants received the treatment while maintaining the double-blind. The eight week OL extension followed the same format as the initial RCT phase, with participants taking one probiotic sachet daily and completing fortnightly online questionnaires online. A final meeting was then conducted at week 16 (end of OL) where the clinician-rated measures were completed and participants completed end of study self-report questionnaires and monitoring questions on an online survey at the appointment. All participants were provided with a \$10 petrol voucher at all meetings at the university to reimburse travel costs.

4.8.3 Six month follow-up. All participants were contacted and asked to complete an online follow-up survey approximately 6 months post-trial, regardless of compliance with the study protocol. This survey contained all self-report measures used in the trial as well as a study review which included questions on participant experience and acceptability of treatment.

4.9 Statistical Analysis

The three primary outcome measures defined *a priori* were the MADRS, the iCGI and the QIDS. IBM SPSS Statistics version 20 was used for all statistical analyses. Data were checked for normal distribution and were \log_{10} transformed if non-normal distributions were found. All significance tests were two-tailed and p-values less than 0.05 were considered statistically significant.

4.9.1 Baseline biomarker analysis. Levels of baseline levels of blood-based biomarkers were tested to determine whether the inflammation levels and vitamin D levels in the current sample were consistent with the existing literature on depression (i.e., whether elevated inflammatory markers and low levels of vitamin D were observed as expected). Biomarker variables found to be not normally distributed were \log_{10} transformed due to the skewed nature of the data. Pearson's *r* correlations and independent t-tests were employed to test whether there were any

associations between baseline biomarker levels and age, gender, factors relating to history of low mood or severity on psychological or IBS outcome measures. ANOVA was employed to test for mean differences in baseline levels of biomarkers between groups, based on categorical baseline variables with more than two levels (e.g., season at time of recruitment). Post-hoc tests using the Bonferroni correction were used to check pairwise comparisons while adjusting for the increase in the rate of Type 1 error. Participants who did not provide baseline blood data were excluded from the baseline biomarker comparisons.

4.9.2 Randomised phase. The changes from baseline to the end of treatment were compared using ANCOVA with the baseline level as the covariate. Change measures (iCGI-I ratings) assessed at the end of treatment were compared using one-way ANOVA. The treatment effects were summarised by mean differences and 95% confidence intervals derived from the ANCOVA/ANOVA models. Adverse events occurring in $\geq 5\%$ of the sample were compared between groups using Fisher's exact tests. Analyses were completed on an intention-to-treat basis: each patient who provided informed consent and was randomised to either the active treatment or placebo group were included in their allocated group for analysis, regardless of compliance with treatment. The 'last-observation-carried-forward' technique was used to impute missing values. Secondary analyses were undertaken on all psychological outcome variables using the per protocol analysis set, defined as those randomised participants who took their allocated product for the full 8 week study period with at least 80% compliance, and who did not violate any of the exclusion criteria outlined above during the study period. Blood-based biomarker variables were \log_{10} transformed and changes from baseline to the end of treatment were compared using ANCOVA with the baseline level as the covariate. These analyses were completed using the per protocol data set of participants who had provided blood samples at baseline and endpoint. Factorial repeated-measures ANCOVA, with the baseline level as a covariate, were employed to assess changes over time on fortnightly measures between the treatment groups.

To determine whether the intervention was more effective for some patients than for others (i.e., whether treatment response was moderated by baseline blood-based biomarkers) exploratory hierarchical multiple regression analyses were employed. All blood-based biomarkers were tested as possible moderators. In each regression analysis, the baseline value of the dependent variable was entered in Step 1, followed by experimental group (active vs. placebo) and moderator variable in Step 2, and the interaction term between group and moderator was added in Step 3 as the critical term identifying the presence of a moderating effect. To avoid high inter-correlations between predictors and the interaction term, moderator variables were centred and group was coded as -1 (active treatment group) and 1 (placebo group) (Aiken & West, 1991). Any variables found to be confounded with biomarker variables were entered as control variables in step 1. Interaction effects were further examined by calculating regression slopes one standard deviation above and below the mean of the moderator, and simple slope analyses were conducted to examine whether the regression slopes differed significantly from zero.

4.9.3 Open label phase. Baseline characteristics of those who completed the OL phase were compared to those who did not complete the OL phase using a series of chi-square tests for categorical variables and independent t-tests for continuous variables, to ascertain whether any factor was associated with increased likelihood of discontinuation. The per protocol data set—those who successfully completed the OL phase protocol with at least 80% compliance and without violating any of the exclusion criteria outlined above during the study period—was used for all further analyses of effectiveness for the OL phase. The changes from week 8 (end of RCT) to end of OL phase were compared using paired samples t-tests. Responders from both phases were visually compared to ascertain whether there was any disparity between the response rates and any treatment effect observed in the RCT phase. The allocation groups from the RCT phase were compared on outcome measures using ANCOVA, with the baseline level as the covariate, to investigate whether a longer time of exposure to treatment was associated with different outcomes.

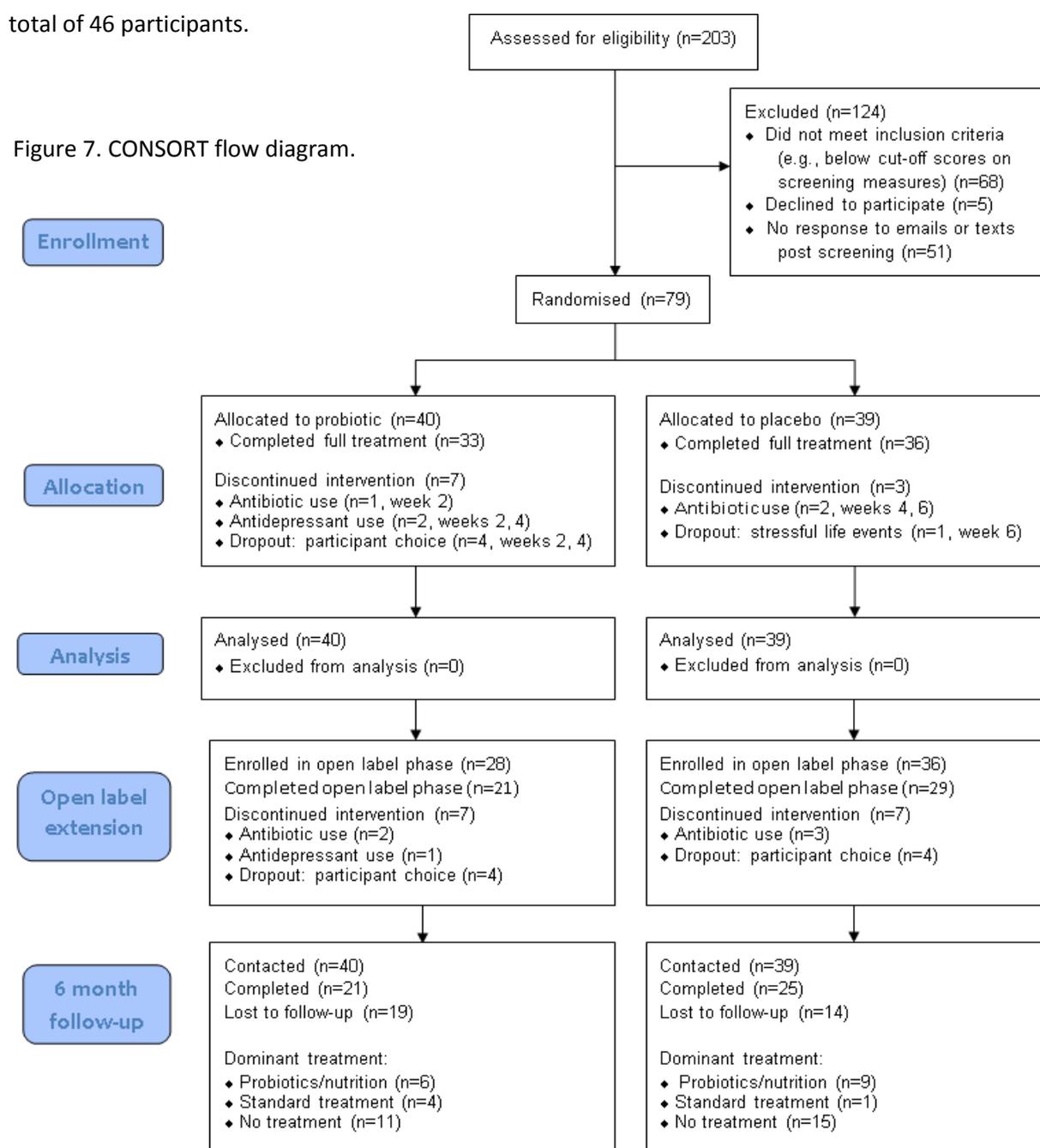
4.9.4 Six month follow-up. Those who completed the 6 month follow-up survey were compared to those who did not complete the survey on baseline characteristics using a series of chi-square tests (or Fisher's exact tests for low values) and independent t-tests, to ascertain whether any factor was associated with increased likelihood of nonresponse to the follow-up survey. Those who completed the follow-up survey made up the per protocol data set which was used for all further analyses. The changes from baseline to follow-up and the end of OL treatment to follow-up were compared using paired samples t-tests. The sample was then divided into three groups based on their self-reported dominant treatment at point of 6 month follow-up (standard treatment, probiotics/other nutritional supplement, or no treatment). To determine dominant treatment, participants were asked a series of questions about whether they were currently taking probiotics or any other supplement for their low mood, whether they were taking antidepressants, and whether they were currently receiving psychotherapy for low mood. Follow-up questions determined the duration and schedule of each treatment. In order to be considered a dominant treatment, the participant must have been taking/receiving the treatment regularly (i.e. every day for supplements/medications and on a regular schedule such as weekly or fortnightly for psychotherapy) for at least one month prior to the point of 6 month follow-up. Baseline factors and scores on outcome measures at end of OL were compared between dominant treatment groups using independent t-tests and chi-square tests. Changes from end of OL to the 6 month follow-up were compared among the three groups using ANCOVA, with the pre-change level (end OL) as a covariate. Mixed ANOVA analyses were employed and plotted to visually compare change over time among the three dominant treatment groups.

Chapter 5: Results

5.1 Overall Study Population

From 203 referrals between May 2013 and May 2014, 79 adults (age ≥ 16 years) with at least moderate symptoms of low mood completed informed consent and were assigned to the active treatment group (n=40) or placebo group (n=39) for the RCT phase (see Figure 7 for CONSORT diagram). A total of 64 participants continued to the OL phase. The 6 month follow-up questionnaire was sent to all participants regardless of compliance with the study protocol and was completed by a total of 46 participants.

Figure 7. CONSORT flow diagram.



5.2 Baseline Biomarker Results

Baseline blood data were available for 77 participants. Non-normally distributed biomarker data were \log_{10} transformed (see Appendix 2 for histograms); biomarkers transformed were hsCRP, IL-1 β , IL-6, TNF- α and BDNF. See Table 4 for the baseline biomarker levels in participants who provided baseline blood samples, presented as geometric mean and range for transformed biomarkers. In total, 58 (75%) of the participants had at least one marker of inflammation (hsCRP, IL-1 β , IL-6 or TNF- α) elevated outside the reference range at baseline (reference ranges shown in Table 4). Mean vitamin D was approaching the deficient level.

Table 4. Baseline biomarker levels for all participants for whom baseline blood data were available, reported as geometric mean and range for biomarkers showing non-normal distribution, and mean and standard deviation for normally distributed biomarkers (n=77).

Sample with baseline blood data (n=77)				
Biomarker (\log_{10} transformed)	GM	Range	Deficient (below reference range): n (%)	Elevated (above reference range): n (%)
hsCRP (mg/L): reference range: 0.00-3.00	1.64	0.15-37.30	NA	23 (30)
IL-1 β (pg/mL): reference range: 0-0.201	0.23	0.01-8.00	NA	49 (64)
IL-6 (pg/mL): reference range: 0.45-9.96	1.92	0.43-10.00	2 (3)	3 (4)
TNF- α (pg/mL): reference range: 0.55-2.82	0.99	0.03-3.62	14 (18)	4 (5)
BDNF (pg/mL): reference range: 6186.0-42580.0	26188.4	5228.0-48188.0	1 (1)	2 (3)
Biomarker	M	SD	Deficient (below reference range): n (%)	Elevated (above reference range): n (%)
Vitamin D (nmol/L) reference range: 50.0-150.0	58.9	24.8	31 (40)	0 (0)

Abbreviations: GM=geometric mean; M=mean; SD=standard deviation; hsCRP=high sensitivity C-reactive protein; IL=interleukin; TNF=tumor necrosis factor; BDNF=brain derived neurotrophic factor.

In the group who provided baseline blood data (n=77), correlations (Pearson's r) were found among several inflammatory biomarkers at baseline (Table 5). HsCRP correlated significantly with IL-1 β , IL-6 and TNF- α . IL-6 correlated significantly with IL-1 β and TNF- α .

Table 5. Pearson’s *r* correlations between biomarkers at baseline in all participants who provided baseline blood data (*n*=77).

Biomarker	hsCRP	IL-1β	IL-6	TNF-α	BDNF	Vitamin D
IL-1β	0.25*	-	0.31**	0.17	-0.15	-0.004
IL-6	0.58***	0.31**	-	0.48***	-0.04	-0.18
TNF-α	0.25*	0.17	0.48***	-	-0.04	-0.06
BDNF	-0.11	0.15	-0.04	-0.04	-	0.10
Vitamin D	-0.11	-0.004	-0.18	-0.06	0.10	-

Abbreviations: hsCRP=high sensitivity C-reactive protein; IL=interleukin; TNF=tumor necrosis factor; BDNF=brain derived neurotrophic factor; **p*<0.05; ***p*<0.01; ****p*<0.001

5.2.1 Relationship between baseline biomarker levels and baseline scores

on outcome measures. Correlations were tested among biomarker levels at baseline and baseline score on psychological outcome measures, to ascertain whether there was any relationship between severity of low mood, or gut function, and biomarker levels at baseline. Baseline vitamin D levels showed a negative correlation with baseline DAS scores, meaning that, at baseline, those with lower vitamin D levels had higher scores on the DAS (indicating more dysfunctional attitudes), *r*=-0.31, *p*<0.01. No correlation was found between the baseline level of any biomarker and any other psychological outcome variable (Table 6).

Table 6. Pearson’s *r* correlations between biomarkers at baseline and scores on outcome measures at baseline in all participants who provided baseline blood data (*n*=77).

Biomarker	MADRS	iCGI-S	QIDS	GAF	DASS	DASS-D	DASS-A	DASS-S	DAS	ATQ	IBS-SSS
hsCRP	0.04	-0.01	0.10	0.04	0.09	0.001	0.17	0.11	0.09	0.14	0.07
IL-1β	-0.02	-0.07	0.06	0.11	0.04	0.05	0.04	0.02	-0.13	0.04	0.09
IL-6	0.14	0.003	0.12	0.13	0.01	-0.01	0.03	-0.01	-0.17	0.05	0.10
TNF-α	-0.02	0.03	-0.07	0.16	-0.11	-0.14	-0.05	-0.10	-0.16	0.04	0.09
BDNF	-0.01	-0.09	0.01	0.05	0.03	0.11	0.08	-0.03	-0.17	0.01	-0.16
Vitamin D	-0.09	0.19	-0.04	-0.04	-0.02	-0.05	-0.05	-0.07	-0.31**	-0.13	-0.13

Abbreviations: MADRS=Montgomery-Åsberg Depression Rating Scale; iCGI-S=improved Clinical Global Impression Scale (Severity scale); iCGI-I=improved Clinical Global Impression Scale (Improvement scale); QIDS=Quick Inventory of Depressive Symptomatology; GAF=Global Assessment of Functioning; DASS=Depression, Anxiety and Stress Scale; DAS=Dysfunctional Attitude Scale; ATQ=Automatic Thoughts Questionnaire; IBS-SSS=Irritable Bowel Syndrome Severity Scoring Scale; hsCRP=high sensitivity C-reactive protein; IL=interleukin; TNF=tumor necrosis factor; BDNF=brain derived neurotrophic factor; ***p*<0.01

5.2.2 Relationship between biomarker levels and other baseline factors.

Independent t-tests (based on \log_{10} scale) revealed that, of the participants who provided baseline blood data ($n=77$), those who had used antidepressants in the past ($n=45$) had significantly lower baseline levels of TNF- α at baseline (GM=0.88, range=3.40) than those who had not used antidepressants in the past (GM=1.17pg/mL, range=3.29), $t(75)=-2.03$, $p<0.05$. Women ($n=61$) had higher baseline IL-6 (GM=2.08pg/mL, range=9.57) than men (GM=1.41pg/mL, range=5.71), $t(36)=-2.23$, $p<0.05$. Levene's test indicated unequal variances ($F=7.00$, $p<0.05$), so degrees of freedom were adjusted from 75 to 36. ANOVA indicated that baseline vitamin D levels varied significantly based on season at time of recruitment, $F(3,73)=6.545$, $p<0.01$. Post-hoc tests using the Bonferroni correction revealed that those participants recruited in winter had significantly lower baseline vitamin D levels (M=44.4nmol/L, SD=23.4) than those recruited in both summer and autumn (M=74.0nmol/L, SD=22.9 and M=66.9nmol/L, SD=21.5 respectively; $p<0.01$). No difference was found when mean levels of baseline biomarkers were compared based on other baseline variables, including total length of low mood, length of current episode, chronic depression (yes/ no; ≥ 2 years continuing symptoms), recurrent depression (≥ 2 depressive episodes), past diagnosis of MDD, and presence or severity of IBS at baseline (as measured on the IBS-SSS).

5.3 Results: Randomised Phase

5.3.1 Study population. As shown in Figure 7, seven people from the active treatment group and three people from the placebo group dropped out prior to finishing the randomised (RCT) phase of the trial (see Figure 7 for individual time points and reasons for dropout). Data from the last observation for each participant were carried forward as per the intention-to-treat protocol for the RCT phase. Overall, the two groups were well matched in clinical and demographic baseline characteristics (Table 7). However, there was a significant imbalance in history of antidepressant usage, with 70% of the active treatment group having used antidepressant medications previously,

compared with 46% of the placebo group, $\chi^2(1, n=79)=4.62, p<0.05$. No group differences were observed in other clinical and demographic baseline characteristics (all p-values >0.05).

Table 7. Baseline demographic and clinical characteristics of study participants.

Characteristic	Probiotic group (n=40)	Placebo group (n=39)
<i>Demographics</i>		
Age, years, mean (SD)	35.8 (14.0)	35.1 (14.5)
Male, n (%)	8 (20)	9 (23)
Socioeconomic status ^a , mean (SD)	45.0 (11.5)	41.6 (13.2)
Education, n (%)		
No high school certificate	3 (8)	7 (18)
Completed high school	13 (33)	10 (26)
Post-secondary (e.g. trade certificate)	15 (38)	13 (33)
University degree	9 (23)	9 (23)
Ethnic origin, n (%)		
New Zealanders of European descent	30 (75)	31 (80)
New Zealand Māori	2 (5)	1 (3)
Other	8 (20)	7 (18)
<i>Clinical</i>		
Meets criteria for MDD ^b , n (%)	33 (83)	26 (67)
Past diagnosis of any mood disorder ^c , n (%)	23 (57)	17 (44)
Baseline MADRS score, mean (SD)	28.3 (6.1)	27.0 (6.3)
Chronic depression ^d , n (%)	31 (78)	24 (62)
Anxiety disorder ^e , n (%)		
Current	20 (50)	16 (41)
Past	21 (53)	18 (46)
Alcohol/ substance misuse or dependence ^e , n (%)		
Current	4 (10)	1 (3)
Past	6 (15)	8 (21)
Any co-occurring disorder ^e , n (%)		
Current	23 (57)	18 (46)
Past	24 (60)	21 (54)
History of antidepressant use ^e , n (%)	28 (70)	18 (46)
Current therapy ^f , n (%)	3 (8)	3 (8)
IBS severity ^g , mean (SD)	150.7 (121.2)	88.0 (68.0)
Severity of IBS by category, n (%)		
None	16 (40)	19 (49)
Mild	10 (25)	15 (39)
Moderate	8 (20)	5 (13)
Severe	6 (15)	0 (0)

Abbreviations: MDD=major depressive disorder; MADRS=Montgomery-Åsberg Depression Rating Scale; IBS=irritable bowel syndrome.

- Based on the New Zealand Socio-Economic Index 2006 (NZSEI-06) (Milne et al., 2013)
- Meets DSM-5 criteria for a current major depressive episode (American Psychiatric Association, 2013a)
- Number self-reporting a past diagnosis of any mood disorder (e.g. major depressive disorder, dysthymia, bipolar disorder)
- Chronic depression defined as >2 years continuous symptoms
- Established from self-report and retrospective self-report
- Currently receiving any form of psychotherapy (self-report). NB, must be regularly for >6 months in order to meet entry criteria.
- Score on Irritable Bowel Syndrome Symptom Severity Scale at baseline (0-500)

5.3.2 Efficacy outcomes. Intent-to-treat analysis showed no significant group differences on any outcome measure (Table 8). Secondary per protocol analyses based on the per protocol sample (n=69) yielded similar results, with no primary or secondary outcomes approaching significance (Table 9).

