



# *Effect of 4 weeks daily wild blueberry supplementation on symptoms of depression in adolescents*

Article

Accepted Version

Fisk, J., Khalid, S., Reynolds, S. and Williams, C. (2020) Effect of 4 weeks daily wild blueberry supplementation on symptoms of depression in adolescents. *British Journal of Nutrition*, 124 (2). pp. 181-188. ISSN 0007-1145 doi: <https://doi.org/10.1017/S0007114520000926> Available at <http://centaur.reading.ac.uk/89214/>

It is advisable to refer to the publisher's version if you intend to cite from the work. See [Guidance on citing](#).

To link to this article DOI: <http://dx.doi.org/10.1017/S0007114520000926>

Publisher: Cambridge University Press

All outputs in CentAUR are protected by Intellectual Property Rights law, including copyright law. Copyright and IPR is retained by the creators or other copyright holders. Terms and conditions for use of this material are defined in

the [End User Agreement](#).

[www.reading.ac.uk/centaur](http://www.reading.ac.uk/centaur)

## **CentAUR**

Central Archive at the University of Reading

Reading's research outputs online

1 Effect of 4 weeks daily wild blueberry supplementation on symptoms of depression in adolescents

2 Jeni Fisk<sup>1¶</sup>, Sundus Khalid<sup>1¶^</sup>, Shirley A Reynolds<sup>1\*</sup> & Claire M Williams<sup>1\*\*^</sup>

3 <sup>1</sup> School of Psychology & Clinical Language Sciences, University of Reading, Berkshire, UK.

4  
5 ¶ These authors contributed equally to the work, \* These authors also contributed equally to the  
6 work

7  
8 ^ Corresponding authors:

9  
10 Sundus Khalid: Email: [sundus.khalid@pgr.reading.ac.uk](mailto:sundus.khalid@pgr.reading.ac.uk), Phone: +44 746 0471618

11 Mailing address: School of Psychology & Clinical Language Sciences, University of Reading,  
12 Harry Pitt Building, Early Gate, Reading, RG6 7BE

13  
14 Claire Williams: Email: [Claire.williams@reading.ac.uk](mailto:Claire.williams@reading.ac.uk), Phone: +44(0)118 378 7540

15 Mailing address: School of Psychology & Clinical Language Sciences, University of Reading,  
16 Harry Pitt Building, Early Gate, Reading, RG6 7BE

17  
18 Shortened title: Wild blueberry and depression in adolescents

19  
20 Keywords: blueberry, flavonoid, depression, anxiety, adolescent

21

**22 Abstract:**

23 Adolescence is an important period for cognitive maturation and emotional regulation and this age  
24 group is particularly vulnerable to developing depression. Diets rich in fruits and vegetables have  
25 been associated with decreased risk of developing depressive disorders across the lifespan, an  
26 association that may be due to the high flavonoid content of these foods. Previously we have  
27 shown increases in transient positive affect in both children and young adults two hours after  
28 administration of a wild blueberry intervention. Here, using a randomized double-blind, placebo-  
29 controlled trial, we investigated the effects of four weeks, daily wild blueberry supplementation  
30 (containing ~253mg anthocyanins) on transient and chronic mood in adolescents. Healthy 12-17-  
31 year old (N = 64, 35 females) were recruited and randomly assigned to receive either a wild  
32 blueberry or matched placebo supplementation. Depression and anxiety symptoms were assessed  
33 before and after the intervention period using the Mood and Feelings Questionnaire and Revised  
34 Child Anxiety and Depression Scale. Transient affect was assessed before, two weeks, and at four  
35 weeks using the Positive and Negative Affect Schedule. Following the intervention period there  
36 were significantly fewer self-reported depression symptoms in participants who were supplemented  
37 with the wild blueberry intervention compared to those who received the matched placebo ( $p=0.02$ ,  
38 95% CI -6.71 to -5.35). There was no between group effect on anxiety symptoms or on transient  
39 affect. Further investigation is required to identify specific mechanisms that link flavonoids  
40 consumption and mood. If replicated, the observed effects of wild blueberry supplementation may  
41 be a potential prevention strategy for adolescent depression and may have benefits for public mental  
42 health.

43

44

**45 Introduction:**

46 Puberty is a complex biologically driven process that has an impact on emotional and behavioral  
47 wellbeing, resulting in a period with increased risk of developing emotional disorders and risk-  
48 taking behavior. The brain undergoes cognitive maturation via synaptic remodeling well into the  
49 20s. The limbic system, responsible for governing reward processing, appetite and pleasure seeking,  
50 matures before the prefrontal cortex, which is responsible for executive functioning such as  
51 problem solving, planning, emotional regulation and multitasking. This difference in cortical  
52 maturity is hypothesized to create a developmental imbalance, making teens vulnerable to  
53 behavioral and mental health problems, such as depression <sup>(1)</sup>.

54  
55 An episode of major depressive disorder (MDD) during adolescence is a major personal and public  
56 health problem across the world <sup>(2)</sup>. The disorder has many acute and long-term adverse  
57 consequences on adolescents' education and occupational success, relationships and family life and  
58 on their future physical and mental health <sup>(3)</sup>. Each year around 7.5% of adolescents aged 13 to 18  
59 years' experience an episode of MDD <sup>(4-6)</sup>. Symptoms of MDD are distressing and include sleep and  
60 cognitive problems, low mood, irritability, feelings of worthlessness and lack of pleasure <sup>(7)</sup>. Sub-  
61 clinical MDD is even more common: recent surveys in the UK suggest that ~25% of young people  
62 report elevated symptoms of depression in any given year <sup>(4,8)</sup>, including depressive symptoms that  
63 are not sufficient in number or severe enough to meet diagnostic criteria. Sub-clinical symptoms  
64 have a major impact on daily functioning and are associated with increased risk of developing the  
65 disorder <sup>(4)</sup>.

