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To cite this article: Nicole Tressa Stringham, Philip V. Holmes & James M. Stringham (2017): Supplementation with macular carotenoids reduces psychological stress, serum cortisol, and sub-optimal symptoms of physical and emotional health in young adults, Nutritional Neuroscience, DOI: 10.1080/1028415X.2017.1286445

To link to this article: http://dx.doi.org/10.1080/1028415X.2017.1286445

Published online: 15 Feb 2017.
Supplementation with macular carotenoids reduces psychological stress, serum cortisol, and sub-optimal symptoms of physical and emotional health in young adults

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Purpose: Oxidative stress and systemic inflammation are the root cause of several deleterious effects of chronic psychological stress. We hypothesize that the antioxidant and anti-inflammatory capabilities of the macular carotenoids (MCs) lutein, zeaxanthin, and meso-zeaxanthin could, via daily supplementation, provide a dietary means of benefit.

Methods: A total of 59 young healthy subjects participated in a 12-month, double-blind, placebo-controlled trial to evaluate the effects of MC supplementation on blood cortisol, psychological stress ratings, behavioural measures of mood, and symptoms of sub-optimal health. Subjects were randomly assigned to one of three groups: placebo, 13 mg, or 27 mg/day total MCs. All parameters were assessed at baseline, 6 months, and 12 months. Serum MCs were determined via HPLC, serum cortisol via ELISA, and macular pigment optical density (MPOD) via customized heterochromatic flicker photometry. Behavioural data were obtained via questionnaire.

Results: Significant baseline correlations were found between MPOD and Beck anxiety scores ($r = -0.28; P = 0.032$), MPOD and Brief Symptom Inventory scores ($r = 0.27; P = 0.037$), and serum cortisol and psychological stress scores ($r = 0.46; P < 0.001$). Supplementation for 6 months improved psychological stress, serum cortisol, and measures of emotional and physical health ($P < 0.05$ for all), versus placebo. These outcomes were either maintained or improved further at 12 months.

Conclusions: Supplementation with the MCs significantly reduces stress, cortisol, and symptoms of sub-optimal emotional and physical health. Determining the basis for these effects, whether systemic or a more central (i.e. brain) is a question that warrants further study.

Keywords: Lutein, Zeaxanthin, Macular pigment, Stress, Cortisol, Anxiety, Depression, Health

Introduction

The basis for all stress responses is the disruption of some homeostatic set point, be it physical, physiological, or psychological. Physiologically, stress is associated with activation of autonomic and endocrine systems, which involves limbic, hypothalamic, and brainstem circuits. Chronic activation of these systems may manifest as anxiety disorders or other stress-related disorders, such as depression. Indeed, the link between anxiety and chronic activation of the limbic structures, such as the amygdaloid complex, has been well established. Psychologically, the basis of anxiety and depression may involve heightened vulnerability to stress or the inability to cope with life stressors and appears to be mediated by dysregulation in cortico-limbic circuitry.

The convergence of physiological, psychological, and neurocognitive data in the case of stress, anxiety, and depression is compelling. In fact, the basis for the Depression Anxiety Stress Scales 21 (DASS-21) is the ‘tripartite model,’ in which anxiety and depression are both related to psychological stress: Anxiety arises out of physiological hyperarousal, whereas depression arises from low positive affectivity, both being impacted by the negative affect brought on by stress. It appears, therefore, that although anxiety and depression are somewhat discrete phenomena, they both share a common root in psychological stress. This idea is further supported by comorbidity rates exceeding 50%. Susceptibility to stress could, therefore, be
considered to be a risk factor for those conditions, such as anxiety and depression, that appear to result from excessive psychological stress.

It has been suggested that dietary differences could modulate susceptibility to stress. Benton noted that effects of minor nutritional deficiencies would manifest first as sub-clinical disruption of brain function, given the complexity and metabolic demands of the brain. In general support of this idea, there have been recent human and animal studies that report stress-reducing effects of supplementation of specific nutrients, such as curcumin, alpha tocopherol, and docosahexaenoic acid (DHA). In each case, supplementation appears to lead to reduced psychological stress and physiological parameters of stress (e.g. blood cortisol). Additionally, Long and Benton conducted a meta-analysis of studies on the effects of vitamin and mineral supplementation on stress and mood in sub-clinical populations, and found a general trend towards stress reduction and improvement in mood.

