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Effects of vitamin supplementation on inflammatory markers and psychological wellbeing among distressed women: a randomized controlled trial

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ABSTRACT

BACKGROUND: Multivitamins are a popular supplement taken to promote physical and mental health. During periods of stress, they may have a protective role for health and wellbeing, although the current evidence of their efficacy is mixed.

OBJECTIVE: To determine whether multivitamin supplementation impacts psychological and inflammatory markers of women who are experiencing psychological distress.

DESIGN, SETTING, PARTICIPANTS AND INTERVENTIONS: An 8-week randomized controlled trial was conducted to assess changes in both psychological state and pro-inflammatory markers of patients receiving multivitamins or placebo. The sample comprised women who reported elevated psychological distress in the previous 4 weeks.

MAIN OUTCOME MEASURES: Psychological state was assessed using Spielberger’s State-Trait Personality Inventory to assess anxiety, curiosity, depression and anger. Pro-inflammatory markers comprised interleukin (IL)-1β, IL-5, IL-6, tumour necrosis factor (TNF)-α and TNF-β.

RESULTS: Improvements across time were observed for all psychological measures and cytokines, except IL-5, but were independent of the active intervention. Only TNF-β demonstrated a significant differential change between groups over the course of the
intervention, in favour of multivitamin supplementation (active group mean rank decreased from 11.1 to 7.1; placebo group mean rank decreased from 8.9 to 7.8).

**CONCLUSION:** The results suggest that administration of multivitamins was not effective in improving psychological state. However, some evidence supported the positive impact of multivitamin supplementation on pro-inflammatory cytokine profiles of women currently experiencing stress.

**TRIAL REGISTRATION:** The trial was registered in the Therapeutic Goods Administration (BR040502).

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1. Introduction

Psychological wellbeing refers to the balance between an individual’s biopsychosocial resources and the biopsychosocial challenges [1]. The use of vitamin supplementation to enhance wellbeing is an increasing trend worldwide, and multivitamins are particularly popular [2–4], which usually comprise a mixture of antioxidants, micronutrients and minerals in a variety of combinations and dosages. Evidence suggests both physiological and psychological factors can impact their supplementation. Antioxidants have been shown to increase suboptimal nutrient status in healthy older adults [5], while deficiencies in key micronutrients such as vitamin B12 and folic acid have been linked to poor mood state in men [6–8]. Improved ratings of stress, mental health and vigour have also been observed in multivitamin-supplemented men over an eight-week period [9].

