Nutrition 57 (2019) 183–193

Contents lists available at ScienceDirect

Nutrition

journal homepage: www.nutritionjrnl.com

Applied nutritional investigation

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

Neil B. Boyle Ph.D. [a,*], Louise Dye Ph.D. [a], Karin Arkbåge Ph.D. [b], Lars Thorell M.Sc. [b], Pernille Frederiksen M.Sc. [c], Fiona Croden M.Sc. [a], Clare Lawton Ph.D. [a]

[a] School of Psychology, The University of Leeds, Leeds, United Kingdom
[b] Arla Strategic Innovation Centre, Arla Foods, Stockholm, Sweden
[c] Arla Foods Ingredients Group, Viby, Denmark

ARTICLE INFO

Article History:
Received 23 October 2017
Received in revised form 23 April 2018
Accepted 4 May 2018

Keywords:
Phospholipid
Psychosocial stress
Cognitive performance
Cortisol
Subjective stress

ABSTRACT

Objectives: The aim of this study was to examine the stress-buffering potential of phospholipid (PL) intake on cognitive performance and neuroendocrine and psychological responses under conditions of psychosocial stress in a high-stress vulnerable (perfectionist) sample.
Methods: Fifty-four high-perfectionist men consumed a 6-wk daily intake of a bovine milk–derived PL (2.7 g/d) or placebo drink in a randomized, 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 6-wk PL or placebo intake.
Results: PL intake improved post-stress reaction time performance on an attention-switching task ($P = 0.01$). No significant attenuation of the salivary cortisol stress response was shown. PL intake significantly increased mid-stress induction energetic arousal ($P = 0.03$). A non-significant reduction in anticipatory subjective stress was reported after PL intake ($P = 0.06$). Systolic and diastolic blood pressures ($P < 0.04$ and $P = 0.01$, respectively) were significantly augmented in the PL condition.
Conclusions: Dietary intake of bovine milk PLs conferred cognitive performance benefit under conditions of psychosocial stress but failed to moderate cortisol response. Moderation of subjective response to stress exposure may have underpinned this performance protection.

Introduction

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 is found in high quantities in the brain and neural tissues [4], and phosphatidylcholine 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 hypothalamic–pituitary–adrenal 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 men [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 also have 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

* Corresponding author. Tel.: +44 113 343 1403; fax: +44 113 343 5749.
E-mail address: N.b.boyle@leeds.ac.uk (N.B. Boyle).

https://doi.org/10.1016/j.nut.2018.05.002
0899-9007/

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 a primary moderator of the acute effects of stress on cognitive function [14,15]. The moderation of cognitive performance under stress often is only demonstrated when significant cortisol elevations are elicited [16,17]. This encourages comparison of cognitive performance across a post hoc median split of cortisol responder types (i.e., high versus 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 impairment 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 et al. [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 [20].

The effects of PL supplementation may be limited to individuals characterized by some form of increased "stress vulnerability." Benton et al. [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 characterized by a normalization of the cortisol response dependent on responder type. For example, an ω-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) also have demonstrated a consistent positive association between salivary cortisol responsivity and a subdimension of the Frost Multidimensional Perfectionism Scale (FMPS) [26].

Inconsistency in the impairing effect of stress on cognitive performance also may be influenced by the divergent sensitivities of the tests of cognitive performance employed. WM is 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 6 wk 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 toward 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 with the intake of a placebo.

Methods

Design

This was a randomized, double-blind, placebo-controlled, parallel groups design study examining cognitive performance and acute physiological/subjective responses to an acute psychosocial stressor before (stress visit 1) and after (stress visit 2) completion of 6-wk daily intake of a BM-PL or a matched placebo drink. The study was registered on ClinicalTrials.gov before commencement (ClinicalTrials.gov Identifier: NCT01879813). The study was approved by the University of Leeds' School of Psychology Research Ethics Committee 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 (body mass index [BMI] < 30 kg/m^2), medication-free, adult men 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-wk supplementation with the PL or placebo drink. The CONSORT diagram of study recruitment is shown in 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]; subscale score >8) [34,35] and endocrine, cardiovascular, or other chronic diseases. Participation in a clinical study within 1 mo before screening and previous participation in a stress-induction study also were 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 [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-min 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°C) for as long as possible (≤3 min) while 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. Before this visit, participants were not explicitly told what stress visit 2 would entail, only that they would complete two challenging tasks. Our laboratory previously demonstrated that this combined psychosocial stressor could 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 1 min to ensure adequate saliva absorption. Saliva was extracted from cotton wool swabs by centrifugation (2500*g* for 5 min) and frozen at −20°C until assay. Salivary-free cortisol concentrations were determined using a Salivary Cortisol Enzyme Immunoassay kit (Sarstedt, Nümbrecht, Germany). Intra- and interassay variability was less than 6.60% and 9.97%, respectively. A Spacelabs ambulatory blood pressure monitor (model 90207; Spacelabs Healthcare, OSI Systems, Inc, Snoqualmie, 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.

