Article:
Boyle, NB, Dye, L orcid.org/0000-0002-2331-4227, Arkbåge, K et al. (4 more authors) (2019) Effects of milk-based phospholipids on cognitive performance and subjective responses to psychosocial stress: A randomized, double-blind, placebo-controlled trial in high-perfectionist men. Nutrition, 57. pp. 183-193. ISSN 0899-9007

https://doi.org/10.1016/j.nut.2018.05.002

Accepted Manuscript

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PII: S0899-9007(18)30362-9
DOI: https://doi.org/10.1016/j.nut.2018.05.002
Reference: NUT 10202

To appear in: *Nutrition*

Received date: 23-10-2017
Revised date: 23-4-2018
Accepted date: 4-5-2018

Please cite this article as: Boyle N.B., Dye L., Arkbåge K., Thorell L., Frederiksen P.D., Croden F., Lawton C.L., The effects of milk-based phospholipids on cognitive performance and subjective responses to psychosocial stress: a randomised, double-blind, placebo controlled trial in high-perfectionist men, *Nutrition* (2018), https://doi.org/10.1016/j.nut.2018.05.002.

The Effects of Milk-Based Phospholipids on Cognitive Performance and Subjective Responses to Psychosocial Stress: A Randomised, Double-Blind, Placebo Controlled Trial in High-Perfectionist Men

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Highlights

- A phospholipid drink conferred a small benefit to RT performance under stress conditions
- Phospholipid intake significantly increased subjective energetic arousal during stress
- Blood pressure responses to stress was significantly raised after phospholipid intake
- Phospholipid intake had no significant effect on working memory or cortisol response

Abstract

Objective

The stress buffering potential of phospholipid (PL) intake on cognitive performance and neuroendocrine and psychological responses under conditions of psychosocial stress were examined in a high stress vulnerable (perfectionist) sample.

Methods

Fifty four high perfectionist males consumed a six week daily intake of a bovine milk-derived PL (2.7g/day) or placebo drink in a randomised, double-blind, placebo controlled, parallel groups design. Working memory, executive control function and acute physiological/subjective responses to an acute psychosocial stressor were examined before and after the six weeks PL or placebo intake.

Results

PL intake improved post-stress RT performance on an attention switching task (p = .01). No significant attenuation of the salivary cortisol stress response was shown. PL intake significantly increased mid-stress induction energetic arousal (p = .03). A non-significant reduction in anticipatory subjective stress was reported after PL intake (p = .06). Systolic (p < .04) and diastolic blood (p = .01) pressures were significantly augmented in the PL condition.

Conclusions

Dietary intake of bovine milk phospholipids conferred cognitive performance benefits under conditions of psychosocial stress, but failed to moderate cortisol response. Moderation of subjective response to stress exposure may have underpinned this performance protection.

Keywords: Phospholipid; psychosocial stress; cognitive performance; cortisol; subjective stress

Phospholipids (PLs) perform a variety of cell membrane structural and regulatory functions. Phosphatidylserine (PS) is crucial in the determination of the surface potential of neuronal membranes essential for intercellular communication [1-3]. Sphingomyelin (SPH) is found in high quantities in the brain and neural tissues [4] and phosphatidylcholine (PC) is the major dietary source of choline (a precursor of acetylcholine synthesis) and also plays a vital role in neuronal membranes [3,5]. Such physiological properties underpin interest in the potential functional benefits of dietary PL intake.

The stress-buffering effects of PLs have been demonstrated via the attenuation of HPA axis-mediated responses to stress. Early research examining PLs extracted from the bovine cortex reported that PS reduced exhaustive exercise induced cortisol activation in well-trained males [6,7]. The transfer of bovine spongiform encephalopathy associated with extraction of PS from bovine cortex prompted examination of the functional properties of PLs extracted from alternative sources; predominantly soy PS (S-PS) and bovine milk PLs (BM-PL). Attenuated cortisol responses to exhaustive exercise [8,9], and psychosocial stress [10] have been demonstrated after S-PS intake. Reduced subjective stress responses to psychosocial challenge have also been reported in those supplemented with S-PS [10] and BM-PLs [11].

The moderation of cognitive performance by stress is well established. Acute stress can have both enhancing and impairing effects on performance. The direction of effect is mediated by a number of variables, including proximity of the stressor to specific cognitive processes (e.g. memory consolidation or retrieval), individual stress responsivity, and cognitive domain [12]. Cognitive processes that are not directly relevant to the stressor faced tend to be impaired. For example, cognitive processes extraneous to the immediate threat (e.g. peripheral attention, retrieval of non-stress relevant information) may be negatively affected. Conversely, enhancement of attentional resources needed to process the threat, and memory consolidation of stress-related information likely to permit future adaptive coping may be shown [13]. Glucocorticoids (primarily cortisol in humans) have been identified as the primary moderator of the acute effects of stress on cognitive function [14,15]. The moderation of cognitive performance under stress is often only demonstrated when significant cortisol elevations are elicited [16,17]. This often encourages comparison of cognitive performance across a post-hoc median split of cortisol responder types (i.e., high vs. low).

Evidence of the stress-buffering effects of PLs raises the hypothesis that supplementation may offer protective effects on cognitive performance that is vulnerable to being impaired under conditions of stress. Evidence to date for the protective effects of PL intake on cognitive performance under stress has been modest and inconsistent. Hellhammer et al. [11] found a trend for improved working memory (WM) reaction time (RT) after BM-PL intake. Additionally, Schubert, Contreras, Franz, and Hellhammer [18] reported improved visuospatial memory in a post-hoc split of high stress load older adults after intake of a similar PL drink. Furthermore, supplementation with S-PS has been shown to

improve serial subtraction test accuracy and completion time in young males [19]. However, these effects were independent of cortisol or subjective stress response. Other studies have found no effects of PLs on cognitive performance (e.g., [20]).

The effects of PL supplementation may be limited to individuals characterised by some form of increased 'stress vulnerability'. Benton, Donohoe, Sillance, & Nabb [21] demonstrated that S-PS reduced subjective stress responses and improved mood in participants scoring highly on a neuroticism scale. The action of PLs may also be characterised by a normalisation of the cortisol response dependent upon responder type. For example, an omega-3 PL-rich capsule resulted in a trend for attenuated cortisol in high cortisol responders and increased cortisol in low cortisol responders [22].

This study aimed to address some of the inconsistencies in the existing evidence for effects of PLs on cognitive performance under stress. Considering the impairment of cognitive performance specifically during high cortisol elevations, and evidence of the stress buffering effects of PLs being moderated by some form of stress vulnerability, a proxy indicator of increased cortisol responsivity was adopted to identify a stress vulnerable sample. Perfectionism, the cognitive pattern of excessive standards, self-criticism, and need for order, has been associated with increased fear of failure and social-evaluative threat [23,24]. It has also been associated with increased cortisol responsivity [25], when faced with performing a task in a social context. Previous studies conducted in our laboratory (Boyle et al., in preparation) have also demonstrated a consistent positive association between salivary cortisol responsivity and a sub-dimension of the Frost Multidimensional Perfectionism Scale (FMPS; [26]).

Inconsistency in the impairing effect of stress on cognitive performance may also be influenced by the divergent sensitivities of the tests of cognitive performance employed. Working memory has been shown to be sensitive to the impairing effects of cortisol [17,27-30], and tests engaging multiple WM components (e.g., *n*-back) may be particularly sensitive [17,29]. Emerging evidence suggests executive function is another prefrontal cortex-associated domain of cognitive performance vulnerable to stress [31-33]. Therefore, performance on the *n*-back and an attention switching paradigm were considered appropriate tests to examine the effects of stress and PL intake.