66  
67 Treatment for MDD in this age group includes psychological therapies and anti-depressant  
68 medication; however, these are only moderately effective and are often inaccessible to young  
69 people due to limited public health service resources <sup>(8)</sup>. A recent meta-analysis of psychological  
70 treatments for children and young people with mental health problems found that the effect size of  
71 treatment for depression was small ( $d = 0.29$ ) and was lower than effects of treatment for other  
72 common mental health problems <sup>(9)</sup>. Many young people with MDD do not receive an evidence-  
73 based treatment and the prevention of adolescent depression is, therefore, a highly valued goal <sup>(10)</sup>.  
74 One potential way to prevent the onset of MDD and sub-clinical depression is through diet. Diet  
75 and depression symptoms are significantly associated in adults, although this relationship is  
76 complex and potentially bidirectional, i.e. unhealthy diet leading to low mood and vice versa <sup>(11)</sup>. A  
77 recent systematic review of the association between depression symptoms and diet in adolescents  
78 found that 'healthy' diets (i.e. consumption of fruits and vegetables) were associated with lower

79 depression symptoms; whilst ‘unhealthy’ diets (i.e. consumption of junk foods and saturated fats)  
80 were associated with higher depression symptoms <sup>(12)</sup>. A large well-controlled epidemiological  
81 study examining associations between habitual intakes of dietary flavonoids and depression risk  
82 showed that individuals consuming diets higher in flavonoids presented a lower depression risk,  
83 particularly amongst older women <sup>(13)</sup>. A similar study assessed symptoms of depression and the  
84 total habitual intake of polyphenols among the participants and found that higher dietary intake of  
85 flavonoids was inversely associated with depressive symptoms <sup>(14)</sup>. Thus, diets rich in fruits and  
86 vegetables are associated with low depression symptoms. Dietary flavonoids are present in  
87 substantial concentrations in commonly consumed fruits and vegetables and may be a potential  
88 mediator for the anti-depressant action of diets rich in fruits and vegetables.

89  
90 The hypothesis that there is a causal relationship between diet and depression symptoms and the  
91 onset of MDD has recently been strengthened by number of intervention studies. Acute purple  
92 grape juice intervention resulted in increase in self-reported ratings of ‘calm’ in healthy young  
93 adults <sup>(15)</sup>. Similarly, acute consumption of flavonoid-rich wild blueberry improved short-term  
94 positive mood in children aged 7-10 years and in young adults aged 18-25 years <sup>(16)</sup>. In a recent  
95 randomized controlled trial with 67 depressed adults <sup>(17)</sup>, participants randomized to an intervention  
96 promoting a healthy diet with at least nine portions of fruit and vegetables each day reported  
97 significantly less depression symptoms at twelve weeks than those randomized to receive social  
98 support. Anti-depressive effects of flavonoid rich plants and their extracts have also been  
99 investigated. *Hypericum perforatum* (also known as Saint John’s wort, derived from a flowering  
100 plant in the Hypericaceae family) extract intervention studies show its effectiveness as treatment for  
101 mild/moderate depression when compared to placebo and have similar effects to pharmacological  
102 treatments <sup>(18-21)</sup>. Similarly, saffron (*Crocus sativus*, derived from the saffron spice of the flowering  
103 plant of *Crocus* genus) extract consumption had equivalent effect as pharmacological treatment for  
104 depression and was significantly more effective than the matched placebo <sup>(22-24)</sup>.

105  
106 The specific effects of sustained wild blueberry flavonoid consumption on symptoms of depression  
107 in adolescents have not yet been tested. Here, we designed a double-blind, placebo-controlled  
108 experiment to test the effect of consuming a flavonoid-rich wild blueberry intervention for four  
109 weeks on symptoms of depression, anxiety and transient affect in healthy adolescents. Participants  
110 were randomly assigned to a wild blueberry or a matched placebo drink with transient affect and  
111 symptoms of depression and anxiety assessed before and after the four-week intervention period.

112

113

**114 Method***115 Ethics*

116 This research was reviewed and given a favorable ethical opinion for conduct by the University of  
117 Reading Research Ethics Committee (UREC 16/55). The study was registered at clinicaltrials.gov  
118 NCT03119597.

119

*120 Participants*

121 An *a priori* power analysis (using G Power 3.1.9.2) based on data from a previous study<sup>(16)</sup>  
122 revealed that 24 participants per group were required to achieve power of 0.8 with alpha set at 0.5  
123 level. Students aged 11-17 years of varying ethnicity, from four schools in Reading Berkshire, UK  
124 were invited to take part in this study. All parents or legal guardians provided informed written  
125 consent for young people under the age of 16. Participants under the age of 16 provided written  
126 assent and those over 16 gave written consent. All participants were screened for any health  
127 conditions (including mental health), any treatment they were receiving and food related allergies  
128 that would exclude them from the study. We screened 82 young people, of whom 18 dropped out  
129 after the first screening session. Sixty four participants were randomly assigned to either a wild  
130 blueberry drink or a matched placebo drink. The randomized allocation of participants to treatment  
131 was generated using excel. The groups were coded A and B and the sequence was saved in a  
132 password protected spreadsheet. Both the researchers and the participant were blind to treatment  
133 group and participants were told the study was investigating effects of different fruit drinks so were  
134 not aware of the study hypothesis.

135

*136 Interventions*

137 Both interventions (wild blueberry and placebo) were measured and packaged into silver opaque  
138 sachets at the University of Reading. Sachets were identical for the wild blueberry and the placebo  
139 drink and neither the researchers nor the participants knew what their sachets contained. Wild  
140 Blueberry Association of North America provided the blueberry powder whilst the matched sugars  
141 and vitamin C (placebo) was obtained from Bulk Powders. The packets of wild blueberry contained  
142 13g of freeze-dried wild blueberry (WBB) powder (containing ~253mg anthocyanins). Placebo  
143 packets were matched to the WBB for sugars (4.52g glucose and 4.79g fructose) and vitamin C (4  
144 mg). Each participant was given 14 days' supply of their requisite intervention, along with written  
145 and video instructions for their parents/guardians on how to prepare the intervention. Each  
146 intervention was prepared daily, by adding 30 ml of low-flavonoid 'Rock's Organic Orange

147 Squash' and 170 ml of water and the contents of the sachet to the opaque cup provided. Each  
148 participant was given a checklist to record the dates and times when they consumed the drink each  
149 day and the name of the person who prepared the drinks. Participants were also asked to bring back  
150 their used sachets after two weeks as a measure of compliance. The remaining 14 days' supply of  
151 each intervention was given to the participants two weeks into the intervention period. The true aim  
152 of the study was not disclosed to the participants, they were informed that it was a fruit drink study,  
153 to avoid revealing the contents of the drink.