5.3.3 Treatment response. At end-point, 15 (38%) of those in the active treatment group showed a $\geq 50\%$ change on the MADRS (responders), compared to 14 (36%) of those in the placebo group, $\chi^2(1, n=79)=0.02, p=ns$. In total, 10 (13%) participants were considered in remission, with a score of < 8 on the MADRS at week 8 (active treatment, n=4 (10%); placebo, n=6 (15%); $\chi^2(1, n=79)=0.52, p=ns$). Similarly on the iCGI improvement scale, 16 (40%) participants from the active treatment group were considered to have experienced “considerable”, “very considerable” or “ideal” improvement at end point, as opposed to 14 (36%) participants in the placebo group, $\chi^2(1, n=79)=0.14, p=ns$.

Table 8. Baseline and post 8-week data on primary and secondary outcome measures in the intent-to-treat population (n=79).

Outcome variable	Active treatment group (n=40)			Placebo group (n=39)			Difference [95% CI]	p	ES ^b d
	Baseline Mean (SD)	Post Mean (SD)	Change from baseline ^a	Baseline Mean (SD)	Post Mean (SD)	Change from baseline ^a			
Primary									
MADRS	28.3 (6.1)	19.3 (1.4)	-8.7	27.0 (6.3)	17.6 (9.5)	-9.7	-1.0 [-4.9 to 3.0]	0.62	0.11
iCGI-S	3.8 (0.5)	2.9 (0.2)	-0.9	3.7 (0.6)	2.7 (1.0)	-0.9	-0.1 [-0.5 to 0.4]	0.75	0.07
iCGI-I ^c		2.4 (0.3)	2.4		2.7 (2.0)	2.7	-0.3 [-1.2 to 0.6]	0.52	0.16
QIDS	15.2 (3.9)	8.5 (8.7)	-6.3	13.2 (3.8)	8.0 (4.8)	-5.6	0.7 [-1.5 to 2.8]	0.53	0.15
Secondary									
GAF	61.2 (5.6)	67.5 (1.3)	6.0	63.1 (5.4)	69.3 (7.1)	6.6	0.7 [-2.6 to 3.9]	0.68	0.1
DASS	64.3 (23.9)	37.9 (4.6)	-23.4	50.0 (20.6)	29.5 (20.8)	-23.6	-0.2 [-10.6 to 10.1]	0.97	0.01
<i>Depression</i>	24.2 (9.09)	13.2 (1.8)	-9.9	19.0 (10.5)	10.3 (8.8)	-9.9	-0.03 [-4.1 to 4.1]	0.99	0.003
<i>Anxiety</i>	15.2 (10.4)	8.9 (1.5)	-5.2	10.6 (6.6)	6.0 (6.0)	-5.7	-0.4 [-3.5 to 2.6]	0.78	0.07
<i>Stress</i>	25.0 (9.2)	15.9 (1.6)	-8.1	20.4 (8.4)	13.2 (8.3)	-8.3	-0.2 [-4.0 to 3.6]	0.92	0.02
DAS	162.5 (41.0)	144.9 (39.0)	-17.1	154.3 (40.4)	140.0 (47.9)	-14.9	-2.2 [-13.9 to 10.0]	0.75	0.09
ATQ	98.1 (28.2)	65.5 (32.7)	-29.2	82.7 (29.5)	59.8 (25.0)	-26.4	-2.8 [-9.4 to 13.8]	0.62	0.11
IBS-SSS	150.7 (121.2)	92.1 (99.3)	-43.8	88.0 (68.0)	62.7 (51.0)	-40.4	3.4 [-25.2 to 31.9]	0.82	0.05

Abbreviations: ES=Effect Size; MADRS=Montgomery-Åsberg Depression Rating Scale; iCGI-S=improved Clinical Global Impression Scale (Severity scale); iCGI-I=improved Clinical Global Impression Scale (Improvement scale); QIDS=Quick Inventory of Depressive Symptomatology; GAF=Global Assessment of Functioning; DASS=Depression, Anxiety and Stress Scale; DAS=Dysfunctional Attitude Scale; ATQ=Automatic Thoughts Questionnaire; IBS-SSS=Irritable Bowel Syndrome Severity Scoring Scale.

a. Adjusted for baseline.

b. Cohen's *d* effect size, measured as the mean difference in change divided by the pooled standard deviation of the change, based on values adjusted for baseline

c. Assesses change only, therefore not measured at baseline

Table 9. Baseline and post 8-week data on primary and secondary outcome measures in the per protocol population (n=69).

Outcome variable	Active treatment group (n=33)			Placebo group (n=36)			Difference [95% CI]	p	ES ^b d
	Baseline Mean (SD)	Post Mean (SD)	Change from baseline ^a	Baseline Mean (SD)	Post Mean (SD)	Change from baseline ^a			
Primary									
MADRS	28.0 (6.1)	17.0 (7.7)	-10.6	26.9 (6.3)	16.7 (9.2)	-10.5	1.0 [-3.9 to 4.1]	0.96	0.01
iCGI-S	3.8 (0.5)	2.7 (1.1)	-1.1	3.6 (0.6)	2.6 (1.0)	-1.0	0.03 [-0.4 to 0.5]	0.90	0.03
iCGI-I ^c		2.9 (2.0)	2.9		2.9 (1.9)	2.9	-0.02 [-1.0 to 0.9]	0.96	0.01
QIDS	14.5 (3.6)	7.3 (4.6)	-6.8	13.1 (3.9)	7.8 (4.8)	-5.7	1.1 [-1.1 to 3.3]	0.32	0.25
Secondary									
GAF	61.2 (8.3)	68.9 (8.3)	7.2	63.1 (5.6)	69.8 (7.1)	7.2	-0.07 [-3.5 to 3.4]	0.97	0.01
DASS	60.4 (21.7)	28.3 (18.8)	-27.7	49.1 (19.8)	26.8 (17.8)	-26.3	1.4 [-7.4 to 10.2]	0.75	0.08
<i>Depression</i>	22.8 (8.1)	9.4 (7.7)	-11.8	18.6 (10.4)	9.1 (7.5)	-10.9	1.0 [-2.5 to 4.5]	0.58	0.14
<i>Anxiety</i>	13.2 (9.7)	5.6 (5.9)	-6.5	10.4 (6.5)	5.4 (5.5)	-6.0	0.6 [-2.0 to 3.1]	0.65	0.11
<i>Stress</i>	24.4 (9.2)	13.3 (8.9)	-9.7	20.1 (8.3)	12.3 (7.5)	-9.0	0.7 [-3.0 to 4.4]	0.69	0.10
DAS	156.0 (38.8)	137.5 (33.2)	-18.4	154.0 (41.6)	137.5 (48.4)	-16.6	1.8 [-10.0 to 13.6]	0.76	0.07
ATQ	92.3 (26.9)	58.8 (26.1)	-30.6	81.8 (29.9)	59.8 (25.7)	-24.8	5.8 [-5.2 to 16.8]	0.30	0.26
IBS-SSS	157.0 (121.9)	86.0 (94.8)	-51.2	85.6 (70.2)	58.3 (50.3)	-45.5	5.6 [-24.9 to 36.2]	0.71	0.09

Abbreviations: ES=Effect size; MADRS=Montgomery-Åsberg Depression Rating Scale; iCGI-S=improved Clinical Global Impression Scale (Severity scale); iCGI-I=improved Clinical Global Impression Scale (Improvement scale); QIDS=Quick Inventory of Depressive Symptomatology; GAF=Global Assessment of Functioning; DASS=Depression, Anxiety and Stress Scale; DAS=Dysfunctional Attitude Scale; ATQ=Automatic Thoughts Questionnaire; IBS-SSS=Irritable Bowel Syndrome Severity Scoring Scale.

a. Adjusted for baseline.

b. Cohen's *d* effect size, measured as the mean difference in change divided by the pooled standard deviation of the change based on values adjusted for baseline

c. Assesses change only, therefore not measured at baseline

5.3.4 Biomarker outcomes. There were no significant differences in biomarker levels between the active treatment and placebo groups at

baseline. Changes in serum levels of biomarkers over time were not significantly different between the active treatment and placebo groups (Table 10).

Table 10. Baseline and post 8-week biomarker levels in participants for whom full blood data were available (n=65).

Biomarker (log₁₀ transformed)	Probiotic group (n=29)			Placebo (n=36)			Difference [95% CI]	<i>p</i>	ES ^b <i>d</i>
	Baseline GM (range)	Post GM (range)	Change from BL ^a	Baseline GM (range)	Post GM (range)	Change from BL ^a			
hsCRP (mg/L)	1.49 (37.15)	1.89 (24.14)	0.10	1.68 (21.05)	1.93 (33.34)	0.06	-0.04 [-0.22 to 0.14]	0.65	0.12
IL-1β (pg/mL)	0.24 (1.71)	0.27 (1.35)	0.07	0.21 (0.92)	0.28 (4.17)	0.27	0.19 [-0.17 to 0.55]	0.29	0.27
IL-6 (pg/mL)	1.57 (7.70)	1.40 (5.65)	-0.75	1.92 (9.51)	2.06 (9.63)	-0.82	-0.07 [-0.36 to 0.23]	0.64	0.12
TNF-α (pg/mL)	0.87 (3.40)	0.79 (2.64)	0.04	1.08 (3.30)	1.00 (5.06)	-0.04	0.01 [-0.15 to 0.15]	0.99	0.003
BDNF (pg/mL)	25599.7 (42960.0)	29164.5 (24968.6)	0.06	24968.6 (30910.0)	24013.3 (42450.0)	-0.02	-0.1 [-0.2 to 0.01]	0.07	0.46
	Baseline Mean (SD)	Post Mean (SD)	Change from BL ^c	Baseline Mean (SD)	Post Mean (SD)	Change from BL ^c	Difference [95% CI]	<i>p</i>	ES ^d <i>d</i>
Vitamin D (nmol/L)	57.6 (22.1)	57.1 (21.8)	-1.2	60.4 (24.3)	57.1 (23.0)	-2.9	-1.7 [-10.2 to 6.8]	0.69	0.10

Abbreviations: GM=geometric mean; BL=baseline; ES=Effect size; hsCRP=high sensitivity C-reactive protein; IL=interleukin; TNF=tumor necrosis factor; BDNF=brain derived neurotrophic factor. hsCRP (mg/L): reference range: 0.00-3.00

- Based on log₁₀ scale and adjusted for baseline
- Cohen's *d* effect size, measured as the mean difference in change divided by the pooled standard deviation of the change, based on values adjusted for baseline and log₁₀ transformed
- Adjusted for baseline
- Cohen's *d* effect size, measured as the mean difference in change divided by the pooled standard deviation of the change, based on values adjusted for baseline

5.3.5 Change over time. Change over time was assessed using factorial repeated-measures ANOVA for all measures rated fortnightly (the QIDS, the DAS and the ATQ). Graphs were plotted showing the change over time between groups on all measures that were measured fortnightly (Figures 8-10).

For the QIDS, the interaction effect between time and group was not significant, $F(4,74)=1.69, p=ns$ (Figure 8).

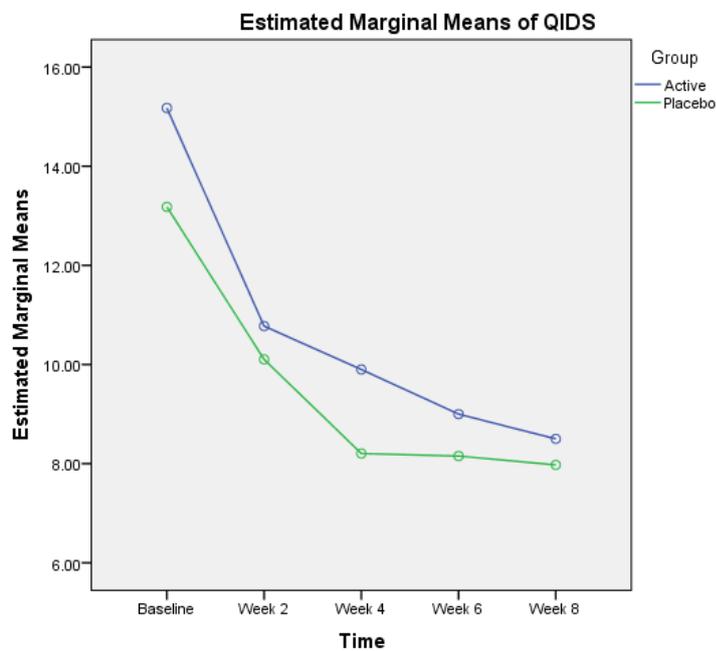


Figure 8. Change over time on the Quick Inventory of Depressive Symptomatology (QIDS) in both groups.

Similarly, on the DAS, the interaction effect between time and group was not significant, $F(4,74)=0.87, p=ns$ (Figure 9).

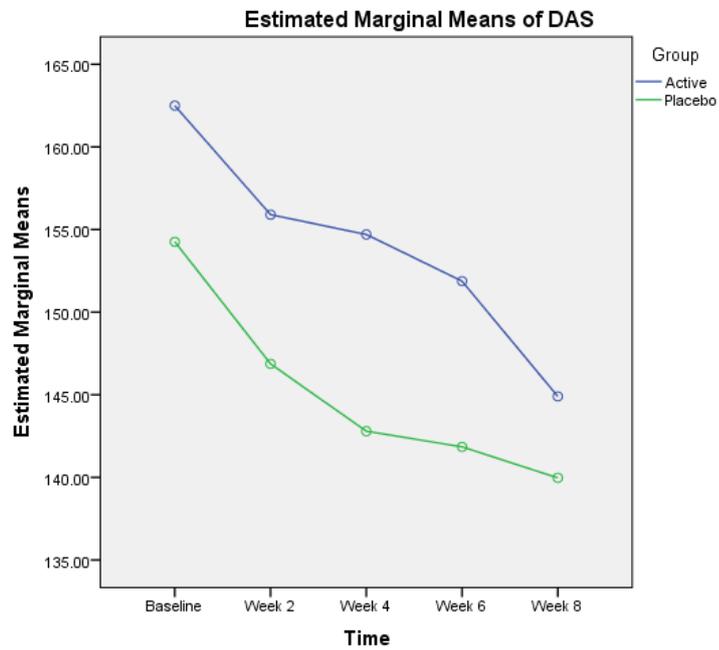


Figure 9. Change over time on the Dysfunctional Attitude Scale (DAS) in both groups.

On the ATQ, the interaction effect between time and group was also not significant, $F(4,74)=1.05, p=ns$ (Figure 10).

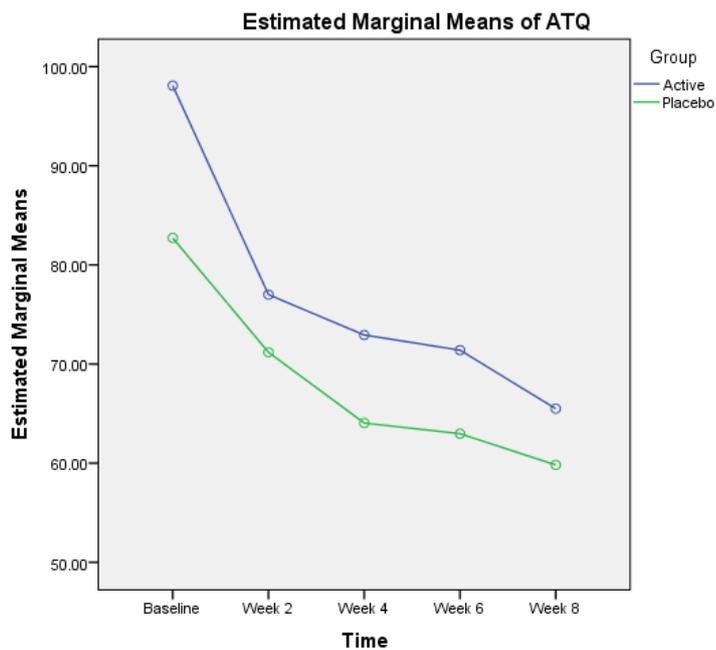


Figure 10. Change over time on the Automatic Thoughts Questionnaire (ATQ) in both groups.

These figures show that the scores of both groups improve very similarly over time. Combined with the relatively small difference in points between the groups at any time and the small, non-significant F-values observed, these graphs do not appear present compelling evidence for a differential effect of treatment over time.

5.3.6 Safety and adherence. No adverse events were observed in the active treatment group that were not also observed in the placebo groups. There were significantly more occurrences of two adverse events in the placebo group (Fisher’s exact tests: dry mouth, $p<0.05$; sleep disruption, $p<0.05$). Given that all ingredients contained in the placebo are also contained in the active treatment it is unlikely that these events were related to the study protocol. The rates of all other adverse events did not differ significantly between the active treatment and placebo groups (Table 11). There were more instances of gastrointestinal disturbances (an expected side effect of the treatment) in the active treatment group, but this did not reach statistical significance ($p=0.15$).

Table 11. Treatment-emergent adverse events reported by at least 5% of participants during the trial, by treatment group.

Event	Probiotic group (n=40)	Placebo group (n=39)	<i>p</i>
Constipation	7	11	0.29
Change in appetite	7	10	0.42
Nausea	6	7	0.77
Loss of libido	5	6	0.76
Weight gain	4	7	0.35
Dry mouth	2	8	0.048
Nightmares	6	4	0.74
Abdominal pain	5	4	1.00
Anxiety	5	4	1.00
Urinary retention	5	4	1.00
Gastrointestinal disturbances	7	2	0.15
Headache	4	5	0.74
Agitation	4	3	1.00
Skin rash	4	2	0.68
Blurred vision	2	3	0.68
Inability to achieve orgasm	3	2	1.00
Sleep disruption	0	5	0.03
Sedation/lethargy	1	3	0.36

There were three serious adverse events over the course of the trial. All three serious events were suicide attempts by one participant from the placebo group. There were no serious adverse events in the active treatment group.

Overall adherence to the study protocol was good, with 97% of required doses taken as measured by post-study sachet counts. There was no significant difference in compliance rates between groups (number who took a minimum of 95% of required doses), $\chi^2(1, n=79)=0.004, p=ns$.

5.3.7 Other variables. Over the study period, there were no group differences in rates of alcohol misusers (defined as more than two standard drinks per day for women and three standard drinks per day for men; active treatment group, $n=4$ (10%); placebo group, $n=2$ (5%); $p=ns$), smokers (active treatment group, $n=8$ (20%); placebo group, $n=10$ (35%); $p=ns$) or cannabis users (active treatment group, $n=6$ (15%); placebo group, $n=3$ (10%); $p=ns$).

5.3.8 Exploratory analyses. Despite there being no difference in response rates between the active treatment and placebo groups, some exploratory analyses were performed to attempt to provide some insights for future directions in this very new area of research.

5.3.8.1 Moderation analysis. Hierarchical multiple regression was employed to test whether baseline blood-based biomarkers moderated treatment response (i.e., whether the intervention was more effective for some patients than for others). Baseline levels of all blood-based biomarkers were tested for possible moderating effects, as the most likely moderators of treatment response given the biological models outlined in Chapters 1 and 2 and possible mechanisms of action of probiotics outlined in Chapter 3. Two participants were excluded from the analyses as they did not provide baseline blood data. Season at time of recruitment was confounded with vitamin D; therefore, season was controlled for in all analyses with Vitamin D as the moderator. No significant moderator effects were found for hsCRP, IL-1 β , IL-6, BDNF or TNF- α on any outcome measure. However, significant interaction effects were found for baseline vitamin D levels with group on several outcome measures: the iCGI-S, the QIDS and the GAF (Table 12). No significant moderator effects were found for vitamin D on the MADRS, DASS, ATQ, DAS or IBS-SSS ($p=ns$).

Table 12. Change in outcome measures (iCGI-S, QIDS and GAF) regressed on outcome measure at baseline, season at time of recruitment, group and baseline vitamin D.