This study examines the effect of six-weeks of daily PL intake on neuroendocrine and subjective stress responses to an acute psychosocial stressor and subsequent cognitive performance in individuals with an increased tendency towards high cortisol responsivity. Supplementation with PLs was expected to dampen the stress response and confer protective effects on cognitive performance sensitive to stress induction compared to the intake of a placebo.

Methods

Design

The study conformed to a randomised, double-blind, placebo controlled, parallel groups design examining cognitive performance after, and acute physiological/subjective responses to, an acute

psychosocial stressor pre- (stress visit 1) and post- (stress visit 2) completion of a six week daily intake of a BM-PL or a matched placebo drink. The study was registered on ClinicalTrials.gov prior to study commencement (ClinicalTrials.gov Identifier: NCT01879813). The study was approved by the University of Leeds' School of Psychology Research Ethics Committee (Ref: 12-0163) and undertaken in accordance with the principles expressed in the Declaration of Helsinki. An honorarium of £120 was paid upon completion of the study.

Participants

Fifty-four healthy, non-smoking, non-obese (BMI < 30 kg/m^2; WHO, 2013), medication-free, adult males were included in the study. Participants were recruited via the University of Leeds participant database and recruitment posters displayed on campus and around the local community. After eligibility screening, participants were randomly assigned to 6 weeks supplementation with the PL or placebo drink. The CONSORT diagram of study recruitment is shown in Figure 1.

<FIGURE 1>

An initial online screening questionnaire was employed to exclude individuals reporting current psychological affective/mood disorders (defined as a Hospital Anxiety and Depression [HADS; [34] subscale score > 8; [35]) and endocrine, cardiovascular, or other chronic diseases. Participation in a clinical study within a month prior to screening and previous participation in a stress induction study were also included as exclusion criteria. The FMPS [26] was administered at screening and scores on the Perfectionism: Organisation subscale were employed to permit selection of individuals with potential for increased cortisol responsivity to acute stress. A median split of Perfectionism: Organisation scores collected over previous studies undertaken in our laboratory, with an analogous sample population (*N* = 57), was used to identify the Organisation score for the top 50th percentile of participants. Accordingly, only individuals scoring ≥ 13 on the Perfectionism: Organisation subscale were considered eligible for participation.

Measures

Stress protocol. The stress induction protocol combined the speech task of the Trier Social Stress Test (TSST; [36]) and the socially evaluated cold-pressor test (SECPT; [37]). The protocols for both stress protocols have been outlined in detail in the respective original papers. Briefly, participants were required to give an unexpected 5 minute speech presenting themselves as a job candidate (stress visit 1) or describe their personality (stress visit 2) to an unresponsive, social-evaluative, opposite sex panel. Upon completion of the speech participants completed a cold pressor test in front of the social-evaluative panel. The SECPT requires the submersion of the hand above the wrist in ice cold water (0 – 4 ºC) for as long as possible (a maximum of three minutes) whilst maintaining eye contact with the panel. Participants were falsely informed that performance on both tasks would be video and audio recorded for further analysis

To reduce the level of habituation in stress responses across repeated stress exposures a number of contextual changes were made to the stress induction protocol across stress visits 1 and 2. The primary researcher, panel members, stress induction room and speech tasks were changed across visits. Prior to this visit, participants were not explicitly told what stress visit 2 would entail, only that they would complete two challenging tasks. Our laboratory has previously demonstrated that this combined psychosocial stressor can be employed over repeated exposures without significant habituation in salivary cortisol or cardiovascular response [38].

Physiological measures. Salivary cortisol samples were collected using a Salivette® device (Sarstedt, Nümbrecht, Germany). Participants were instructed to chew the cotton wool swab for one minute to ensure adequate saliva absorption. Saliva was extracted from cotton wool swabs by centrifugation (2500 rpm for 5 minutes) and frozen at – 20ºC until assay. Salivary-free cortisol concentrations were determined using a Salivary Cortisol Enzyme Immunoassay kit (EIA; Sarstedt; Nümbrecht, Germany;). Intra- and inter-assay variability was below 6.60% and 9.97% respectively. A Spacelabs ambulatory blood pressure monitor (model 90207; Spacelabs Healthcare, OSI Systems, Inc, WA, USA) was used to measure systolic blood pressure (SBP) and diastolic BP (DBP). Two measurements were taken at each time point and the average of the readings used in all analyses

Subjective and psychometric measures. The Stress and Arousal Checklist (SACL; [39]) is a 30-item adjective list of self-reported feelings of stress (18 items) and arousal (12 items). Respondents rate the extent to which each adjective (e.g., stimulated, apprehensive, up tight) describes how they are feeling at the time of completion. Responses are made with reference to a four-point Likert scale: *definitely describes your feelings (++), more or less describes your feelings (+), cannot decide whether it describes how you feel (?),* and *does not describe the way you feel (-*). The long scoring method was employed (++ = 4; + = 3; ? = 2; - = 1). Alternative ordered versions of the SACL were administered at each time point to reduce habituation in response.

The FMPS [26] is a 35-item questionnaire that assesses multiple aspects of perfectionism. The FMPS comprises six subscales: *Concern over Mistakes* (9 items), *Personal Standards* (7 items), *Parental Expectations* (5 items), *Parental Criticism* (4 items), *Doubts about Actions* (4 items) and *Organisation* (6 items). Respondents rate the extent to which a scale item describes them (e.g., "I am a neat person") with reference to a five-point Likert scale: *strongly disagree* = 1*, disagree* = 2*, neither agree nor disagree* = 3*, agree* = 4, and *strongly agree* = 5.

The Perceived Stress Scale (PSS; [40]) was employed to assess pre- and post-intervention chronic stress levels. The PSS is a 10-item self-report scale that assesses how frequently respondents have experienced an uncontrollable, unpredictable or overloading situation during the last month, and the perceived effectiveness of individual ability to cope with this stress (e.g., "In the last month, how often have you felt that you were unable to control the important things in your life?"). Responses were made in reference to a five-point Likert scale: *never* = 0*, almost never* = 1*, sometimes* = 2*, fairly often* = 3, and *very often* = 4.

Cognitive Tests

All cognitive tests were presented using E prime software (Psychology Software Tools, Inc, PA, USA) on a Dell Optiplex 760 desktop computer with a 17" monitor (screen resolution 1280 x 800 pixels).

2-back. Performance on the *n*-back task both engages multiple WM components and is impaired by stress induced cortisol elevations [28]. The n-back is a continuous performance task that measures monitoring, manipulation, and updating WM processes [41]. The task requires respondents to continuously monitor a stimulus sequence and identify if stimuli presented matches the stimuli presented *n* items back in the sequence or not. The load factor *n* can be adjusted to vary task difficulty. A series of digits from 0 to 9 (Palatino Linotype, bold, font size: 30), were presented in a quasi-random sequence in trial blocks of 50 stimuli (inter-stimulus delay 850 ms). Participants were required to decide if the digit presented was a target (matched the digit presented *2* steps back) or a non-target (did not match the digit *2* steps back). Responses were made on a keyboard using the "1" key to record a target and the "2" key for a non-target stimulus. Target stimuli were presented randomly with a probability of 33%. The first three stimuli in each trial block were not targets.