154

#### 155 *Measures*

156 The Mood and Feelings Questionnaire (MFQ) was used to measure symptoms of depression <sup>(25)</sup>.  
157 The MFQ is considered to be the gold standard self-report measure for depression in young people  
158 (NICE, 2015). It is a standardized and well-validated 33-item self-report measure of the severity of  
159 depression symptoms in adolescents. Each item relates to a symptom or experience associated with  
160 depression. Participants are asked to rate each item in relation to their symptoms in the past 2  
161 weeks on a 3-point Likert scale (not true = 0, sometimes = 1, true = 2). Total MFQ scores range  
162 from 0 to 66 where higher scores indicate greater risk of depression. The clinical cut off for the  
163 MFQ is 27, with scores above 27 indicating significant risk of a diagnosis of MDD <sup>(25)</sup>.

164

165 Anxiety symptoms were assessed using the anxiety sub-scale of the Revised Child Anxiety and  
166 Depression Scale (RCADS) <sup>(26)</sup>, a standardized and validated measure of anxiety symptoms in  
167 young people used routinely in UK NHS mental health services. The anxiety sub-scale of RCADS  
168 consists of 37 items, each rated on a 4-point Likert scale (never =1, sometimes = 2, often = 3,  
169 always = 4). Total scores range from 37 to 148 with higher scores indicating increased risk of an  
170 anxiety disorder. Again, participants were asked to rate the items keeping the past two weeks in  
171 mind.

172

173 Current mood (i.e. transient affect) was assessed using the Positive and Negative Affect Schedule-  
174 NOW (PANAS-NOW) at screening, and at two and four weeks. As the term suggests this is a  
175 measure of transient mood. The PANAS is a valid and reliable 20 self-report measure of positive  
176 affect (PA – 10 items) and negative affect (NA - 10 items) that can be used on multiple test  
177 occasions <sup>(27,28)</sup>. Participants rated the degree to which they were currently experiencing each item  
178 on a 5-point Likert scale ranging from 'very slightly' to 'extremely'. Ratings of positive and  
179 negative items were summed to calculate an overall positive affect and overall negative affect score,  
180 each ranging from 10-50 where lower scores indicate lower levels of positive or negative affect.



181

182 Habitual fruit and vegetable consumption were assessed using EPIC-Norfolk food frequency  
183 questionnaire, a semi-quantitative paper-based questionnaire, which includes 130 food items, each  
184 rated on 9-point Likert scale (never or less than a month-1 to 6+perday-9). FETA software was used  
185 to analyse the data collected to calculate 46 nutrient and 14 food group values including average  
186 daily fruit and vegetable intake <sup>(29)</sup>.

187

188 *Other measures i.e. working memory, verbal fluency, cognitive accuracy and reaction time were*  
189 *assessed and are reported elsewhere* <sup>(30)</sup>.

190

191

### 192 *Procedure*

193 As outlined in Figure 1, participants were seen by the researchers four times across a five weeks  
194 period. All participants did not attend all assessment – the number of participants assessed at each  
195 timepoint is indicated in Figure 1. Research sessions took place either at the University of Reading  
196 or at the participant's school. Sessions were scheduled at the same time of day for each participant.  
197 The first two sessions, scheduled 48 hours apart, were screening sessions where participants  
198 completed a battery of questionnaires: MFQ, RCADS, (screening session 1) PANAS, EPIC-  
199 Norfolk food frequency questionnaire and a questionnaire about their health status (screening  
200 session 2). Screening sessions were limited to 30 minutes to fit with the school timetable and to  
201 maintain high levels of participant engagement in both sessions. Parents were also asked to  
202 complete a demographic questionnaire. Participants started the intervention the day after the  
203 second screening session was completed. Two weeks later they returned their used drink sachets,  
204 were given a new checklist and completed the PANAS (Test session 1). Participants were also  
205 asked if they were experiencing any adverse effects of the drink and feedback on its palatability.  
206 They then returned two weeks later (Test session 2), returned their drink sachets, completed the  
207 PANAS, MFQ and RCADS and were debriefed. For each test session, participants were instructed  
208 not to consume their allocated intervention before the test session to ensure that chronic, not acute,  
209 effects of the intervention were being measured.

210

### 211 *Statistical Analysis*

212 Statistical analyses were conducted using IBM SPSS version 22. T-test was used to investigate  
213 differences in symptoms of depression, anxiety and fruit and vegetable intake between the two  
214 groups at baseline. Effects of intervention on transient affect was analysed using Linear Mixed

215 Modelling (LMM) using an unstructured covariance matrix to model successive repeat test sessions,  
216 with subjects included as random effects. Data from two weeks and four weeks measures of the  
217 PANAS and treatment group were included as fixed factors, with baseline PANAS scores included  
218 as a covariate. LMM deals with data that is missing at random and with multiple measurement  
219 points, giving unbiased estimates of each of the means. To test the effects of the intervention on  
220 anxiety and depressive symptoms at four weeks, data were analysed using Analysis of Covariance  
221 (ANCOVA) with drink (Placebo, WBB) as an independent variable and MFQ and RCADS scores  
222 at 4 weeks as dependent variable. Baseline measures of depression and anxiety were used as  
223 covariates and Bonferroni corrected t-tests were used to investigate all fixed effects and  
224 interactions.

225

## 226 **Results**

### 227 *Sample characteristics*

228 Sixty-four participants were randomised (35 females, 29 males) aged 12-17 years (M = 14.20 years,  
229 SD = 1.71). Thirty-five participants were randomly allocated to receive the placebo drink and  
230 twenty-nine to the WBB intervention. Participants' demographic data, baseline mood scores and  
231 habitual fruit and vegetable intakes are reported in table 1. There were no significant differences  
232 between groups in the amount of daily fruit  $t(51) = 0.14, p = 0.89$  or vegetables  $t(51) = 1.45, p =$   
233  $0.15$  consumed. One sample t-test revealed that the mean fruit and vegetable consumption by the  
234 participants was significantly lower than the 400g per day as recommended by WHO; fruit:  $t(52) =$   
235  $11.20, p < 0.005$ , vegetables  $t(52) = 7.12, p < 0.005$ .

236

237 At baseline mean depression and anxiety scores were 12.35 (SD = 9.31) and 23.19 (SD = 13.80)  
238 respectively, both below the clinical threshold. There was no significant group difference in  
239 symptoms at baseline; MFQ  $t(60) = 0.60, p = 0.55$ , RCADS  $t(40) = 0.45, p = 0.66$  and no group  
240 difference in mean positive and negative affect;  $t(62) = 1.40, p = 0.17$  and  $t(62) = 0.80, p = 0.98$   
241 respectively. A minority of participants (9.38%) reported depression symptoms above the clinical  
242 cut-off of 27 on the MFQ (11.4% in the placebo group, 3.4% in the intervention group). No  
243 participants reported anxiety symptoms above the clinical threshold. No participants reported a  
244 diagnosis of depression or anxiety, or that they were receiving treatment for these disorders.