A recent report by El Ansari et al. on a large (n = 3706), generally healthy population of college-aged adults examined, via survey, dietary patterns and stress / depressive symptoms. They found a significant relationship between consumption of healthy foods (fresh fruits, salads, and cooked vegetables) and reduced perceived psychological stress. Conversely, consumption of ‘junk’ food was associated with increased perceived psychological stress. Although these findings were correlational, there is a physiological rationale to account for how healthy foods may reduce psychological stress: consumption of antioxidants. It has been shown that systemic oxidative stress is induced by psychological stress (e.g. in medical students), and it appears that reduction of systemic oxidative stress significantly reduces indicators of psychological stress (via alpha-tocopherol administration; and by lutein). Of particular relevance to the present study, Yajima et al. reported that lutein (L) supplementation produced an anxiolytic-like effect in mice exposed to constant illumination stress. Taken together, these findings suggest a role for dietary antioxidants in reducing psychological stress.

Based on the idea that any form of homeostatic upset will produce a similar stress response in the body, it is perhaps the case that a situation, favouring oxidative stress within the body, may produce an ‘alarm state’, which may ultimately be interpreted psychologically as uneasiness, or stress. Although the specific neurophysiological mechanisms are undoubtedly complex, we believe this general idea to be plausible, based on what is known in the literature on the matter.

Carotenoids, such as L, comprise a fairly large proportion of dietary antioxidants for humans with a reasonably healthy diet that includes daily consumption of fruits and vegetables. Along with L, two other yellow-orange carotenoids, zeaxanthin (Z), and meso-zeaxanthin (MZ) are deposited in rich concentration in the central retina, where they form the macular pigment (MP). MP is most dense in the metabolically intense central retina (fovea), where its powerful antioxidant and high-energy short-wave light filtration properties appear to protect the macula from acute damage, protect against cumulative damage resulting in age-related macular disease, and maintain visual sensitivity over a lifetime. MP is strictly derived via diet, and so a person’s level of MP is dependent upon his or her consumption of foods that contain these carotenoids; for example, dark leafy-green vegetables, such as kale and spinach, are excellent sources of L. L and Z also accumulate in the brain, where they may influence cognitive performance, especially in aged individuals. In a manner apparently similar to the retina, L and Z cross the blood–brain barrier and accumulate in the brain regions that maintain relatively high metabolism (e.g. frontal and occipital lobes, and hippocampus), and are therefore at higher risk for oxidative stress and inflammation. Importantly, MPOD has been shown to be significantly correlated with brain levels of L and Z, which suggests similar mechanisms of uptake, and supports the idea that there is preferential deposition of these powerful antioxidants / anti-inflammatories in neural tissues that maintain high metabolism and therefore concomitant oxygen tension and potential for oxidative stress and inflammation.

There were two goals of the present study: (1) To determine, in healthy young adults, the relationship at baseline between MPOD and psychological stress level, serum cortisol, and symptoms of sub-optimal emotional and physical health, and (2) To determine the effect of 12 months’ L, Z, and MZ supplementation on the aforementioned parameters. Once deposited in retinal tissue, L and Z (the two primary dietary components of macular pigment) are quite stable in the absence of high oxidative stress, e.g. such as that brought on by smoking or diabetes. Therefore, a person’s macular pigment level is generally thought to reflect his or her lifelong consumption of L and Z. The baseline assessment of MPOD and psychological stress, and physical / emotional health status would thereby enable the analysis of potential cumulative effects of diet on these outcome parameters. In contrast, the 12-month supplementation trial enabled the analysis of potential acute effects of MC supplementation.