Attention switch task. The ability to switch between tasks is a fundamental function of executive control [42,43]. Attention switch tasks typically require respondents to repeatedly perform a task on some trials then switch to another task when cued to do so. Performance on repeated trials (same task) is typically superior to performance on switch trials (different task). This decrement in performance is the *switch cost* which reflects the time and effort needed to switch between the two tasks [44]. An attention switch task, originally devised by Wylie et al. [44] that combines a task-switch paradigm with a Go/noGo task was employed. Letter-number pairs (Arial, bold, font size: 40) were presented on a horizontal plane in the centre of the screen for 1 sec (120 ms inter-stimulus). Each character was 1° to the left or right of the central fixation point (randomly determined). Letters were taken from a set containing 4 vowels (A, E, I, and U) and four consonants (G, K, M, and R). The numbers were taken from a set containing 4 even (2, 4, 6, and 8) and 4 odd numbers (3, 5, 7, and 9). The letter-number pairs were presented in two alternating colours every three trials. Respondents were required to make a Go/noGo choice based upon the colour of the letter-number pairs. The change in colour cued the switch in task-set. For example, when letter-number pairs were red, respondents were required to respond when the letter was a vowel (Go), but not when the letter was a consonant (noGo). Alternatively, when the letter-number pairs switched to blue, respondents were required to respond when the number was even (Go), but not when the number was odd (noGo). The three trials in each task-set are split into switch, nested, and pre-switch trials. Switch trials are the first letter-number pairs presented after the task-switch (i.e., the Go/noGo colour switch). Nested and pre-switch are the subsequent repeat trials within the same task-set. (see Figure 2 for stimulus configuration).

<FIGURE 2>

In total 144 trials were presented with target trials randomly presented with a probability of 50%. Responses were made on a keyboard spacebar. Parallel versions of the task were employed differing only with respect to colours used to cue the task-switch. The cost of switching between switch and repeat trials was examined by calculating the difference between the accuracy and RT performance on switch and pre-switch trials relative to nested trial performance. The nested trial was selected as a baseline as this initial repeat trial is less contaminated by preparation to switch to the new task-set than the pre-switch trial [44]. Accuracy and RT *switch costs* (switch trial – nested trial) and *repeat* costs (pre-switch trial – nested trial) were calculated as a measure of switching performance on switch and repeat trials. The accuracy costs are presented as a percentage of the total number of targets (e.g., switch trial – nested trial/total number of targets [72]*100).

Study Drinks

Water-based, isovolumetric (250 ml) BM-PL and placebo drinks were produced with milk protein concentrates. The macronutrient content of both products was similar (Table 1). The PL drink was formulated using a milk protein concentrate rich in PS, SPH, PC, and phosphatidylethanolamine (PE; Arla Foods Ingredients, Denmark) which provided a daily dose of 2.7 g of PLs (including 300 mg PS). The placebo drink did not contain any PLs. The fat content of the placebo drink was matched with the PL drink by adding butteroil which contains only triglycerides. Drinks were provided in a plain white TetraBrik® carton and flavoured with vanilla and nougat and contained 1.5 % added sucrose to give a comparable taste. Participants consumed one drink each morning providing approximately 140 kcal per daily portion (250 ml). Experimenters were blind to the drink conditions until all data were entered and checked and statistical analyses were completed. Intervention drinks were distinguishable only by a condition code.

<TABLE 1>

Procedure

Participants attended an initial familiarisation visit prior to providing written informed consent. Study eligibility was confirmed during this visit and individuals exhibiting raised BP (> 140/90 mmHg over four measures) were excluded. Cognitive performance practice effects are most pronounced during early test exposures, often reaching asymptote by the third exposure [45-48]. Accordingly, participants completed the 2-back and attention switch task twice at the familiarisation visit to reduce early practice effects influencing performance during intervention study visits.

Participants were randomly allocated to the PL or placebo drink condition at study entry using a SAS generated (Version 9.2; SAS Institute, Inc., Cary, NC) randomisation schedule produced by an independent statistician. All study visits commenced between 1100 hr and 1600 hr (stress induction was completed between the hours of 1200 hr and 1400 hr for all participants). In acknowledgement of evidence demonstrating the moderation of cortisol responsivity by nutritional status [49-51], a test meal was consumed upon arrival at the laboratory to standardise baseline nutritional state. After completion of a 60 minute relaxation period an ambulatory BP monitor was fitted to the upper non-

dominant arm of each participant. Salivary cortisol, cardiovascular and subjective response measures (SACL) were collected at timed intervals across each stress visit (see Figure 3 for measurement time points). Following completion of the stress induction period the cognitive tests were completed in serial order.

<FIGURE 3>

A partial debrief was given to participants following completion of stress visit 1 explaining that none of the 'recorded' data would be analysed until completion of stress visit 2. An initial two week supply of drinks was provided after stress visit 1. A daily study diary was completed by participants to monitor drink compliance and medication intake. A face to face meeting was completed every 2 weeks during drink restock visits to check adherence to study protocols. Participants returned six weeks (± 2 days) after stress visit 1 to complete stress visit 2. The start time of the stress visits was matched within 1 hour to control for any time of day effects. A full debrief was provided upon completion of stress visit 2

Statistical Analyses

All statistical analyses were performed using SAS (Statistical Analysis System, Version 9.2; SAS Institute, Inc., Cary, NC). Cortisol data were skewed and normalized using logarithmic transformations. Cortisol delta increase was calculated by subtracting the baseline (0 minutes) cortisol from the peak post-stress level. The area under the curve with respect to ground (AUCg) was calculated using the trapezoid method [52]. One participant from the PL condition was removed from the study due to non-compliance with drink intake. All data from this participant were removed from analysis. The final sample comprised 27 participants in the placebo condition and 26 in the PL drink condition. One placebo participant's data was removed from analysis for both cognitive tests due to performance being > 4 SDs below the sample mean.

The SAS mixed models procedure was employed to analyse the effects of stress exposure across cognitive performance outcomes, and on salivary cortisol, cardiovascular (SBP and DBP), and subjective stress (SACL) responses. Participant ID was entered as a random effect; drink condition, visit (stress visit 1 and 2), time (time study measures were collected: e.g. 0, + 10, +20), and attention switch trial (switch and repeat costs), were fixed effects. Age, BMI, and PSS scores (prior to stress visits 1 and 2) were initially entered as covariates but subsequently removed from all models due to non-significance. The corresponding measure of each dependent variable at stress visit 1 (pre-intervention) were employed as control variables to assess differences between drink conditions for salivary cortisol, cardiovascular, subjective stress, and cognitive performance outcomes at stress visit 2 (post-intervention). Tukey-Kramer-adjusted *p* values [53] were employed to compare least-squares mean responses across and between the profiles of each drink condition. All values (text and figures) are presented as mean and standard error of the mean *(SEM).*

Results

Sample

The characteristics of participants randomised to each drink condition are shown in Table 2. Participants randomised to the PL and placebo drink conditions did not significantly differ in age, BMI, HADS-A, PSS (prior to stress visits 1 or 2), or Perfectionism: Organisation (*p* values all > .14). A significant difference in HADS-D score across condition was however revealed, $t(51) = 2.22$, $p = .03$. Participants randomised to the PL condition ($\bar{x}$ = 2.80 ± 0.41) reported higher depression ratings compared to those in the placebo condition ($\bar{x}$ = 1.52 ± 0.28). However, the HADS-D scores for both conditions were well within the 'non-caseness' range (< 8; [35]) and likely inconsequential. The duration of SECPT hand submersion across drink condition at stress visit 1, $t(51) = -0.31$, $p = .75$, and stress visit 2, $t(51) = 0.04$, $p = .97$, did not significantly differ. Furthermore, the number of drinks consumed (self-reported compliance) was not significantly different across condition, $t(51) = -1.08$, $p = .29$ (PL $\bar{x}$ = 41.12 ± 0.43; placebo $\bar{x}$ = 41.76 ± 0.32).