245

### 246 *Hypothesis testing*

247 At four weeks 59 participants provided self-report data on anxiety (RCADS) and depression (MFQ)  
248 symptoms; 26 from the intervention group and 33 from the placebo group. As shown in Figure 2a,

249 after four weeks of the intervention, the mean MFQ score for participants who consumed WBB was  
250 significantly lower than the mean MFQ score for participants who consumed the placebo drink.  
251 This was significant  $F(1,57)=5.52$ ,  $p=0.02$  95%CI -6.71 to -5.35 with a medium effect size ( $d = 0.$   
252 65). The change in the depression scores for each participant including regression line for both  
253 treatments is shown in figure 3. There was no significant effect of WBB on symptoms of anxiety  
254 (Figure 2b) after four weeks of supplementation  $F(1,34) = 2.1$ ,  $p=0.16$ ; mean RCADS score for  
255 participants in the WBB group was 13.90, (SD = 8.39) and the mean RCADS for the placebo group  
256 was 19.3, (SD = 11.31).

257

258 We also examined the effect of intervention on positive affect and negative affect (PANAS) after  
259 two and four weeks (see Figure 4). There was no significant effect of Drink,  $F(1,64.33) = 0.26$ ,  
260  $p=0.62$ , Repeated trial,  $F(1,62.22) = 2.95$ ,  $p=0.09$ , or any Drink x Repeated trial interaction  $F$   
261  $(1,62.22) = 3.686$ ,  $p=0.06$  on transient positive affect. Figure 4a shows the mean PA scores  
262 following intervention of WBB and placebo at two weeks and at four weeks. There was also no  
263 significant effect of the intervention on NA; Repeated trial,  $F(1,59.3) = 0.66$   $p=0.42$ , Drink,  $F$   
264  $(1,63.79) = 0.24$   $p=0.63$  or Repeated trial  $\times$  Drink interaction,  $F(1,59.30) = 1.17$ ,  $p=0.28$ . As shown  
265 in Figure 4b, NA was not significantly different after consuming the WBB drink or the placebo  
266 drink.

267

## 268 Discussion

269 This randomized, placebo controlled, double blinded trial investigated the effects of 4 weeks  
270 consumption of a flavonoid-rich WBB drink on symptoms of depression and anxiety and on  
271 transient affect in a community sample of healthy 12-17-year old. The results demonstrated that  
272 after four weeks of daily WBB intervention there was a between groups difference in self-reported  
273 depressive symptoms; participants randomised to the WBB intervention reported significantly  
274 lower scores on the measure of depression symptoms than participants who were randomised to the  
275 placebo drink. There was no significant effect of the intervention on anxiety symptoms or on  
276 positive affect or negative affect (i.e. transient affect). The data suggest that flavonoid  
277 supplementation may be beneficial in reducing depressive symptoms in healthy adolescents.

278

279 This is, to our knowledge, the first randomized double blinded study to show the effects of chronic  
280 WBB flavonoids on depression symptoms in teenagers. The participants in the study were healthy  
281 but at baseline assessment were consuming sub-optimal habitual levels of flavonoids, i.e. their daily  
282 consumption of fruit (44.87%) and vegetable (57.46%) was well below the WHO recommended

283 amount of 400g/day<sup>(31,32)</sup>. This is consistent with the typical diet of young people in the UK, where  
284 only 18% of adolescents meet the recommended daily requirement, and the average daily  
285 consumption within this age group is 256g (3.5 portions) of fruit and vegetables<sup>(33)</sup>. Levels of  
286 depression and anxiety were similar to community norms on gold standard self-report measures.  
287 Importantly, because the effects of the intervention were observed in a community sample, these  
288 effects cannot necessarily be generalised to adolescents with more severe symptoms of depression  
289 or a diagnosis of depression.

290

291 Within this community sample the effect size of the flavonoid intervention compared to the control  
292 group on the measure of depression symptoms, the MFQ, was  $d = 0.65$ , a medium effect size. To  
293 put this into context, two recent meta-analyses have examined the effects of psychological  
294 treatments for depression and the prevention of depression. Ecksthtain et al., (2019) concluded that  
295 the treatment effect size of psychological treatments for adolescents with depression was  $d = .36$   
296<sup>(34)</sup>. In a review of interventions to prevent depression Ssegonia et al., (2019) reported an effect size  
297 of  $d = .22$ <sup>(35)</sup>. In relation to the specific measure of depression used in this study the reduction of  
298 the 4 points on mean MFQ scores in the intervention group indicates complete amelioration of 2  
299 items on the scale or a reduction (from 2 to 1, or 1 to 0) of 4 items. Because each item reflects a  
300 symptom or adverse effect of depression, clinically this would be likely to reflect a meaningful  
301 reduction in the impact of depression on the young person<sup>(36)</sup>.

302

303 Previously the effects of flavonoids from different sources such as apples, cocoa and grape juice  
304 showed no effects on depression in healthy adults<sup>(37-40)</sup>. However, our results are consistent with  
305 previous animal and epidemiological studies that suggest anti-depressive effects of a flavonoid rich  
306 diet<sup>(13,41-44)</sup>. They also are in keeping with experimental data on the acute effects of WBB on  
307 positive mood in children and young adults<sup>(15,16)</sup>, and the acute effect of grape juice on mood in  
308 healthy adults<sup>(45)</sup>. Unlike a previous acute intervention study, we did not observe a significant  
309 effect of WBB on momentary mood (i.e. transitory affect). However, the interval between  
310 consuming the WBB drink and assessing NA and PA was variable, unlike the standard 2-hour  
311 interval used in previous studies. In addition, the four-week assessment (our end point) was  
312 conducted during the first week of school after the summer holidays. Unlike symptoms of  
313 depression (and anxiety) which were measured over a minimum two-week period and which are  
314 conceptualised as relatively stable, positive and negative affect are conceived as short-lived events  
315 that have rapid decay after elicitation<sup>(46)</sup>. It is therefore possible that this external event (returning  
316 to school) had a measurable impact on participants' momentary affect.