DV (n=77)	iCGI-S				QIDS				GAF			
	B	SE B	β	ΔR^2	B	SE B	β	ΔR^2	B	SE B	β	ΔR^2
<i>Step 1</i>				0.19***				0.24***				0.22***
Constant	-0.31	0.79			-1.35	2.06			32.05	9.13		
DV at baseline	0.72	0.21	0.37		0.57	0.14	0.44		0.63	0.15	0.44	
Season	0.21	0.11	0.21		0.75	0.50	0.16		-1.20	0.76	-0.16	
<i>Step 2</i>				0.09				0.03				0.02
Constant	-0.14	0.82			-1.56	2.11			33.26	9.29		
DV at baseline	0.68	0.21	0.34		0.59	0.14	0.46		0.60	0.15	0.43	
Season	0.21	0.11	0.21		0.70	0.49	0.15		-1.14	0.76	-0.15	
Group	-0.04	0.11	-0.04		0.15	0.53	0.03		0.46	0.83	0.06	
Vitamin D	0.004	0.01	0.10		0.04	0.02	0.17		-0.04	0.03	-0.13	
<i>Step 3</i>				0.06*				0.04*				0.05*
Constant	0.21	0.81			-0.83	2.09			36.36	9.16		
DV at baseline	0.60	0.21	0.31		0.56	0.14	0.43		0.55	0.15	0.40	
Season	0.19	0.10	0.19		0.63	0.48	0.13		-0.96	0.75	-0.13	
Group	-0.05	0.11	-0.05		0.10	0.52	0.02		0.55	0.81	0.07	
Vitamin D	0.01	0.01	0.14		0.04	0.02	0.19		-0.05	0.03	-0.16	
Group x Vitamin D	-0.01	0.01	-0.25		-0.04	0.02	-0.20		0.07	0.03	0.23	

Abbreviations: DV=dependent variable; iCGI-S=improved Clinical Global Impressions scale, severity subscale; QIDS=Quick Inventory of Depressive Symptoms; GAF=Global Assessment of Functioning; * p <0.05; ** p <0.01; p <0.001

Regression slopes were calculated for both groups and plotted for “high” (+ 1SD) and “low” (- 1SD) values of vitamin D on each measure (Figures 11-13). The high value of vitamin D (83.73nmol/L) is within the normal reference range (50-150nmol/L) and the low value (34.07nmol/L) is in the deficient range. Simple slope analyses revealed that the association between baseline vitamin D and change on the iCGI-S was significant for the active treatment group ($t(73)=2.26$, $p<0.05$), but not for the placebo group ($t(73)=-0.76$, $p=ns$), showing that, among those participants randomised to the active treatment group, those who had high vitamin D at baseline showed more improvement than those who had low vitamin D at baseline (Figure 11).

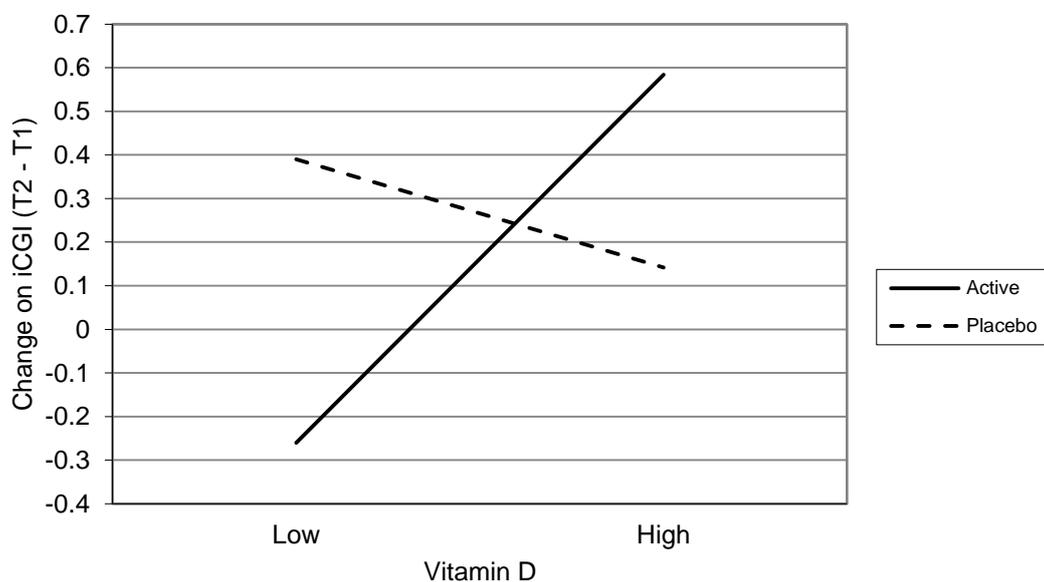


Figure 11. Interactive effect of group and baseline vitamin D levels on change in iCGI-S scores (Time 2 – Time 1).

Similarly on the QIDS (Figure 12), a significant association was observed for the active group ($t(73)=2.60$, $p<0.05$) but not for the placebo group ($t(73)=-0.07$, $p=ns$), meaning that, among those randomised to the active treatment group, those who had high vitamin D at baseline showed more improvement in mood than those who had low vitamin D at baseline.

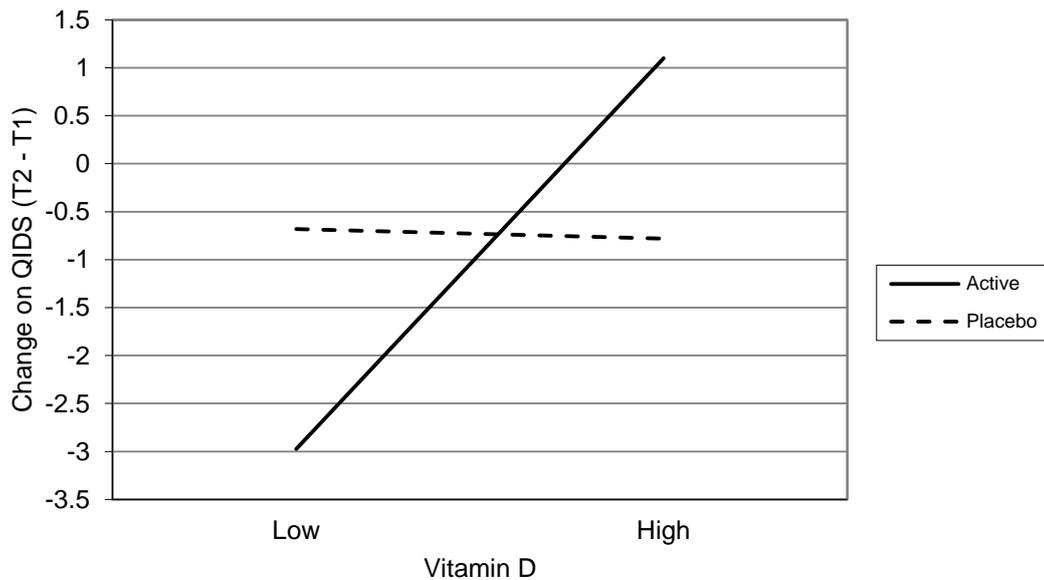


Figure 12. Interactive effect of group and baseline vitamin D levels on change in QIDS scores (Time 2 – Time 1).

Similar results were observed for the GAF (Figure 13), with a significant association for the active group ($t(73)=-2.66, p<0.05$) but not for the placebo group ($t(73)=-0.47, p=ns$). A higher score on the GAF indicates better functioning, meaning that among those randomised to the active treatment group, those who had high vitamin D at baseline showed more improvement in functioning (as measured on the GAF) than those who had low vitamin D at baseline.

It appears that participants with high levels of vitamin D at baseline are more likely to benefit from probiotic treatment than those with low vitamin D, after controlling for baseline severity on outcome measures and season at time of recruitment.

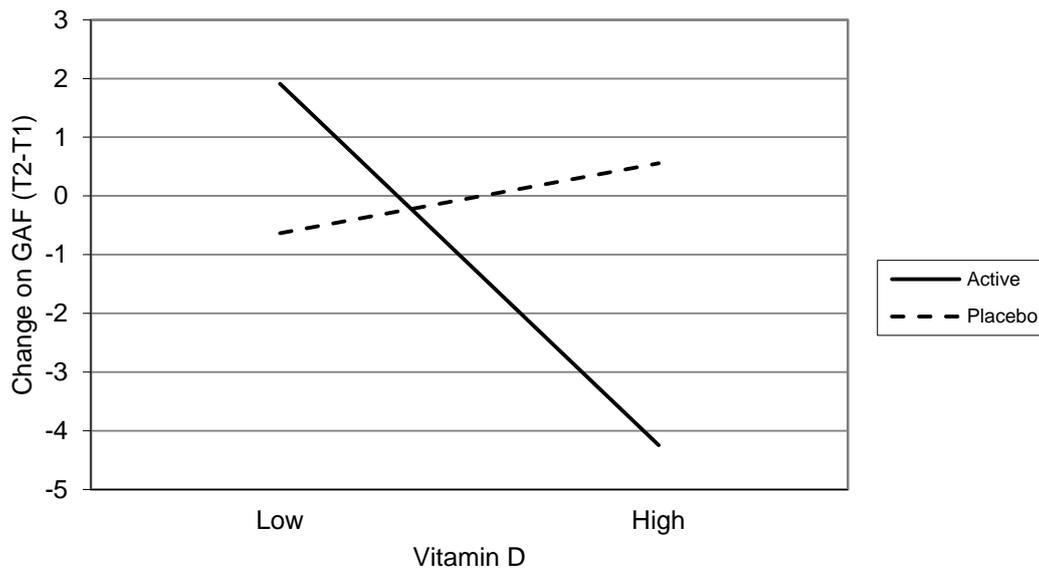


Figure 13. Interactive effect of group and baseline vitamin D levels on change in GAF scores (Time 2 - Time 1).

5.4 Results: Open Label Phase

5.4.1 Open label study population. As shown in Figure 7 (CONSORT flow diagram), 64 participants in total elected to continue to the OL intervention phase: 28 from the active treatment group in the first phase, and 36 from the placebo group. Of the 15 participants who did not progress to the OL phase, 10 participants had discontinued treatment in the first phase, and 5 chose not to continue with the OL phase. Reasons for discontinuing the study after completion of the RCT phase included: possible side effects of treatment (headaches; n=1); found the study time consuming (n=2); did not wish to give a reason (n=2). Overall, 49 participants completed the OL phase. Reasons for discontinuing the study during the OL phase included dropout (participant choice; n=9), use of antibiotics (n=5) and use of antidepressants (n=1). There were no significant differences in baseline or other characteristics between those who completed the OL phase and those who did not (Table 13). The 49 participants who completed the OL phase make up the per protocol data set; further analyses for the OL phase will be completed using these data only. Overall adherence to the study protocol was good, with 97% of required doses taken as measured by post-study sachet counts.

Table 13. Demographic and clinical features of those participants who completed and did not complete the open label phase.

Characteristic	Completed OL (n=49)	Did not complete OL (n=30)
<i>Demographics</i>		
Age, years, mean (SD)	35.9 (13.9)	34.8 (14.7)
Male, n (%)	12 (25)	5 (17)
Socioeconomic status ^a , mean (SD)	44.0 (12.2)	42.2 (12.9)
Education, n (%)		
No high school certificate	4 (8)	6 (20)
Completed high school	12 (24)	11 (37)
Post-secondary (e.g. trade certificate)	18 (37)	10 (33)
University degree	15 (31)	3 (10)
Ethnic origin, n (%)		
New Zealanders of European descent	38 (78)	23 (77)
New Zealand Māori	2 (4)	1 (3)
Other	9 (18)	6 (20)
<i>Clinical</i>		
Meets criteria for MDD ^b , n (%)	36 (74)	23 (77)
Past diagnosis of any mood disorder ^c , n (%)	24 (49)	13 (43)
Baseline MADRS score, mean (SD)	27.4 (6.1)	28.2 (6.3)
Chronic depression ^d , n (%)	32 (65)	23 (77)
Anxiety disorder ^e , n (%)		
Current	22 (45)	14 (47)
Past	24 (49)	15 (50)
Alcohol/ substance misuse or dependence ^e , n (%)		
Current	1 (2)	4 (13)
Past	7 (14)	7 (23)
Any co-occurring disorder ^e , n (%)		
Current	26 (53)	15 (50)
Past	29 (59)	16 (53)
History of antidepressant use ^e , n (%)	27 (55)	19 (63)
Current therapy ^f , n (%)	4 (8)	2 (7)
IBS severity ^g , mean (SD)	116.8 (106.4)	124.4 (98.6)
Severity of IBS by category, n (%)		
None	27 (55)	8 (27)
Mild	12 (25)	13 (43)
Moderate	6 (12)	7 (23)
Severe	4 (8)	2 (7)

Abbreviations: OL=open label; MDD=major depressive disorder; MADRS=Montgomery-Åsberg Depression Rating Scale; IBS=irritable bowel syndrome.

- a. Based on the New Zealand Socio-Economic Index 2006 (NZSEI-06) (Milne et al., 2013)
- b. Meets DSM-5 criteria for a current major depressive episode (American Psychiatric Association, 2013a)
- c. Number self-reporting a past diagnosis of any mood disorder (e.g. major depressive disorder, dysthymia, bipolar disorder)
- d. Chronic depression defined as >2 years continuous symptoms
- e. Established from self-report and retrospective self-report
- f. Currently receiving any form of psychotherapy (self-report). NB, must be regularly for >6 months in order to meet entry criteria.
- g. Score on Irritable Bowel Syndrome Symptom Severity Scale at baseline (0-500)

5.4.2 Pre to post analyses. Paired samples t-tests on pre-OL and post-OL scores on outcome measures revealed significant improvements in scores on six measures over time (Table 14), including two primary outcome measures.

Table 14. Pre- and post-OL data on primary and secondary outcome measures in per protocol population (n=49).

Outcome variable	End RCT Mean (SD)	Post OL Mean (SD)	Change pre to post	<i>p</i>	ES ^a <i>d</i> _{RM}
Primary					
MADRS	17.3 (8.5)	14.1 (10.4)	-3.2 [-5.2 to -1.2]	0.003	0.47
iCGI-S	2.8 (1.0)	2.3 (1.3)	-0.5 [-0.7 to -0.2]	0.004	0.44
iCGI-I	2.8 (2.0)	3.4 (2.6)	0.6 [-0.03 to 1.3]	0.06	0.28
QIDS	7.7 (4.6)	6.8 (4.6)	-1.0 [-2.0 to 0.1]	0.07	0.27
Secondary					
GAF	69.1 (7.8)	69.5 (12.8)	0.4 [-3.1 to 3.9]	0.83	0.03
DASS	28.8 (17.5)	22.53 (21.6)	-6.2 [-11.3 to -1.2]	0.02	0.36
<i>Depression</i>	10.2 (7.1)	8.5 (10.2)	-1.7 [-3.9 to 0.4]	0.12	0.25
<i>Anxiety</i>	5.3 (5.6)	4.3 (5.2)	-1.0 [-2.6 to 0.6]	0.22	0.18
<i>Stress</i>	13.3 (8.0)	9.8 (8.7)	-3.5 [-5.4 to -1.6]	0.001	0.52
DAS	142.1 (42.1)	136.0 (41.4)	-6.2 [-10.7 to -1.7]	0.01	0.40
ATQ	59.7 (23.6)	54.1 (27.4)	-5.6 [-11.0 to -0.2]	0.04	0.30
IBS-SSS	72.2 (77.9)	61.6 (74.4)	10.6 [-25.8 to 4.7]	0.17	0.20

Abbreviations: ES=effect size; MADRS=Montgomery-Åsberg Depression Rating Scale; iCGI-S=improved Clinical Global Impression Scale (Severity scale); iCGI-I=improved Clinical Global Impression Scale (Improvement scale); QIDS=Quick Inventory of Depressive Symptomatology; GAF=Global Assessment of Functioning; DASS=Depression, Anxiety and Stress Scale; DAS=Dysfunctional Attitude Scale; ATQ=Automatic Thoughts Questionnaire; IBS-SSS=Irritable Bowel Syndrome Severity Scoring Scale.

a. Effect size, *d*_{RM}(repeated measures) which corrects for dependence between means, as described by Morris and DeShon (2002)

5.4.3 Treatment response. In total, 10 participants (20%) experienced a ≥50 change on the MADRS over the course of the OL phase and were classified as responders in this phase. Thirty (61%) of the participants who completed the OL phase were considered to have experienced “considerable”, “very considerable” or “ideal” improvement compared with baseline by the end of

OL. In total, 15 (31%) of the participants who completed the OL phase were considered in remission with a score of <8 on the MADRS at the end of OL, which includes 7 participants who were in remission at the end of the RCT phase.

Given the lack of any group difference in the RCT phase, responders to treatment in the OL phase would be expected to be responding only due to placebo effects. To ascertain whether the response rates in the RCT phase and OL phase were consistent with the lack of treatment effect observed in the RCT phase (i.e., whether there were a disproportionate number of non-placebo responders who went on to respond in the OL phase), the individual change of those who had been in the placebo group in the RCT phase who then went on to respond in the OL phase responders was visually compared. Of the 49 participants who completed the OL phase per protocol, 28 participants had been in the placebo group in the RCT phase. Of these, 17 participants did not respond to the placebo in the RCT phase, and five of these non-placebo responders responded to the active treatment in the OL phase. The individual MADRS scores of these five participants at three time points are presented in Figure 14.

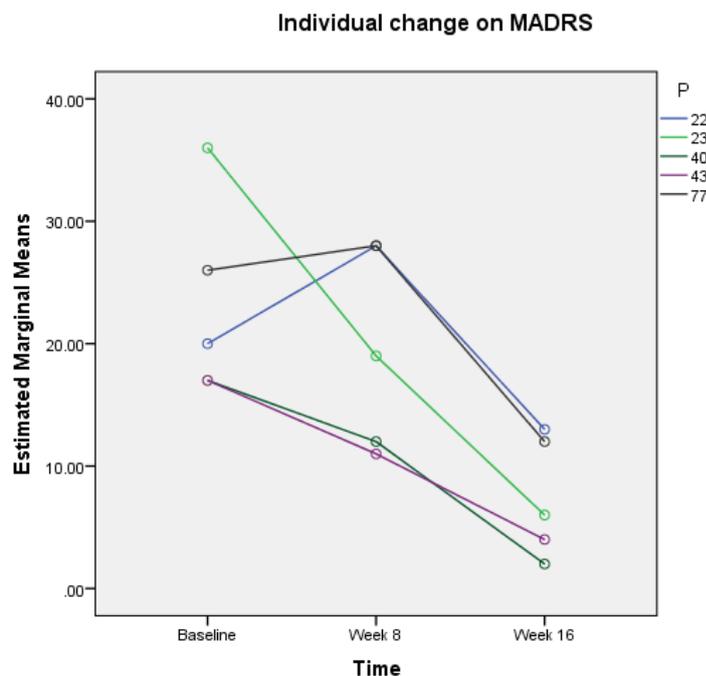


Figure 14. Individual change on MADRS of non-responders to placebo who responded in the OL phase (n=5).

As can be seen in Figure 14, three of the non-responders to placebo in the RCT phase show a relatively continuous gradient of response over the trial, experiencing a 29-47% change on the MADRS in the RCT phase (P.23, 40 and 43). Two participants (P.22 and 77) experienced a worsening of symptoms while taking the placebo in the RCT phase, with an increase in MADRS score of 8% and 40% respectively, and went on to respond in the OL phase. Given that these two participants make up 4% of the total per protocol sample from the OL phase, and that a slight increase in placebo responding is expected when participants are aware that they are taking the active product, it appears that there is no major disparity between rates of OL phase responding and the lack of treatment effect in the RCT phase.