<TABLE 2>

Cognitive Performance

2-back. Pre-intervention 2-back performance at stress visit 1 was a significant predictor of target accuracy, $F(1,48) = 102.96$, $p < .001$, target RT, $F(1,48) = 41.43$, $p < .001$, and non-target RT, $F(1,48) = 83.19$, $p < .001$, post-intervention at stress visit 2. A significant pre-intervention (stress visit 1)×condition interaction, $F(1,48) = 9.38$, $p = .04$, and a significant main effect of drink condition, $F(1,48) = 10.16$, $p = .03$, were revealed for target accuracy. However, post-hoc comparisons revealed no significant differences in performance post-intervention across drink conditions. The significant effects were indicative of higher target accuracy performance in the PL-drink condition pre- and post-intervention (a summary of 2-back data is shown in supplementary materials).

Attention switch task. A significant main effect of attention switch trial (switch cost vs. repeat cost) was revealed for accuracy, $F(1.51) = 35.69$, $p < .001$, and RT, $F(1,51) = 122.04$, $p < .001$. Accuracy and RT switch costs were significantly higher than the repeat costs across both drink conditions pre- and post-intervention (all significant at $p < .001$; Figure 4). This is indicative of lower performance (i.e., less accurate and slower) on switch vs. repeat trials.

<FIGURE 4>

Controlling for performance at stress visit 1 revealed a significant main effect of drink condition on RT repeat cost, $F(1,48) = 6.66$, $p = .01$. Post-hoc comparisons revealed the RT repeat cost (performance decrement) was significantly higher for participants in the placebo condition (Figure 5). PL participants incurred significantly lower performance costs on repeat trials than placebo participants ($p = .01$). No significant differences between drink conditions were revealed for attention switch accuracy.

<FIGURE 5>

Cortisol Response

A significant condition×visit×time interaction, $F(16,248) = 2.25$, $p = .01$, and main effects of time, $F(5,260) = 57.38$, $p < .001$, and visit, $F(1,52) = 9.18$, $p = .01$, were demonstrated for salivary cortisol response (Figure 6).

<FIGURE 6>

A higher post-stress cortisol response trajectory and peak (+ 35, + 45, and + 55 minutes) was demonstrated during stress visit 1 in the PL condition. However, no significant differences between the drink condition response profiles were evident at this visit. The significant interaction reflects an increase in salivary cortisol in anticipation of stress induction (0 and + 10 minutes) at stress visit 2. Whilst this tendency was demonstrated in both drink conditions, this response sensitisation (stress visit 2 > stress visit 1) only reached significance in the PL condition. Salivary cortisol levels at 0 minutes were significantly higher at stress visit 2 than corresponding levels at stress visit 1 for this drink condition ($p = .04$).

No significant post-intervention differences in cortisol response between drink conditions at stress visit 2 were revealed when cortisol responses at stress visit 1 were controlled for. Cortisol levels at stress visit 1 were the only significant predictor of post-intervention cortisol levels at + 10, + 25, + 35, + 45, and + 55, minutes (smallest $F(1,49) = 8.24$, $p < .001$).

Higher pre-stress induction cortisol levels and a subsequent less pronounced rise to peak at stress visit 2 resulted in a smaller delta increase in cortisol for both drink conditions (Figure 7). This difference was significant for the PL condition reflected by a main significant effect of visit, $F(1,51) = 9.35$, $p = .003$. Post-hoc comparisons revealed significantly lower delta increases in the PL condition at stress visit 2 compared to stress visit 1 ($p < .03$). A comparable response pattern in the placebo condition did not reach significance. No significant differences in salivary cortisol AUCg across stress visits or between drink conditions were demonstrated (Figure 7). No significant post-intervention differences in cortisol delta increase or AUCg between drink conditions at stress visit 2 were revealed when aggregated cortisol levels at stress visit 1 were controlled for. AUCg cortisol response at stress visit 1 was the only significant predictor of post-intervention AUCg cortisol at stress visit 2, $F(1,49) = 34.49$, $p < .001$.

<FIGURE 7>

Subjective Response

Stress (SACL). A significant condition×time×visit interaction, $F(16,248) = 10.78$, $p < .001$, and significant main effect of time, $F(5,260) = 47.03$, $p < .001$, were revealed for subjective stress responses. The significant interaction reflected different response profiles across the stress visits.

Pre-stress induction ratings were higher, and post-stress induction ratings (mid-stress - + 45 minutes) lower, at stress visit 2 compared to stress visit 1 (Figure 8).

Subjective stress ratings at + 25, and + 35 minutes were significantly higher than pre-stress ratings at 0 and + 10 minutes in both conditions pre-intervention at stress visit 1 (all significant at p <.001).

<FIGURE 8>

Post-intervention subjective stress rating at stress visit 2 peaked mid-stress (+ 25 minutes) in the PL condition, and was significantly higher than post-stress ratings at + 45 and + 55 minutes (both significant at p < .02). Conversely, a pre-stress induction peak was demonstrated in the placebo condition at stress visit 2. This resulted in stress ratings at 0 and + 10 minutes being significantly higher than post-stress levels at + 35 and + 45 minutes (all significant at p < .01). For PL participants, subjective stress at + 10 minutes was significantly higher than the corresponding ratings during stress visit 1 (both significant at p < .01). A more consistent pre-stress induction subjective stress response in placebo participants resulted in ratings at both 0 and + 10 minutes being significantly higher at stress visit 2 compared to corresponding ratings during stress visit 1 (p < .001). A mid-stress (+ 25) habituated response was shown in both conditions post-intervention at stress visit 2.

Controlling for performance at stress visit 1 revealed a significant pre-intervention (stress visit 1)×condition interaction, $F(1,50) = 6.12$, $p = .02$, for subjective stress ratings at 0 minutes. Participants consuming the PL-drink demonstrated lower post-intervention subjective stress ratings at 0 minutes during stress visit 2 than those consuming the placebo ($p = .06$). Pre-intervention stress ratings at stress visit 1 were also a significant predictor of stress ratings at 0, + 10, + 25, + 35, + 45, and + 55 minutes post-intervention at stress visit 2 (smallest $F(1,49) = 18.09$, $p < .001$).

Arousal (SACL). A significant main effect of time, $F(5,260) = 18.73$, $p < .001$, was revealed for subjective arousal response across the two stress visits (Figure 8). No significant differences were revealed across the subjective arousal response profile between drink conditions at stress visit 1 suggesting a comparable response across the drink conditions pre-intervention. Post-hoc comparisons revealed no significant differences across the response profile during stress visit 1 in the PL condition. However, in the placebo condition peak arousal ratings at + 25 minutes were significantly higher than pre-stress ratings a 0, and + 10 minutes, and post-stress ratings at + 35, + 45, and + 55 minutes (all significant at p < .03). This relationship was reversed post-intervention at stress visit 2. Peak arousal ratings at + 25 minutes were significantly higher than pre-stress ratings at 0 and + 10 minutes in the PL condition (both significant at p < .03), whilst no significant differences were found across the placebo condition response profile.

Controlling for arousal ratings at stress visit 1 revealed a significant main effect of drink condition, $F(1,49) = 7.49$, $p = .01$, for mid-stress subjective arousal ratings at + 25 minutes. PL participants

reported significantly higher mid-stress subjective arousal post-drink intervention at stress visit 2 (p = .01). Pre-intervention arousal ratings at stress visit 1 were a significant predictor of arousal ratings at 0, + 10, + 25, + 35, + 45, and + 55 minutes post intervention at stress visit 2 (smallest $F(1,49)$ = 13.60, $p < .001$).

Cardiovascular Response

A significant main effect of time and visit were revealed for SBP (time: $F(7,364) = 143.84$, $p < .001$; visit: $F(1,52) = 11.16$, $p < .001$) and DBP (time: $F(7,364) = 93.07$, $p < .001$; visit: $F(1,52) = 9.86$, $p < .002$) across the two stress visits. No significant differences were found across the SBP or DBP profiles between drink conditions at stress visit 1 suggesting a comparable response pre-intervention (Figure 9).