317

318 Although anxiety and depression are frequently co-morbid in young people and share some  
319 symptoms (e.g. fatigue, low concentration and sleep disturbances), the results of this intervention  
320 study suggest that flavonoids may reduce symptoms that are more prominent in depression than  
321 anxiety, e.g. low mood, anhedonia, feelings of guilt, and worthlessness and do not reduce symptoms  
322 that are specific to anxiety. However, it is also possible that the effect of flavonoids on anxiety is  
323 smaller than the effect on depression and that a larger sample, with greater power, might result in a  
324 significant effect.

325

326 Some authors have proposed that flavonoids increase cerebral blood flow to the dorsolateral  
327 prefrontal cortex, a site that is highly associated with cognitive and emotional regulation, including  
328 rumination, a cognitive process of repetitive thinking that may exacerbate feelings of guilt and  
329 worthlessness<sup>(47-49)</sup>. This suggests that there may be an indirect pathway between flavonoid  
330 consumption and depression whereby flavonoid consumption enhance cerebral blood flow, which  
331 boosts executive functioning; in turn improved executive functioning helps to enhance cognitive  
332 control, inhibits rumination and thus reduces depression. Adolescents with depression have  
333 impaired executive function compared to non-depressed and anxious young people<sup>(50)</sup> and therefore  
334 the benefits of flavonoid consumption may be more prominent in these young people. However,  
335 potentially any positive effects of flavonoid consumption on executive function would have benefits  
336 for more young people because executive function is critical for academic achievement<sup>(51)</sup>.

337

338 A plausible direct pathway between flavonoid consumption and mood is the effects of flavonoids  
339 on Monoamine Oxidase (MAO)<sup>(52)</sup>. MAO inhibitors have been used to treat mood disorders and  
340 flavonoids may mimic their effects<sup>(52,53)</sup>. A recent study showed that consuming fruits high in  
341 flavonoids i.e. blackcurrants significantly reduces MAO activity and increases the circulating  
342 monoamines and thereby elevates mood<sup>(52)</sup>. Another possible mechanism by which flavonoids may  
343 affect mood is by mimicking anxiolytic-like effects by binding to benzodiazepine receptors,  
344 enhancing the effect of GABA via GABAA receptors<sup>(34,54,55)</sup>. However, in line with a previous  
345 study<sup>(16)</sup> that showed no changes in negative affect (an indicator of anxiety) after acute flavonoid  
346 intervention, here there was no significant of flavonoid consumption on anxiety.

347

348 Although the mechanisms of action require further investigation there is accumulating evidence of a  
349 causal relationship between flavonoid consumption and depression symptoms. This evidence has  
350 been published by independent research groups using different research designs, including

351 epidemiology, clinical trials and experiments. However, the research is preliminary and requires  
352 robust replication and extension, with larger samples, longer time scales and careful tests of  
353 mechanisms of action. Our study examined the effects of flavonoids on healthy young people, some  
354 of whom had elevated symptoms of depression. We did not have adequate power to conduct sub-  
355 group analysis but clearly it is important to identify if the change in depression symptoms is driven  
356 by improvements in those with relatively elevated symptoms, or if the effects are similar across all  
357 levels of baseline depression. This distinction is important because flavonoids may have the  
358 potential to prevent depression in those at risk (i.e. those with elevated symptoms) or may have a  
359 more general effect. The former would suggest that dietary interventions could be used for early  
360 intervention in those exhibiting symptoms of depression; the latter that dietary interventions could  
361 have a broader benefit to public mental health.

362

### 363 **Conclusions**

364 This randomized double-blind study demonstrated the chronic effects of wild blueberry flavonoid  
365 consumption on reducing symptoms of depression in a community sample of adolescents. Dietary  
366 flavonoid interventions may have potential to reduce symptoms of depression in adolescents. This  
367 study requires replication, not only in healthy participants, but also in clinically referred samples to  
368 assess the potential of flavonoids to be used as a practical and cost-effective intervention. In  
369 addition to this, studies focused on investigating biochemical changes and investigating the  
370 mechanistic pathways in which flavonoids decrease depressive symptoms in humans is essential.

371

### 372 **Acknowledgements**

373 We are grateful to the Wild Blueberry Association of North America who provided the freeze-dried  
374 wild blueberry powder used for this study. The authors would also like to acknowledge the  
375 contribution of the staff and participants of the EPIC-Norfolk Study. EPIC-Norfolk has been  
376 supported by the Medical Research Council programme grants (G9502233, G0401527, G1000143)  
377 and Cancer Research UK programme grants (SP2024/0201, SP2024/0204, C865/A2883,  
378 C864/A8257, C864/A14136)"

379

### 380 **Authorship**

381 All the authors were involved in the design of the experiments; S.K, and J.F performed the  
382 experiments and analysed the data. S.K, J.F, C.W and S.R were involved in the writing and revisions  
383 of the manuscript.

384

385 **Conflicts of Interest**

386 The authors declare no conflicts of interest arising from the conclusions of this work.

387

388 **References**

- 389 1. Hawton K, Saunders KE, O'Connor RC (2012) Self-harm and suicide in  
 390 adolescents. *Lancet* **379**,2373–2382.
- 391 2. World Health Organization (2017) Depression – fact sheet.  
 392 <http://www.who.int/mediacentre/factsheets/fs369/en/> (accessed November 2018)
- 393 3. Clayborne ZM, Varin M, Colman I (2019) Systematic Review and Meta-Analysis:  
 394 Adolescent Depression and Long-Term Psychosocial Outcomes. *J Am Acad Child Adolesc*  
 395 *Psychiatry*, **58** 72–79.
- 396 4. Avenonoli S, Swendsen J, Jian-Ping H *et al.* (2015) Major depression in the national co-  
 397 morbidity survey – adolescent supplement. Prevalence, correlates and treatment. *J Am*  
 398 *Acad Child Adolesc Psychiatry* **54**, 37-44.
- 399 5. Polanczyk GV, Salum GA, Sugaya LS *et al.* (2015) Annual Research Review: A meta-  
 400 analysis of the worldwide prevalence of mental disorders in children and adolescents. *J*  
 401 *Child Psychol Psychiatry* **56**,3.
- 402 6. Jane Costello E, Erkanli A, Angold A (2006). Is there an epidemic of child or adolescent  
 403 depression? *J Child Psychol Psychiatry* 27,1469-7610
- 404 7. APA. (2013). Diagnostic and Statistical Manual of Mental Disorders (DSM-5®): American  
 405 Psychiatric Pub
- 406 8. National Institute for Health and Care Excellence (2015) Depression in children and young  
 407 people: identification and management. [https://www.nice.org.uk/guidance/cg28/](https://www.nice.org.uk/guidance/cg28/chapter/ftn.footnote_2)  
 408 [chapter/ftn.footnote\\_2](https://www.nice.org.uk/guidance/cg28/chapter/ftn.footnote_2) (accessed November 2018)
- 409 9. Brent, D. A., Gibbons, R. D., Wilkinson, P., & Dubicka, B. (2018,). Antidepressants in  
 410 paediatric depression: Do not look back in anger but around in awareness. *BJPsych Bull.*  
 411 <https://doi.org/10.1192/bjb.2017.2>
- 412 10. Children's Society (2008) The Good Childhood Inquiry: health research evidence. London:  
 413 Children's Society
- 414 11. Murakami K, & Sasaki S (2010). Dietary intake and depressive symptoms: a systematic  
 415 review of observational studies. *Mol Nutr Food Res* **54**, 471–488.
- 416 12. Khalid S, Williams C, Reynolds S (2016). Is there an association between diet and  
 417 depression in children and adolescents? A systematic review. *Br J of Nutr* **116**, 2097-2108.