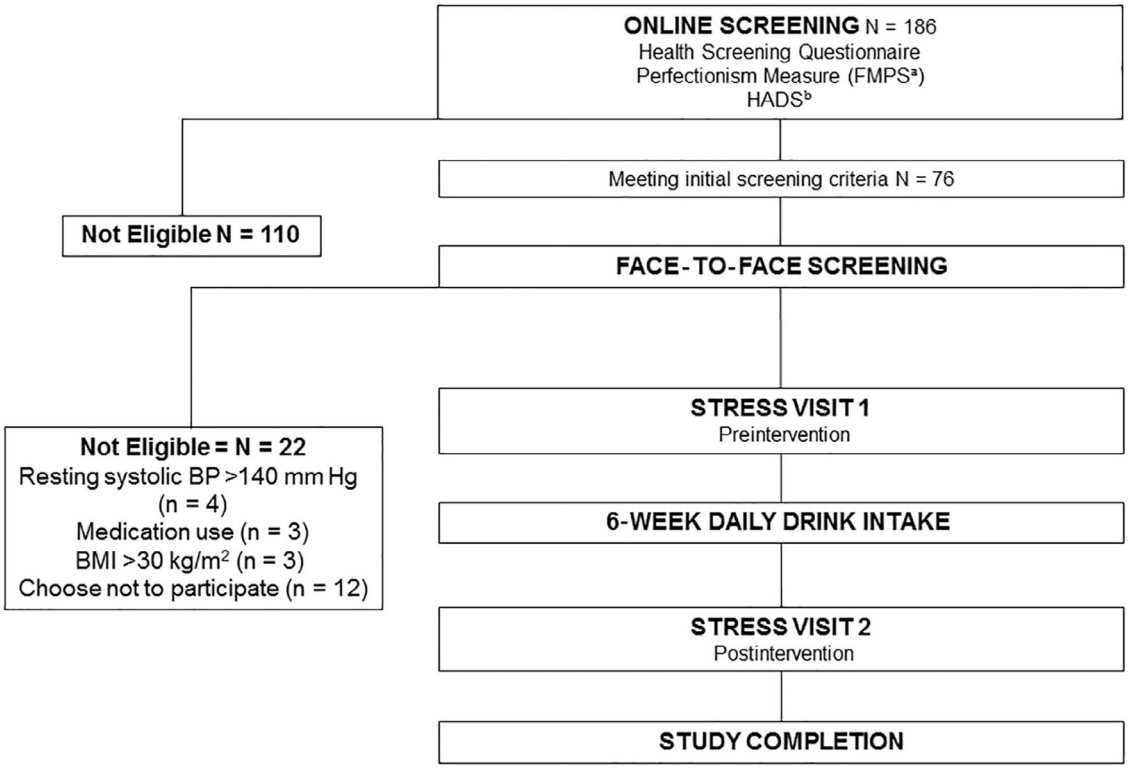

Fig. 1. Study CONSORT diagram. BMI, body mass index; BP, blood pressure; FMPS, Frost Multidimensional Perfectionism Scale; HADS, Hospital Anxiety and Depression Scale.

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, uptight) describes how they are feeling at the time of completion. Responses are made with reference to a 4-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 (nine items), Personal Standards (seven items), Parental Expectations (five items), Parental Criticism (four items), Doubts about Actions (four items), and Organisation (six items). Respondents rate the extent to which a scale item describes them (e.g., *I am a neat person*) with reference to a 5-point Likert scale where 1 is *strongly disagree*, 2 *disagree*, 3 *neither agree nor disagree*, 4 *agree*, and 5 *strongly agree*.

The Perceived Stress Scale (PSS) [40] was employed to assess pre- and postintervention 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 previous 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 5-point Likert scale where 0 is *never*, 1 *almost never*, 2 *sometimes*, 3 *fairly often*, and 4 *very often*.

Cognitive tests

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

N-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 match the stimuli presented *n* items back in the sequence. 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 (interstimulus delay 850 ms). Participants were required to decide if the digit presented was a target (matched the digit presented two steps back) or a non-target (did not match the digit two 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/no Go task was employed. Letter–number pairs (Arial, bold, font size 40) were presented on a horizontal plane in the center of the screen for 1 s (120 ms interstimulus delay). Each character was 1 degree to the left or right of the central fixation point (randomly determined). Letters were taken from a set containing four vowels (A, E, I, and U) and four consonants (G, K, M, and R). The numbers were taken from a set containing four even (2, 4, 6, and 8) and four odd numbers (3, 5, 7, and 9). The letter–number pairs were presented in two alternating colors every three trials. Respondents were required to make a Go/no Go choice based on the color of the letter–number pairs. The change in color 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 (no Go). 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 (no Go). The three trials in each task set were split into switch, nested, and preswitch trials. Switch trials were the first letter–number pairs presented after the task switch (i.e., the Go/no Go color switch). Nested and preswitch were the subsequent repeat trials within the same task set (see Fig. 2 for stimulus configuration).

In total, 144 trials were presented with target trials randomly shown with a probability of 50%. Responses were made on a keyboard spacebar. Parallel versions of the task were employed differing only with respect to colors 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 preswitch 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 preswitch trial [44]. Accuracy and RT switch costs (switch trial—nested trial) and repeat costs (preswitch 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 rinks

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, sphingomyelin, hosphatidylcholine, and phosphatidylethanolamine (Arla Foods Ingredients, Viby, 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 triacylglycerols. Drinks were provided in a plain white TetraBrik carton and flavored with vanilla and nougat and contained 1.5% added sucrose to give a comparable taste. Participants consumed one drink each morning, providing ~140 kcal per daily portion (250 mL). Experimenters were blinded 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.