An analogous SBP and DBP response profile was demonstrated across stress visits in both drink conditions. Blood pressure was significantly elevated above pre-stress (0 and + 10 minutes) levels after introduction to the stressor at + 20 minutes and remained significantly raised until + 35 minutes when BP levels declined towards pre-stress levels ($p < .001$). However, heightened cardiovascular responses in anticipation of stress induction (0 and + 10 minutes) at stress visit 2 were demonstrated in the PL condition for SBP and DBP. This SBP response was significantly higher than the corresponding stress visit 1 SBP measures at + 10 minutes (p = .01).

<Figure 9>

Controlling for pre-intervention (stress visit 1) BP levels revealed significant main effects of pre-intervention SBP and drink condition at 0 minutes (pre-intervention, $F(1,50) = 20.21$, $p = .001$, condition, $F(1,50) = 4.27$, $p = .04$) and + 35 minutes (pre-intervention, $F(1,50) = 47.57$, $p = .001$, condition, $F(1,50) = 4.72$, $p = .03$). Significant main effects of pre-intervention DBP, $F(1,50) = 18.28$, $p = .001$, and drink condition, $F(1,50) = 3.83$, $p = .02$, were also revealed for DBP at + 45 minutes. Post-hoc comparisons revealed participants consuming the PL drink had significantly higher SBP (0 and + 35 minutes; both $p < .04$) and DBP (+ 45 minutes; $p = .01$) during stress visit 2 compared to placebo participants.

DISCUSSION

Despite the lack of attenuation of cortisol response, PL intake was associated with improved RT performance on a task of executive function (attention switch task). Executive control is required in situations that involve the rapid and flexible switching between tasks, actions, or goals, when cued to do so. The cost of switching to a new task (requiring the inhibition of the previous task action) versus the cost of task repetition is considered a measure of cognitive control efficiency. This is a well characterised effect under normal conditions [41,43], and has been demonstrated to be augmented under stress [33,32]. In this study, performance decrements (accuracy and RT) between switch and repeat trials were demonstrated in both drink conditions. The differentiation in RT performance was

within trial type (repeat) rather than across the task switch set. Attention switch trial accuracy did not differ across condition so improved RT following PL intake was not indicative of a speed-accuracy trade-off. Moderation of performance across the task switch or improved performance specific to switch trials, which are central to performance cost effects, would be expected if executive control performance was being influenced. Consequently, the potential for PL intake to protect cognitive performance under stress may relate to RT performance on tasks requiring sustained attention rather than executive function per se. A level of cognitive specificity of this improved RT is implied considering the lack of moderation of WM RT performance. A trend for improved RT performance independent of attenuation of cortisol response has previously been demonstrated following intake of an analogous PL drink [11]. Therefore, RT performance on specific tasks under conditions of stress may benefit from PL intake.

The effect of PL intake on cortisol response demonstrated in the present study is inconsistent with the hypothesised stress attenuating capacity of these lipids. Indeed, an increased cortisol response in anticipation of stress induction was demonstrated after supplementation with PLs. Trends towards elevated anticipatory cortisol responses following PL intake have been reported previously [11,18]. However, this contradicts previous evidence of the potential of PLs to attenuate cortisol responses to acute stress [8,10,6,7,9]. It is worth noting that a heightened pre-stress induction cortisol response was also evident in the placebo condition at stress visit 2, and no significant differences between post-intervention cortisol levels were demonstrated once the pre-intervention response at stress visit 1 was controlled for. This suggests that an anticipatory effect specific to repeated stress exposure and/or individual variability in cortisol responses of individuals randomised to drink conditions may account for the observed effects. Indeed, heightened anticipatory cortisol response appears to be a characteristic of repeated exposure to a homotypic stressor [54,55], and physically challenging stressors in particular have previously been associated with increased anticipatory cortisol responses [56-58].

These findings can be seen to add to the existing heterogeneous evidence of the potential for dietary PLs to moderate cortisol response to stress. More clearly defined mechanisms for hypothesised actions of PLs on psychoneuroendocrine function are required to better understand why these lipids demonstrate an inconsistent capacity to attenuate and augment cortisol responses to stress. The mechanisms via which PLs are incorporated into cellular membranes are complex and not fully characterised; particularly *in vivo* in humans [59]. Mechanisms by which PLs may exert effects on HPA axis-mediated stress responses are particularly poorly explicated. Furthermore, further clarification is needed regarding the intake period necessary to sufficiently alter brain levels of PLs and the bioavailability of these lipids during dietary intake. Early research suggested the amount of PLs that reach the CNS after oral or intraperitoneal administration may be very small [60]. For example, only 0.01% of PS was detected in the rat brain after acute intraperitoneal injection [61]. However, more recent evidence demonstrates dietary PLs are readily absorbed and distributed to

multiple tissues, including the brain (rat model; [62]), and can affect neonatal brain growth (gray and white matter), structure, and chemistry after 28 days of intake (piglet model; [63]).

The psychological stress buffering potential of PLs received modest support with only a trend towards attenuated anticipatory subjective stress response in PL supplemented participants. Whilst marginal, the direction of effect is in line with previous evidence of the potential psychological stress-buffering effects of PLs [21,8,10,11]. The mechanism underpinning such effects is unclear. Higher cortisol levels have been previously associated with reduced negative mood and lower levels of anxiety [64,65]. Therefore, higher anticipatory cortisol response evident in the PL condition may explain this attenuated subjective response. Intake of PL also heightened subjective arousal mid-stress. The SACL arousal dimension is primarily a measure of energetic arousal exemplified by the adjective ratings: *activated, vigorous, energetic, stimulated* [39]. Thus, PL intake increased subjective levels of energy and arousal during peak stress exposure which may be hypothesised to increase stress coping potential. The capacity of PL intake to reduce subjective anticipatory stress and increase peak stress energetic arousal is comparable to previous evidence demonstrating reduced perceived stress and increased perceived stress controllability [11]. Improved cognitive performance in the absence of attenuated cortisol response may, therefore, be underpinned by the subjective stress-buffering effects of PL intake.

An unexpected effect of PL intake was increased cardiovascular response. This increased responsivity was evident both in anticipation of stress induction (0 minutes [SBP]) and response recovery (+ 35 [SBP] and + 45 [DBP]). No moderation of cardiovascular parameters by PL intake has been reported in previous stress induction studies [10,22,6,7,19]. Indeed, PL intake has previously been associated with reduced basal BP [66] and positive moderation of markers related to cardiovascular function (e.g., lowered blood cholesterol; [59]). No significant differences in cold pressor hand submersion were demonstrated so this cannot account for the differences in cardiovascular tone.

The action of cortisol on the cardiovascular response to stress may have contributed to the divergent post-intervention BP response. The permissive effects of glucocorticoids (GCs) on BP and cardiac output have been demonstrated in humans and animal models [67]. In most cases (predator avoidance being one exception), GCs act to 'permit' catecholamines and other vasoconstrictors to exert their full actions by augmenting cardiovascular activation during stress [68]. Mechanisms include a positive inotropic effect on vascular and cardiac tissues [69], the inhibition of catecholamine reuptake and peripheral catechol-*O*-methyltransferase and monoamine oxidase (catecholamine degrading enzymes; [70,71]), and increased cardiovascular sensitivity to catecholamines [67]. Higher cortisol response demonstrated by participants in the PL condition may have augmented the cardiovascular response in this condition compared to the placebo. However, this finding should be treated with caution considering the lack of any previous evidence for this effect of PL intake.