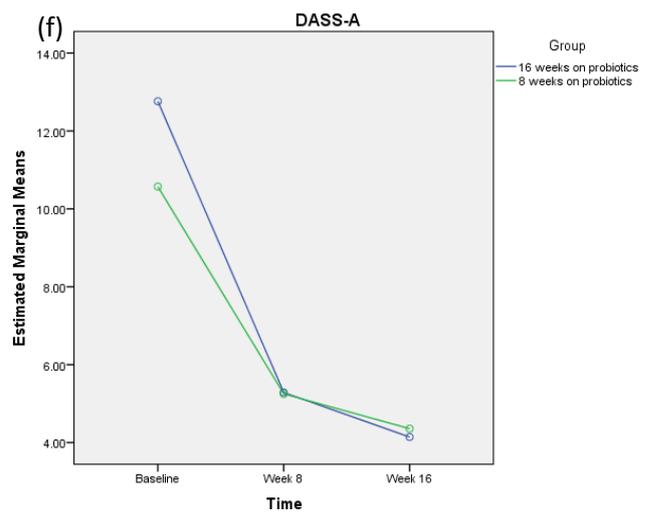
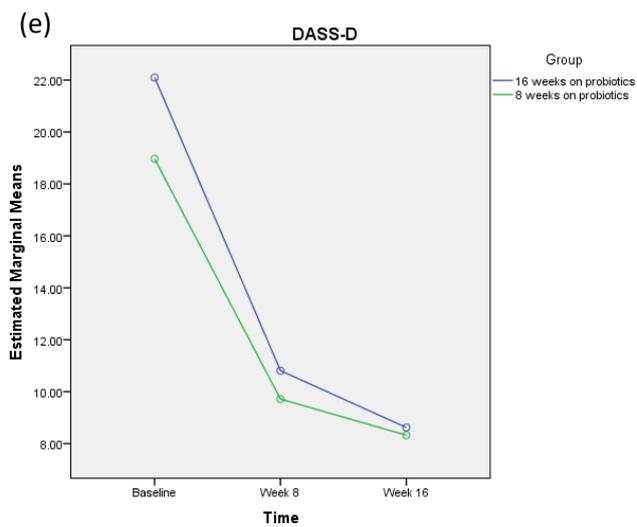
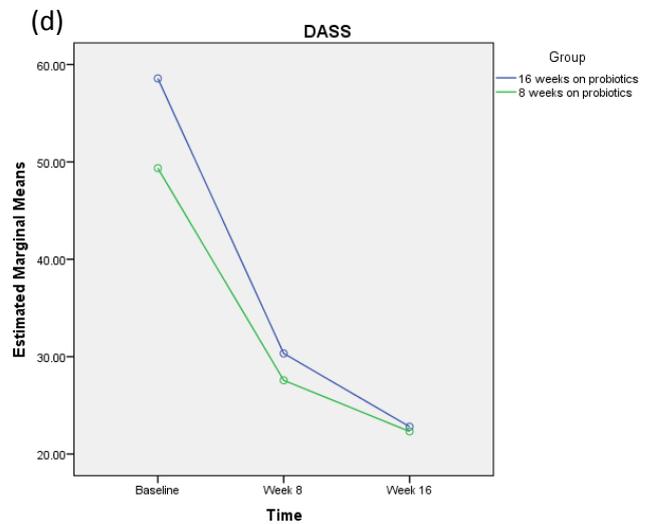
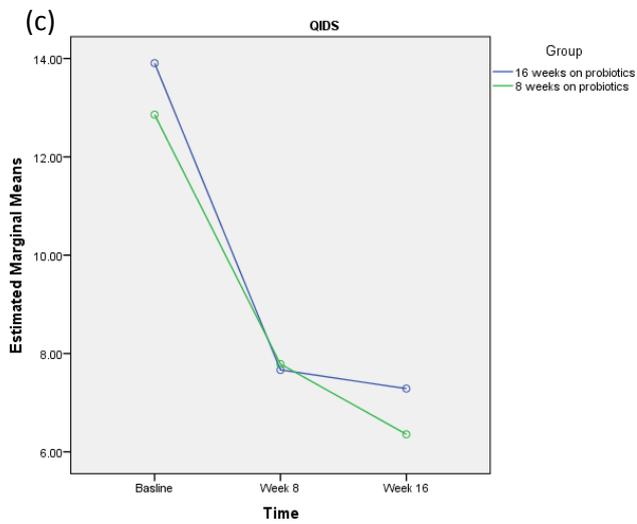
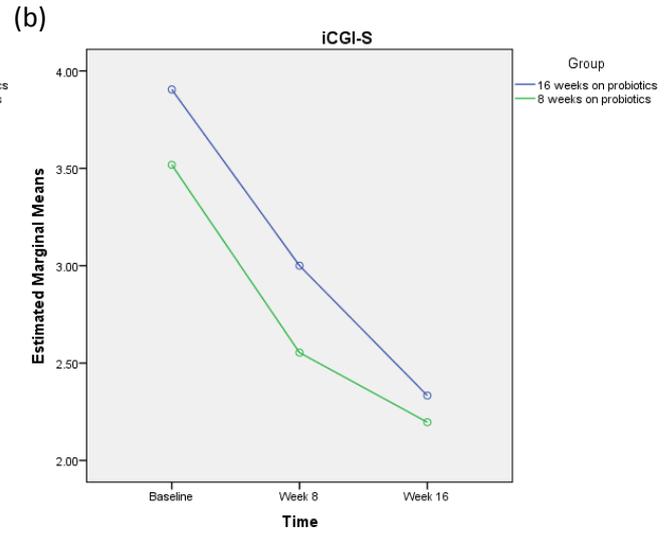
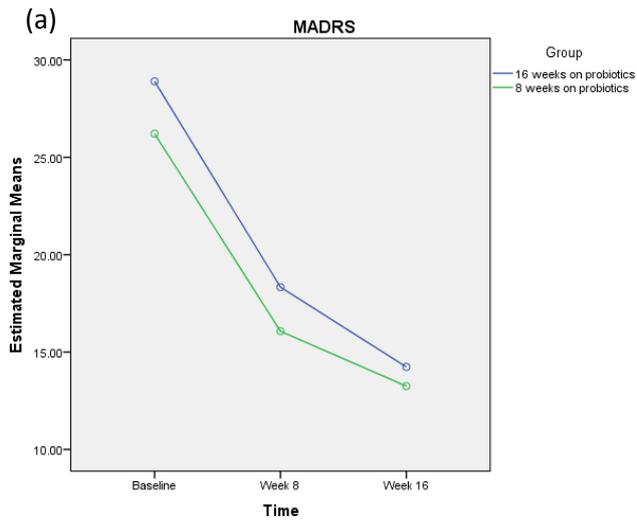
5.4.4 Effect of time of exposure to probiotics. Of the 49 participants who completed the OL phase, those who had been in the active treatment group in the RCT phase (n=21) had been taking the active product for 16 weeks at the end of the OL phase, while those who had been in the placebo group (n=28) had been taking the probiotic for 8 weeks at the end of the OL phase. Change scores—baseline to post-OL phase—on all outcome measures were compared between the randomisation groups from the first phase using ANCOVA with the baseline level as a covariate, to ascertain whether length of exposure to the active product had any effect on outcome measures. No group difference was observed for any psychological or IBS outcome measure (see Table 15). Change over time in both groups is presented for all measures in Figure 15(a-j) as repeated-measures ANOVA graphs for baseline, week 8 (end of RCT phase) and week 16 (end of OL), displaying similar gradients of response in both groups over time.

Table 15. Baseline and post-OL (week 16) data on primary and secondary outcome measures in the per protocol population from OL phase (n=49).

Outcome variable	Group 16 weeks on probiotics (n=21)			Group 8 weeks on probiotics (n=28)			Difference [95% CI]	p	ES ^b d
	Baseline Mean (SD)	Post-OL Mean (SD)	Change from baseline ^a	Baseline Mean (SD)	Post-OL Mean (SD)	Change from baseline ^a			
Primary									
MADRS	28.9 (6.2)	14.2 (9.2)	-13.9	26.2 (6.0)	13.3 (10.6)	-13.5	-0.4 [-6.1 to 5.4]	0.90	0.04
iCGI-S	3.9 (0.4)	2.3 (1.1)	-1.6	3.5 (0.5)	2.2 (1.3)	-1.3	-0.3 [-1.0 to 0.3]	0.31	0.32
iCGI-I ^c		3.3 (2.7)	3.3		3.7 (2.3)	3.7	-0.4 [-1.8 to 1.1]	0.61	0.15
QIDS	13.9 (3.6)	7.3 (4.8)	-6.4	12.9 (3.9)	6.4 (6.3)	-6.7	0.3 [-2.8 to 3.4]	0.85	0.06
Secondary									
GAF	60.4 (6.5)	70.6 (9.7)	10.1	63.4 (5.4)	69.3 (14.7)	6.0	4.2 [-2.7 to 11.1]	0.23	0.36
DASS	58.6 (23.3)	22.8 (19.4)	-31.6	49.4 (20.1)	22.32 (23.5)	-30.1	-1.5 [14.3 to 11.3]	0.81	0.07
<i>Depression</i>	22.1 (8.7)	8.6 (9.7)	-12.6	19.0 (10.4)	8.3 (10.7)	-11.3	-1.3 [-6.6 to 4.1]	0.64	0.14
<i>Anxiety</i>	12.8 (10.8)	4.1 (4.2)	-7.5	10.6 (6.8)	4.4 (5.9)	-7.1	-0.5 [-3.5 to 2.6]	0.77	0.09
<i>Stress</i>	23.7 (9.7)	10.0 (8.8)	-12.2	19.8 (8.4)	9.6 (8.8)	-11.3	-0.8 [-5.8 to 4.2]	0.74	0.10
DAS	158.9 (38.4)	142.2 (37.3)	-16.3	156.5 (37.7)	131.3 (44.2)	-25.5	9.1 [-8.2 to 26.5]	0.29	0.31
ATQ	90.6 (25.9)	52.7 (24.0)	-34.4	80.4 (29.4)	55.2 (30.1)	-27.9	-6.5 [-21.7 to 8.8]	0.40	0.25
IBS-SSS	171.4 (128.5)	88.5 (94.0)	-51.4	75.9 (61.8)	41.4 (48.1)	-58.1	-6.7 [-31.9 to 45.2]	0.73	0.11

Abbreviations: OL=Open label; ES=Effect size; MADRS=Montgomery-Åsberg Depression Rating Scale; iCGI-S=improved Clinical Global Impression Scale (Severity scale); iCGI-I=improved Clinical Global Impression Scale (Improvement scale); QIDS=Quick Inventory of Depressive Symptomatology; GAF=Global Assessment of Functioning; DASS=Depression, Anxiety and Stress Scale; DAS=Dysfunctional Attitude Scale; ATQ=Automatic Thoughts Questionnaire; IBS-SSS=Irritable Bowel Syndrome Severity Scoring Scale.

- a. Adjusted for baseline.
- b. Cohen's *d* effect size, measured as the mean difference in change divided by the pooled standard deviation of the change based on values adjusted for baseline
- c. Assesses change only, therefore not measured at baseline.



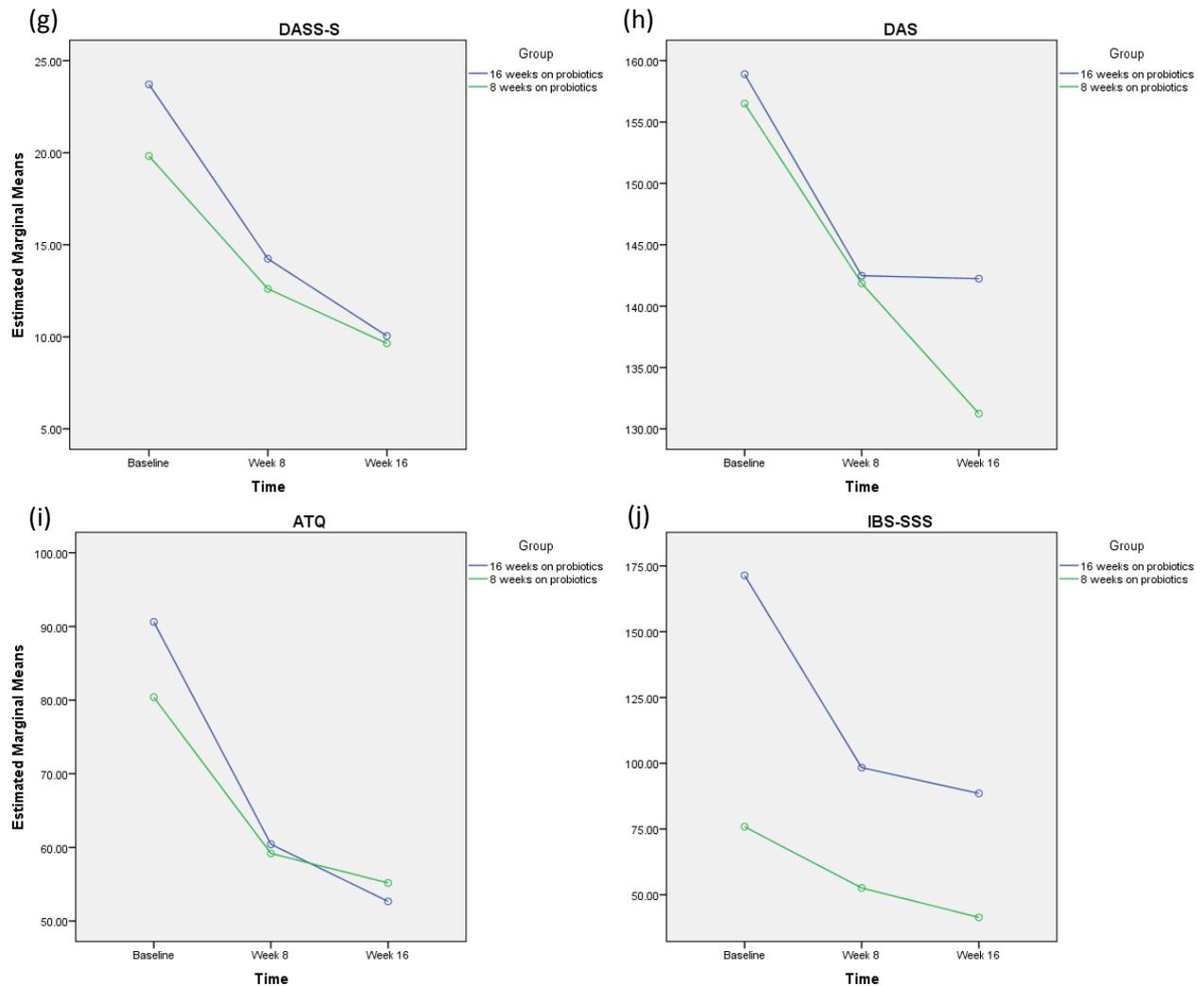


Figure 15(a-j). Repeated measures ANCOVA data showing change over time at baseline, week 8 (end of RCT phase) and week 16 (end of OL), in the two groups that had been taking the active probiotic for 8 and 16 weeks respectively.

5.5 Results: 6 Month Follow-up

5.5.1 Those followed versus those lost to follow-up. As shown in Figure 1

(CONSORT flow diagram), all 79 participants who were randomised to receive either the active treatment or placebo in the RCT phase were asked to complete the follow-up questionnaire six months after exiting the trial, regardless of compliance with treatment. In total, 46 responses were received (58%) and these make up the per protocol data set for the 6 month follow-up. Overall, 36 of these responses (78%) were from participants who successfully completed the entire study protocol (both the randomised and OL phases) compared with those who did not complete the study protocol, $\chi^2(1, n=79)=12.324, p<0.01$.

See Table 16 for demographic and clinical features of those who completed the follow-up survey versus those who did not. The mean age of those who completed the follow-up questionnaire was significantly higher than those who did not complete follow-up, $t(76)=2.772, p<0.01$. Levene's test indicated unequal variances ($F=4.704, p<0.01$) so degrees of freedom were adjusted from 77 to 76.

Table 16. Demographic and clinical features of those participants from the RCT included and not included in the 6 month follow-up.

Characteristic	Followed up (n=46)	Not followed up (n=33)
<i>Demographics</i>		
Age, years: mean (SD)	38.5 (15.0)	31.3 (11.8)
Male, n (%)	10 (22)	7 (21)
Socioeconomic status ^a , mean (SD)	43.2 (11.3)	43.5 (2.4)
Education, n (%)		
No high school certificate	5 (11)	5 (15)
Completed high school	13 (28)	10 (30)
Post-secondary (e.g., trade certificate)	17 (37)	11 (33)
University degree	11 (24)	7 (21)
Ethnic origin, n (%)		
New Zealanders of European descent	35 (76)	26 (79)
New Zealand Maori	2 (4)	1 (3)
Other	9 (19)	6 (18)
<i>Clinical</i>		
Meets criteria for MDD ^b , n (%)	32 (70)	27 (82)
Past diagnosis of any mood disorder ^c , n (%)	19 (41)	18 (55)
Baseline MADRS score, mean (SD)	26.9 (5.6)	28.7 (7.0)
Chronic depression ^d , n (%)	29 (63)	26 (79)
Anxiety disorder ^e , n (%)		
Current	18 (39)	18 (54)
Past	20 (43)	19 (58)
Alcohol/ substance misuse or dependence ^e , n (%)		
Current	1 (2)	4 (12)
Past	5 (11)	9 (27)
Any co-occurring disorder ^e , n (%)		
Current	25 (54)	20 (61)
Past	21 (46)	20 (61)
History of antidepressant use ^e , n (%)	25 (54)	21 (64)
IBS severity ^f , mean (SD)	112.1 (103.5)	130.2 (102.7)
Severity of IBS by category, n (%)		
None	24 (52)	11 (33)
Mild	12 (26)	13 (39)
Moderate	7 (15)	6 (18)
Severe	3 (7)	3 (9)

Abbreviations: MDD=major depressive disorder; MADRS=Montgomery-Åsberg Depression Rating Scale; IBS=irritable bowel syndrome.

- Based on the New Zealand Socio-Economic Index 2006 (NZSEI-06) (Milne, Byun, & Lee, 2013)
- Meets DSM-5 criteria for a current major depressive episode (American Psychiatric Association, 2013a)
- Number self-reporting a past diagnosis of any mood disorder (e.g. major depressive disorder, dysthymia, bipolar disorder)
- Chronic depression defined as >2 years continuous symptoms
- Established from self-report and retrospective self-report
- Score on Irritable Bowel Syndrome Symptom Severity Scale at baseline (0-500)

5.5.2 Scores on outcome measures at 6 months post-trial. There was significant regression from end of OL to 6 month follow-up on the

DASS global score, DASS-S and IBS-SSS; however, participants were still significantly improved from baseline on all psychological measures, but not on the measure of IBS symptoms (Table 17).

Table 17. Baseline, end of open label and 6 month follow-up data on outcome measures in those who completed 6 month follow-up (n=46).

Variable	Total follow-up sample (n=49)						
	BL	End OL	6 month FU	Change from BL to 6 month FU	Change from end of OL to 6 month FU		
	M (SD)	M (SD)	M (SD)	Mean difference [95% CI]	ES ^a	Mean difference [95% CI]	ES ^a
					d_{RM}		d_{RM}
QIDS	13.6 (3.7)	5.6 (4.3)	7.6 (4.9)	-5.9** [-7.5 to -4.3]	1.15	0.9 [-1.1 to 2.8]	0.15
DASS	52.0 (22.0)	18.6 (17.0)	30.6 (20.1)	-22.2** [-30.0 to -14.5]	0.86	7.7* [0.4 to 15.1]	0.31
Depression	19.4 (9.8)	7.1 (8.5)	11.5 (9.8)	-8.3** [-11.6 to -5.9]	0.57	3.1 [-0.3 to 6.6]	0.33
Anxiety	11.4 (8.1)	3.2 (3.8)	6.4 (6.4)	-5.2** [-7.8 to -2.6]	0.61	1.7 [-0.2 to 3.6]	0.35
Stress	21.2 (9.4)	8.5 (7.0)	12.8 (7.4)	-8.7** [-11.6 to -5.9]	0.93	2.8* [0.1 to 5.6]	0.35
DAS	157.8 (38.6)	134.9 (40.9)	139.3 (41.1)	-18.5** [-27.8 to -9.2]	0.59	1.1 [-5.9 to 8.1]	0.05
ATQ	83.9 (30.0)	51.4 (24.9)	55.6 (21.9)	-28.3** [-37.5 to -19.1]	0.93	1.3 [-7.8 to 10.4]	0.05
IBS-SSS	112.1 (103.5)	56.3 (69.2)	97.4 (92.5)	-22.8 [-48.9 to 3.4]	0.27	27.3* [0.2 to 54.3]	0.38

Abbreviations: BL=baseline; OL=open label; FU=follow-up; CI=confidence interval; QIDS=Quick Inventory Depressive Symptomatology; GAF=Global Assessment of Functioning; DASS=Depression, Anxiety and Stress Scale; DAS=Dysfunctional Attitude Scale; ATQ=Automatic Thoughts Questionnaire; IBS-SSS=Irritable Bowel Syndrome Severity Scoring Scale; *p<0.05; **p<0.01.

a. Effect size, d_{RM} (repeated measures) which corrects for dependence between means, as described by Morris and DeShon (2002).

5.5.3 Dominant treatment at 6 month follow-up. Participants were asked if they had continued taking probiotics or another nutritional supplement for their low mood over the 6-month follow-up period, whether they had taken any antidepressant medication since the study, or whether they had received any counselling or psychotherapy since the study. Based on responses, participants were divided into three groups by dominant treatment, defined as (a) probiotics or other nutritional supplement (probiotics/nutrition group), (b) antidepressant medication or counselling/psychotherapy (standard treatment group) and (c) no continuing treatment or therapy (no treatment group).

Of the 46 participants who completed the 6 month follow-up, 15 (33%) were continuing to regularly take a probiotic supplement (any brand) or yoghurt (n=12) or another nutritional product (micronutrient formula, n=3) regularly at the point of follow-up. Of the reasons cited for discontinuing probiotics after the study, the most commonly cited reason was cost, with 10 participants (31%) rating this as “much” or “very much” a reason for discontinuing (6 or 7 on a 7-point Likert scale). Nine participants were included in the standard treatment group because they reported predominantly taking an antidepressant since the study (n=4), or having received counselling or psychotherapy (individual or group) regularly for a minimum of 2 months before follow-up (n=2), or both taking antidepressants and receiving psychotherapy (n=3). In total, 22 participants did not report any continuing treatment for their depression.

Among the three groups, there were significant differences in the number of people who met DSM-5 criteria for MDD at baseline (probiotics/nutrition group, n=14 (93%); standard treatment group, n=6 (67%); no treatment group, n=12 (55%)), $\chi^2(2, n=46)=6.382, p<0.05$. There were no other differences in baseline characteristics among the groups. There was no group difference in scores on outcome measures at the end of the open label phase (Table 18).

