Author's response to reviews

**Title:** Reducing Occupational Stress with a B-Vitamin Focussed Intervention: A Randomized Clinical Trial - Study Protocol

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**Author's response to reviews:** see over
Response to reviewers comments: Reducing Occupational Stress with a B-Vitamin Focussed Intervention: A Randomized Clinical Trial - Study Protocol

We thank the reviewer’s for their comments, and acknowledge that attention to each of their suggestions will make for a stronger manuscript. We have replied to each of their comments below.

Editor’s comment: Title page: Please include a title page as page 1. The title page should include the title, authors, author affiliations and corresponding author details.

- Each of these details appears on the cover page.

Reviewer's report
Title: Reducing Occupational Stress with a B-Vitamin Focussed Intervention: A Randomized Clinical Trial - Study Protocol
Version: 1
Date: 19 September 2014
Reviewer: Jolanta Gromadzinska
Reviewers's report:
In my opinion manuscript should be divided into 2 parts, and only this version will be acceptable for me.

1. Review of existing publications concerning long-lasting occupational stress and their consequences for health of employees and role of vitamins, metabolites and genetic factors on quality of life and development of pathologies.

As requested, the manuscript is now clearly divided into the ‘review’ of the literature, and the study protocol sections.

Page 5. To improve the flow of the manuscript we have added a new section with the title “Dietary Vitamins in the Central Nervous System”. This section now reviews general mechanisms of dietary antioxidants and B Vitamins. We have moved paragraphs from the first draft describing B Vitamin mechanisms into this section.

Dietary Vitamins in the Central Nervous System
The central nervous system is dependent upon the nutrients supplied through a good, varied, healthy diet. Good nutrition is imperative, particularly for maintaining the brain’s structure and normal cognitive function [5]. Vitamins and micronutrients play a significant biochemical role in maintaining cognitive processes within the brain. The role of antioxidants and B vitamins contained in food and their relationship to maintaining cognitive processes and general health is discussed below.

i. Antioxidants
Fresh fruits, green vegetables and berries contain a rich source of vitamins A, C and E. These food sources contain dietary antioxidants that can prevent, inhibit or repair damage caused by oxidative stress [5, 6]. Oxidative stress is representative of an imbalance or disruption in the redox state (oxidation/reduction reactions) within cells causing impaired signalling and regulation, resulting in impaired functioning [7]. The maintenance of redox homeostasis is essential for healthy cellular function.

Antioxidant vitamin C levels are particularly high in the brain and are needed for the transformation of dopamine into noradrenalin as well as the production of some neurotransmitters [5]. Vitamin E is a lipid soluble antioxidant which has
neuroprotective effects against free radical damage, preventing cellular injuries to the brain related to oxidative stress [8]. Vitamins E and A protect against lipid peroxidation, a damaging process affecting the permeability of cell membranes [9]. Vitamin C works synergistically with B group vitamins and is vital for the metabolism and utilization of folate/folic acid [10].

i. B Vitamins
B group vitamins can be found in a wide range of foods like whole grains, bananas, beans and meats. Folate/folic acid, B6 and B12 represent some of the B group vitamins. Folate, B6 and B12 vitamins are known to contribute to the regulation of healthy levels of the amino acid HCY, and share a synergistic role in the maintenance of cardiovascular and neural health, and are also vital for energy metabolism [5, 8, 9]. Cross sectional and prospective studies have identified that elevated plasma HCY levels is associated with the promotion of spontaneous cell death (apoptosis), incidence of stroke, brain atrophy, Alzheimer’s disease, bone fracture and is considered a risk factor for cerebrovascular disease [11, 12].

Folate/folic acid, B6 and B12 vitamins are essential for the methylation of HCY to methionine in the central nervous system. Methionine plays a crucial role in one-carbon metabolism; biological processes for DNA synthesis, repair and other methylation reactions [13]. If HCY is not sufficiently converted back to methionine, the methylation process will be inhibited resulting in a build-up of HCY. An elevated level of HCY increases the likelihood for oxidative stress, leading to negative events like mitochondrial membrane damage and DNA strand breakage [14, 15]. The role of HCY in disease pathogenesis remains unclear. HCY could play a direct role in the disease process or be simply a marker of folate, B6 and or B12 vitamin deficiency. However, research has identified that chronic stress depletes vitamin B6 [16] while supplementation with B6 vitamins could be a therapeutic strategy in reducing stress [17]. Therefore, one mechanism by which B group vitamin intervention may aid the reduction of stress and fatigue ratings of those in full time employment is through the uninhibited clearance of HCY.

In addition, neuroimaging studies suggest that high levels of plasma HCY and low levels of B vitamins are linked to a higher incidence of brain atrophy and degeneration [18, 19]. This type of data suggests that HCY plays a role in brain ageing, by contributing to subclinical brain changes in what we would believe to be an otherwise healthy population.