Procedure

Participants attended an initial familiarization visit before providing written informed consent. Study eligibility was confirmed during this visit, and individuals exhibiting raised BP (>140/90 mm Hg 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 n-back and attention-switch tasks twice at the familiarization 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, USA) randomization schedule produced by an independent statistician. All study visits commenced between 1100 and 1600 (stress induction was completed between the hours of 1200 and 1400 for all participants). In acknowledgment of evidence demonstrating the moderation of cortisol responsivity by nutritional status [49–51], a test meal was consumed upon arrival at the laboratory to standardize baseline nutritional state. After completion of a 60-min 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 Fig. 3 for measurement time points). After completion of the stress-induction period, the cognitive tests were completed in serial order.

A partial debriefing was given to participants after completion of stress visit 1 explaining that none of the "recorded" data would be analyzed until completion of stress visit 2. An initial 2-wk 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 wk during drink restock visits to check adherence to study protocols. Participants returned 6 wk (±2 d) after stress visit 1 to complete stress visit 2. The start time of the stress visits was matched within 1 h to control for any time-of-day effects. A full debriefing was provided at completion of stress visit 2.

Statistical analyses

All statistical analyses were performed using SAS Version 9.2. Cortisol data were skewed and normalized using logarithmic transformations. Cortisol delta increase was calculated by subtracting the baseline (0 min) 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 analyze 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 visits 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 (before stress visits 1 and 2) were initially entered as covariates but subsequently were removed from all models due to non-significance. The corresponding measure of each dependent variable at stress visit 1 (preintervention) 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 (postintervention). 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 SEM.

Results

Sample

The characteristics of participants randomized to each drink condition are shown in Table 2. Participants randomized to the PL and placebo drink conditions did not significantly differ in age,

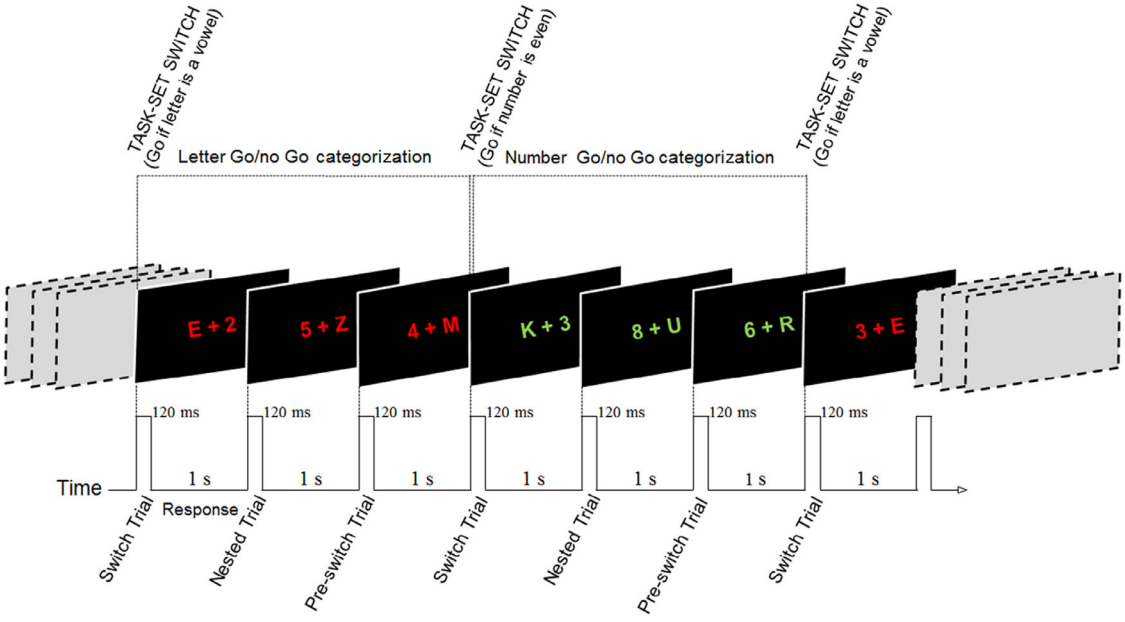

Fig. 2. Attention-switching task stimulus configuration (adapted with permission from Wylie et al., 2003 [44]) showing seven consecutive trials. Participants were required to make a Go/no Go response if the letter shown was a vowel or consonant (red stimuli) and if the number shown was odd or even (green stimuli). The task set switched between the two categorization Go/no Go tasks every three trials.

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

	PL drink	Placebo drink
Macronutrients	per 100 g	
Protein, g	3.2	3.4
Carbohydrates, g	5	4.8
Fat, g	2	1.8

BMI, HADS-A, PSS (before stress visits 1 or 2), or Perfectionism: Organisation (all $P > 0.14$). A significant difference in HADS-D score across condition was revealed ($t(51) = 2.22$, $P = 0.03$). Participants randomized to the PL condition ($x = 2.80 \pm 0.41$) reported higher depression ratings compared with those in the placebo condition ($x = 1.52 \pm 0.28$). 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 ($t(51) = -0.31$, $P = 0.75$) and stress visit 2 ($t(51) = 0.04$, $P = 0.97$) did not significantly differ. Furthermore, the number of drinks consumed (self-reported compliance) was not significantly different across condition ($t(51) = -1.08$, $P = 0.29$; PL $x = 41.12 \pm 0.43$; placebo $x = 41.76 \pm 0.32$).