Table 18. Comparison of scores on outcome measures at end of open label by dominant treatment group.

	Probiotics/ nutrition (n=15)	Standard treatment (n=9)	No treatment (n=22)		
	M (SD)	M (SD)	M (SD)	<i>F</i>	<i>p</i>
QIDS	6.2 (5.1)	6.8 (4.3)	4.6 (3.6)	0.84	0.44
DASS	20.4 (19.0)	19.5 (18.4)	16.8 (15.5)	0.17	0.84
<i>Depression</i>	8.7 (9.7)	5.7 (7.7)	6.3 (8.0)	0.40	0.68
<i>Anxiety</i>	3.6 (5.0)	3.3 (2.8)	2.8 (3.0)	0.20	0.82
<i>Stress</i>	8.0 (6.8)	10.5 (8.8)	7.7 (6.8)	0.36	0.70
DAS	131.2 (36.3)	144.2 (46.8)	134.6 (44.5)	0.20	0.82
ATQ	58.9 (31.1)	49.8 (26.4)	45.3 (16.6)	1.14	0.33
IBS-SSS	60.2 (74.6)	37.0 (27.5)	60.2 (76.9)	0.27	0.77

Abbreviations: QIDS=Quick Inventory Depressive Symptomatology; DASS=Depression, Anxiety and Stress Scale; DAS=Dysfunctional Attitude Scale; ATQ=Automatic Thoughts Questionnaire; IBS-SSS=Irritable Bowel Syndrome Severity Scoring Scale

Table 19 shows the comparison across the three dominant treatment groups in change on outcome measures from end of open label to the point of 6 month follow-up. There were no significant group differences on any measure.

Table 19. Comparison of change on outcome measures from end of open label to 6 month follow-up by dominant treatment at follow-up.

Variable	End of OL			6 month FU			Change from end of OL to 6 month follow-up			F	p
	Probiotics/ nutrition (n=15)	Standard treatment (n=9)	No treatment (n=22)	Probiotics/ nutrition (n=15)	Standard treatment (n=9)	No treatment (n=22)	Probiotics/ nutrition (n=15)	Standard treatment (n=9)	No treatment (n=22)		
	M (SD)	M (SD)	M (SD)	M (SD)	M (SD)	M (SD)	Difference ^a [95% CI]	Difference ^a [95% CI]	Difference ^a [95% CI]		
QIDS	6.2 (5.1)	6.8 (4.3)	4.6 (3.6)	5.9 (4.6)	9.1 (4.6)	8.1 (5.1)	0.8 [-2.5 to 2.6]	1.3 [-2.5 to 5.1]	1.3 [-1.0 to 3.7]	0.30	0.74
DASS	20.4 (19.0)	19.5 (18.4)	16.8 (15.5)	25.6 (18.0)	37.6 (26.1)	31.2 (18.6)	6.0 [-4.0 to 16.0]	9.1 [-6.1 to 24.3]	8.8 [-0.9 to 18.4]	0.11	0.90
Depression	8.7 (9.7)	5.7 (7.7)	6.3 (8.0)	9.6 (9.8)	12.7 (12.7)	12.3 (8.6)	2.3 [-2.5 to 7.1]	1.3 [-6.1 to 8.6]	4.7 [0.02 to 9.3]	0.42	0.66
Anxiety	3.6 (5.0)	3.3 (2.8)	2.8 (3.0)	4.8 (5.9)	8 (7.1)	6.9 (6.4)	0.7 [-2.3 to 3.6]	2.4 [-2.1 to 6.9]	2.5 [-0.3 to 5.3]	0.46	0.63
Stress	8 (6.8)	10.5 (8.8)	7.7 (6.8)	11.2 (6.3)	16.9 (9.2)	12.1 (7.1)	2.8 [-0.8 to 6.4]	5.6 [0.1 to 11.1]	1.8 [-1.7 to 5.2]	0.72	0.50
DAS	131.2 (36.3)	144.2 (46.8)	134.6 (44.5)	129.7 (39.2)	156.1 (42.9)	139.0 (41.4)	-3.3 [-14.5 to 7.7]	10.1 [-7.0 to 27.3]	1.6 [-8.8 to 12.0]	0.90	0.42
ATQ	58.9 (31.1)	49.8 (26.4)	45.3 (16.6)	54.4 (25.8)	60.9 (23.7)	54.2 (18.9)	0.2 [-12.0 to 12.3]	1.6 [-16.6 to 19.9]	2.1 [-9.2 to 13.4]	0.03	0.97
IBS-SSS	60.2 (74.6)	37 (27.5)	60.2 (77.0)	77.9 (90.9)	126.6 (85.3)	101.8 (97.2)	17.6 [-24.8 to 60.0]	27.4 [-52.1 to 106.9]	36.8 [-5.7 to 79.3]	0.21	0.81

Abbreviations: BL=baseline; OL=open label; FU=follow-up; CI=confidence interval; QIDS=Quick Inventory Depressive Symptomatology; DASS=Depression, Anxiety and Stress scale; DAS=Dysfunctional Attitude Scale; ATQ=Automatic Thoughts Questionnaire; IBS-SSS=Irritable Bowel Syndrome Severity Scoring Scale.

a. Adjusted for pre-change (end of open label) score

5.5.3.1 Change over course of trial by dominant treatment group. Although there were no significant differences over time among the dominant treatment groups from the end of OL to the 6 month follow-up, it was thought that further investigation into the treatment response of each dominant treatment group might provide some insight into any patterns in responding among the groups. This was completed by visually presenting the change over time across the entire study in each of the dominant treatment groups for all measures taken at 6 month follow-up, from mixed ANOVA analyses (Figures 16-21).

On the QIDS, the interaction between time and group was not significant, $F(6,96)=1.41$, $p=ns$. Visually, the group who elected to continue with no treatment appeared to score slightly lower at the end of the OL phase (although, as discussed above, there were no significant group differences at this point when adjusted for baseline) and the standard treatment and probiotics/nutrition groups scored similarly at end of OL (Figure 16). The no treatment group appears to score slightly lower on the QIDS at the end of OL and also show the biggest regression in QIDS scores at the point of 6 month follow-up. The probiotics/nutrition group were the only group not to regress in QIDS score at 6 month follow-up.

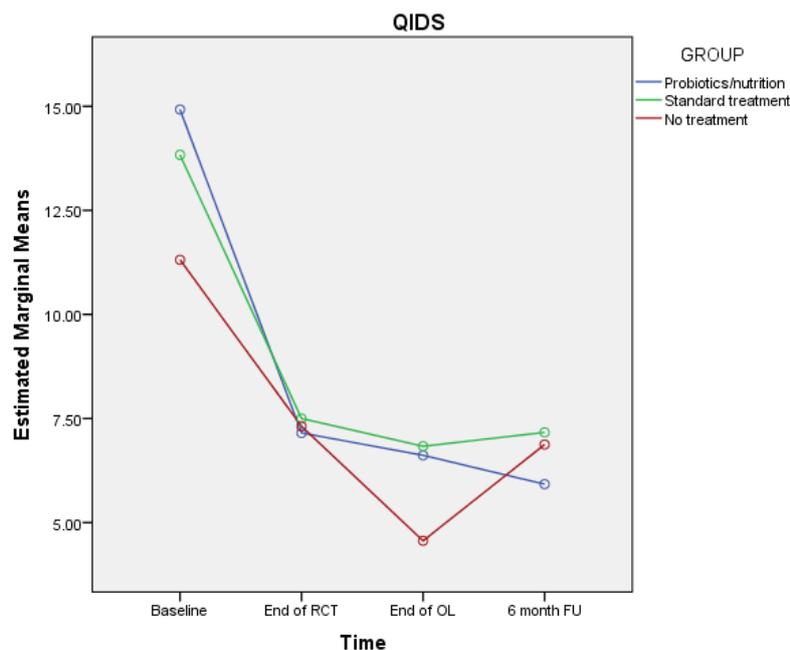


Figure 16. Change over time across the entire study in each of the dominant treatment groups on the QIDS.

On the DASS total score, the interaction between time and group was not significant, $F(6,96)=0.77, p=ns$. In keeping with this, the difference among the groups at both the end of OL and the point of 6 month follow-up appears very small (Figure 17).

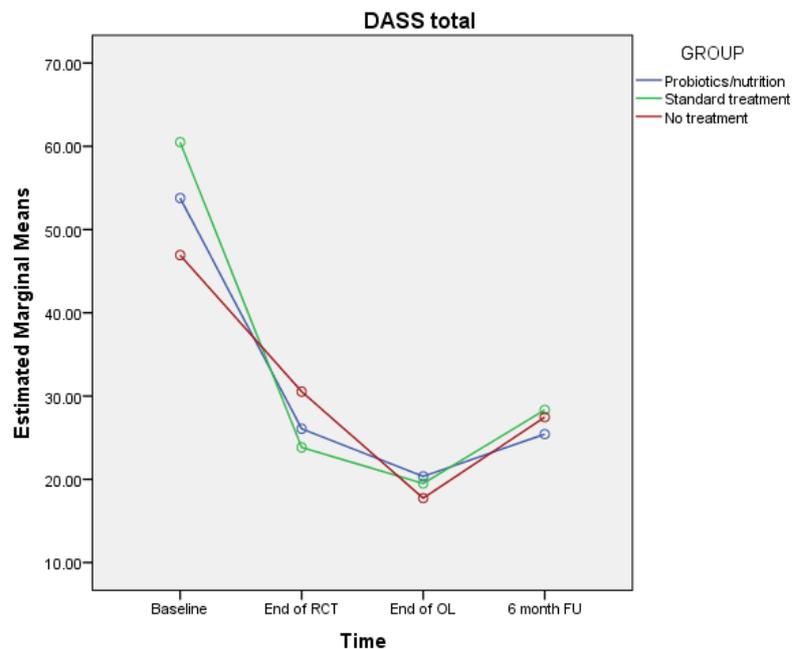


Figure 17. Change over time across the entire study in each of the dominant treatment groups on the DASS total.

Figure 18(a-c) show the changes over time in the three groups on the three subscales of the DASS: the DASS-D, DASS-A, and DASS-S. The interaction between time and group was not significant on the Depression subscale ($F(6,96)=0.70, p=ns$), the Anxiety subscale ($F(6,96)=0.80, p=ns$) or the Stress subscale ($F(6,96)=0.66, p=ns$). On the depression subscale, the standard treatment group appears to score slightly lower than the other two groups at the end of OL, but regression appears similar among the three groups at 6 month follow-up. On the anxiety subscale, response and regression at 6 month follow-up appear very similar among the three dominant treatment groups. On the stress subscale regression in all groups appears very similar at 6 month follow-up, with the standard treatment group scoring slightly higher than the other two groups at the end of OL.

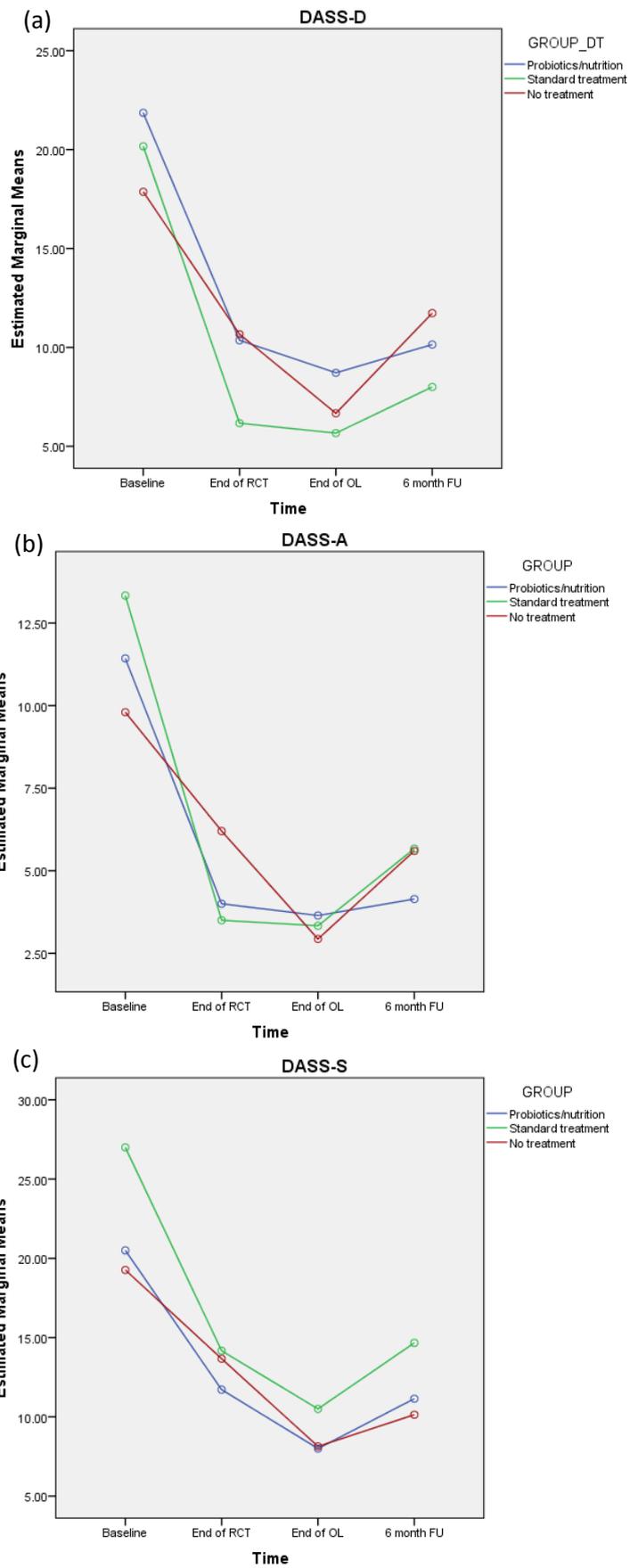


Figure 18(a-c). Change over time across the entire study in each of the dominant treatment groups on the DASS subscales.

Despite no significant interaction between group and time on the DAS ($F(6,99)=1.51, p=ns$), there appears to be an interesting pattern of responding among the three groups, with the standard treatment group scoring consistently higher than the other groups throughout the study and regressing more up to the point of 6 month follow-up (Figure 19). The probiotic group score higher at baseline and appear to improve more than the other groups throughout the study and continue to improve in score from the end of OL to the point of 6 month follow-up. However, it is important to note that these differences, and the interaction, are not significant.

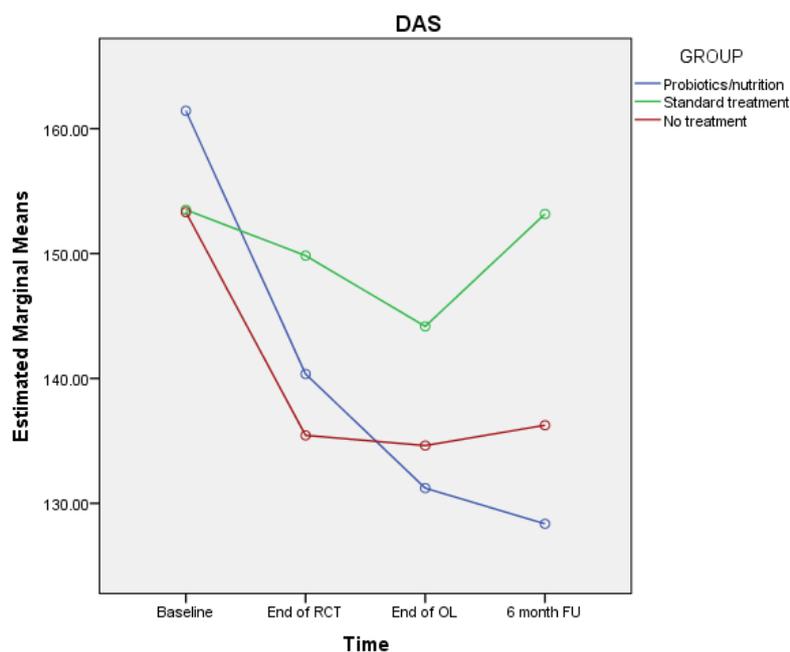


Figure 19. Change over time across the entire study in each of the dominant treatment groups on the DAS.

On the other measure of cognition (the ATQ), there no significant interaction between group and time, $F(6,99)=0.43, p=ns$. Visually, the probiotic group appear to score slightly higher at the end of OL, but all three groups score very similarly at the point of 6 month follow-up (Figure 20).

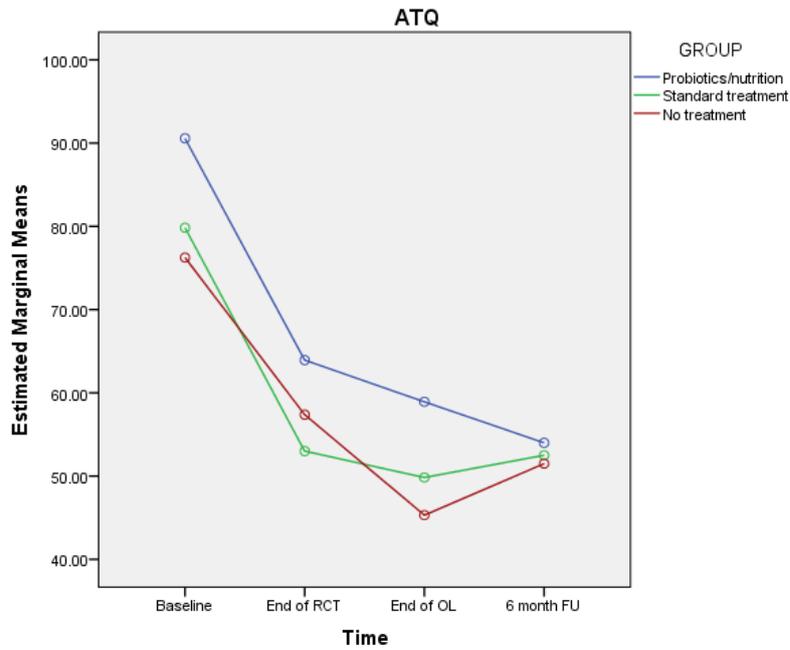


Figure 20. Change over time across the entire study in each of the dominant treatment groups on the ATQ.

On the measure of IBS symptoms, the interaction between group and time was once again not significant ($F(6,99)=1.06, p=ns$), but the group who elected to discontinue any treatment for their low mood appear to score slightly higher than the other groups on IBS symptoms at the end of OL (Figure 21). The no treatment group also regress more in score at the point of 6 month follow-up (almost back to their baseline level), while the other groups appear to respond and regress similarly to each other.

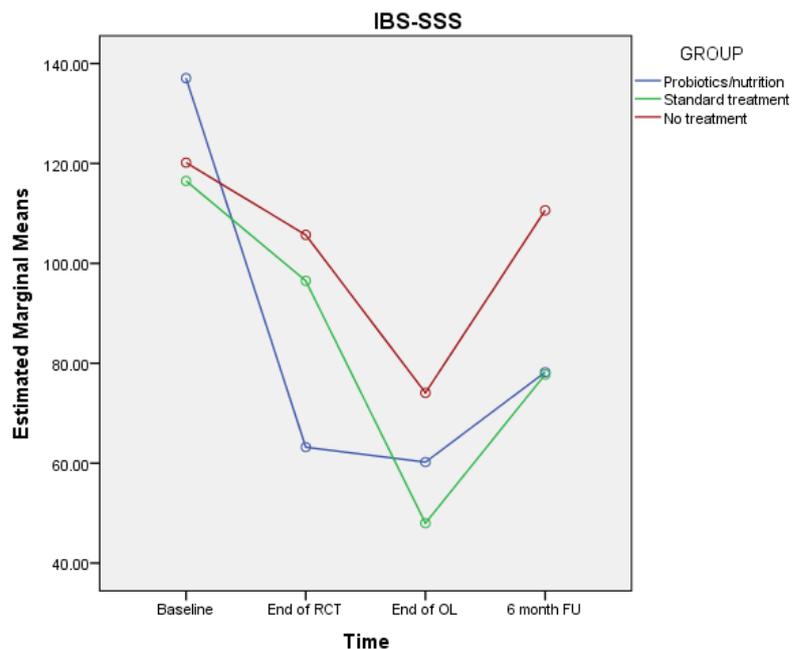


Figure 21. Change over time across the entire study in each of the dominant treatment groups on the IBS-SSS.

5.6 Summary of Results

The current study recruited 79 participants and randomised them to receive either the active probiotic supplement or a matched placebo for eight weeks. Of these 79 participants, 49 participants then completed a further eight week OL phase, taking the active study product. All participants were followed up at 6 months post-trial, with 46 participants completing the 6 month follow-up questionnaire.