- 418 13. Chang SC, Cassidy A, Willett WC et al (2016). Dietary flavonoid intake and risk of  
419 incident depression in midlife and older women. *Am J Clin Nutr* **104**, 704-714
- 420 14. Godos J, Castellano S, Ray S, et al (2018). Dietary Polyphenol Intake and Depression:  
421 Results from the Mediterranean Healthy Eating, Lifestyle and Aging (MEAL) Study.  
422 *Molecules*, **23**, 999.
- 423 15. Haskell-Ramsay CF, Stuart RC, Okello EJ, et al (2017). Cognitive and mood improvements  
424 following acute supplementation with purple grape juice in healthy young adults. *Eur J*  
425 *Nutr* **56**, 2621–2631.
- 426 16. Khalid S, Barfoot K L, May G, et al (2017). Effects of Acute Blueberry Flavonoids on  
427 Mood in Children and Young Adults. *Nutrients* **9**, 158.
- 428 17. Jacka FN, O’Neil A, Opie R et al (2017). A randomised controlled trial of dietary  
429 improvement for adults with major depression (the “SMILES” trial). *BMC Med* **15**, 23
- 430 18. Brattström A (2009) Long-term effects of St. John's wort (*Hypericum perforatum*)  
431 treatment: a 1-year safety study in mild to moderate depression. *Phytomedicine* **16**, 277–  
432 283.
- 433 19. Clement K, Covertson C., Johnson MJ et al (2006). St. John's wort and the treatment of  
434 mild to moderate depression: a systematic review. *Holist Nurs Pract* **20**, 197–203.
- 435 20. Kasper S, Anghelescu IG, Szegedi A, et al (2006). Superior efficacy of St John's wort  
436 extract WS 5570 compared to placebo in patients with major depression: a randomized,  
437 double-blind, placebo-controlled, multi-center trial [ISRCTN77277298]. *BMC Med* **23**,4–  
438 14.
- 439 21. Mannel M, Kuhn U, Schmidt U, et al (2010). St. John's wort extract LI160 for the treatment  
440 of depression with atypical features - a double-blind, randomized, and placebo-controlled  
441 trial. *J Psychiatr Res* **44**,760–767.
- 442 22. Moshiri E, Basti AA, Noorbala AA et al (2006). *Crocus sativus* L. (petal) in the treatment  
443 of mild-to-moderate depression: a double-blind, randomized and placebo-controlled trial.  
444 *Phytomedicine* **13**, 607–611.
- 445 23. Noorbala AA, Akhondzadeh S, Tahmacebi-Pour N, et al (2005). Hydro-alcoholic extract of  
446 *Crocus sativus* L. versus fluoxetine in the treatment of mild to moderate depression: a  
447 double-blind, randomized pilot trial. *J Ethnopharmacol* **97**, 281–284.
- 448 24. Shahmansouri N, Farokhnia M, Abbasi SH et al (2014). A randomized, double-blind,  
449 clinical trial comparing the efficacy and safety of *Crocus sativus* L. with fluoxetine for  
450 improving mild to moderate depression in post percutaneous coronary intervention patients.  
451 *J Affect Disord* **155**, 216–222.



- 452 25. Angold A, Costello EJ, Messer SC, et al (1995) The development of a short questionnaire  
453 for use in epidemiological studies of depression in children and adolescents. *Int J Methods*  
454 *Psychiatr Res* **5**, 237 - 249.
- 455 26. Chorpita BF, Yim L, Moffitt C, et al (2000). Assessment of symptoms of DSM-IV anxiety  
456 and depression in children: a revised child anxiety and depression scale. *Behav Res Ther*  
457 **38**,835-855.
- 458 27. Watson D, Clark LA, Tellegen A (1988). Development and validation of brief measures of  
459 positive and negative affect: The PANAS scales. *J Pers So. Psychol* **54**, 1063–1070.
- 460 28. Crawford JR & Henry JD (2004). The Positive and Negative Affect Schedule (PANAS);  
461 Construct validity, measurement properties and normative data in a large non-clinical  
462 sample. *Br J Clin Psychol* **43**, 245–265.
- 463 29. Angela AM, Robert NL, Amit B et al (2014). A new tool for converting food frequency  
464 questionnaire data into nutrient and food group values: FETA research methods and  
465 availability. *BMJ* **27**, e004503.
- 466 30. Khalid S (2020). *The Effects of Wild Blueberry Flavonoids on Mood and Cognition in*  
467 *Young Adults*. Ph.D Thesis. University of Reading.
- 468 31. World Health Organization (2017) Global Strategy on Diet, Physical Activity and Health-  
469 Information Sheet. <https://www.who.int/dietphysicalactivity/fruit/en/index2.html> (accessed  
470 December 2018)
- 471 32. Vereecken C, Pedersen TP, Ojala K et al (2015). Fruit and vegetable consumption trends  
472 among adolescents from 2002 to 2010 in 33 countries. *Eur J Public Health*, **25**,16–19.
- 473 33. NHS Digital (2017) Health Survey for England. [https://digital.nhs.uk/data-and-](https://digital.nhs.uk/data-and-information/publications/statistical/health-survey-for-england/2017)  
474 [information/publications/statistical/health-survey-for-england/2017](https://digital.nhs.uk/data-and-information/publications/statistical/health-survey-for-england/2017) (accessed December  
475 2018).
- 476 34. Eckstain, D., Kuppens, S., Ugueto, A., Ng, M. Y., Vaughn-Coaxum, R., Corteselli, K., &  
477 Weisz, J. R. (2019). Meta-Analysis: 13-Year Follow-up of Psychotherapy Effects on Youth  
478 Depression. *Journal of the American Academy of Child & Adolescent Psychiatry*.  
479 <https://doi.org/10.1016/j.jaac.2019.04.002>
- 480 35. Ssegonja, R., Nystrand, C., Feldman, I., Sarkadi, A., Langenskiöld, S., & Jonsson,  
481 U. (2019). Indicated preventive interventions for depression in children and  
482 adolescents: A meta-analysis and meta-regression. *Preventive medicine*, 118, 7-15.
- 483 36. McCarty, C.A., Violette, H.D., Duong, M.T., Cruz, R.A. and McCauley, E., 2013. A  
484 randomized trial of the positive thoughts and action program for depression among early  
485 adolescents. *Journal of Clinical Child & Adolescent Psychology*, 42(4), pp.554-563.