Blake-Mortimer and colleagues [10–12] have proposed two biochemical mechanisms which may serve to explain the role of antioxidant and nutrient levels on both pro-oxidant and pro-inflammatory markers. Firstly, periods of psychological distress result in a prolonged release
of cortisol and subsequent elevated activation of immune cells, specifically neutrophil, as part of the innate stress response. Excess neutrophils lead to the generation of oxidants. In regular circumstances, neutrophils are beneficial with levels fluctuating as needed. In excess, however, free radicals deplete (oxidize) the body’s antioxidant stores, damaging biological molecules and key cellular components, and compounding the oxidative imbalance. Secondly, during prolonged psychological distress, lymphoid tissue becomes desensitized to the ability of cortisol to down-regulate the stress response. Repeated activation of the hypothalamic-pituitary-adrenal axis leads to interconnected systems producing by-products to compensate for dysfunction in other systems (i.e., decreasing lymphocytes compensated by increased pro-inflammatory processes). This pathway specifically results in inflammation. Indeed, growing evidence suggests that inflammation may be a link between psychological distress and poor health [13]. Some cytokines, such as interleukin (IL)-5, primarily control and contain immune responses. Others, such as IL-6 and tumour necrosis factor (TNF)-α, induce inflammation [14]. Psychological distress and depression reliably increase inflammation in both naturalistic and laboratory contexts. Conversely, pro-inflammatory cytokine administration induces “sickness behaviours”; these are behavioural changes that resemble somatic symptoms associated with depression, such as anhedonia and lethargy [15]. Evidence suggests that pathways between negative moods and inflammation are bidirectional [16]. Meta-analyses show depression to be reliably associated with elevated inflammatory markers (e.g., IL-6 and IL-1) and changes in immune cell numbers [17,18], which are indicative of a pro-inflammatory reaction. More recently, evidence has suggested that emotional symptoms (affective feelings, cognitions and physiological arousal) are partly products of inflammation, gut dysbiosis, oxidative stress and compromised mitochondrial energy production [1]. Further, a systematic review by Rucklidge and Kaplan [19] finds support for the use of broad-based multivitamin formulas for the treatment of a wide range of symptoms ranging from depression to psychological distress to psychiatric and antisocial behaviours. The current trial aimed to build on the observational study of Hapuarachchi et al. [12] noted above. Their cohort (N = 43; 23 men, 20 women), which reported chronic psychological distress, was divided into high (multivitamins three or more times weekly) and low (did not use vitamins) antioxidant groups. The high antioxidant group was found to have significantly lower indications of oxidation and pro-inflammatory response as measured by C-reactive protein. However, self-reported vitamin use was not standardized in terms of content, dosage, or duration. Such variation would likely have affected outcomes. For example, duration of
supplementation has been shown to influence both in vivo markers of inflammation and measures of mood even over short-dosage durations such as 6 weeks [20,21] and 33 days [9]. Further, both men and women were included [12]. Yet the inflammation reactions of men and women may vary due to hormonal differences in response to stress [22]. Postmenopausal hormonal changes may also influence the inflammatory profile [23]. In general, studies of women in this area are rare, despite the suggestion that nutrient deficiencies in the 19 to 34 age range are common [24]. The focus of this study was governed by this evidence, along with the public belief that vitamin supplements have value for managing stress. In contrast, the current trial comprised a randomized controlled trial (RCT) of a targeted group of pre-menopausal women, controlling for the level of stress and duration and dosage of supplementation. Further, inflammatory cytokines rather than oxidative markers were evaluated. These comprised IL-1β, IL-5, IL-6, TNF-α and TNF-β. Psychological responses (i.e., anxiety, anger, depression and curiosity) were also recorded. While anxiety and depression are common psychological indicators of wellbeing, maladaptive effects of anger are traditionally emphasised as a contributor to the aetiology of neuroses and depression and linked to the pathogenesis of hypertension. In contrast, curiosity contributes to effective personal judgment and successful adaptation to environmental stimuli [25]. This trial tested the utility of a widely-available multivitamin supplement for women from the general population experiencing psychological distress.

2. Materials and methods

2.1. Participants

Women aged 25 to 45 were invited to participate via newspaper advertisements, posters and radio interviews in Adelaide, South Australia. The trial was approved by the University of Adelaide Human Research Ethics Committee and was registered in the Therapeutic Goods Administration (BR040502). All participants were given written informed consent. Eighty-one women contacted the principal researcher (JOB) and were screened using the short-form General Health Questionnaire (GHQ-12) [26]. Participants were included if they reported moderate to severe psychological distress (≥ 3). This cut-off is considered optimal for determining non-psychotic psychiatric disorder in community settings [27,28]. Exclusion was on the basis of self-reported infection (e.g., colds and flus) or inflammatory disease (i.e., heart disease or diabetes), autoimmune disease (e.g., rheumatoid arthritis, Addison’s disease, and Cushing’s disease), severe psychotic disorders or cancer, use of immunosuppressive
medication (e.g., cortisone), blood-thinning agents (e.g., warfarin), or vitamin supplements, pregnancy or current breastfeeding. Any irregularities in full blood examinations (FBEs) at baseline were reported to participants, who were excluded from the trial and referred to their medical practitioner.

2.2. Randomisation and blinding strategies
Following assessment, the remaining women were allocated to the active or placebo group using a computer-generated block randomization technique. The principal researcher, responsible for all data collection, was blinded to allocation until results were analysed. Supplement manufacturers were blinded as to who the participants were and had no contact. All staff (e.g., biochemists) who undertook pre- and post-intervention assays were blinded to group allocation, demographic and psychological outcomes for the entire study.