At baseline, a high proportion of the sample was found to have elevated levels of inflammatory markers, and mean vitamin D was approaching the deficient level. All inflammatory biomarkers were correlated with at least one other inflammatory marker. Vitamin D correlated with baseline severity on the DAS. No other biomarker correlated with baseline severity on any other psychological or IBS outcome measure. Several correlations were found between several baseline factors (history of antidepressant usage and gender) and levels of inflammatory biomarkers, and a seasonal pattern was observed for vitamin D levels.

For the RCT phase, no group differences were found between the active treatment and placebo groups over time in either the intent-to-treat or per protocol data sets. There was a high level of placebo responding (36%) in the RCT phase. There were no group differences in change in biomarkers over the course of the RCT phase; however, levels of BDNF changed more in the probiotic group than the placebo group, with a result approaching significance at $p=0.07$. Graphs showing change over time on fortnightly measures did not present consistent or compelling evidence for a differential effect over time. Adherence to the study protocol and compliance with treatment were good, and no adverse events were observed in the active treatment group that were not also observed in the placebo group. The drop-out rate in the RCT phase was 13%. Hierarchical multiple regression analyses showed a moderating effect of vitamin D across three measures, showing that participants with high vitamin D at baseline who were randomised to the active treatment group experienced more improvement than those who had low vitamin D at baseline, with no effect on responding in the placebo group.

A high drop-out before and during the OL phase meant that only 49 participants (62% of the initial sample) completed the OL phase. Significant improvements were observed on a number of measures over the OL phase, with 10 participants responding to treatment (≥ 50 change on the MADRS) in this phase. No disparity was evident between rates of responding in the OL phase and the lack of treatment effect in the RCT phase. Time of exposure to the probiotics did not appear to have a significant impact on treatment response.

In total, responses to the 6 month follow-up questionnaire were received from 46 (58%) of the initial sample. In these participants, there was significant regression on several outcome measures from end of trial to 6 month follow-up, but participants were still, on average, significantly improved from baseline at the point of 6 month follow-up; however, it is possible that this is due to regression to the mean. Participants who completed the 6 month follow-up questionnaire were divided into three groups based on their self-reported dominant treatment post-trial: probiotics/other nutritional supplement, standard treatments or no treatment. There was a significant difference among the groups in the number who met criteria for MDD at baseline, with the probiotics/nutrition group having the highest proportion at 93%, followed by the standard treatment group (67%) and the no treatment group (55%). When controlling for scores at the end of the trial (end of OL phase) there were no significant group differences in change on any measure from end of trial to 6 month follow-up. When change over the course of the whole trial was plotted (controlling for baseline levels on each measure), some interesting patterns were visually apparent, particularly on the DAS, but no significant interactions were observed between time and group on any measure. This may be a function of a lack of statistical power.

Chapter 6: Discussion

The existing literature suggests that depression is associated with increased permeability of the gut wall (Maes et al., 2012), increased immune and inflammatory activation (Berk et al., 2013; Maes, 2011) and gut disorders such as irritable bowel syndrome (IBS; Fond et al., 2014). These underlying biological pathways support a proposal made by Logan and Katzman (2005), who suggested that probiotics could be used as an adjuvant treatment for depression, a concept which is supported by evidence from animal studies and some human research in healthy population samples. However, the existing evidence base of human research investigating the effects of probiotic treatment on mood and other mental health outcomes was found by systematic review (Chapter 3) to be lacking in completeness and applicability.

This thesis presents the first randomised controlled trial to test whether supplemented probiotic bacteria affect mood and other psychological outcomes in a population recruited for low mood. This discussion will restate the study aims and hypotheses, and will then outline the results of the study sectioned by hypothesis. The strengths and limitations of this study will then be addressed, followed by the general implications of the trial and ideas for directions for future research in the area.

6.1 Thesis Objectives and Hypotheses

The current study aimed to strengthen and grow the field of research through providing the first randomised controlled trial to assess whether probiotics could affect mood and other psychological outcomes in a sample presenting with low mood. One objective of the trial was to determine whether there was increased inflammatory activation or evidence of alterations in other blood-based biomarkers in the sample presenting with low mood at baseline, and whether the levels of these biomarkers were related to mood or other baseline factors. Further, the study aimed to discover whether the presence, absence or severity of IBS symptoms, and levels of proinflammatory

cytokines, BDNF and vitamin D, would predict or impact on treatment response, or whether these variables would be affected by probiotic treatment.

Based on the existing evidence from gut-brain axis research, and on models linking depression with inflammation, immune activation, low vitamin D levels, and the state of the gut microbiota, the following hypotheses were formed (as outlined in section 3.7.1):

1. The sample would have elevated levels of inflammatory biomarkers and low levels of vitamin D at baseline, which may be associated with scores on psychological and IBS outcome measures;
2. When the active probiotic product was acutely administered, versus placebo, to participants with at least moderate symptoms of depression, group differences in scores on measures of depressive symptoms, general functioning and symptoms of anxiety and stress would be observed;
3. Group differences (active treatment versus placebo) in blood levels of proinflammatory cytokines, hsCRP, vitamin D and BDNF, and scores on a measure of gut function/IBS would also be observed, and levels of these variables may predict or impact on treatment response;
4. Group differences would be observed at the point of the 6-month follow-up between those who continued to take the probiotic and those who discontinued probiotic use;
5. The investigational product would have minimal side effects.

6.2 Summary of Findings

6.2.1 Hypothesis 1: Baseline biomarker levels. In agreement with the first hypothesis, 75% of participants in the current trial who provided baseline blood data had at least one inflammatory biomarker (hsCRP, IL-1 β , IL-6 or TNF- α) elevated outside the normal reference range at baseline. Several inflammatory biomarkers were significantly correlated: hsCRP was significantly correlated with all proinflammatory cytokines (IL-1 β , IL-6 and TNF- α), and IL-6 was also

significantly correlated with IL-1 β and TNF- α . These correlations appear to provide evidence in support of systemic elevations in inflammation in the sample. These results support the existing literature in the field which associates depression with increased levels of proinflammatory cytokines (Dowlati et al., 2010) and (hs)CRP (Copeland et al., 2012; Pasco et al., 2010). As discussed in Chapter 1, a direction of causality has not been firmly established for the association between depression and inflammation, with studies finding associations in both directions (e.g., Copeland et al., 2012; Pasco et al., 2010), and other studies suggesting that the relationship is more complex than simply causal in one direction or the other, with other factors such as childhood adversity being implicated in the relationship (Danese et al., 2008). It has been suggested that activation of the inflammatory response system could be a mediator of environmental risk factors for depression (G. E. Miller & Cole, 2012).

Interestingly, those who had used antidepressant medications in the past had significantly lower baseline TNF- α than those who had not. This could be due to the immunoregulatory effects of antidepressants, particularly SSRIs which have been shown by meta-analysis to have significant effects on proinflammatory cytokines including TNF- α (Hannestad et al., 2011). The participants in this sample confirmed that they had not been taking any antidepressant medication for a minimum of 4 weeks prior to the study; however, the question of whether the immunoregulatory effects of antidepressants may continue after ceasing antidepressant use does not appear to have been addressed by the literature.

Women had higher baseline IL-6 than men. Although many studies have assessed the relationship between IL-6 levels and age, and the effect of gender on this relationship, there have not been any studies which have compared IL-6 levels in men and women in a similar population to the current study, nor in a healthy sample. Age was not correlated with IL-6 levels in either men or women in the current study. It is noteworthy that both the group sizes and the variances were

unequal in the IL-6 by gender analysis, which makes the t-test more prone to Type 1 error (de Winter, 2013), making this result somewhat unreliable.

The levels of inflammatory biomarkers did not correlate with severity on any psychological or IBS outcome measure. Some studies have suggested that levels of inflammatory markers are related to the severity of depressive symptoms (e.g., Alesci et al., 2005), but this study does not provide any evidence in support of this notion. That the current study did not find any association between depression severity and levels of proinflammatory cytokines is consistent with other studies in the literature which have found a general association between depression and raised levels of proinflammatory cytokines, but have not found any correlation with severity of depressive symptoms (e.g., Schlatter et al., 2004). Raison et al. (2006) state that these inconsistencies in the literature suggest that inflammation may contribute to some cases of depression but not others, and advise against strong pronouncements about the role of the immune system in depression.

The mean vitamin D level of the current study sample at baseline was approaching deficient at 58.9nmol/L (SD=24.8), with the normal reference range being 50-150nmol/L. In total, 40% of participants were deficient (<50nmol/L) in vitamin D. This finding is in keeping with the literature which has associated low vitamin D levels with low mood (Anglin et al., 2013). Baseline vitamin D levels were also negatively correlated with baseline DAS scores, indicating that those with low vitamin D levels at baseline were more prone to dysfunctional attitudes as measured on the DAS. The literature examining the relationship between vitamin D and mood appears to mainly focus on vitamin D as a dichotomous variable (deficient versus non-deficient) and the associated risk of developing depression. The few studies which appear to have examined a correlation between vitamin D levels and depression severity have not found a correlation. For example, the study by Jorde et al. (2008) discussed in Chapter 1—investigating supplemented vitamin D as an intervention for low mood in overweight and obese patients—found in their sample that baseline scores on the

BDI were higher in those deficient in vitamin D, but that vitamin D levels did not correlate with depression severity.

In the current study, baseline Vitamin D levels varied depending on season at time of recruitment, with those recruited in winter having lower vitamin D than those recruited in any other season, and significantly lower than those recruited in summer and autumn. This result suggests a seasonal pattern of vitamin D levels, which is likely due to a decrease in sun exposure (and therefore vitamin D absorption via skin synthesis) in winter (Holick, 2007). However, this result is contradictory to some studies which have found no relationship between vitamin D levels and time of year (e.g., Jorde et al., 2008).

6.2.2 Hypothesis 2: Treatment effects of probiotics on psychological

outcomes. Contrary to the second hypothesis, in the RCT phase of the trial, no group differences were observed between the active treatment and placebo groups on any psychological outcome measure over time. The lack of any statistically significant group difference in the RCT phase is supported by the visual analysis of the change in each group on fortnightly outcome measures which did not suggest a differential effect of the active treatment and placebo over time. The lack of group difference on any psychological outcome measure appears contrary to the suggestion of the many narrative reviews on the topic (e.g., Bested et al., 2013b; Cryan & Dinan, 2012; Cryan & O'Mahony, 2011; Dinan & Quigley, 2011; Dinan et al., 2013), beginning with the seminal review by Logan and Katzman (2005) that suggested probiotics as an adjuvant treatment for depression. However, in retrospect, the lack of any effect of probiotics on psychological outcomes is perhaps not surprising given the incipient nature of this area of research and the sparse support from human randomised controlled trials discussed in Chapter 3.

There are many possible reasons for the lack of effect of probiotics on psychological outcomes in the current study. For example, it is possible that the lack of effect observed here is an issue of bacterial strain. Although the study product used has previously shown positive outcomes

for both emotional behaviour in animal studies (Arseneault-Breard et al., 2012; Messaoudi et al., 2010), and psychological outcomes in one human study (Messaoudi et al., 2011), it is possible that different strains, more colony-forming units (CFU) or a broader range of strains combined into a single product may be more effective. Chapman, Gibson, and Rowland (2011) have conducted a non-systematic review on this topic: they state that some human studies have shown that combining a broader range of strains from specific genera can increase the effectiveness of probiotic formulations in altering gut microbiota when compared to monospecies supplements, but whether this is due to synergistic interactions between strains or the additive effects of strain-specific properties (e.g., colonisation of different niches, effects on GI pH range), or merely a consequence of a higher probiotic dose, is unclear from the small number of studies which have investigated this matter to date. Further research in this area is warranted.

It is possible that the current study was underpowered for a clinical sample, given that the previous human probiotics studies which have found changes in mood after supplementation have used healthy population samples (Benton et al., 2007; Messaoudi et al., 2010). The only prior probiotics study to date assessing psychiatric symptoms in a clinical sample was conducted in 65 patients with schizophrenia, and no changes were observed in psychiatric symptoms of schizophrenia in that study (Dickerson et al., 2014). However, change on psychological outcome measures in the present study was remarkably similar in both groups (p-values for change ranged from 0.52 to 0.99), with the placebo group experiencing slightly bigger change on several outcome measures. The lack of any signal in the data suggests that it is unlikely that the lack of any group difference was an issue of power alone. The trial by (Messaoudi et al., 2010) found a moderate effect of probiotics on two mood-related subscales of the HSCL-90 after 30 days of probiotic supplementation. Although this was a trial in a healthy population, given the present study used the same probiotic intervention and almost doubled the intervention period used in the (Messaoudi et al., 2010) study, it did not appear unreasonable to expect that a moderate effect could be observed in the present trial; however, in retrospect, had the severity and chronicity of the sample been

anticipated, then powering for a small effect may have been more appropriate. Whether a small effect would remain clinically significant is another issue that would have to be taken into consideration. Future studies using probiotics could be conducted in mildly depressed samples, as a small effect would then be more likely to remain clinically significant, given that antidepressant drugs are not recommended for mild depression because the risk-benefit ratio is poor (National Institute for Clinical Excellence, 2004): an efficacious treatment with a better side effect profile than antidepressant drugs may be a more appropriate treatment for mild depression even if the effect was small.

It is possible that the results of the current study were restricted by the length of the intervention period. Some studies which have found changes in mood after administration of probiotics have used shorter intervention periods than the present study, including Messaoudi et al. (2010) and Benton et al. (2007) (30 and 20 days respectively); however, both of these studies were conducted on healthy population samples. It is possible that any changes in mood would take longer in those with low mood at baseline, and particularly in those who meet criteria for MDD as 74% of the present study sample did. As mentioned above in the context of statistical power, the severity and chronicity of the sample is another consideration. The mean baseline MADRS score of the sample was at the high end of the moderate range, and 70% of the total sample had been depressed for more than 2 years: it is possible that probiotics may be more effective in shorter term or less severe depression, or that a longer intervention period would be needed to effect change in a chronic, severe sample such as this one. However, there was no group difference between those exposed to the active treatment for 8 weeks versus 16 weeks at the end of the OL phase, which suggests that a longer intervention period may not affect the rate of response to treatment; however, this may have been due to a lack of statistical power, as the power of this analysis was affected by the dropout rate before and during the OL phase. Visually, the changes over the course of the trial in the two groups taking the active product for 8 weeks and 16 weeks look very similar, which supports the lack of any group difference. However, 16 weeks is not a particularly long

intervention period compared to other probiotics studies with intervention periods of 6 months or longer (e.g., Kajander, Hatakka, Poussa, Farkkila, & Korpela, 2005).

The rate of placebo responding ($\geq 50\%$ change on the MADRS) in the RCT phase was very high at 36%. Placebo responses in antidepressant trials have been found by meta-analysis to average around 30% (Walsh, Seidman, Sysko, & Gould, 2002). It is possible that this high rate of placebo responding masked any treatment effects that may otherwise have been observed. The high placebo response was somewhat unexpected given that the RCT phase of the trial was designed to minimise non-specific trial effects by limiting participant-researcher contact, with participants meeting a researcher at baseline and endpoint only. In total, 13% of participants achieved remission (MADRS score < 8) in the RCT phase, with no significant difference between groups. Rates of remission in efficacy trials of individual antidepressant drugs appear to be higher than those observed in the current trial at around 30% (Warden et al., 2007). However, the disparity between the remission rate in the current trial and those usually found in antidepressant trials could be due to the more representative population of the current trial: Warden et al. (2007) state that the high rates of remission seen in antidepressant efficacy trials may not generalise to patients typically seen at clinical practices because these trials often exclude patients with chronic depression or comorbid psychiatric conditions, and that rates of remission in effectiveness studies with more representative populations are usually lower at between 11-30%. The population of the current trial is more closely matched to the populations of effectiveness studies given that the presence of comorbid psychiatric conditions and chronic depression were not exclusion criteria for the current trial, and that the proportion of participants in the present study with chronic depression or at least one other current comorbid psychiatric disorder was high (70% and 52% respectively).

The results of the open label trial also do not support the second hypothesis. Although the group showed significant changes on several outcome measures over the course of the OL phase, given the high placebo response in the RCT phase it is likely that this change is due to placebo

responding. Ten participants responded in the open label phase, with five of these participants being placebo non-responders from the RCT phase. The individual change of these participants on the MADRS over the course of both phases was visually compared, and it was evident that three of these participants had experienced a relatively continuous response gradient over the trial, with a 29-47% change on the MADRS in the RCT phase. The fact that two participants did not show any improvement on the placebo in the RCT phase and went on to respond in the OL phase does not suggest a disparity in response rates between the two phases, as a slight increase in placebo responding is to be expected when participants are aware that they are taking the active product.

6.2.3 Hypothesis 3: Treatment effects of probiotics on biomarkers and gut function, and the impact of these factors on treatment response. Contrary to the first part of the third hypothesis, no group differences (active treatment versus placebo) were found in the level of any blood-based biomarker, or in change in score on the measure of gut function, from baseline to endpoint. The result for BDNF was trending towards being different between the groups ($p < 0.1$), with the active treatment group experiencing a bigger change in BDNF than the placebo group, but this difference was not statistically significant. The rationale for measuring BDNF levels in the current trial was that probiotics have been shown to reduce levels of inflammation, and that BDNF secretion has been shown to be affected by levels of inflammation (A. H. Miller et al., 2009; O'Toole & Cooney, 2008). It was hypothesised that probiotics may affect levels of BDNF through lowering the levels of proinflammatory cytokines, thereby allowing for increased secretion of BDNF. Given this rationale for measuring BDNF as a possible mechanism of action, the lack of any effect of probiotics on inflammatory biomarkers means that the trend towards a group difference in BDNF levels is not supported by other results. Given that the result is not statistically significant, as well as the lack of mechanistic support from other results, it appears likely that the trend observed towards a group difference in BDNF levels is due to chance.

The second part of the third hypothesis states that the levels of biomarkers and scores on the measure of gut function would predict or impact on treatment response. In support of this hypothesis, the exploratory analyses appeared to provide some support for a mechanistic role for vitamin D. These analyses appear to show a moderation effect of vitamin D on treatment response: in the active treatment group, participants with high levels of vitamin D at the beginning of the study experienced significantly more improvement on several psychological outcomes over time than those with low vitamin D at baseline, even after controlling for seasonal variation. Baseline vitamin D had no effect on responding in the placebo group. Given the modulatory effects of vitamin D on the immune system (e.g., Battersby et al., 2012) and its role in immune cell development and function (Griffin et al., 2003), it is possible that the vitamin D status of the host could have an effect on the relationship between the gut microbiota and the immune system and inflammatory responses, as suggested by Ly et al. (2011). If vitamin D does indeed modulate systemic inflammation and the communication between the gut microbiota and the immune system, this provides a plausible explanation for the moderation effect of vitamin D on treatment outcomes in the active treatment group, as a deficiency in vitamin D could therefore be a limiting factor in treatment response to probiotics. However, this result is not supported by any other results in the study. No association was found between vitamin D and the level of any inflammatory marker, and levels of inflammation did not moderate treatment response. Although vitamin D appears to show a robust moderating effect across three measures of psychological outcomes, it is possible that the recurrence of the result across several outcome measures is simply indicative of high inter-correlation among these three measures. Circulating vitamin D levels may also be affected by body mass index (BMI), as vitamin D is fat soluble. We did not measure BMI, meaning that we cannot assess whether BMI had any impact on the moderation effect of vitamin D. However, despite the tenuous nature of this result, given the research associating vitamin D with inflammation and possibly with the gut microbiota, measuring vitamin D levels as a possible moderator of treatment effect may be valuable for future probiotics studies. It is also possible that assessing levels of biomarkers of cell-mediated

immunity (such as IL-2Ra; Maes, 2011) rather than inflammatory markers may provide more insight into the possible mechanisms of this association.

Contrary to the second part of the third hypothesis, no moderation effect was found for any inflammatory biomarker. In addition, controlling for presence and severity of IBS symptoms did not have any impact on the results of the efficacy analyses. The lack of any treatment effects in the RCT phase made examining these factors as mechanisms of action on treatment response problematic.

6.2.4 Hypothesis 4: 6 month follow-up. Overall, in the group who completed the 6 month follow-up survey—who were, as a group, older than those who did not complete the 6 month follow-up questionnaire, and of whom a majority (78%) were those who successfully completed both phases of the trial—there was significant regression in scores on two psychological outcome measures and the measure of gut function from the end of the OL phase of the trial to the point of 6 month follow-up. However, scores on all psychological outcome measures were still significantly improved at the point of 6 month follow-up compared with baseline. The fourth hypothesis, that group differences would be observed at the point of the 6 month follow-up between those who continued to take the probiotic and those who discontinued probiotic use, is not supported by the data from the 6 month follow-up survey. When the group who completed the 6 month follow-up were allocated to groups based on their self-reported dominant treatment since the trial (probiotics/nutrition, standard treatment and no treatment), there were no differences among the three treatment groups at the point of 6 month follow-up. The absence of any group difference is somewhat surprising: almost 50% of the sample who completed the follow-up survey had not continued with any treatment for their depression after the trial, but were not experiencing worse symptoms at the point of follow-up than those who had elected to receive a standard treatment (antidepressant medications or psychotherapy) or those who continued with a nutritional supplement for their low mood.