- 486 37. Khan H, Perviz S, Sureda A et al (2018). Current standing of plant derived flavonoids as an  
487 antidepressant. *Food Chem Toxicol*, **119**, 176–188.
- 488 38. Hendrickson SJ & Mattes RD (2008). No acute effects of grape juice on appetite, implicit  
489 memory and mood. *Food Nutr Res*, **52**,1-5
- 490 39. Bondonno CP, Downey LA, Croft KD et al (2014). The acute effect of flavonoid-rich  
491 apples and nitrate-rich spinach on cognitive performance and mood in healthy men and  
492 women. *Food Funct* **5**, 849–858
- 493 40. Scholey AB, Haskell CF, French SJ, et al (2009). Consumption of cocoa flavanols results in  
494 acute improvements in mood and cognitive performance during sustained mental effort. *J*  
495 *Psychopharmacol* **24**,1505-14
- 496 41. Mihrshahi S, Dobson AJ, Mishra GD, (2015). Fruit and vegetable consumption and  
497 prevalence and incidence of depressive symptoms in mid-age women: results from the  
498 Australian longitudinal study on women's health. *Eur J Clin Nutr* **69**, 585–589
- 499 42. Pase MP, Scholey AB, Pipingas A et al (2013). Cocoa polyphenols enhance positive mood  
500 states but not cognitive performance: a randomized, placebo-controlled trial. *J*  
501 *Psychopharmacol* **27**,451–458.
- 502 43. Bouayed J (2010). Polyphenols: a potential new strategy for the prevention and treatment  
503 of anxiety and depression. *Curr Nutr Food Sci* **6**, 13–18.
- 504 44. Brattström, A (2009). Long-term effects of St. John's wort (*Hypericum perforatum*)  
505 treatment: a 1-year safety study in mild to moderate depression. *Phytomedicine* **16**, 277–  
506 283.
- 507 45. Haskell C, Stuart RRC, Okello EEJ et al. (2017). Cognitive and mood improvements  
508 following acute supplementation with purple grape juice in healthy young adults. *Eur J*  
509 *Nutr* **56**,26021-2631
- 510 46. Qiao-Tasserit E, Garcia Quesada M, Antico L, et al (2017). Transient emotional events and  
511 individual affective traits affect emotion recognition in a perceptual decision-making task.  
512 *PLOS ONE*, **12**,e0171375.
- 513 47. Vauzour D, Vafeiadou K, Rodriguez-Mateos A, et al (2008).The neuroprotective potential  
514 of flavonoids: A multiplicity of effects. *Genes Nutr* **3**, 115–126.
- 515 48. Miller EK (2000) The prefrontal cortex and cognitive control. *Nat Rev Neurosci* **1**, 59–65
- 516 49. Schore AN (2016) Affect Regulation and the Origin of Self: The Neurobiology of  
517 Emotional Development, Classic ed., [Routledge] New York.
- 518 50. Fisk J, Ellis JA, Reynolds SA (2019) A test of the CaR-FA-X mechanisms and depression  
519 in adolescents. *Memory*, **27** 455-464.

- 520 51. St Clair-Thompson, H. L., & Gathercole, S. E. (2006) Executive functions and achievements  
521 in school: Shifting, updating, inhibition, and working memory. *The quarterly journal of*  
522 *experimental psychology*, 59(4), 745-759
- 523 52. Watson AW, Haskell-Ramsay CF, Kennedy DO, et al (2015) Acute supplementation with  
524 blackcurrant extracts modulates cognitive functioning and inhibits monoamine oxidase-B in  
525 healthy young adults. *J Funct Foods* 17, 524–539.
- 526 53. Carradori, S., Gidaro, M. C., Petzer, A., Costa, G., et al (2016). Inhibition of Human  
527 Monoamine Oxidase: Biological and Molecular Modeling Studies on Selected Natural  
528 Flavonoids. *J Agr Food Chem*, 64(47), 9004–9011. <https://doi.org/10.1021/acs.jafc.6b03529>
- 529 54. Hanrahan JR, Chebib M, Johnston GAR (2011) Flavonoid modulation of GABA(A)  
530 receptors. *B. J Pharmacol*, 163, 234–245.
- 531 55. Wasowski C & Marder M (2012) Flavonoids as GABAA receptor ligands: The whole  
532 story? *J Exp Pharmacol* 4, 9–24.  
533

534 **TABLES**

535 Table 1: Demographic details, mean fruit and vegetable intake and mean depression and anxiety  
 536 scores at baseline for both intervention groups.