2.3. Multivitamin supplement
The manufacturer provided identical capsule containers numbered sequentially to implement the random allocation sequence. Participants allocated to the treatment (active) group received an 8-week supply of Blackmores Women’s D-Stress® multivitamin supplements. The combination of ingredients (Table 1) is suggested by the manufacturer, to balance systems in the body that respond to stress and replace nutrients depleted during chronic stress that protect cells from free radical damage and oxidative stress. Further details of this product can be obtained from the Australian Register of Therapeutic Goods [29]. This supplement contains low-risk levels (active, excipient and homoeopathic preparation ingredients) of each vitamin or mineral, indicated to be at least 25% of the Australian recommended dietary intake (RDI) as specified by Australian Regulatory Guidelines from the National Health and Medical Research Council [30]. Doses were consistent with this indication. The placebo group was provided with an 8-week supply of placebo capsules comprising unreactive ingredients (a formulation of calcium dihydrogen phosphate). At the completion of the trial the manufacturer provided information to determine only group membership (i.e., group A or group B). Change in folate levels was assessed as an adherence check. Participants were also requested to return supplement containers after intervention as an adherence check.

Table 1. Composition of the multivitamin supplement (per tablet).

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>RDI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin B1 (thiamine nitrate) 12.5 mg</td>
<td>1.1 mg/d</td>
</tr>
<tr>
<td>Vitamin B2 (riboflavin) 12.5 mg</td>
<td>1.1 mg/d</td>
</tr>
<tr>
<td>Nicotinamide (niacin) 25 mg</td>
<td>35 mg/d</td>
</tr>
<tr>
<td>Vitamin B5 (calcium pantothenate) 37.5 mg</td>
<td>4 mg/d</td>
</tr>
<tr>
<td>Vitamin B6 (pyridoxine hydrochloride) 25 mg</td>
<td>1.3 mg/d</td>
</tr>
<tr>
<td>Vitamin B12 (cyanocobalamin) 25 µg</td>
<td>2.4 µg/d</td>
</tr>
</tbody>
</table>
Biotin 37.5 µg 30 µg/d†
Folic acid 150 µg 400 µg/d
Vitamin C (ascorbic acid) 75 mg 45 mg/d
Magnesium oxide (magnesium 62.5 mg) 109 mg 310 mg/d
Zinc amino acid chelate (zinc 6 mg) 30 mg 8 mg/d
Withania somnifera (winter cherry) extract (equivalent to dry root 1.5 g) Not available†

RDI: recommended dietary intake.
* These data are adequate intakes as RDI cannot be determined.
† Withania somnifera is a traditional Ayurvedic medicine used in times of stress.

2.4. Data collection
Baseline data collection was conducted prior to randomization. Self-reported demographic and health behaviour measures were sought at baseline only. Standardized psychological measures and biomarkers (blood assays) were collected both pre- and post-intervention. Data collection occurred at the same time of day (between 11:30 and 14:00) to control for possible circadian fluctuations of markers.

2.5. Measures
2.5.1. Demographic and health behaviour measures
Standard demographic information (e.g., age, marital status, education and employment details) and the following health behaviour data were recorded at baseline. The hazardous alcohol use subscale of the WHO Alcohol Use Disorders Identification Test (AUDIT) [31] provides an accurate measure of alcohol risk across gender, age and culture [32]. Scores range from 0–12 with higher scores indicating more hazardous alcohol use patterns (i.e., no alcohol-free days and binge drinking). Smoking status (smoker or non-smoker) was determined using the assessment of dependence and motivation to stop smoking [33].

2.5.2. Screening
The GHQ-12 [26] was used only for screening (as described above). Participants use a 4-point scale (“not at all,” “same as usual,” “more than usual” and “much more than usual”) to describe their experience of each item. The dichotomous scoring method was employed (range 0–12) and provided sound internal reliability (α = 0.88).

2.5.3. Psychological measures
Three state constructs from the State-Trait Personality Inventory (STPI) [34] were included as outcome measures. Anxiety, depression and curiosity are considered major indicators of psychological distress and wellbeing, and the STPI is a reliable and valid tool with which to assess these constructs [25]. Each comprises 10 items which are assessed on a 4-point scale (“almost never,” “sometimes,” “often” and “almost always”), with summed scores ranging from 10 to 40. The internal consistencies (α) of the baseline data for the total sample were
0.86 (depression), 0.87 (anxiety) and 0.81 (curiosity). The distribution of the fourth construct (anger) demonstrated a substantial floor effect and is therefore not reported.