**Methods**

Fifty-nine subjects participated in this 12-month, double-blind, randomized, placebo-controlled...
supplementation trial. Subjects were generally healthy, college-aged (18–25, mean = 21.5 years; 27 males / 32 female) non-smokers with a BMI < 27. Subjects were instructed to maintain their current diet; those who were planning on changing their diet (for whatever reason) were excluded from consideration for the trial. In consideration of macular pigment testing, all subjects had uncorrected or contact lens-corrected visual acuity of 20/20 or better in the test (right) eye, and had no current or previous history of ocular pathology. Subjects were recruited from the population of students at the University of Georgia in Athens, Georgia. Informed consent was obtained from each subject and the study adhered to the tenets of the Declaration of Helsinki. The study was approved by the Institutional Review Board of the University of Georgia.

Several parameters were assessed over the course of the study, including retinal status of MCs, serum cortisol, serum lutein, serum zeaxanthin isomers. Symptoms of sub-optimal health, psychological health, and emotional health were assessed via questionnaire (see Table 1 for a summary of questionnaires used in the study and order of administration). All measures were taken at baseline, 6 months, and 12 months. Laboratory visits included (in order): blood draw, questionnaire completion, and vision testing.

**Macular carotenoid supplementation**

Subjects were randomly assigned to one of three groups: placebo, n = 10; 13 mg/day MC, n = 24; or 27 mg/day MC. Pills were brown coloured, soft gelatin capsules, with L, Z, and MZ suspended in safflower oil. Independent analysis indicated that the 13 mg supplement contained 10.86 mg lutein / 2.27 mg zeaxanthin isomers, and the 27 mg supplement contained 22.33 mg lutein / 4.70 mg zeaxanthin isomers. Placebos contained no L or Z isomers, only safflower oil. Z and MZ were found in roughly equal amounts in the active supplements. All reported values were within ± 5% variability. Subjects were instructed to ingest one pill with a meal (preferably lunch or dinner) every day. Compliance was ensured with weekly phone calls and pill counts.

**Measurement of macular pigment optical density (MPOD)**

The concentration of MCs in the central retina (MPOD) was assessed with a non-invasive, perceptual task called heterochromatic flicker photometry (HFP). A densitometer (Macular Metrics Corp., Rehoboth, MA) described by Wooten et al.40 was used for this purpose. The densitometer, detailed measurement procedures, and the principle of HFP have been fully described in earlier publications.41,42 Briefly, subjects are presented with two superimposed lights that are temporally alternated in square-wave counterphase. This gives the subject an impression on a flickering disc of light. The peak (550 nm) of the spectral composition of one of the lights is chosen to bypass the absorption of MP, and the other (460 nm) is strongly absorbed by MP. The subject’s task is to adjust the relative radiance of the two lights until a percept of no flicker is achieved. All other factors being equal, a subject that requires more short-wave (i.e. 460 nm) relative to middle-wave (i.e. 550 nm) light to achieve null flicker has higher MPOD. This task is performed for the locations of interest within the fovea, which presumably contain MP, and for a reference location in the parafovea that does not (about 7° eccentricity). To obtain a measure of MPOD at a given test locus, the logarithmic ratio of short- to middle-wave radiance (for null flicker) at the reference location is subtracted from the corresponding logarithmic ratio found at the test locus.

**Blood collection**

Fasting blood was collected between 9 am and 11 am, by a licensed phlebotomist, at baseline, 6-month, and 12-month visits. Subjects’ whole blood was collected into a serum separator vacutainer tube (SST) via venipuncture. Blood was allowed to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 × g. Serum was then removed and stored in microvials at −20°C until analysis.