	PLACEBO GROUP	WILD BLUEBERRY GROUP	P VALUES
MEAN AGE	14.5 (SD=1.804)	13.82(SD=1.54)	P=0.11
MALE %	48.6	41.4	P=0.57
FEMALE %	51.4	58.6	P=0.57
BRITISH %	60	52.4	P=0.52
ASIAN%	11.4	12.5	P=0.52
MIXED%	5.8	12.6	P=0.52
AFRICAN	2.9	8.3	P=0.52
CHINESE	2.9	4.2	P=0.52
MEAN FRUIT INTAKE (GRAMS/DAY)	188 (SD=168.3)	176 (SD=98.0)	P=0.89
MEAN VEGETABLES (GRAMS/DAY)	257.6 (SD= 187.0)	187.5 (SD=144.6)	P=0.15
MEAN DEPRESSION (MFQ)	13.0 (SD= 10.0)	11.3 (SD= 8.5)	P=0.55
MEAN ANXIETY (RCADS)	24.2 (SD= 14.90)	22.3 (SD=13.0)	P=0.66
MEAN POSITIVE AFFECT	28.0 (SD=7.7)	25.3 (SD=8.0)	P=0.17
MEAN NEGATIVE AFFECT	15.1 (SD=5.24)	14.1 (SD=4.38)	P=0.98

537

538

539

540 FIGURES

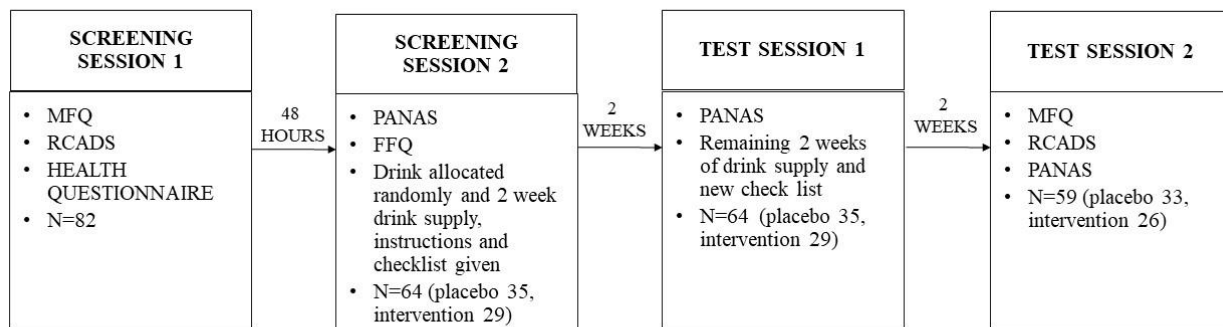


Figure 1. A schematic of the study procedure

541

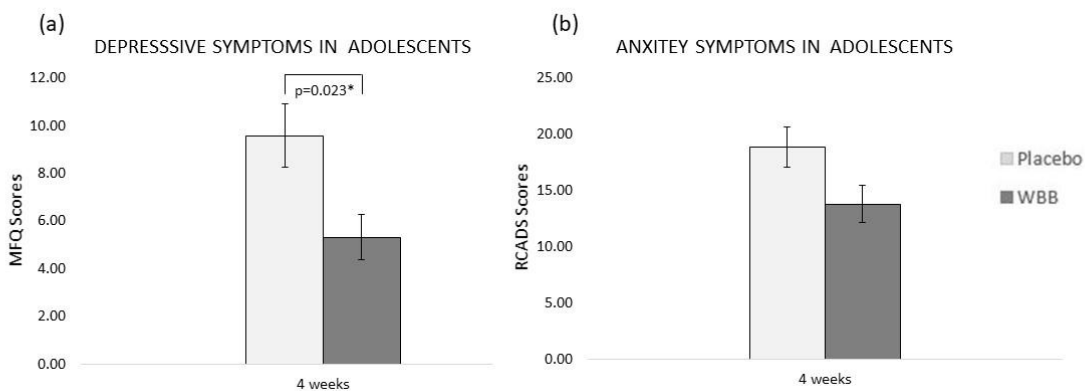


Figure 2. Mean scores ( $\pm$  standard error of the mean) in adolescents aged 11-17 years (a) Mean MFQ scores after 4 weeks consumption of placebo and intervention drinks. (b) Mean RCADS scores after 4 weeks consumption of placebo and intervention drinks.

542

543

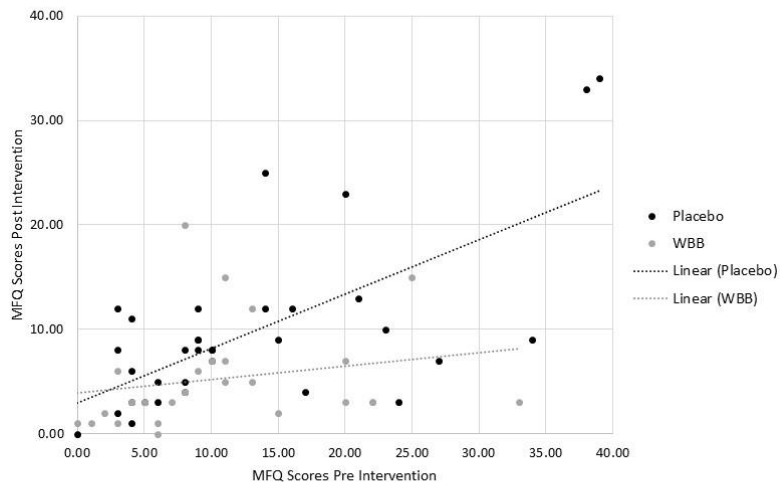


Figure 3. Scatterplot showing the MFQ scores at baseline and 4-week post intervention

544

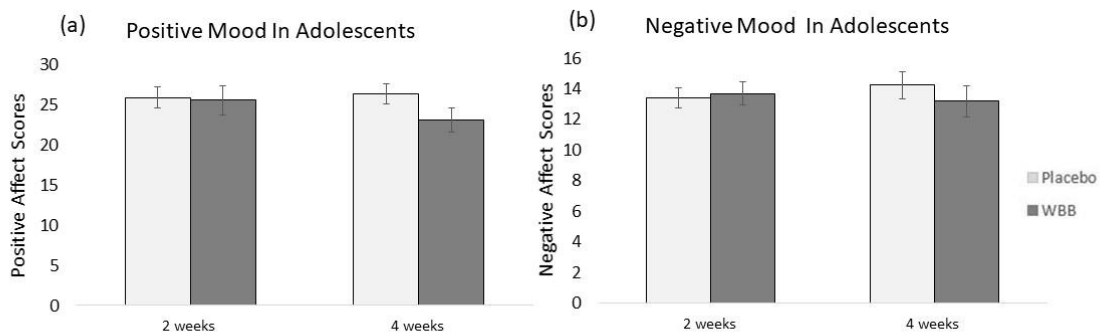


Figure 4. Mean PANAS-NOW Mood scores ( $\pm$  standard error of the mean) in adolescents aged 11-17 years: (a) Mean PA scores 2 and 4 weeks post-consumption of placebo and intervention drinks. (b) Mean NA scores 2 and 4 weeks post-consumption of placebo and intervention drinks.

545