2.5.4. Laboratory blood assays

FBEs and folic acid assays were conducted in Southpath Pathology, Adelaide and complied with the standards of the National Pathology Accreditation Advisory Council [35]. Serum cytokine levels for IL-1β, IL-5, IL-6, TNF-α and TNF-β were measured by the Immunology Department of the Women’s and Children’s Hospital, Adelaide. Samples were stored at –20 °C. Cytokines were measured by fluorescent cytokine-capturing beads with the assistance of the BD™ Cytometric Bead Array Flex Set System (Becton Dickinson, California). Serum samples were diluted to 40 pg/mL, according to the requirement of the BD array system [35].

2.6. Data analysis

Data were analysed using SPSS (version 22.0). An a priori sample size calculation for the interaction between group and time (the effect of key interest) was conducted using G*Power 3 [36]. For α of 0.05 and power of 80%, it was determined that a small change in cytokine levels (effect size of $\eta^2_p = 0.02$; equivalent to Cohen’s $f = 0.14$) would require 30 participants per group. However, due to the potential for skewed distributions in the observed variables, assumptions of normality were investigated prior to final analysis. Substantial skew was evident among both psychological and pro-inflammatory cytokines. Transformations were not performed so as not to interfere unnecessarily with “real life” patterns [37]. For example, cytokine levels reflected normal patterns of inflammation. Rather, baseline checks for randomization were conducted using Mann-Whitney tests, while the Scheirer-Ray-Hare extension of the Kruskal Wallis test [38,39] was utilized in place of standard 2 (group) × 2 (time) analysis of variance and analysis of covariance (ANCOVA) (controlling for folic acid intake). It is acknowledged that this decision conflicts with the sample size calculation described above. However, such conflict will operate against hypothesis. That is, H is a more conservative test than F (power is reduced). Median and interquartile range (IQR) for raw data are reported, but all variables were converted to ranks for analysis. H values were then calculated for each factor and interaction term.

3. Results

Fifty women completed both baseline and post-intervention assessments (Fig. 1), representing an 80% completion rate. Post-intervention data were collected approximately 8 weeks after they started taking the supplement (median = 63.3 days, range: 48–83). Post-
intervention data collection did not differ between the study groups ($U = 409.0, P = 0.54$). Non-completion was predominantly due to loss of interest and scheduling difficulties. All available data from participants were analysed in the groups to which they were allocated, regardless of whether the intervention was followed.

![Fig. 1. CONSORT flowchart of trial recruitment, allocation, and participation.](image)

### 3.1. Randomization check

Randomization produced balanced groups in terms of demographic and health variables (Table 2). Overall, the majority of the sample were tertiary-educated (80%), working full-time (72%), living with partners (78%) and had children (64%). Participants reported low levels of both alcohol and cigarette consumption. The median rank age of participants (active = 40, IQR = 10; placebo = 38, IQR = 11; $U = 254.5, P = 0.47$), and median alcohol use (active = 4, IQR = 4; placebo = 4, IQR = 2; $U = 242.5, P = 0.32$) were also equivalent.

Importantly, median GHQ scores, which were the basis of study inclusion, were balanced across the groups (active = 29.13, IQR = 6; placebo = 31.85, IQR = 8; $U = 409.0, P = 0.54$). Comparisons of folic acid and both psychological and pro-inflammatory markers also suggested group equivalence at baseline (Table 3).

### Table 2. Demographic and health behaviour characteristics.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Active group ($n = 30$)</th>
<th>Placebo group ($n = 30$)</th>
<th>$\chi^2$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Partner ($n, yes %$)</td>
<td>20 (66.7)</td>
<td>19 (63.3)</td>
<td>0.07</td>
<td>0.79</td>
</tr>
<tr>
<td>Children ($n, yes %$)</td>
<td>22 (73.3)</td>
<td>19 (63.3)</td>
<td>0.31</td>
<td>0.58</td>
</tr>
<tr>
<td>Tertiary-educated ($n, yes %$)</td>
<td>18 (60.0)</td>
<td>22 (73.3)</td>
<td>1.20</td>
<td>0.27</td>
</tr>
<tr>
<td>Full-time work ($n, yes %$)</td>
<td>19 (63.3)</td>
<td>17 (56.7)</td>
<td>0.28</td>
<td>0.60</td>
</tr>
</tbody>
</table>
Table 3. Baseline comparison of active and placebo groups.