Cognitive performance

N-back

Preintervention n-back performance at stress visit 1 was a significant predictor of target accuracy ($F(1,48) = 102.96$, $P<0.001$; target RT, $F(1,48) = 41.43$, $P<0.001$) and non-target RT ($F(1,48) = 83.19$, $P<0.001$) postintervention at stress visit 2. A significant preintervention (stress visit 1) × condition interaction ($F(1,48) = 9.38$, $P = 0.04$) and a significant main effect of drink condition ($F(1,48) = 10.16$, $P = 0.03$) were revealed for target accuracy. However, post hoc comparisons revealed no significant differences in performance postintervention across drink conditions. The significant effects were indicative of higher target accuracy performance in the PL-drink condition pre- and postintervention (a summary of n-back data is shown in the supplementary materials).

Attention-switch task

A significant main effect of the attention-switch trial (switch cost versus repeat cost) was revealed for accuracy ($F(1.51) = 35.69$, $P<0.001$) and RT ($F(1,51) = 122.04$, $P<0.001$). Accuracy and RT switch costs were significantly higher than the repeat costs across both drink conditions pre- and postintervention (all significant at $P<0.001$; Fig. 4). This is indicative of lower performance (i.e., less accurate and slower) on switch versus repeat trials.

Controlling for performance at stress visit 1 revealed a significant main effect of drink condition on RT repeat cost ($F(1,48) = 6.66$, $P = 0.01$). Post hoc comparisons revealed the RT repeat cost (performance decrement) was significantly higher for participants in the placebo condition (Fig. 5). PL participants incurred significantly lower performance costs on repeat trials than placebo participants ($P = 0.01$). No significant differences between drink conditions were revealed for attention switch accuracy.

Cortisol response

A significant condition × visit × time interaction ($F(16,248) = 2.25$, $P = 0.01$) and main effects of time ($F(5,260) = 57.38$, $P<0.001$) and visit ($F(1,52) = 9.18$, $P = 0.01$) were demonstrated for salivary cortisol response (Fig. 6).

A higher post-stress cortisol response trajectory and peak (+35, +45, and +55 min) 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 min) at stress visit 2. Although this tendency was demonstrated in both drink conditions, this response sensitisation (stress visit 2 greater then stress visit 1) only reached significance in the PL condition. Salivary cortisol levels at 0 min were significantly higher at stress visit 2 than corresponding levels at stress visit 1 for this drink condition ($P = 0.04$).

No significant postintervention differences in cortisol response between drink conditions at stress visit 2 were revealed when controlling for cortisol responses at stress visit 1. Cortisol levels at stress visit 1 were the only significant predictor of postintervention cortisol levels at +10, +25, +35, +45, and +55 min [smallest $F(1,49) = 8.24$, $P<0.001$].

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 (Fig. 7). This difference was significant for the PL condition reflected by a main significant effect of visit ($F(1,51) = 9.35$, $P = 0.003$). Post hoc comparisons revealed significantly lower delta increases in the PL condition at stress visit 2 compared with stress visit 1 ($P<0.03$). 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

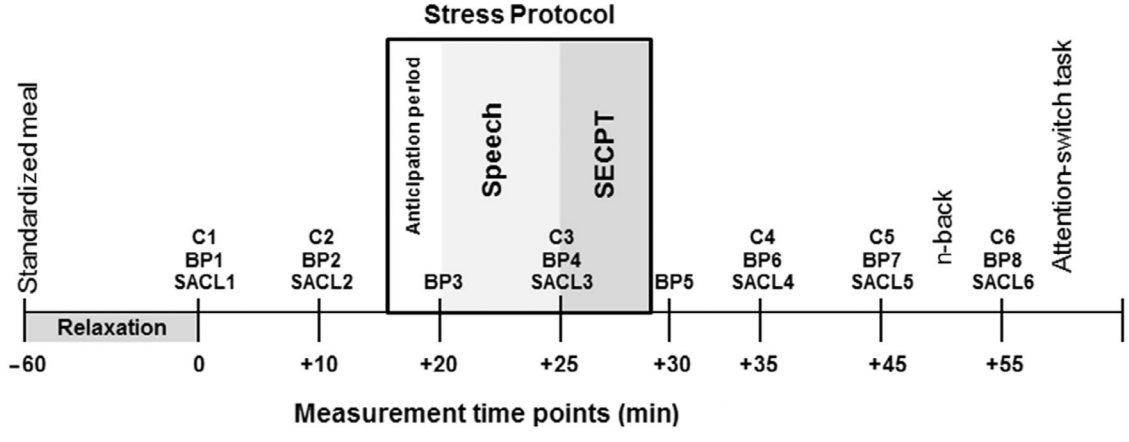

Fig. 3. Procedural time line showing study measurements and time points. BP, blood pressure; C, salivary cortisol; SACL, Stress and Arousal Checklist; SECPT, socially evaluated cold pressor test.