**High-performance liquid chromatography (HPLC)**

Sample extractions and analyses were completed under yellow light. Serum proteins were precipitated with an equal volume of ethanol (1% BHT), containing the internal standard, trans-β-apo-8′-carotenal. After centrifugation, samples were extracted three times with n-hexanes, mixing, and centrifugation. Organic layers were pooled and evaporated to dryness with nitrogen and re-suspended in the mobile phase. An Agilent 1200 series HPLC system, consisting of a quaternary pump with degasser, autosampler, thermostated column compartment, UV–vis diode array detection (DAD) with standard flow cell, and 3D ChemStation software (Agilent Technologies, Santa Clara, CA, USA), was employed for the chromatography. A reversed-phase YMC C30 column (4.6 × 250 mm, 5-μm particle size) was utilized. A stepwise elution consisting of mobile phase A (95% methanol) and mobile phase B (methyl tert-butyl ether) from 15 to 85% B over a 27-minute period at a flow rate of 1 mL/min was employed. A volume of 100 μL was injected for each of the serum samples. Detection wavelengths were λ = 447 nm (L) and 450 nm (Z isomers).
Enzyme-linked immunosorbent assay (ELISA)
Serum was diluted and processed according to the manufacturer’s instructions for the Parameter Cortisol Human ELISA kit (KGE008, R&D Systems, Minneapolis MN, USA). Wells were read at 450 nm (MiniReader MR590, Dynatech Instruments, Inc, Santa Monica CA, USA), averaged across duplicates, and a curve of best fit was used to calibrate to standards. Cortisol concentration data are reported as ng/mL. All coefficient of variability values were under 10%.

Psychological stress measure
Subjects’ psychological stress level was assessed via questionnaire with the 9-item Psychological Stress Measure (PSM-9).43

Brief symptom inventory
Subjects’ current psychological distress was assessed with the Brief Symptom Inventory (BSI),44 a 53-item, self-report instrument developed from the longer SCL-90-R.

Beck anxiety inventory
Subjects’ symptoms of anxiety were assessed with the Beck Anxiety Inventory (BAI),45 a 21-item self-report instrument that is validated for measuring the severity of anxiety.

Beck depression inventory
Subjects’ symptoms of depression were assessed with the Beck Depression Inventory (BDI),46 a 21-item self-report instrument that is validated for measuring the severity of depression.

General health status
The number of physical symptoms of sub-optimal health was determined via self-report questionnaire, using the 25-item Suboptimal Health Status Questionnaire (SHSQ-25).47

Table 1 Summary of self-report questionnaires used during the course of the study

<table>
<thead>
<tr>
<th>Instrument</th>
<th>Items</th>
<th>Outcome measure</th>
<th>Range of scores</th>
<th>Cronbach’s α</th>
<th>Test/retest reliability</th>
<th>Published</th>
<th>Order</th>
</tr>
</thead>
<tbody>
<tr>
<td>PSM-9 Psychological Stress Measure 9</td>
<td>9</td>
<td>Stress in general population</td>
<td>9–72</td>
<td>0.95</td>
<td>0.68–8</td>
<td>Lemyre et al.43</td>
<td>5</td>
</tr>
<tr>
<td>BSI Brief Symptom Inventory</td>
<td>53</td>
<td>Current psychological distress</td>
<td>0–212</td>
<td>0.71–0.85</td>
<td>0.68–91</td>
<td>Derogatis and Melisaratos44</td>
<td>1</td>
</tr>
<tr>
<td>BAI Beck Anxiety Inventory</td>
<td>21</td>
<td>Severity of anxiety</td>
<td>0–63</td>
<td>0.92</td>
<td>0.75</td>
<td>Beck et al.45</td>
<td>2</td>
</tr>
<tr>
<td>BDI Beck Depression Inventory</td>
<td>21</td>
<td>Severity of depression</td>
<td>0–63</td>
<td>0.86</td>
<td>0.93</td>
<td>Beck et al.46</td>
<td>3</td>
</tr>
<tr>
<td>SHSQ-25 Suboptimal Health Status Questionnaire</td>
<td>25</td>
<td>Suboptimal Health Status</td>
<td>25–125</td>
<td>0.93</td>
<td>0.89–98</td>
<td>Yan et al.47</td>
<td>4</td>
</tr>
</tbody>
</table>

Statistical analysis
Graphs and statistical analysis, including descriptive statistics, Pearson product-moment correlations, dependent-samples t-tests, and Repeated-Measures ANOVA were generated using Origin software (Northampton, MA, USA). Statistical significance was determined at the P = 0.05 level. The number of subjects required to detect effects (if present) was calculated via power analysis, which was based on a 20% change in the composite outcome measure of psychological stress / cortisol, and assumed a placebo group with n = 10.