<table>
<thead>
<tr>
<th>Item</th>
<th>Active group (n = 30)</th>
<th>Placebo group (n = 30)</th>
<th>U</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nutrient biomarker</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Folic acid (nmol/L)</td>
<td>26.6 (15.4)</td>
<td>28.3 (16.7)</td>
<td>404.5</td>
<td>0.86</td>
</tr>
<tr>
<td>Psychological measures</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anxiety</td>
<td>19.5 (6.0)</td>
<td>21.0 (9.0)</td>
<td>441.0</td>
<td>0.22</td>
</tr>
<tr>
<td>Curiosity</td>
<td>24.5 (6.0)</td>
<td>24.0 (6.0)</td>
<td>427.5</td>
<td>0.68</td>
</tr>
<tr>
<td>Depression</td>
<td>18.5 (10.0)</td>
<td>19.0 (7.0)</td>
<td>425.5</td>
<td>0.86</td>
</tr>
<tr>
<td>Inflammatory markers</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-1β</td>
<td>25.5 (65.2)</td>
<td>37.0 (56.4)</td>
<td>375.5</td>
<td>0.49</td>
</tr>
<tr>
<td>IL-5</td>
<td>7.6 (4.3)</td>
<td>8.0 (5.3)</td>
<td>392.5</td>
<td>0.67</td>
</tr>
<tr>
<td>IL-6</td>
<td>12.3 (10.5)</td>
<td>18.3 (9.9)</td>
<td>379.0</td>
<td>0.40</td>
</tr>
<tr>
<td>TNF-α</td>
<td>15.6 (16.6)</td>
<td>17.2 (16.9)</td>
<td>390.0</td>
<td>0.48</td>
</tr>
<tr>
<td>TNF-β</td>
<td>9.9 (5.0)</td>
<td>9.0 (7.0)</td>
<td>420.0</td>
<td>0.82</td>
</tr>
</tbody>
</table>

Data were showed as median (IQR). IL-1β: interleukin-1β; IL-5: interleukin-5; IL-6: interleukin-6; TNF-α: tumour necrosis factor-α; TNF-β: tumour necrosis factor-β; IQR: interquartile range.

3.2. Adherence check

Folic acid was assessed for differential change over the trial that was presumed to be attributable to the intervention (Table 4). While there were overall significant effects for both group and time, of more importance are any significant interaction effects demonstrating an increase in folic acid over time among the active group, relative to the placebo group, suggesting that the intervention was effective. Therefore, analyses of change are reported first without, and then with, folic acid as a covariate.

Table 4. Evaluation of change in nutrient biomarkers.

<table>
<thead>
<tr>
<th>Item</th>
<th>Baseline (Med (IQR))</th>
<th>Post-intervention (Med (IQR))</th>
<th>ANOVAs (H value) †</th>
<th>ANCOVAs (H value) †</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Active group (n = 22)</td>
<td>Placebo group (n = 28)</td>
<td>Group</td>
<td>Time</td>
</tr>
<tr>
<td>Folic acid (nmol/L)</td>
<td>27.1 (13.9)</td>
<td>28.2 (16.7)</td>
<td>8.02*</td>
<td>1.15***</td>
</tr>
<tr>
<td>Anxiety</td>
<td>19.0 (4.0)</td>
<td>21.0 (9.0)</td>
<td>0.75</td>
<td>11.59***</td>
</tr>
<tr>
<td>Curiosity</td>
<td>25.0 (7.0)</td>
<td>24.0 (6.0)</td>
<td>0.00</td>
<td>5.64**</td>
</tr>
<tr>
<td>Depression</td>
<td>18.0 (9.0)</td>
<td>19.0 (7.0)</td>
<td>0.07</td>
<td>10.70***</td>
</tr>
<tr>
<td>IL-1β (pg/mL)</td>
<td>44.6 (86.5)</td>
<td>51.9 (57.4)</td>
<td>0.91</td>
<td>16.47***</td>
</tr>
<tr>
<td>IL-5 (pg/mL)</td>
<td>7.6 (2.9)</td>
<td>8.0 (5.1)</td>
<td>0.01</td>
<td>0.00</td>
</tr>
<tr>
<td>IL-6 (pg/mL)</td>
<td>17.2 (23.4)</td>
<td>18.4 (10.4)</td>
<td>1.16</td>
<td>6.50**</td>
</tr>
<tr>
<td>TNF-α (pg/mL)</td>
<td>16.0 (21.5)</td>
<td>18.2 (17.4)</td>
<td>2.88</td>
<td>5.68**</td>
</tr>
<tr>
<td>TNF-β (pg/mL)</td>
<td>11.1 (13.8)</td>
<td>8.9 (6.6)</td>
<td>0.32</td>
<td>5.14*</td>
</tr>
</tbody>
</table>