It is possible that the group not receiving any treatment for their depression after the study were less severe in their depression than the other groups. There are some data which appear to support this possibility: although the MADRS scores of the three groups were not significantly different at the end of OL phase, there was a significant imbalance in the number of participants who met DSM-5 criteria for MDD at baseline among the three groups. The probiotics/nutrition group had the highest proportion of people who had met criteria for MDD at baseline (93%), followed by the standard treatment group (67%) and then the group receiving no treatment after the study (55%). This supports the idea that those receiving no treatment for their low mood at the point of 6 month follow-up had presented as less severe at baseline (in terms of not meeting criteria for MDD) than the other groups. However, if the number of people who met criteria for MDD at baseline is taken as an indicator of the severity of the group, then it should also be noted that the group who elected to receive standard therapy for their depression were less severe at baseline than those who elected to continue with probiotics or another nutritional supplement for their low mood, yet at the point of follow-up there was no group difference between these treatments on any psychological outcome.

When the unadjusted data were presented as repeated measures ANCOVA graphs for the three dominant treatment groups over the course of the whole trial, there did not appear to be any pattern of differential responding among the three groups on most measures. The only psychological outcome measure which appeared to show a slight (although non-significant) pattern was the DAS: on this measure, the probiotics/nutrition group appear to improve continuously throughout the trial, while both of the other groups appear to experience a regression in score from end of OL to the point of 6 month follow-up. The standard treatment group appears to score higher than the other two groups on this measure through the entire trial. That differential responding (albeit with no statistically significant differences or interactions) was visually observed on the DAS and not on any other measure is interesting in light of the results of a recent probiotics trial which was published after the systematic review of Chapter 3. The trial, by Steenbergen, Sellaro, van Hemert, Bosch, and

Colzato (2015), reported that a multispecies probiotic formulation reduced cognitive reactivity to sad mood in a healthy population sample significantly more than the placebo with moderate to large effect sizes (d range=0.63 to 1.56). Cognitive reactivity is the activation of dysfunctional patterns of thinking that are triggered by subtle changes in mood, and play a central role in the development, maintenance, and recurrence of depression (Beck, 1967). The authors state that future studies should test the applicability of their result to clinical and high risk populations. Although the current trial did not find any treatment effect of probiotics on cognitions, given the similarity between the measure of cognitive reactivity and the cognitive outcomes measured by the DAS, it appears that measuring effects on cognition is a plausible way forward for future probiotics studies.

The lack of any difference among the dominant treatment groups at 6 months post-trial suggests that outcomes are similar at this point regardless of treatment chosen. However, these results may be affected by the low response rate to the 6 month follow-up questionnaire, which is discussed in more detail below in the context of the limitations of the current research.

6.2.5 Hypothesis 5: Side effects and feasibility. The final hypothesis was supported by this research: no side effects were observed in the active treatment group that were not also observed in the placebo group. The rates of compliance in the current study were exceptionally good, with 97% of required doses taken. This compliance result may not be entirely accurate: it is possible that participants could have returned fewer sachets than were actually remaining post-trial in order to appear more compliant with treatment (social desirability bias). However, the calculated compliance rate is somewhat supported by the results of the acceptability of treatment questionnaire from the 6 month follow-up survey, where participants reported the sachets to be easy to take and easy to remember. This suggests that this mode of delivery of the intervention (one sachet of pleasant-tasting orodispersible powder per day) may be a practical asset to future probiotic studies, as similarly good rates of compliance may be seen in future studies using the same product.

6.3 Research Strengths

The main strength of the current study was the rigorous study design of the first phase, which was placebo-controlled, and was double-blinded and randomised using an appropriately thorough method. The method of the study was prospectively registered on the Australia New Zealand Clinical Trials Registry, ensuring that the original, pre-specified primary outcome measures, methods of statistical analysis, and other key design features of the study were not changed after registration. The efficacy analyses were completed by an independent statistician after the database was locked. These measures were put in place with the aim of removing any potential bias.

Another methodological strength of the current study was the inclusion of an open label extension, which ensured that all participants had the option to receive the active study product after the first phase while maintaining the double blind. It also allowed for the comparison between the two groups, to ascertain whether exposure to the probiotics for a longer period (16 weeks; twice the length of the RCT phase) resulted in any differential effect on psychological outcomes when compared with those who had only taken the active product for 8 weeks in total. Following participants up at 6 months post-trial allowed for comparisons between those who continued to take a probiotic product and those who discontinued probiotic use after the study. It also allowed for comparisons between probiotics and the current standard treatments for depression.

The broad inclusion criteria are a strength of the current study as they add external validity to the results. People with comorbid psychiatric disorders were not excluded from the current study unless there were concerns for the safety of the participant. This led to a relatively varied sample with better generalisability to the general population, given the high co-occurrence between depression and other psychiatric disorders, particularly anxiety disorders (Kessler et al., 2003).

There were several strengths in the implementation of the current study. Firstly, the use of the online method of monitoring participants over the course of the trial ensured a low level of

missing data, whilst also limiting participant-researcher contact during the trial, thereby potentially minimising non-specific trial effects. The high rate of compliance is also a strength of the current study, and was likely achieved because of the convenient mode of delivery of the study products, which was one sachet of orodispersible powder per day. The high compliance rate observed in the current study suggests that this mode of delivery may potentially be valuable for future probiotics studies. The bacteria in the study product were also freeze-dried, meaning that the bacteria were protected from heat and stomach acid, thereby increasing the likelihood of survival through the GI tract and rendering the final study product room temperature stable. The survival of the study product bacteria were confirmed by bacterial enumeration at the end of the study. Finally, the high level of participant retention is a strength of the trial, with 87% of participants retained until the endpoint of the RCT phase. Factors which may have contributed to this high level of participant retention include the thorough and comprehensive informed consent process, and the small number of visits to the university required by the study design that allowed minimal disruption to the lives of participants whilst ensuring that participants could always contact a researcher if necessary.

6.4 Research Limitations

Although this research employed a rigorous design, with numerous measures taken to reduce bias and ensure reliable results, some limitations are inevitable in research, particularly when researching a clinical population. Some of the possible limitations of the current trial are discussed below.

6.4.1 Oversights in data collection. A major limitation of the current study is that blood samples were not taken in the morning after fasting overnight, which is the method typically used for cytokines due to their circadian rhythm. Circulating T-cells and the production of proinflammatory cytokines peak during the night, whilst the production of anti-inflammatory cytokines peaks during the day (Lange, Dimitrov, & Born, 2010). This oversight may have affected the biomarker outcomes in unpredictable ways, as blood sample collection was undertaken at the

baseline meeting, for which time of day was not restricted. Another limitation of this study is that it did not assess dietary intake or physical activity levels, both of which may affect levels of inflammation (Berk et al., 2013), so it was not possible to control for possible influences that these variables may have had on the final results. As mentioned above, measures of BMI and body fat percentage were not taken, making it impossible to assess whether body weight had any impact on results, particularly on the moderation effect of vitamin D.

6.4.2 Contact with researchers/ therapeutic input. Improvements observed in psychological outcomes in the current study may have been influenced by the therapeutic input involved in research trials. Although the design of the current trial aimed to minimise this input through monitoring participants via online questionnaires rather than in person over the study period, participants may have experienced therapeutic input through contact with the researcher at the beginning of the trial. At the baseline meeting, participants attended a personal interview which included in-depth assessment of symptoms, discussion of the participant's history of low mood, and empathic responses from the researcher. Although researchers were trained to minimise therapeutic input while still gathering the necessary information (participants did not receive any psychological strategies during their appointments, with visits being purely assessment focused) participants could have experienced a reduction in symptomology simply through contact with the researcher (the therapist effect). However, a majority of participants had experienced therapeutic interactions with a health professional prior to the study without experiencing significant improvement in mood. Contact with the researcher was kept to a minimum, with the initial interview generally lasting less than an hour after informed consent, which included the completion of all self-report and clinician-rated measures. However, as mentioned above, the therapist effect may have contributed to the high rates of placebo responding observed in the current trial.

6.4.3 Dropout rate post-randomised phase. Although participant retention was high in the RCT phase of the trial, only 49 participants (62%) completed the open label extension.

One possible reason for the increased dropout rate in the open label phase may be that participants were more likely to discontinue the study product if they did not experience any benefit during the open label phase, when they were aware that they were taking the active study product.

Only 58% of participants completed the 6 month follow-up questionnaire, which limits the generalisability of these results because non-response bias cannot be ruled out: for example, those who did not complete the survey may have been systematically better or worse in terms of depressive symptoms than those who did complete the survey, or more of those participants who did not reply to the follow-up survey may have used standard treatments such as antidepressants since the study.

6.6 General Implications

There has been considerable recent interest in the scientific literature in the possible links between the gut and depression, and the possibility of an interventional role for probiotics in mental health, particularly for low mood. However, as discussed in Chapter 3, the evidence for an interventional role for probiotics in low mood is sparse, and comes mainly from studies in animals and very few human trials. The main implication of the systematic review of the research presented in Chapter 3 is that there is little evidence to support a mental health application for probiotics, and that much care should be taken not to overstate the possible mental health applications of probiotics without supporting evidence from well-controlled clinical trials in populations presenting with elevated scores on psychological outcome measures.

The current trial investigating probiotics as an intervention for psychological outcomes in a sample presenting with low mood did not find any evidence suggesting a treatment effect of probiotics on psychological outcomes; therefore, the theory that probiotics may have antidepressant properties in a population presenting with low mood is not supported by the current research. Of course, the negative outcome of the current trial does not disprove any theory; however, it does

show that, despite preliminary evidence from animal trials and one healthy population human trial suggesting a possible mental health application for this particular probiotic formulation, acute administration of this probiotic for eight weeks does not affect psychological outcomes in a population presenting with low mood. Thus, the current trial begins the process of investigating the possibility of mental health applications for probiotics in clinical samples, but there is much work still to be done to investigate whether this avenue of investigation is worth pursuing, or whether we are merely repeating the mistakes of the past: are we on the cusp of a major clinical breakthrough, or are we “merely in the quicksand of auto-intoxication II?” (Bested et al., 2013b; p.1).

6.7 Directions for Future Research

Despite the lack of any effect of probiotics on psychological outcomes in the present study, future studies in this area should not be discouraged and should seek to broaden and grow the area in order to further develop our understanding of these complex and interrelated topics. Future research should test this application of probiotics in different populations (e.g., less severely depressed, less chronically depressed, or presenting with elevated anxiety rather than low mood), possibly with different and/or a broader range of bacterial strains (preferably those which have shown potential in preclinical trials using animal models of the psychological outcome being investigated) and for a longer intervention period.

The limitations of the current study should be addressed in future research: future studies should use a regular morning fasting blood sampling procedure; measures of BMI, and if possible body fat percentage, should be taken; and measures of dietary intake and physical activity may also be beneficial. Given the associations among vitamin D, gut health and inflammation, measuring vitamin D levels as a possible moderating factor may be valuable for future probiotics studies in any health condition. Faecal sampling may be beneficial in future probiotics studies, to confirm whether the probiotic product is effectively altering microbial balance in the gut and for how long this persists after discontinuation of the probiotic supplement.

The elevated levels of inflammatory biomarkers observed in the current sample provide support for theories of the inflammatory nature of major depressive disorder, which is the premise behind recent research investigating anti-inflammatory drugs for depression, such as the trial by Raison et al. (2013) investigating infliximab, a TNF- α antagonist, for depression. This avenue should be explored further, in particular testing this drug in larger randomised controlled trials in depressed patients with elevated levels of inflammatory biomarkers at baseline. The difference in TNF- α levels between those who had a history of antidepressant usage and those who had never taken antidepressants provides an interesting avenue for research that does not appear to have been addressed by the literature: is it possible that the immunoregulatory effects of antidepressants endure beyond the discontinuation of the drug, and if so, how long are inflammatory pathways affected?

It is possible that probiotics may be more effective as an adjuvant treatment for depression (as suggested by Logan and Katzman, 2005), rather than as a stand-alone treatment. Trials investigating the possibility that probiotics may enhance the treatment effects of antidepressant medication or psychological therapy may be useful, implementing a 2x2 factorial design to test probiotics versus placebo and the standard treatment versus placebo/no therapy. Future studies may also consider probiotics in combination with other nutritional treatment strategies, rather than with the current standard treatments. For example, given the strong associations between diet and depression (e.g., Jacka et al., 2010) there has been a movement towards whole diet interventions for the prevention and treatment of depression (such as the PREDIMED study; Sánchez-Villegas et al., 2013). Given that a healthy gut microbiota is important to the proper absorption and synthesis of nutrients from diet, it is possible that adding a probiotic supplement to future whole diet interventions could enhance the effects of these interventions on depression outcomes. Similarly, priming participants with a probiotic supplement to correct microbial imbalances before supplementing with micronutrients or polyunsaturated fatty acids may be valuable.

It is also worth considering that prevention is better than cure: longitudinal preventative trials giving probiotics in infancy, possibly to those at risk of gut dysbiosis (e.g., those born by caesarean section or bottle fed in infancy), which record any impact on mental health outcomes in later life, may prove more valuable. Pärty, Kalliomäki, Wacklin, Salminen, and Isolauri (2015) recently conducted a trial of this nature—providing probiotics or a placebo for 6 months in infancy and following the group longitudinally—and found a group difference in the occurrence of ADHD and ASD at 13 years of age. If the investigators have ethical approval and funding to follow these participants into adulthood, evaluating rates of episodes of depression between the groups may prove invaluable to the field.

6.8 Conclusion

The current study was the first randomised controlled trial to assess whether probiotics could affect mood and other psychological outcomes in a sample presenting with low mood. It is possible that the lack of effect of probiotics on psychological outcomes is due to the length of the intervention period and the severity and chronicity of the sample. Although it is likely that poor gut health contributes to increased inflammation and immune activation, which may in turn contribute to the development of depression, it is possible that probiotics are not able to reverse these changes in a severe sample in a relatively short time period. However, we must also face the possibility that probiotics may not be an effective treatment for the symptoms of depression. The considerable impact of gut health on systemic health (e.g., rates of atopic dermatitis and allergies; Ly et al., 2011), and likely on mental health (Cryan & Dinan, 2012), does not necessarily indicate an interventional role for probiotics. However, being the first study of its kind to investigate this theory in a sample presenting with low mood, it is important that the results of this trial are not interpreted as a definitive answer. Future studies in the area should attempt to further broaden this field, in particular by recruiting samples with mild or non-chronic depression for interventional studies, or by approaching probiotics as a preventative or adjuvant treatment strategy for depression.

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Appendices



Appendix 1. Participant information sheet and consent form.

INFORMATION SHEET: March 7th 2013, URA/12/05/013

Information Sheet for Randomized Phase

Title of research: An RCT investigating the efficacy of a probiotic supplement in the treatment depressive symptomatology, and its effects on related biomarkers.

Local Investigators: Assoc Prof Julia Rucklidge, Dr Roeline Kuijer & Amy Romijn, PhD student
Department of Psychology, University of Canterbury
Private Bag 4800, Christchurch, 8140
Phone: 03 364 2987 ext 7705

What is the purpose of the study?

You are invited to participate in a study which will evaluate the effect of a probiotic supplement on depressive symptoms. The study will also measure the levels of specific biological indicators (chemicals in the blood) which are involved in depression. These indicators will be measured by collecting blood samples at the beginning and end of the study. The release of the chemicals is a normal response of your body to a condition of stress and depression.

There is a need for new treatments which can act on the symptoms of depression, either to provide complimentary treatments or practical alternatives to antidepressant medication. The supplement being studied here has been shown to have beneficial effects for participants suffering from stress in a recent study. Stress is a significant factor in depression, which supports the theory that this supplement might also be useful in the treatment of depression. Depression has been linked with gut inflammation, which may also be improved by probiotics. Other probiotics have shown positive effects on depression, but this is a relatively new area of research and so needs more solid evidence with human subjects to increase the reliability of these effects. For this reason, the supplement will be tested using a randomised double-blind placebo-controlled trial (RCT) - the most scientifically reliable method of testing.

The supplement being tested is called **Probio'Stick**, and contains two different kinds of bacteria which have known beneficial effects in the human body. This product is manufactured by Institut Rosell-Lallemand - a company which specialises in the development and production of probiotics for use in human nutrition. The manufacturer has provided the supplement free of charge, and is providing some funding towards the costs of running the study. However, the institute is not involved in the study design, application, analysis or publication, and none of the investigators are employed by the company.

What is involved in the study?

You have already been screened for this study using a web-based procedure, which included some brief questions, demographic information and eligibility screens including the completion of a questionnaire to assess your level of depressive symptoms. This initial screening has provided us with an overall assessment of you and your current level of depression. You are eligible for this study because you are experiencing symptoms of depression but are not currently being treated with psychiatric medications. Approximately 80 people in Christchurch will be recruited to participate in this study. Since you are eligible to participate in our study, you have been invited to the Department of Psychology of the University of Canterbury to review the information sheets, provide your written consent to participate, and to give you the opportunity to ask any questions you may have about the study.

If you would like to participate, you will undergo an initial assessment. This will involve:

1. A short interview here today with one of the investigators, which will further assess your depressive symptoms and your history of depression
2. The completion of some computer-based questionnaires.
3. The collection of a blood sample from you. The tissue will be disposed of in a standard manner by the community laboratory which will process the tissue to obtain test results, and will not be stored after the results are obtained. Participants may choose to have a karakia at disposal of samples.

Each person who participates in this study will have a 50% chance of receiving either Probio'Stick or a placebo (an inactive substance which looks, smells and tastes the same as the active one). This method of randomizing is an important part of the standard method used in controlled trials. After the first eight weeks, everyone will be offered the opportunity to take Probio'Stick for the following eight weeks. This part of the study, in which everyone will know that they are taking Probio'Stick, is called an open-label extension. If you choose to continue with the open-label extension, you will be asked to sign a new consent form at that time. The following consent form is devoted to the **first eight weeks only** (the randomized phase). Each phase of the study is expected to last eight weeks, but accommodations may be made for some circumstances if the need arises (e.g., if an appointment needs to be re-scheduled due to illness or a booked holiday).

During the randomized phase in first eight weeks, the study will be carried out with complete 'blinding'. This means neither participants nor investigators will know which participants are taking Probio'Stick or placebo. This double-blinding procedure is another important aspect of a controlled trial. However, **your safety** is of the utmost importance, so, in the event of an emergency (i.e. should you experience serious symptoms after taking the supplement), you should go immediately to the emergency services. The emergency room personnel will be able to call the number on your stick bag to obtain information about the study and the contents of the sticks. In addition, if your treating physician is concerned at any time about your health and safety, it is possible to find out which group you are in (placebo or active strain) so that this information can be used to assist in your care.

Once the initial assessment is completed, we will give you the product you will need for the duration of the trial. During the trial, you will be asked to take one probiotic stick each day **during or just after breakfast** (or the evening meal). The sticks are in the form of a pleasantly sweet tasting orodispersible powder, which can be taken without water. A list of

the probiotic strains and ingredients in Probio'Stick, as well as the ingredients in the placebo formula, is provided at the end of the consent form. This product is room temperature stable for some time, but to ensure the best level of stability we ask that you store it **in the refrigerator**. During the 8 week period, you will be monitored every fortnight via the internet, using the same questionnaires as used at baseline. These questionnaires will take about 45 minutes to complete each time. You will also be asked to keep a daily diary where you can describe any unusual events in your life and to **log any sticks that you miss**.

At the end of this phase you will be asked to attend a re-assessment, which will be similar to the initial assessment, and will involve the collection of a new set of blood samples. We will be happy to provide you with feedback on the results of the testing should you so wish.

Can I carry on as normal whilst involved in the study?

You will be asked **not** to try any alternative medicines or other forms of therapy until your involvement in this study is complete. In addition, you are strongly encouraged to avoid alcohol, marijuana, caffeine, nicotine, and street drugs throughout the study, as these substances may decrease the potential benefits of the treatment. You will be asked to estimate your use of these substances.

If you need to take an antibiotic or antifungal agent orally for a health problem, please contact one of the investigators as it may be necessary for you to withdraw from the study for the time it takes you to complete the course of the drug. This is because antibiotics and antifungal drugs seem to interfere with the absorption of this probiotic supplement.

Members of all cultures will be encouraged to participate in the study. Respect for Maori customs and traditions are of the highest priority and, if necessary, your appointment at the university can include a cultural advisor. The researchers are available to discuss the research with the whanau to assist in developing their understanding of the clinical disorders and how the disorders can impact on te taha hinengaro (mental wellbeing), whanaungatanga (family relationships), taha wairua (spiritual wellbeing) and taha tinana (physical wellbeing).

What are the risks?