2. Publication of original study describing the role of group B vitamins on occupational stress and quality of life performed according to protocol described in submitted manuscript.

An additional heading and introduction has been added to define the beginning of the protocol section of the manuscript.

Study Protocol: Reducing Occupational Stress with a B-Vitamin Focussed Intervention

Given the growing incidence, cost and pervasive effects of stress in the modern workforce, the primary objective of this research is to determine whether
administration of B-vitamins reduces occupational stress (or strain), burnout, and the cost of absenteeism in a population of older workers with high levels of occupational stress. We will examine the relationship between B-vitamin supplementation, workplace stress, cognitive, personality and mood measures, cardiovascular (brachial and aortic systolic and diastolic blood pressures as well as arterial stiffness), biochemical (assays to measure inflammation and safety) as well as genetic assessments (to assess stress processing) and neuroimaging measures (to investigate in vivo mechanisms of action of B vitamins) to identify the mechanisms through which B-vitamin supplementation may improve the well-being of Australian workers.

**Methodology**

The primary aim of this study is to investigate 6 month effects of a B vitamin formulation (Blackmores® Executive B Stress Formula) on mood and workplace stress in a sample of full time, healthy employees. The secondary aim is to examine the mechanisms underpinning any mood or workplace stress enhancing actions of B vitamins by examining relationships between cognitive, biological (biochemical, genetic and brain metabolites) and cardiovascular variables over 6 months. By examining the interrelationship between inflammation, cardiovascular health and cognitive performance, the current study aims to identify modifiable risk factors for workplace stress that can be targeted by supplementation. It is hypothesised that 6 month supplementation with Executive B Stress Formula will improve measures of workplace stress, relative to placebo with the greatest effects observed at 6 months. It is also expected that there will be an improvement in general health factors, cognitive performance, biological variables and cardiovascular health. The primary
psychological outcome measure is the difference between the B-vitamin groups and placebo group over time on the total stress/strain score of the PSQ from the OSI-R. The primary physiological outcomes will be the differences levels of HCY in blood plasma. The secondary outcome measures are the differences between the B vitamin group and placebo group in mean changes over time on: the other OSI-R Questionnaires and their subscales; (2) the GHQ-12, the state-trait anxiety and mood measured by the Profile of Moods Scale (POMS).

The methods state that workers who report feeling stressed will be recruited but this is not clear in the abstract.

First sentence has been changed to reflect that workers who report feeling stressed will be recruited.

Will target sample size recruited allow for dropouts?

The target sample size is will be reached when 200 participants have completed the study, thus it will not need to utilise linear mixed modelling to account for missing data

– particularly important given that ANOVAs are proposed to analyse outcomes, which do not factor in missing data (NB linear mixed modelling is a better method for factoring in missing data)

See above.

Page 12 states that participants are screened for eligibility on their first visit whilst the flow chart states that they will be screened on the phone (preferable to making them come in fasted before eligibility determined!)

We thank the reviewer for noticing this inconsistency. The sentences now read: “Eligible participants are required to attend two testing sessions and commit to completing five online questionnaires. An overview of the testing sessions is provided in the clinical trial flow chart (Figure 1). Participants are initially screened over the telephone for eligibility prior to their first visit. During the first visit participants are again screened for eligibility and for compliance to the study day restrictions which were described above”.

Minor (examples)
Page 4 typo: ‘to make a stress-related workcover claims’

This has been corrected.

‘This data’ should be ‘these data’

This change has been made.

Page 5 first para, last sentence – more effective than what?
Specifically, high doses of B vitamins have been suggested to be effective in improving mood states in both clinical and non-clinical populations.

These sentences have been altered for clarity: "Our research centre has recently examined whether a popular multivitamin supplement available in Australia, (Blackmore’s Executive B Stress Formula) containing a complex of mostly B group vitamins, improved work related stress (the only study to address work stress variables)[23]. The duration of administration of the multivitamin in this study was 90 days, a significant increase in comparison to previous studies of 30 days of multivitamin administration [24-26]. Sixty participants, recruited from the community, completed the 3-month, double-blind, randomized, placebo-controlled trial in which personality[27], work demands, mood, anxiety and strain were assessed. The primary analysis revealed that the Vitamin B group reported significant reduction in Personal Strain (p=0.02), from weeks 4 (M 92.10, SE 2.44) to week 12 (M 85.54, SE 2.27), while the placebo group showed a significant increase in levels of strain from week 4 (M 88.84, SE 4.33) to week 12 (M 93.44, SE 4.03)."

This sentence has been reworded: “Therefore, it is suggested that consumption of vitamin B12 will address the accumulation of HCy, and in turn, alleviate the associated neurological, sensory and motor system symptoms”.

Participants section. This is now on Page 12. We have clarified the standard drinks for men and women. It now reads:

“... current regular alcohol use exceeding 14 standard drinks per week for women or 28 standard drinks per week for men, ..."