Table 2
Participant characteristics (x ± SEM) according to drink condition

	n	Age, y	BMI, kg/m^2	HADS		PSS		Perfectionist
Condition				Anxiety	Depression	Week 1	Week 7	Organization
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)

BMI, body mass index; HADS, Hospital Anxiety and Depression Scale; PL, phospholipid; PSS, Perceived Stress Scale.
*Significantly higher HADS Depression rating in PL groups. Both scores are well below suggested caseness value (<8) [34].

(Fig. 7). No significant postintervention differences in cortisol delta increase or AUCg between drink conditions at stress visit 2 were revealed when controlling for aggregated cortisol levels at stress visit 1. AUCg cortisol response at stress visit 1 was the only significant predictor of postintervention AUCg cortisol at stress visit 2, $F(1,49) = 34.49$, $P<0.001$.

Subjective response

Stress (SACL)

A significant condition × time × visit interaction ($F(16,248) = 10.78$, $P<0.001$) and a significant main effect of time ($F(5,260) = 47.03$, $P<0.001$) 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 min) lower, at stress visit 2 compared with stress visit 1 (Fig. 8).

Subjective stress ratings at +25 and +35 min were significantly higher than pre-stress ratings at 0 and +10 min in both conditions preintervention at stress visit 1 (all significant at $P<0.001$).

Postintervention subjective stress rating at stress visit 2 peaked mid-stress (+25 min) in the PL condition and was significantly higher than post-stress ratings at +45 and +55 min (both significant at $P<0.02$). 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 min being significantly higher than post-stress levels at +35 and +45 min (all significant at $P<0.01$). For PL participants, subjective stress at +10 min was significantly higher than the corresponding ratings during stress visit 1 (both significant at $P<0.01$). A more consistent pre-stress induction subjective stress response in placebo participants resulted in ratings at both 0 and +10 min being significantly higher at stress visit 2 compared with corresponding ratings during stress visit 1 ($P<0.001$). A mid-stress (+25) habituated response was shown in both conditions postintervention at stress visit 2.

Controlling for performance at stress visit 1 revealed a significant preintervention (stress visit 1) × condition interaction ($F(1,50) = 6.12$, $P = 0.02$) for subjective stress ratings at 0 min. Participants consuming the PL drink demonstrated lower postintervention subjective stress ratings at 0 min during stress visit 2 than those consuming the placebo ($P = 0.06$). Preintervention stress ratings at stress visit 1 were also a significant predictor of stress ratings at 0, +10, +25, +35, +45, and +55 min postintervention at stress visit 2 (smallest $F(1,49) = 18.09$, $P<0.001$).

Arousal (SACL)

A significant main effect of time ($F(5,260) = 18.73$, $P<0.001$) was revealed for subjective arousal response across the two stress visits (Fig. 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 preintervention. 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 min were significantly higher than pre-stress ratings a 0, and +10 min, and post-stress ratings at +35, +45, and +55 min (all significant at $P<0.03$). This relationship was reversed in postintervention at stress visit 2. Peak arousal ratings at +25 min were

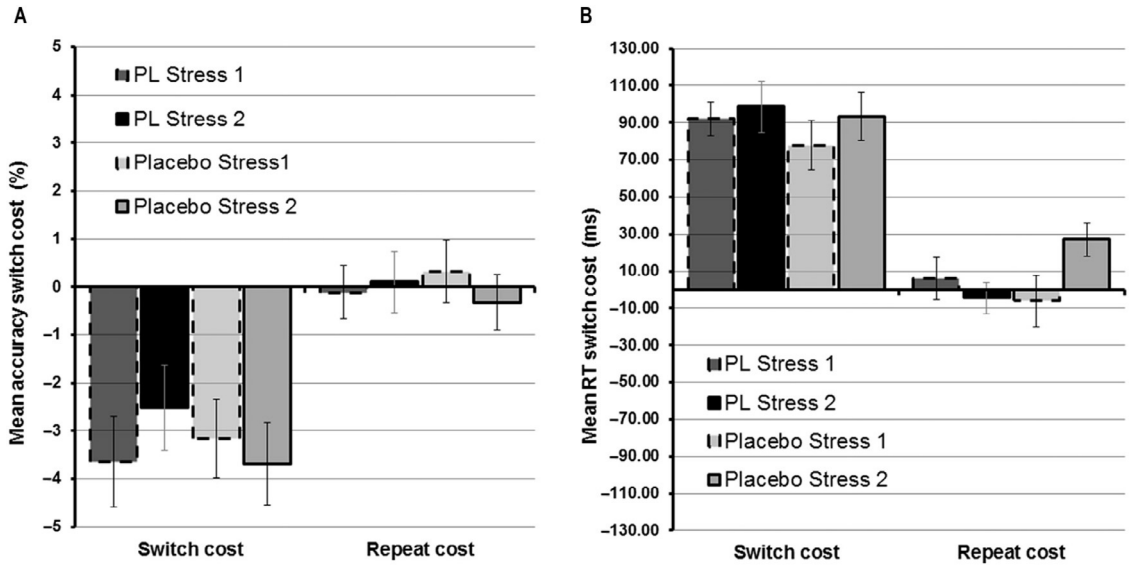

Fig. 4. Mean (± SEM) performance accuracy (A) and reaction time (B) switch and repeat costs before (stress visit 1) and after (stress visit 2) intervention. Performance on switch (switch cost) and repeat (repeat cost) trials is relative to nested trials. X axis denotes nested trial comparator performance level. PL, phospholipid.