We have no reason to suspect that this supplement can harm a physically healthy individual in any way, as there have been no adverse events identified during the previous studies which were not observed in the placebo group. Furthermore, AxPharma pharmacovigilance has not reported any adverse events. No interactions have been shown with other medicines, but you will be invited to note if you are taking any other medications or dietary supplement. While risks associated with blood tests are small, minimal bruising can occur.

Will I benefit if I take part?

There may or may not be a direct medical benefit to you. Your depressive symptoms may be improved during the study, but there is no guarantee that this research will help you. The information we obtain from this study may, however, help us to provide better treatments for patients with depression in the future.

Do I have to participate?

No. If you decide not to participate in this study, or if you decide to withdraw part-way through, you are completely free to do so. This decision will not influence your ongoing healthcare in any way. Similarly, the study investigators might choose to end your

participation in the study at any time for any reason. If new information becomes available that might affect your willingness to participate in the study, you will be informed as soon as possible.

Will I be paid for participating, or do I have to pay for anything?

At each visit, you will receive a \$10 petrol voucher to cover the costs of transportation to and from the University. The sticks that you take during the study will be provided at no cost.

Will my records be kept private?

All information collected in this study will remain strictly confidential. The only people who will have access to the information are the study investigators and designated staff. We are very careful in dealing with confidential information – any information you disclose will be kept in a confidential file, which will be kept in a locked filing cabinet at all times. This data will be stored for 10 years after collection, in accordance with University regulations. With your permission, data from this study may be used in future related studies, which have been given ethical approval from the Health and Disability Ethics Committee. However, all information will be kept as group data - forms will be coded and names removed so that no individual participant can be identified. Confidentiality will be respected and **no material which could personally identify you will be used in any reports on this study**. However, in cases where we are concerned about your safety or the safety of others, we may decide to breach confidentiality.

The results of the tests described above will be used for research purposes only, in the context of this study. We would need your permission and signed consent to send these test scores to another professional involved in your care. We recommend that a psychologist or physician interpret the results of these tests. During this study, it may be necessary for a member of the research team to look at your previous medical records. Please be assured that this will also be handled in a confidential manner. This research is being conducted as a part of a student qualification (PhD in Psychology). A PhD thesis is a public document which will be stored in the library database of the University of Canterbury.

What happens after the study?

At the end of the 8 weeks, you will be invited to participate in an 8 week open-label extension. This means that you will know that you are taking Probio'Stick. Should you decide to participate, we will review consent with you at that point and provide you with a new information sheet. Only at the end of the trial (i.e. when all participants have finished) will you find out whether you were taking the active substance or the placebo.

If I suffer a research-related injury, will I be compensated?

In the unlikely event of a physical injury as a result of your participation in this study, you may be covered by ACC under the Injury Prevention, Rehabilitation and Compensation Act. ACC cover is not automatic, and your case will need to be assessed by ACC according to the provisions of the 2001 Injury Prevention Rehabilitation and Compensation Act. If your claim is accepted by ACC, you still may not get any compensation - this depends on a number of factors. ACC usually provides only partial reimbursement of costs and expenses and there may be no lump sum compensation payable. There is no cover for mental injury unless it is a result of physical injury. If you have ACC cover, generally this will affect your right to sue

the investigators. If you have any questions about ACC, please contact your nearest ACC office, or one of the investigators.

If you have any queries or concerns regarding your rights as a participant in the study you can contact an independent health and disability advocate. This is a free service provided under the Health and Disability Commissioner Act. Telephone (NZ Wide) 0800 555 050, Free Fax (NZ wide): 0800 2787 7678 (0800 2 SUPPORT), Email (NZ wide) advocacy@hdc.org.nz. You can also contact Dr. Julia Rucklidge, the principal investigator, 364-2987 ext. 7959, should you have any questions or concerns about this research. The Human Ethics committee at the University of Canterbury, and the Southern **Health and Disability** Ethics Committee have reviewed and approved this study. We have also consulted with Te Komiti Whakarite.

CONSENT FORM
March 7th 2013, URA/12/05/013

Title of research project: An RCT investigating the efficacy of a probiotic supplement in the treatment depressive symptomatology, and its effects on related biomarkers.

Investigators: Dr. Julia Rucklidge (PI), Amy Romijn, Dr Roeline Kuijer

I have read and understood the information sheet dated March 7th 2013 for participants in the study on the effects of a probiotic formula on depressive symptoms. I have had the opportunity to discuss this study with the investigators and I am satisfied with the answers I have been given. I have had the opportunity to use whanau support or a friend to help me ask questions and understand the study. I understand that taking part in this study is voluntary (my choice). I also understand that I may withdraw from the study at any time, and that this will in no way affect my continuing or future health care. I understand that I may also withdraw any information already provided. I understand that I can request a karakia at the point of disposal of my tissue samples.

I am aware that Institut Rosell-Lallemand, the manufacturer of Probio'Stick, has offered to provide the supplement free of charge and provide some funding towards the costs of running the study. However, the institute is not involved in the design, application, analysis or publication of the study. None of the investigators or research assistants are employed by the company. This research project has been reviewed and approved by the University of Canterbury Human Ethics Committee, and the Southern **Health and Disability** Ethics Committee.

I understand that my participation in this study is confidential and that no material which could personally identify me will be used in any reports on this study. I understand that the treatment, or investigation, will be stopped if it should appear harmful to me. I understand the provisions which will be made for the reimbursement of expenses involved in this study. I have had time to consider whether or not I would like to take part. I know who to contact if I have any side effects due to the study, or if I have any questions about the study.

I consent to supply blood samples as indicated YES/NO

I request a karakia at disposal of sample YES/NO

I wish to receive a copy of the results

YES/NO

A significant delay may occur between data collection and publication of the results.

I agree to my GP or other current provider being informed of my participation in this study/the results of my participation in this study, and being provided with any laboratory reports obtained for the purposes of this study

YES/NO

I consent to **being contacted** approximately 8 weeks following the initial assessment for a review, regardless of whether I decide to discontinue with the supplement/placebo. At the time of the review, I can decide whether I wish to participate in this review.

YES/NO

I consent to **being contacted** approximately 6 months following the initial assessment for a review, regardless of whether I decide to discontinue with the supplement/placebo. At the time of the review, I can decide whether I wish to participate in this review.

YES/NO

I consent to my name being placed in a separate database so that I can be contacted in the future should there be other studies which I might like to participate in, with the understanding that I can choose whether to participate in such studies or not.

YES/NO

I consent to the use of my data for future related studies, which have been given ethical approval from a Health and Disability Ethics Committee

YES/NO

I hereby consent to participate:

Name of Participant

Signature of Participant

Signature of person who gained consent

Date _____

The person who may be contacted about the research is: **Dr. Julia Rucklidge, Principal Investigator, 364-2987 ext 7959.** *A signed copy of this consent form has been given to you to keep for your records and future reference.*

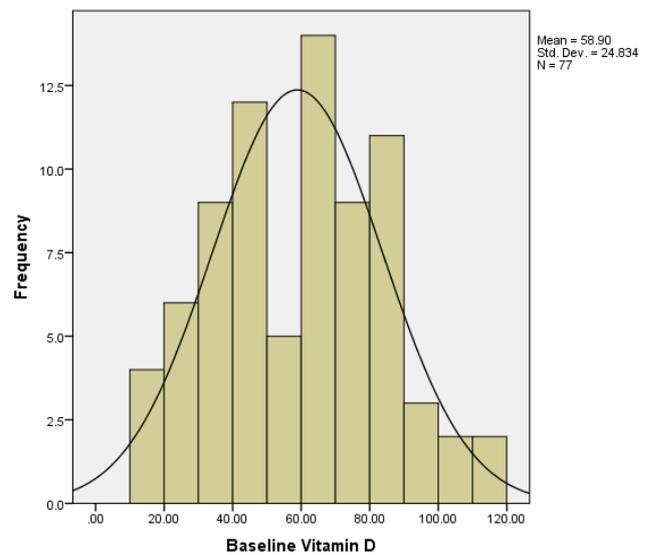
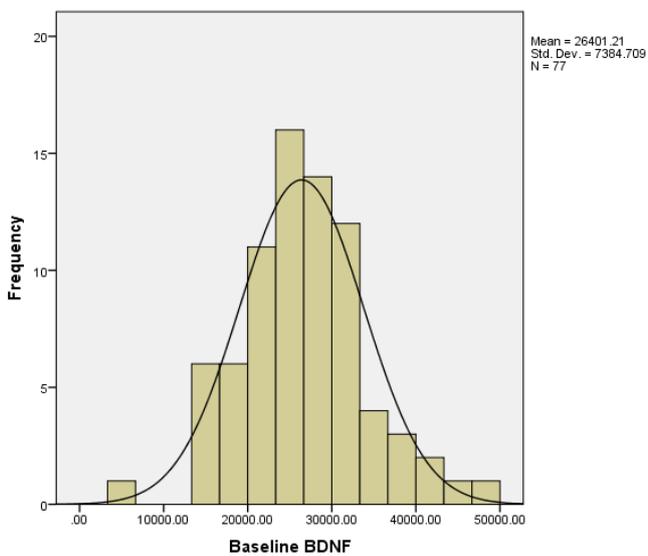
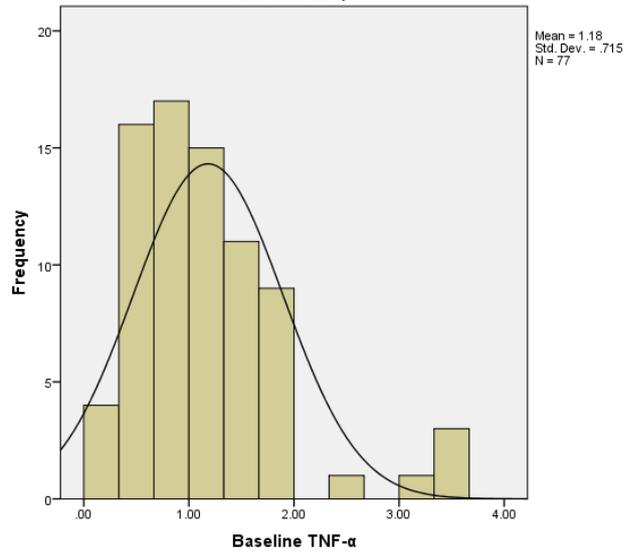
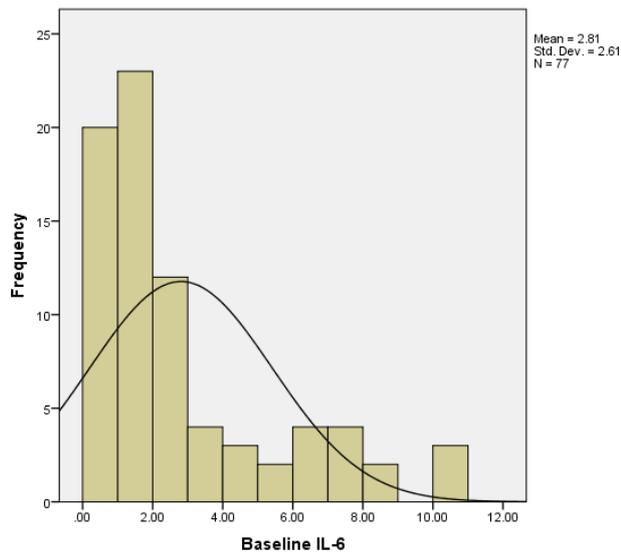
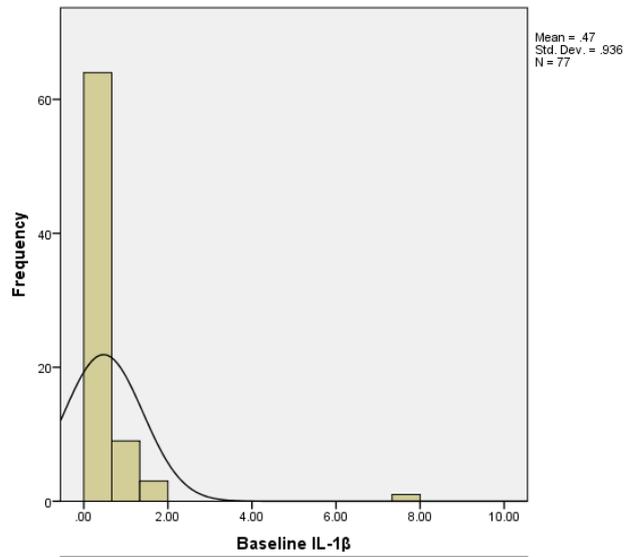
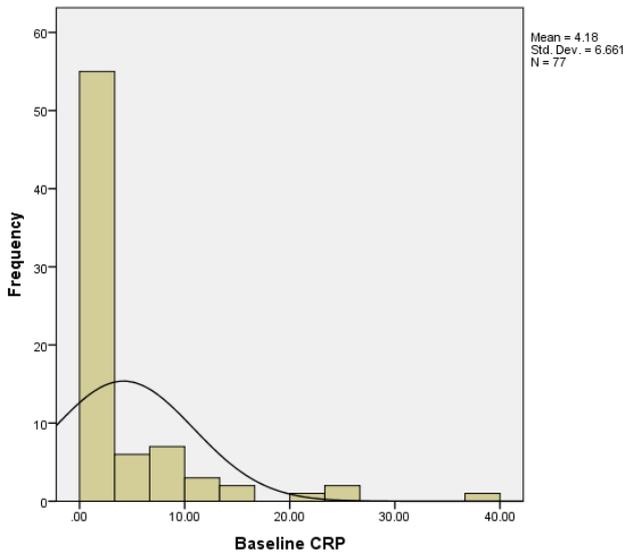
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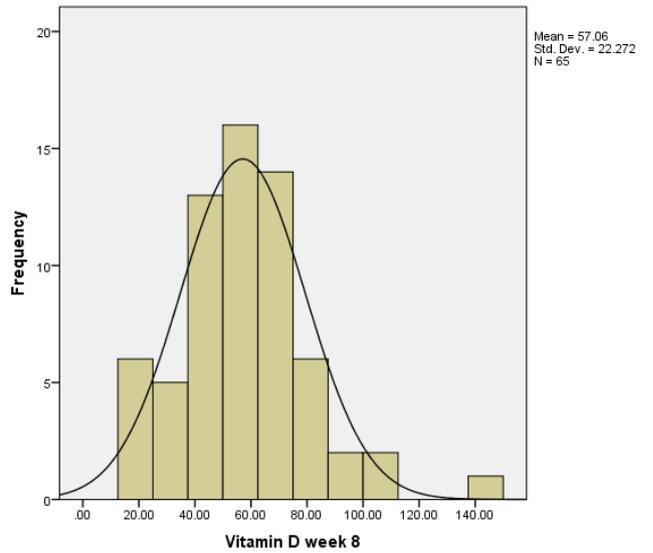
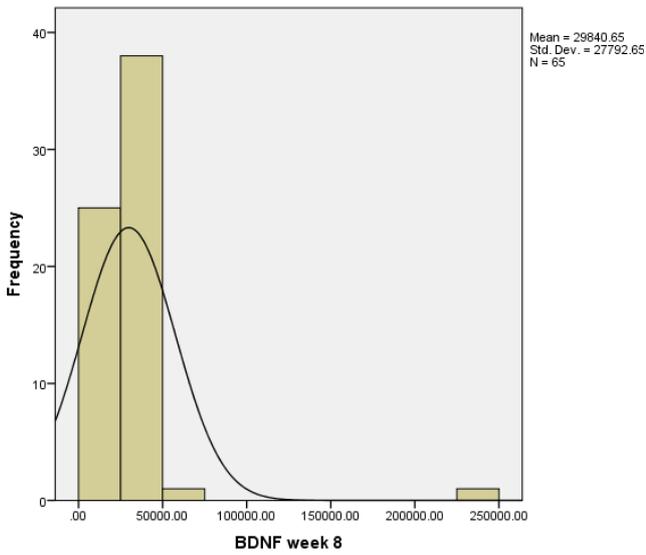
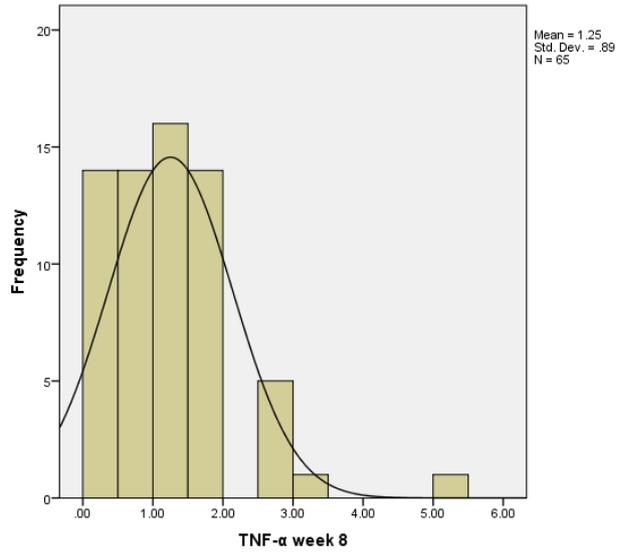
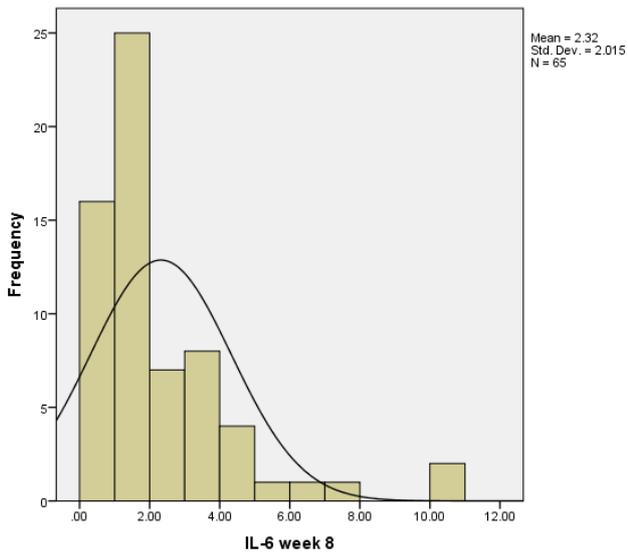
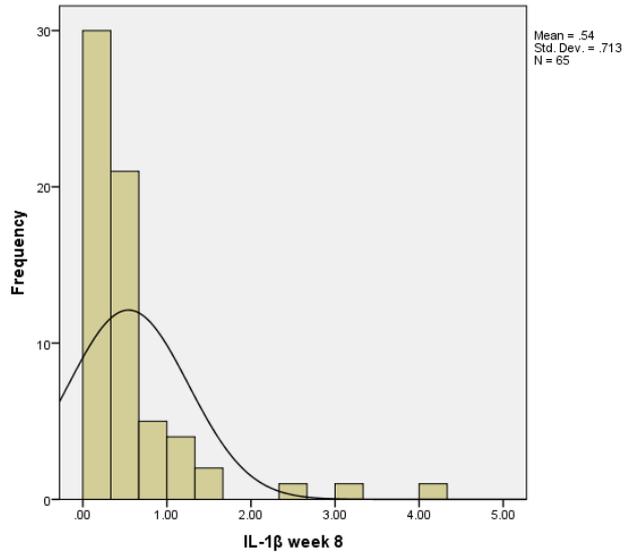
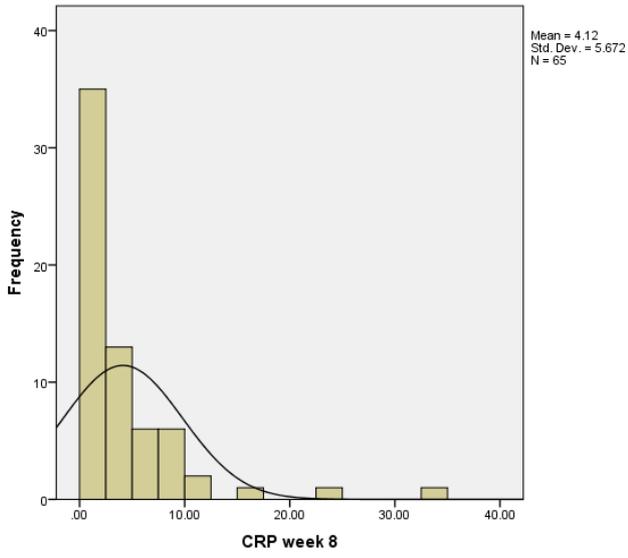
Strains	Per stick (1,5 g)
<i>Lactobacillus helveticus R0052</i> <i>Bifidobacterium longum R0175</i>	3 x 10 ⁹ colony-forming units/stick
<u>Excipients</u> : xylitol, maltodextrin, plum flavour, malic acid	

Placebo formula

Ingredients
<u>Excipients</u> : xylitol, maltodextrin, plum flavour, malic acid

Appendix 2(a). Histograms of biomarker data before log₁₀ transforming.





Appendix 2(b). Histograms of biomarker data after log₁₀ transforming.