Results
At baseline, significant correlations were determined between MPOD and BAI scores (r = −0.28; P = 0.032 – see Table 2), MPOD and BSI scores (r = −0.27; P = 0.037 – see Table 2), and between serum cortisol and PSM-9 scores (r = 0.46; P < 0.001 – see Fig. 1). Although not statistically significant, marginal correlations were determined at baseline for MPOD and serum cortisol (r = −0.202; P = 0.124), MPOD and psychological stress (r = −0.218; P = 0.10), and MPOD and symptoms of sub-optimal health (r = −0.22; P = 0.092). See Table 2 for a summary of baseline and supplementation effects for all behavioural measures.

After 6 months of MC supplementation, repeated-measures ANOVA revealed that there were no significant beneficial changes from baseline in any parameter for the placebo group. At 6 months, however, serum cortisol was found to increase significantly from baseline. This change did not persist, and returned to baseline levels at 12 months (see Fig. 2). For the 13 mg/day group however, we found that MPOD (P < 0.001) was significantly higher (see Fig. 2), and serum cortisol (P < 0.001 – see Fig. 2), BSI scores (P = 0.005), and number of sub-optimal health symptoms (P = 0.0012) were significantly lower compared to baseline. The 27 mg/day group was found to significantly increase in MPOD (P < 0.001 – see Fig. 2), and
<table>
<thead>
<tr>
<th>Measure</th>
<th>Psychological Stress Measure (PSM-9)</th>
<th>Brief Symptom Inventory (BSI)</th>
<th>Beck Anxiety Inventory (BAI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline relation to MPOD</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>( r = -0.218; P = 0.10 )</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time point</td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td></td>
<td>Baseline</td>
<td>6 months</td>
<td>12 months</td>
</tr>
<tr>
<td>0 mg/day MC</td>
<td>26.7</td>
<td>6.43</td>
<td>29.1</td>
</tr>
<tr>
<td>13 mg/day MC</td>
<td>31.83</td>
<td>7.38</td>
<td>31.38</td>
</tr>
<tr>
<td>27 mg/day MC</td>
<td>31.44</td>
<td>10.24</td>
<td>27.76</td>
</tr>
<tr>
<td></td>
<td>Suboptimal Health Status Questionnaire (SHSQ-25)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>( r = 0.078; P = 0.671 )</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time point</td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td></td>
<td>Baseline</td>
<td>6 months</td>
<td>12 months</td>
</tr>
<tr>
<td>0 mg/day MC</td>
<td>5.3</td>
<td>8.01</td>
<td>5.2</td>
</tr>
<tr>
<td>13 mg/day MC</td>
<td>4.83</td>
<td>3.57</td>
<td>3.79</td>
</tr>
<tr>
<td>27 mg/day MC</td>
<td>4.2</td>
<td>4.03</td>
<td>3.36</td>
</tr>
</tbody>
</table>

\(^aP < 0.05\) compared to baseline.

\(^bP < 0.05\) compared to 6 months.
decrease significantly for the BSI ($P = 0.009$ – see Table 2), BAI scores ($P < 0.001$ – see Table 2), psychological stress ($P = 0.05$ – see Fig. 2), serum cortisol ($P = 0.01$ – see Fig. 2), and number of sub-optimal health symptoms ($P < 0.001$ – see Table 2).

There were no significant changes from baseline determined for any measure in the placebo group at 12 months. For the 13 mg/day group, a significant increase from 6 months to 12 months was found for MPOD ($P < 0.001$ – see Fig. 2), and significant decreases were determined for BSI scores ($P = 0.002$ – see Table 2), BAI scores ($P = 0.013$ – see Table 2), psychological stress ($P = 0.018$ – see Fig. 2), serum cortisol ($P = 0.0037$ – see Fig. 2), and number of sub-optimal health symptoms ($P = 0.007$ – see Table 2). Comparing 6- and 12-month measures, the 27 mg/day MC increased significantly in terms of MPOD ($P = 0.0087$ – see Fig. 2), and decreased significantly for the BSI ($P = 0.013$ – see Table 2), the BAI ($P = 0.038$ – see Table 2), serum cortisol ($P = 0.037$ – see Fig. 2), and symptoms of sub-optimal health ($P = 0.041$ – see Table 2). A significant decrease in scores on the PSM-9 and BDI was determined at 12 months for the 27 mg/day MC group when compared to baseline ($P = 0.05$ and 0.025; see Fig. 2 and Table 2, respectively).