The strengths of the reported study lie in the robust methodology adopted. The potential for phospholipids to moderate stress responses and cognitive performance were examined in a randomised, double-blind, placebo controlled design with careful consideration given to potential confounding factors highlighted by previous research (e.g., nutritional status, sensitivity of cognitive domains/tests, habituation to repeated stress induction protocols). However, a number of weaknesses are acknowledged. A formal power calculation was not possible due to the lack of appropriate existing evidence of the protective effects of phospholipid intake on cognitive performance. Therefore interpretation of the findings need to be treated with caution. Informally, the sample size was informed by the sample sizes shown to be sufficient to demonstrate an effect of stress on cognitive performance outcomes. The relative contribution of selecting participants high in perfectionism to the cortisol responses exhibited was not possible to assess without the inclusion of a low perfectionism comparator group. It is also noted that the participants randomly allocated to the PL condition demonstrated a higher post-stress salivary cortisol response trajectory. However, this response did not differ significantly from that of participants randomised to the placebo condition, and was entered as a control variable in relevant statistical models. As with any dietary intervention study carried out in a free-living context, full compliance with the study protocol cannot be assured. Whilst drink intake diaries and face-to-face compliance meetings may have increased the likelihood of compliance, differences in frequency of drink intake across conditions cannot be ruled out. Studies examining acute cortisol responses often exclude female participants from studies owing to the sex dimorphism in HPA axis-mediated stress responses. Males also demonstrate a tendency for higher cortisol stress responses [72]. Therefore, this control measure is commonly adopted in the studies of stress and cognition (e.g., [73,29]), and stress and PL intake [20,21,8,22,11,6,7,19,18,9]. However, considering evidence of the effects of PL intake are almost exclusively confined to male samples, future studies should include female participants. Further, protective effects of dietary interventions on cognitive performance may be more consistently observed in individuals more likely to be cognitively and nutritionally compromised than the young healthy sample reported here. Finally, considering the relatively small RT improvement demonstrated, performance benefits offered by PLs may be particularly relevant to groups for whom smaller margins of performance are important (e.g., athletes).

Conclusions

A six week intake of bovine milk-derived PLs improved RT performance on an attention switching task. This was accompanied, and potentially underpinned, by a trend for an attenuation of heightened subjective anticipatory stress and significantly heightened mid-stress energetic arousal. Working memory performance was unaffected by PL supplementation, suggesting domain-specific benefits of PL intake. Supplementation with PL did not significantly attenuate salivary cortisol responses to psychosocial stress. Rather, intake was associated with a non-significant increase in anticipatory cortisol response.

Ethical Standards

The authors confirm that all participants provided written informed consent prior to inclusion in the study and the anonymity of each was maintained.

Acknowledgements

The first author was supported by a research studentship PhD grant from Arla Foods. The authors acknowledge the vital contributions of Iseli De Waard, Suzie Monk and Vanessa Karsah in the collection of data. The time and austerity of those that formed the stress panels is greatly appreciated.

Conflict of Interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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Figure and Table legends

Figure 1. Study CONSORT diagram

Figure 2. Attention switching task stimulus configuration (adapted with permission from Wylie et al., 2003) showing seven consecutive trials. Participants were required to make a Go/noGo response if the letter shown was a vowel or consonant (red coloured stimuli), and if the number shown was odd or even (green coloured stimuli). The task set switched between the two categorisation Go/noGo tasks every three trials. Wylie GR, Javitt DC, Foxe JJ (2003) Task switching: a high-density electrical mapping study. *Neuroimage* 20 (4):2322-2342. doi:10.1016/j.neuroimage.2003.08.010

Figure 3. Procedural time line showing study measurements and time points

Figure 4. Mean (± *SEM)* performance accuracy (a) and RT (b) switch and repeat costs pre- (stress visit 1) & post- (stress visit 2) intervention. Performance on switch (switch cost) and repeat (repeat cost) trials is relative to nested trials. *X* axis denotes nested trial comparator performance level

Figure 5. Mean (± *SEM)* RT switch cost for repeat trials pre & post-intervention

Figure 6. Mean (± *SEM*) salivary cortisol response (nmol/L) according to drink condition and stress visit

Figure 7. Mean (± *SEM)* aggregated salivary cortisol AUCg (a) and delta increase (b) according to drink condition and stress visit

Figure 8. Mean (± *SEM)* subjective stress (a) and arousal (b) rating (SACL) according to drink condition and stress visit

Figure 9. Mean (± *SEM)* SBP (a) and DBP (b; mmHg) according to drink condition and stress visit

Table 1 Study drinks macronutrient content (g/100g)

	PL drink	Placebo drink
Macronutrients	(per 100g)	
Protein	3.2 g	3.4 g
Carbohydrates	5.0 g	4.8 g
Fat	2.0 g	1.8 g

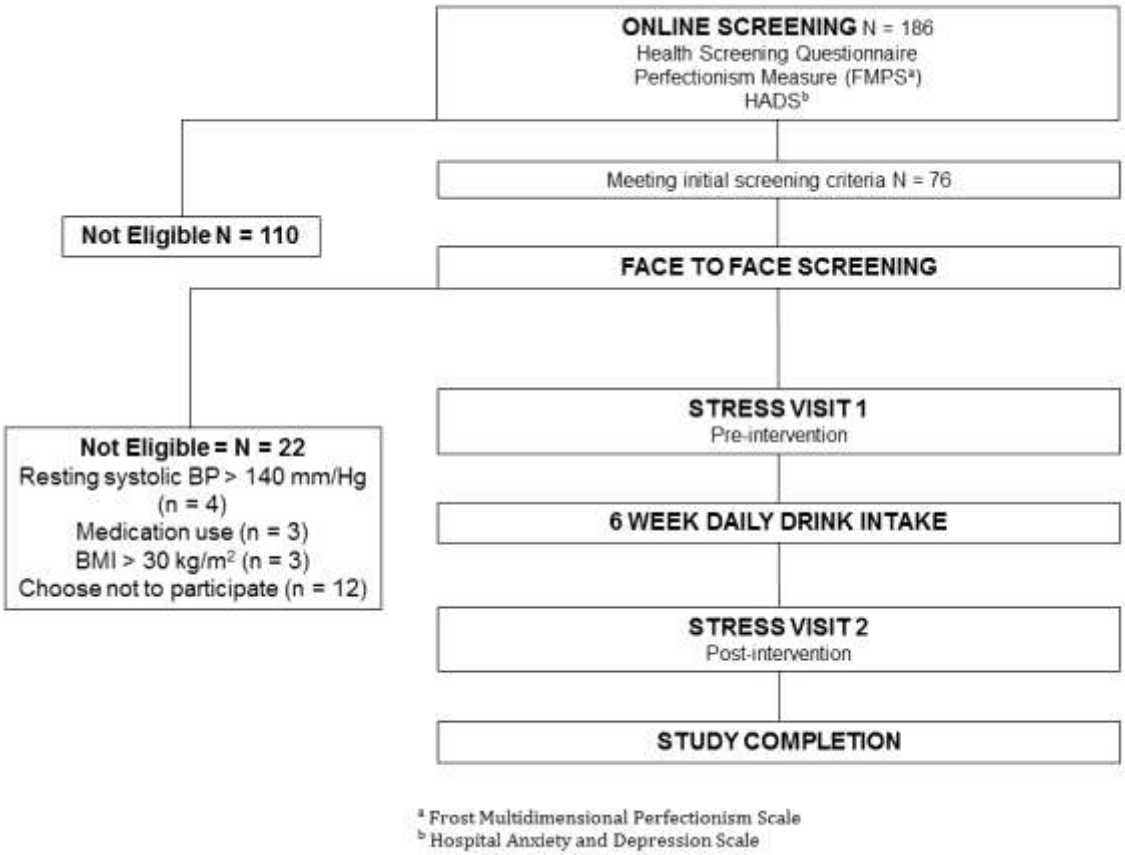
Table 2 Participant characteristics ($\bar{x}$ ± *SEM)* according to drink condition