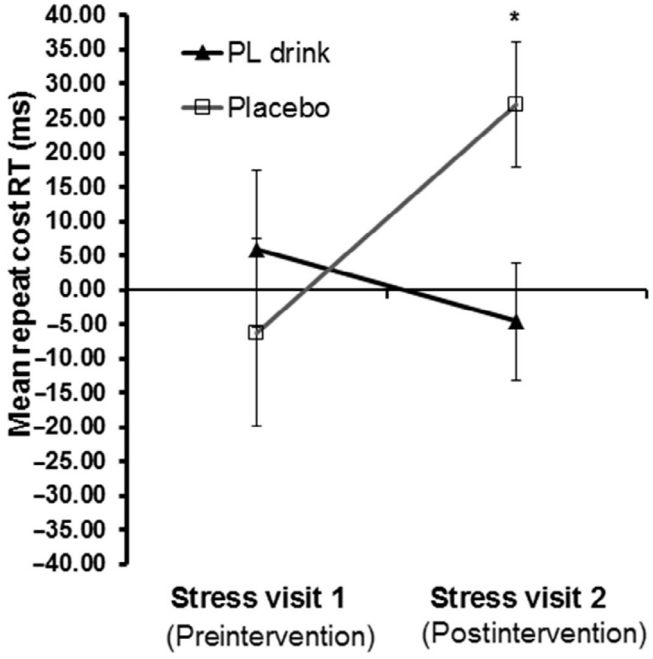

Fig. 5. Mean (± SEM) Reaction time switch cost for repeat trials pre- and postintervention. PL, phospholipid; RT, reaction time. *$P = 0.01$.

significantly higher than pre-stress ratings at 0 and +10 min in the PL condition (both significant at $P<0.03$), although 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 ($F(1,49) = 7.49$, $P = 0.01$) for mid-stress subjective arousal ratings at +25 min. PL participants reported significantly higher mid-stress subjective arousal post-drink intervention at stress visit 2 ($P = 0.01$). Preintervention arousal ratings at stress visit 1 were a significant predictor of arousal ratings at 0, +10, +25, +35, +45, and +55 min postintervention at stress visit 2 (smallest $F(1,49) = 13.60$, $P<0.001$).

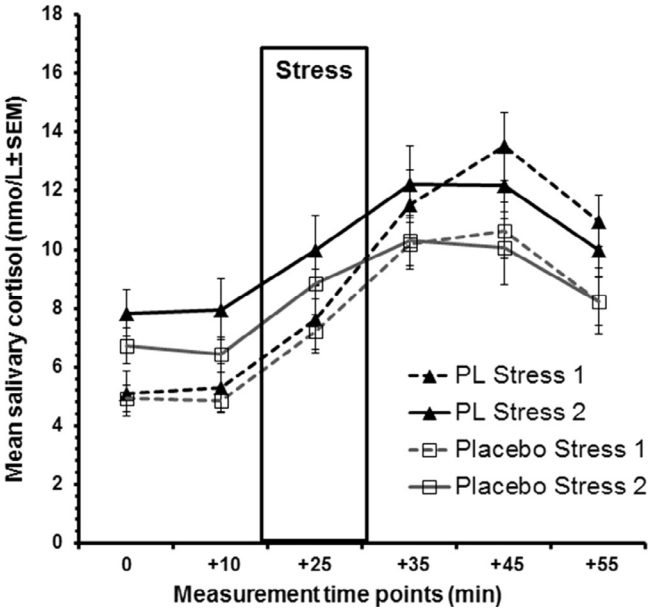

Fig. 6. Mean (± SEM) salivary cortisol response (nmol/L) according to drink condition and stress visit. PL, phospholipid.

Cardiovascular response

A significant main effect of time and visit were revealed for SBP (time: $F(7,364) = 143.84$, $P<0.001$; visit: $F(1,52) = 11.16$, $P<0.001$) and DBP (time: $F(7,364) = 93.07$, $P<0.001$; visit: $F(1,52) = 9.86$, $P<0.002$) 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 preintervention (Fig. 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 min) levels after introduction to the stressor at +20 min and remained significantly raised until +35 min when BP levels declined toward pre-stress levels ($P<0.001$). However, heightened cardiovascular responses in anticipation of stress induction (0 and +10 min) 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 min ($P = 0.01$).

Controlling for preintervention (stress visit 1) BP levels revealed significant main effects of preintervention SBP and drink condition at 0 min (preintervention, $F(1,50) = 20.21$, $P = 0.001$, condition, $F(1,50) = 4.27$, $P = 0.04$) and +35 min (preintervention, $F(1,50) = 47.57$, $P = 0.001$, condition, $F(1,50) = 4.72$, $P = 0.03$). Significant main effects of preintervention DBP ($F(1,50) = 18.28$, $P = 0.001$) and drink condition ($F(1,50) = 3.83$, $P = 0.02$) were also revealed for DBP at +45 min. Post hoc comparisons revealed participants consuming the PL drink had significantly higher SBP (0 and +35 min; both $P<0.04$) and DBP (+45 min; $P = 0.01$) during stress visit 2 compared with 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-characterized effect under normal conditions [41,43] and has been demonstrated to be augmented under stress [32,33]. 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 conditions, 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 were 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 hypothesized stress-attenuating capacity of these lipids. Indeed, an increased cortisol