Repeated-measures ANOVA determined that serum L and Z isomers increased significantly after 6 months of supplementation for both active supplement groups ($P < 0.001$; see Figs 3 and 4, respectively) versus placebo, and maintained an apparent steady-state
level at 12 months. As can be seen in Fig. 3, the steady-state L serum level was found to be roughly $2.25 \, \mu g/mL$ for the 13 mg/day MC group, and $3.25 \, \mu g/mL$ for the 27 mg/day MC group. The placebo group remained at a concentration of approximately $0.25 \, \mu g/mL$ throughout the 12-month study period. The change in serum concentration of Z isomers was also found to be significant at 6 months ($P < 0.001$) and, as in the case of L, maintained the 6-month level through 12 months (see Fig. 4). From Fig. 4, it can be seen that the Z isomer steady-state level for the 13 mg/day MC group was $0.37 \, \mu g/mL$, and $0.47 \, \mu g/mL$ for the 27 mg/day MC group. The placebo group remained at a concentration of roughly $0.10 \, \mu g/mL$ throughout the study.

As noted above, MPOD increased significantly from baseline at 6 months, and from 6 months to 12 months in both 13- and 27- mg/day MC groups (see Fig. 2). Despite double the amount of carotenoid in the 27 mg/day MC group’s supplement (27 mg vs. 13 mg), retinal response across the study period was virtually identical for both groups.

In terms of change in measures over the 12-month study period, the relationship between increases in MPOD and decreases in serum cortisol was found to be significant ($r = -0.454; \, P < 0.001$; see Fig. 5). This same relationship was found for psychological stress, where increases in MPOD were significantly related to reduced PSM-9 scores ($r = 0.398; \, P = 0.002$ – see Fig. 6). This kind of relationship with change in MPOD was not found for the other behavioural measures. There were, however, nearly significant relationships determined between the change in symptoms of sub-optimal health and psychological stress ($P = 0.08$), and cortisol ($P = 0.07$), respectively. The finding of a relationship between cortisol and sub-optimal health symptoms was also determined by Yan et al., using the same scale (SHSQ-25) as the present study.

The finding of a relationship between cortisol and sub-optimal health symptoms was also determined by Yan et al., using the same scale (SHSQ-25) as the present study.

**Discussion**

Given the results of this study, it appears that there is a significant role for diet, specifically the MCs, in reducing stress and improving symptoms of both physical and emotional health. Although similar improvements were determined for all outcome measures over the course of the study in both active supplement groups, measures of stress (serum cortisol and PSM-9) were the only measures that were related directly to increases in MPOD. The mechanism for the stress reduction effects appears, therefore, to be related to the accumulation of the MCs in the retina (and presumably the brain). Given the biochemical properties of the MCs, a plausible mechanism for this finding may involve the direct antioxidant and anti-inflammatory action within specific neural tissues that ultimately leads to production of stress-related hormones. Additionally (as suggested in the Introduction section), it could be that the presumed reduction of systemic or local neural oxidative stress via L, Z, and MZ supplementation effectively produced lower physiological stress, which led to reduced psychological stress. As for the measures related to mood (BAI, BDI, BSI) and physical health (SHSQ-25), there was a clear benefit of supplementation with the MCs, but the improvements were not directly related to the change in MPOD. If retinal / brain deposition of L, Z, and MZ, does not account for the improvements in physical / emotional health symptoms, then it would seem plausible that changes in systemic (i.e. serum) carotenoid levels could explain the effects. But changes in serum carotenoid levels were not directly related to changes in physical / emotional health symptoms. There are several possible reasons for this. It may be that differences in systemic oxidative stress and inflammation among participants served to modify serum carotenoid levels in such a way as to mask any relationship between mood / health scales and serum carotenoid
concentration. Alternatively, retinal and brain carotenoid transport efficiency differs substantially between individuals, and this may have impacted serum levels in a non-systematic way. Additionally, serum carotenoid concentrations for a supplementation trial, such as the present study, tend to saturate by about 12 weeks of daily supplementation. Therefore, correlations involving analysis of change would be limited, due to the fact that our subjects probably reached serum saturation long before their second measure (6 months). Lastly, and perhaps most parsimoniously, the reduced psychological stress levels seen in our treatment groups may have served to reduce symptoms of anxiety, depression, and sub-optimal health symptoms in a manner that is not related to our serum measures of either cortisol or carotenoids. Whatever the case, the effects found in our study are consistent with either systemic or neural tissue elevation of MCs. Based on our supplementation data for serum and MPOD, it appears that deposition in neural tissues requires a consistent, relatively elevated serum concentration of L, Z, and MZ – that the placebo group (which did not exhibit improvements in any outcome parameter) did not increase in either serum or MPOD speaks convincingly to this point.