ANOVA: analyses of variance; ANCOVA: analyses of covariance; IL-1β: interleukin-1β; IL-5: interleukin-5; IL-6: interleukin-6; TNF-α: tumour necrosis factor-α; TNF-β: tumour necrosis factor-β; Med: median; IQR: interquartile range.
† P < 0.10, * P < 0.05, ** P < 0.01, *** P < 0.001.
†† Results are based on non-parametric ANOVAs/ANCOVAs using the H distribution.

3.3. Change in psychological variables

Improvements to psychological wellbeing were observed, with Table 4 indicating significant time effects for all psychological markers. That is, regardless of group, there was a significant
decrease in self-reported anxiety (active group: median from 19.0 to 16.0; placebo group: median from 21.0 to 18.5), and depression (active group: median from 18.0 to 15.0; placebo group: median from 19.0 to 15.5). There was also a significant increase in curiosity post-intervention (active group: median from 25.0 to 27.0; placebo group: median from 24.0 to 27.0). ANCOVA results, which included folic acid as a covariate, largely replicated these effects, although curiosity became non-significant.

3.4. Change in pro-inflammatory markers

Table 4 also includes analyses for pro-inflammatory markers. With the exception of IL-5, all pro-inflammatory cytokine levels decreased across the course of the trial, regardless of group. These changes were IL-1β (active group: median from 44.6 to 13.3; placebo group: median from 51.9 to 23.4), IL-6 (active group: median from 17.2 to 8.2; placebo group: median from 18.4 to 12.8), TNF-α (active group: median from 16.0 to 10.8; placebo group: median from 18.2 to 15.7), and TNF-β (active group: median from 11.1 to 7.1; placebo group: median from 8.9 to 7.8). However, TNF-β also showed a significant interaction effect, indicating differential change across time between the groups. That is, larger decreases were observed for TNF-β in the active group (median from 11.1 to 0.1 post-intervention) than in the placebo group (median from 8.9 to 7.8 post-intervention). ANCOVAs with folic acid, as a covariate, replicated these results, although with evidence of greater discrimination. For example, two additional interaction effects (TNF-α, P = 0.06; IL-1β, P = 0.08) approached significance. As with TNF-β, these indices decreased more in the active group (TNF-α: median from 16.0 to 10.8 post-intervention; IL-1 β: median from 44.6 to 13.3) than in the placebo group (TNF- α: median from 18.2 to 15.7 post-intervention; IL-1 β median from 8.9 to 7.8).

4. Discussion

The aim of this study was to determine whether participating in an 8-week multivitamin supplementation program would improve psychological wellbeing and/or lower pro-inflammatory markers among women reporting moderate to high levels of stress. Participants were effectively randomized, providing comparable groups at baseline. Psychological improvement was observed for all participants across the trial regardless of allocation to the active or placebo group. Specifically, significantly lower anxiety and depression and concurrent higher curiosity levels were identified. Further, significantly different trajectories of change (or trends toward same) were observed for TNF-β, TNF-α and IL-1 levels, suggesting that lower pro-inflammatory responses tended to be exhibited by those consuming
multivitamins in this trial. Fundamental cellular processes modulated by TNF-related cytokines include inflammation, host defence, maintenance of the secondary lymphoid organs (e.g. lymph nodes) and generation of an efficient humoral immune response to various pathogens [40]. Recently this family of cytokines has been targeted therapeutically to treat autoimmune and inflammatory diseases such as rheumatoid arthritis and Crohn’s disease [41,42].