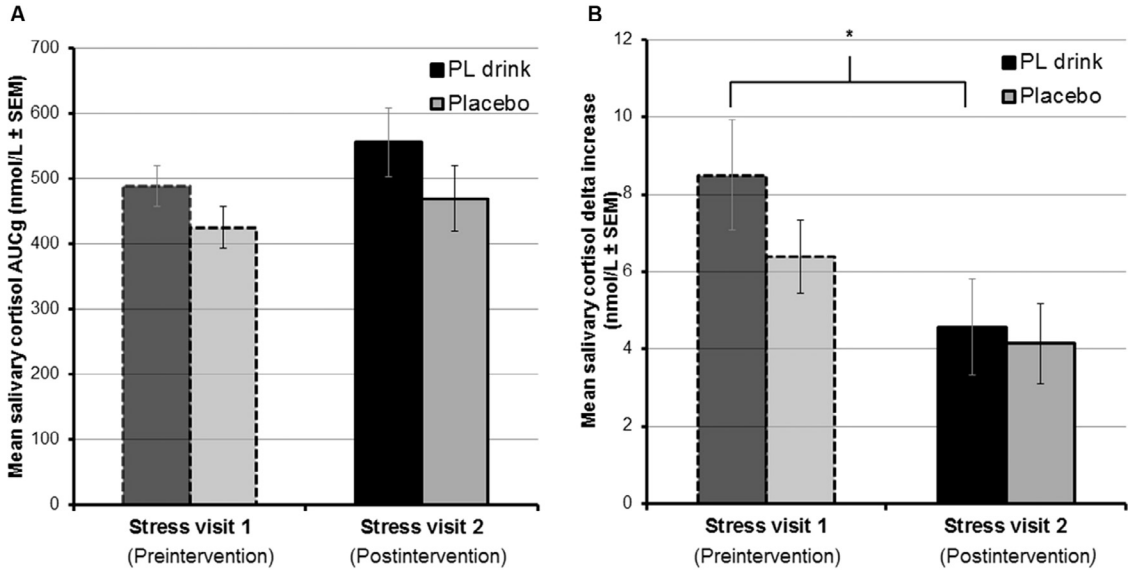

Fig. 7. Mean (± SEM) aggregated salivary cortisol AUCg (A) and delta increase (B) according to drink condition and stress visit. AUCg, area under the curve with respect to ground; PL, PL, phospholipid. *$P = 0.03$.

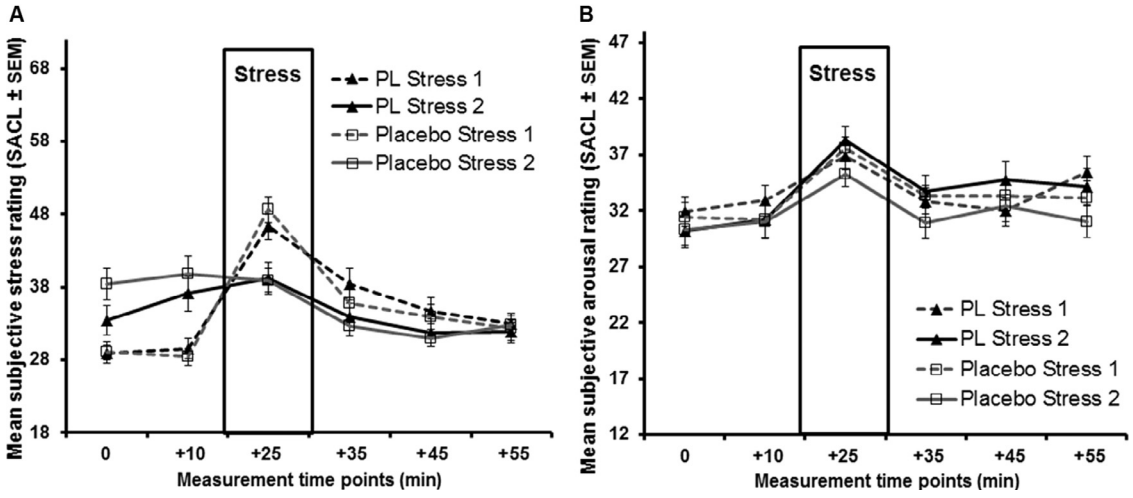

Fig. 8. Mean (± SEM) subjective stress (A) and arousal (B) rating (SACL) according to drink condition and stress visit. PL, phospholipid; SACL, Stress and Arousal Checklist.

response in anticipation of stress induction was demonstrated after supplementation with PLs. Trends toward elevated anticipatory cortisol responses after 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 [6–10]. 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 postintervention cortisol levels were demonstrated once the preintervention 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 randomized 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 hypothesized 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 characterized, 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 central nervous system 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 d of intake (piglet model) [63].