MPOD response to supplementation in both active supplement groups was very similar, despite the higher dose supplement containing roughly double the amount of carotenoids. Serum response was about 30% higher for the 27 mg/day MC group, which indicates that the additional carotenoids either remained higher in serum, or were deposited in other tissues, such as skin or adipose tissue. Another possibility is that, despite random assignment, participants assigned to 13 mg/day MC group tended to (overall) respond more favourably in the retina, compared to those in 27 mg/day MC group. Variability in retinal response to supplementation with retinal carotenoids has been shown previously. Moreover, retinal response typically is found to increase somewhat linearly with increased dose. Although the results of these previous studies are difficult to reconcile with the present results, retinal response was nevertheless robust in both active supplement groups.

Taken together, the cross-sectional and supplementation-trial data make a strong case for the involvement of L, Z, and MZ in psychological stress levels and physical/emotional health symptoms. In terms of stress and physical health, given the well-established relationship between psychological stress and compromised immune function, it is quite possible that the reduction in sub-optimal health symptoms over the period of the study is an effect subsequent to the reduction in stress seen with supplementation. Support for this possibility is provided by the baseline relationship between psychological stress level and number of sub-optimal health symptoms \((r = 0.415; P = 0.0011)\) – see Fig. 7. The nearly significant relationships between change in symptoms of sub-optimal health and change in both serum cortisol \((r = 0.24; P = 0.07)\), and change in psychological stress \((P = 0.08)\) over the study period is further evidence for this idea.

Cortisol is the effector hormone of the hypothalamic-pituitary-adrenal (HPA) axis (the ‘stress’ axis), and widely considered to be an excellent physiological marker for psychological stress. We determined a marginally significant relationship between MPOD and serum cortisol at baseline \((P = 0.124)\), and a strongly significant relationship between change in MPOD and change in serum cortisol for all subjects over the study period \((r = 0.454; P < 0.001)\). As noted above, however, the effect of cortisol reduction was not related to serum carotenoid response. In other words, a subject’s blood response was somewhat similar to the placebo group (which did not exhibit improvements in any outcome parameter) did not increase in either serum or MPOD.

![Figure 6 Change in PSM-9 score over the 12-month study period, as a function of change in MPOD over the same time period. Dotted line least-squares fit to data.](image)

![Figure 7 Baseline SHSQ-25 scores (higher scores = greater number of sub-optimal health symptoms), as a function of baseline PSM-9 scores. Dotted line least-squares fit to data.](image)
independent of cortisol reduction over the study period. This apparent discrepancy could be explained by the fact that the effect of stress reduction is driven by the neural (presumably brain) deposition of these carotenoids, and that this deposition may lead to modulation of the HPA axis. The mechanism for this could involve a local reduction of inflammation, which has been previously shown to be closely linked with stress.\textsuperscript{51} Additionally, corticosteroids generated from the stress response decrease the effectiveness of endogenous antioxidant systems.\textsuperscript{52} Because dietary antioxidants, such as L and Z, supplement endogenous antioxidant systems, such as glutathione and superoxide dismutase,\textsuperscript{53} it could be that local reduction of both oxidation and inflammation (via L and Z) plays a role in the cortisol and stress reduction effects found in our study.