	n	**Age**	**BMI**	**HADSa**		**PSSb**		**Perfectionism**
Condition				*Anxiety*	*Depression*	*Week 1*	*Week 7*	*Organisation*
PL	26	22.04 (0.76)	22.60 (0.39)	4.26 (0.39)	2.80 (0.41)*	14.80 (1.00)	14.07 (1.01)	16.31 (0.81)
Placebo	27	20.81(0.34)	23.18 (0.38)	4.15 (0.48)	1.52 (0.28)	13.45 (0.70)	15.19 (1.05)	16.63 (0.80)

a Hospital Anxiety Depression Scale

b Perceived Stress Scale

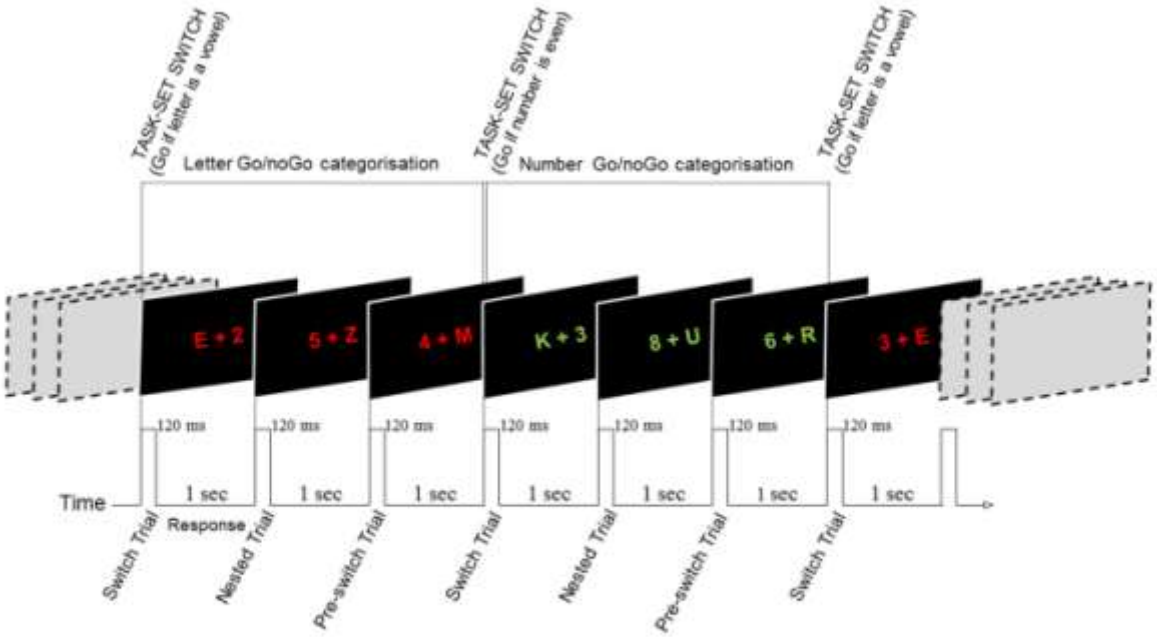
* Significantly higher HADS Depression rating in PL groups. Both scores are well below suggested caseness value (< 8; [34])