Beyond these observations, however, findings were inconclusive as to whether multivitamin supplementation was beneficial. This is not an uncommon result, with studies of multivitamin supplementation for improving psychological wellbeing yielding mixed findings. Stough et al. [43] completed a 90-day RCT of Blackmores’ Executive B Stress Formula®, reporting a positive effect for depression-dejection but not state anxiety. It is notable that these results were for a mixed gender sample. However, in an RCT similar to the current research, Haskell et al. [44] found day-to-day functioning for women, as measured by occupational stress, improved in their active group receiving 9 weeks of a high antioxidant, B-vitamin composition.

To the extent that the current research identified trajectories of change among pro-inflammatory cytokines, such changes may be explained by their ability to interfere with the hypothalamic-pituitary-gonadal axis down-regulation of local inflammatory responses [15,45]. One active ingredient in the test compound that is proposed to have this influence is *Withania somnifera*, which is steroidal in nature, and has demonstrated antioxidant-like effects in animal stress models [46,47].

In general, however, it should be noted that research concerning the benefits of multivitamin supplementation is complex [48]. For example, it is recommended that only biologically relevant doses be considered in healthy populations [49,50]. Exceeding the levels of RDIs should be carefully monitored for adverse side effects. Notably, levels of minerals and nutrients in the supplement used by Haskell et al. [44] were considerably higher than RDIs. These comments identify the need for pragmatic rather than explanatory trials of supplements, allowing such constraints to be acknowledged [51]. While explanatory trials are no doubt effective in evaluating proof of concept, pragmatic trials, such as the approach taken in the current research, using realistic levels of multivitamins, test real-world effectiveness. The use of relevant participant groups in non-clinical settings, may be more appropriate in this context.

The current trial was more rigorous than previous similar work [12] by selecting a single sex sample of women, screening for an adequate level of stress, controlling for the dose of
multivitamin supplementation and duration by providing active and placebo capsules, and assessing compliance by blood assay of folic acid levels. However, the strongest effects reported involved improvement over time irrespective of randomization. This placebo effect may be a function of expectancy, clinical attention or a combination of these. That is, improvement, particularly in psychological state, may have been associated with expectations of the effect of the “supplement” and/or the positive therapeutic alliance forged with the trial personnel. Similar research also points towards possible placebo effects. For example, a 6-week trial, comparing groups taking multivitamins, B-complex vitamins, placebo and a no-treatment control, found no significant difference among stress levels of participants administered multivitamin, B-complex vitamin or the placebo, but those in the control condition reported more depression symptomatology [20]. Interestingly, in the current study the physiological markers moved in concert with psychological states. Future studies may benefit from a non-intervention group, such as a wait-list control, to further disentangle such placebo effects. Whilst non-specific placebo effects are a clear candidate contributing to the observed pattern, there are other possible contributing factors which should be considered. The nature of the GHQ could explain this pattern, as this measure captures symptoms which represent differences from an individual’s normal functioning, hence the observed pattern may be explained by return to normal functioning. Similarly, other causes behind changes in nutrient biomarkers which were not assessed, e.g., dietary intake, may underlie the observed changes.

The study was constrained by low statistical power. While ample participants were initially recruited, the inability to maintain the required number to the end of the trial reduced its effectiveness. Further reduction in power was caused by the need to analyse the data using the more conservative H distribution rather than F. Nevertheless, one inflammatory marker achieved significance and two provided encouraging statistical trends, suggesting that additional participants may have led to even more positive outcomes.

5. Conclusions

Improvements in inflammation may be attributable to multivitamin supplementation, as evidenced by lowered TNF-β, TNF-α and IL-1. However, improvement by way of decreased inflammation and enhanced psychological wellbeing was not constrained to the active group. The reported data reflect an intervention using a readily-available supplement that offered some evidence of efficacy in a pragmatic trial.
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Competing interests

The authors declare that there are no conflicts of interest.

References


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Assessed for eligibility (N = 81)

Excluded (n = 21)
- Failed inclusion criteria (n = 15)
- Declined to participate (n = 5)
- Other reasons (n = 1)

Randomized (n = 60)

Allocated to placebo group (n = 30)

Allocated to active group (n = 30)
- Received allocated intervention (n = 30)
- Did not receive allocated intervention (n = 0)

Lost to follow-up (n = 8)
- Loss of interest (n = 6)
- Pregnancy (n = 1)
- Nausea (n = 1)

Lost to follow-up (n = 2)
- Loss of interest (n = 2)

Analysed (n = 22)

Analysed (n = 28)