Serum cortisol concentration increased significantly from baseline in the placebo group at 6 months, and then returned to baseline levels at 12 months (see Fig. 2). This may be due to a seasonal effect of variation in serum cortisol,\textsuperscript{54} as the 6-month measure fell within the months when cortisol is reportedly elevated in healthy subjects. Nevertheless, both supplementation groups’ serum cortisol decreased at both 6 and 12 months, suggesting an overall long-term effect of L, Z, and MZ supplementation on serum cortisol.

That such specific nutrients are able to confer substantial and meaningful effects over a relatively short time period could be interpreted in several ways. First, it could be that human beings were meant to consume significantly more foods (e.g. leafy-green vegetables) that contain these carotenoids than is currently the case.\textsuperscript{55} Our serum data from the baseline measure of our entire sample are indicative of low intake (overall) of L, Z, and MZ. Indeed, data from the National Health and Nutrition Examination Survey (NHANES, 2003, as cited in Johnson et al.\textsuperscript{55}) indicate that Americans in the age range (19–30 years) corresponding to our subjects’ general age range consume a paltry 1.5 mg of L and Z daily. At such low levels of consumption, the body may use any available carotenoid for more immediate, systemic purposes (e.g. inflammation, or oxidative stress) rather than depositing it in tissues such as the retina or brain (where our data suggest stress-reducing effects).

Perhaps, our intervention simply brought serum MCs, MPOD (and brain carotenoid) levels up to a point that facilitated relatively ‘normal’ function. In terms of psychological stress and cortisol, this point can be argued not only from the standpoint of the intervention but also from the cross-sectional analysis, where subjects with higher levels of MPOD were found to have marginally significantly lower psychological stress levels and serum cortisol (see Table 2).

In addition to low baseline dietary intake of L, Z, and MZ, another consideration for our findings is the level of stress experienced by the study participants. Our subjects were young and healthy, but nevertheless experienced relatively high levels of psychological stress, and reported a fair number of sub-optimal physical and emotional health symptoms. Stressful situations most often noted by subjects were struggles with coursework (e.g. worrying about grades), relationship problems, and worrying about money. It may be the case that college students experience higher-than-average stress (and subsequent negative health symptoms) than the overall population. If the MCs serve a function of reducing serum cortisol and stress, then it follows logically that supplementation in individuals experiencing relatively high levels of stress would produce acute benefits.

As with any study, caution should be exercised before extending these results to other populations. Although there are advantages in terms of experimental control to studying a fairly homogeneous group, it can limit external validity. Our subjects were similar along many dimensions, including age, BMI, education level, and current life status (i.e. college student); our findings may therefore hold true for this group, but may not extend to others. Additionally, it may be tempting to interpret the behavioural data (BAI, BDI, and BSI) as evidence for the ability of MC supplementation to reduce anxiety or depression. None of our subjects were diagnosed with depression or an anxiety disorder. Our results simply suggest that supplementation with the MCs can reduce symptoms (however few) of anxiety and/or depression. In order to address other populations (e.g. clinically anxious or depressed individuals), additional studies would need to be conducted. In the future, we hope to investigate the effects characterized in the present study in subjects with different lifestyle and dietary habits, in different age groups, and different socioeconomic backgrounds.

Disclaimer statement
Contributors Author N.T.S. contributed to experimental design, data collection, blood collection and analysis, data analysis, and writing of the manuscript. Author P.V.H. contributed to experimental design and manuscript writing. Author J.M.S. contributed to experimental design, data collection, data analysis, and writing of the manuscript.

Funding Omniactive Health Technologies, Inc.

Conflict of interest None.

Ethics approval The study was approved by the Institutional Review Board of the University of Georgia.
References
14 Benton D. To establish the parameters of optimal nutrition do we need to consider psychological in addition to physiological parameters? Mol Nutr Food Res 2013;57(1):6–19.
42 Stringham JM, Hammond BR, Nolan JM, Wooten BR, Mammen A, Smollon W, Snodderly DM. The utility of using...


50 Stringham JM, Stringham NT. Serum and retinal responses to three different doses of macular carotenoids over 12 weeks of supplementation. Exp Eye Res 2016;151:1–8.