881

882 Fig 1.tif

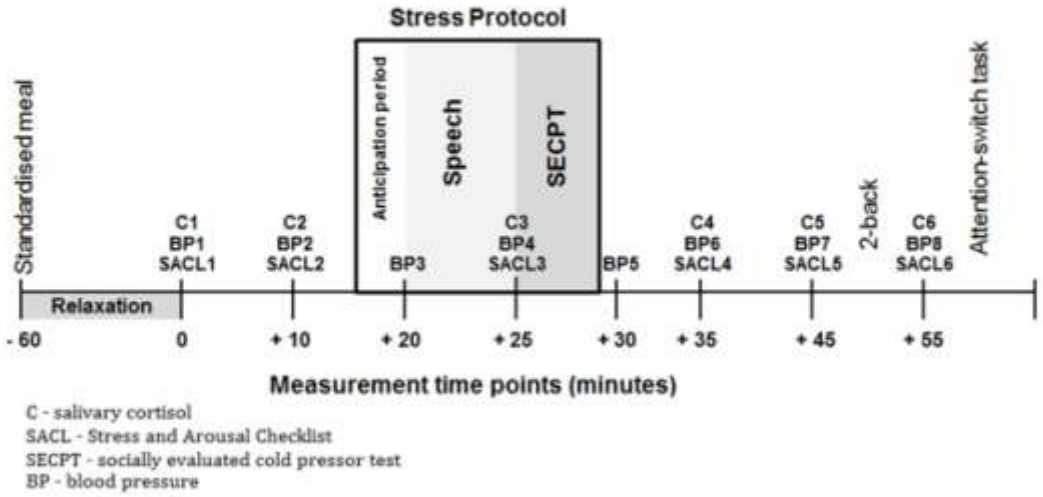
883



884

885 Fig 2.tif

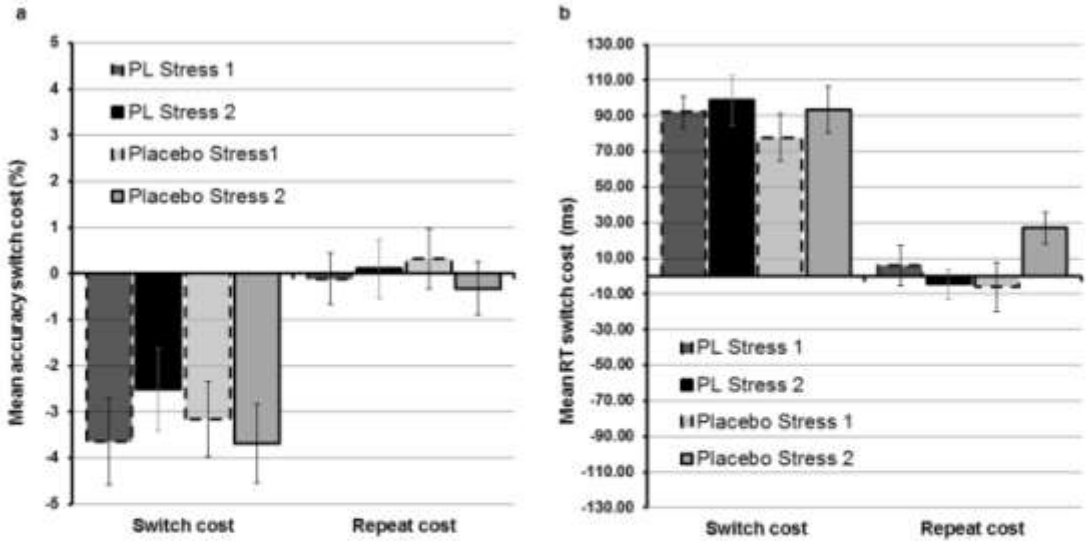
886



887

888 Fig 3.tif

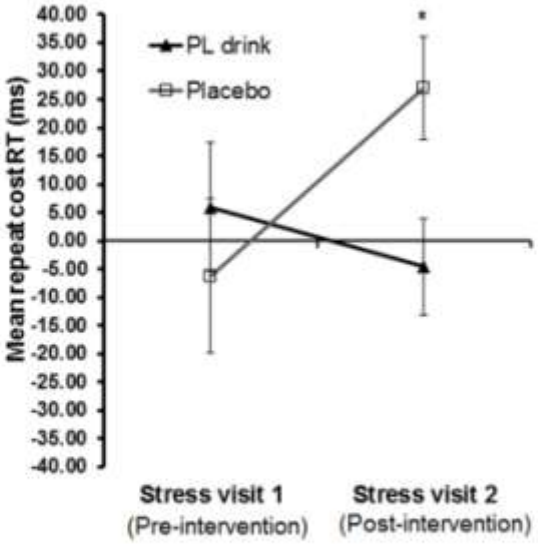
889



890

891 Fig 4.tif

892



*p = .01

893

894 Fig 5.tif

895

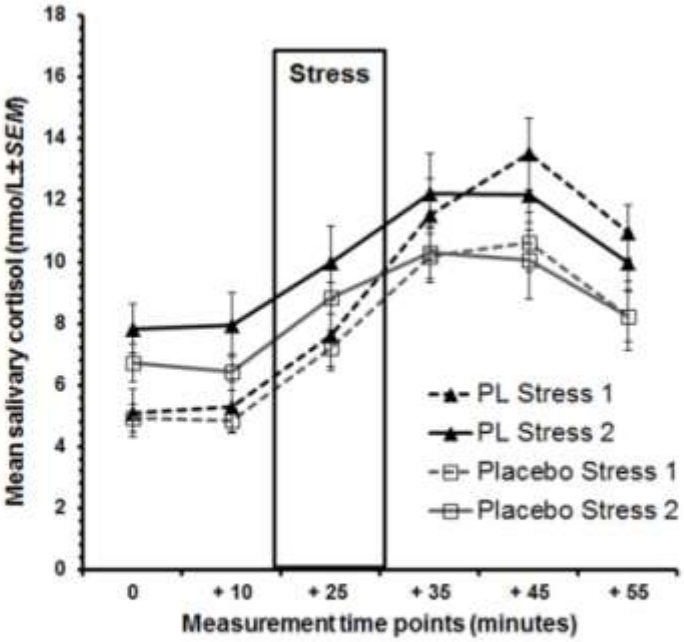
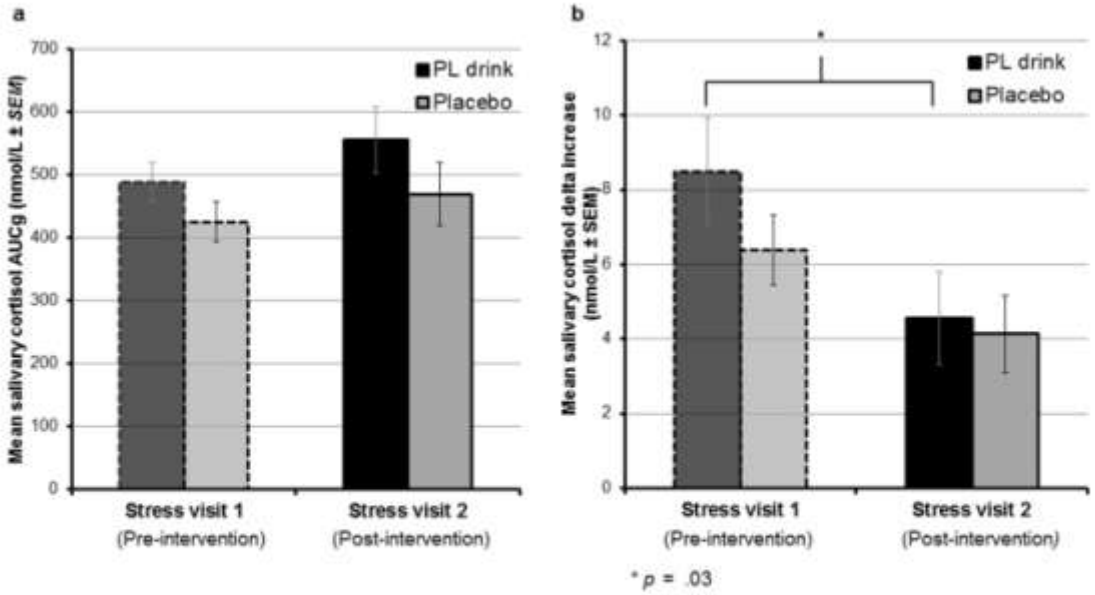


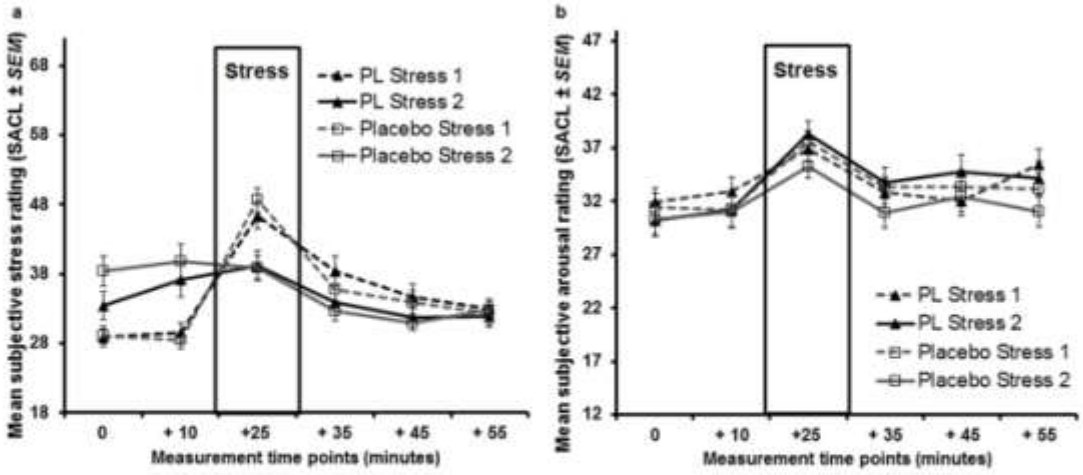
Fig 6.tif



899

900 Fig 7.tif

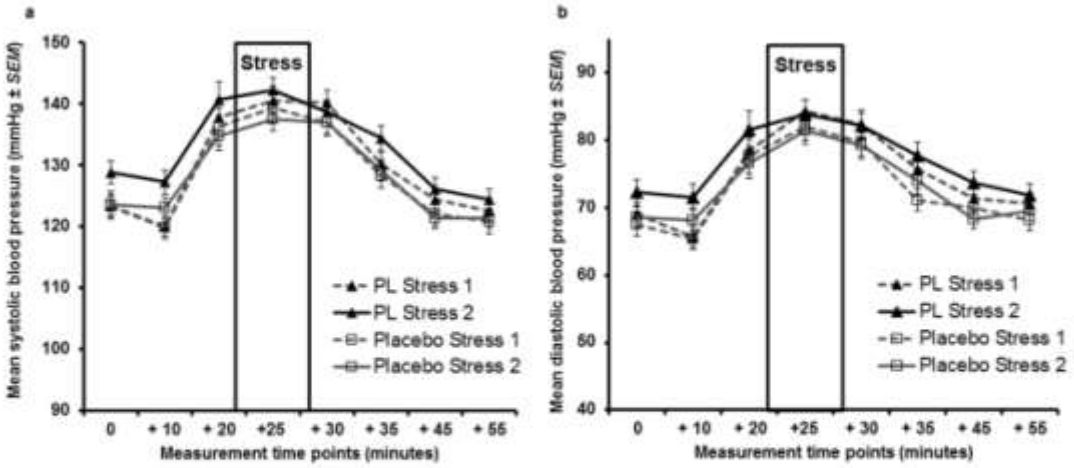
901



902

903 Fig 8.tif

904



905

906 Fig 9.tif