The psychological stress buffering potential of PLs received modest support with only a trend toward attenuated anticipatory

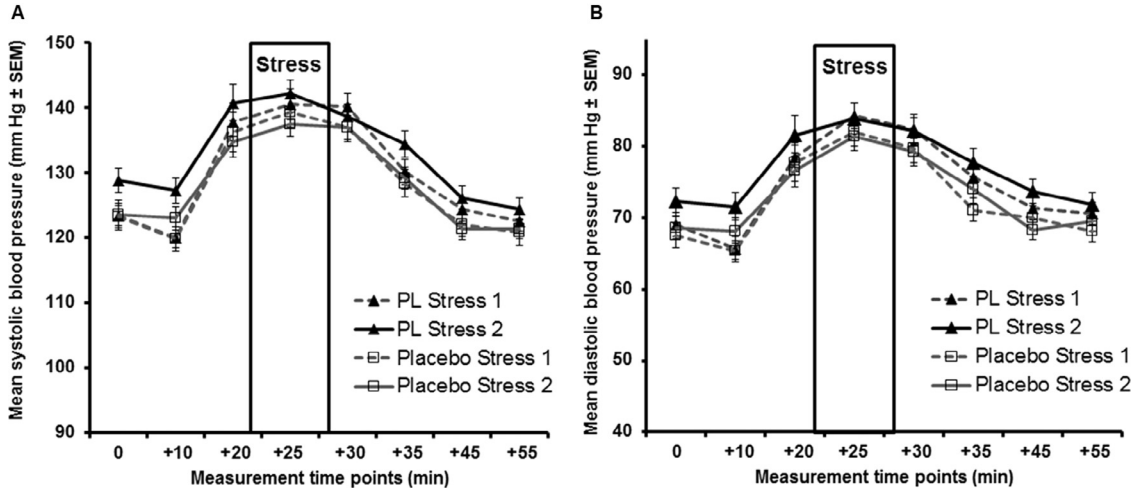

Fig. 9. Mean (± SEM) SBP (A)and DBP (B; mmHg) according to drink condition and stress visit. DBP diastolic blood pressure; SBP, systolic blood pressure.

subjective stress response in PL supplemented participants. Although marginal, the direction of effect is in line with previous evidence of the potential psychological stress-buffering effects of PLs [8,10,11,21]. 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 hypothesized 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 min [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 [6,7,10,19,22]. 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 postintervention BP response. The permissive effects of glucocorticoids on BP and cardiac output have been demonstrated in humans and animal models [67]. In most cases (predator avoidance being one exception), glucocorticoids 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 with 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 randomized, 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 randomized 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. Although 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 because of 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 [29,73] and stress and PL intake [6–9,11,18–22]. However, because evidence of the effects of PL intake is almost exclusively confined to male samples, future studies should include female participants. Furthermore, 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 6-wk intake of BM 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. WM 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.

Acknowledgments

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 who formed the stress panels is greatly appreciated.

Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.nut.2018.05.002.

References

[1] Blokland A, Honig W, Brouns F, Jolles J. Cognition-enhancing properties of subchronic phosphatidylserine (PS) treatment in middle-aged rats: comparison of bovine cortex PS with egg PS and soybean PS. Nutrition 1999;1:778–83.
[2] Casamenti F, Mantovani P, Amaducci L, Pepeu G. Effect of phosphatidylserine on acetylcholine output from the cerebral-cortex of the rat. J Neurochem 1979;32:529–33.
[3] McDaniel MA, Maier SF, Einstein GO. "Brain-specific" nutrients: a memory cure? Nutrition 2003;19:957–75.
[4] Ohlsson L, Burling H, Duan RD, Nilsson A. Effects of a sphingolipid-enriched dairy formulation on postprandial lipid concentrations. Eur J Clin Nutr 2010;64:1344–9.
[5] Pepeu G, Casamenti F, Scali C, Jeglinski W. Effect of serine phospholipids on memory and brain cholinergic mechanisms in aging rats. Neurosci Res Commun 1993;13:S63–6.
[6] Monteleone P, Beinat L, Tanzillo C, Maj M, Kemali D. Effects of phosphatidylserine on the neuroendocrine response to physical stress in humans. Neuroendocrinology 1990;52:243–8.
[7] Monteleone P, Maj M, Beinat L, Natale M, Kemali D. Blunting by chronic phosphatidylserine administration of the stress-induced activation of the hypothalamo-pituitary-adrenal axis in healthy-men. Eur J Clin Pharmacol 1992;42:385–8.
[8] Fahey TD, Pearl MS. The hormonal and perceptive effects of phosphatidylserine administration during two weeks of resistive exercise-induced overtraining. Biol Sport 1998;15:135–44.
[9] Starks MA, Starks SL, Kingsley M, Purpura M, Jaeger R. The effects of phosphatidylserine on endocrine response to moderate intensity exercise. J Int Soc Sports Nutr 2008;5:11.
[10] Hellhammer J, Fries E, Buss C, Engert V, Tuch A, Rutenberg D, et al. Effects of soy lecithin phosphatidic acid and phosphatidylserine complex (PAS) on the endocrine and psychological responses to mental stress. Stress 2004;7:119–26.
[11] Hellhammer J, Waladkhani A-R, Hero T, Buss C. Effects of milk phospholipid on memory and psychological stress response. Brit Food J 2010;112:1124–37.
[12] Lupien SJ, Maheu F, Tu M, Fiocco A, Schramek TE. The effects of stress and stress hormones on human cognition: implications for the field of brain and cognition. Brain Cogn 2007;65:209–37.
[13] Schwabe L, Joëls M, Roozendaal B, Wolf OT, Oitzl MS. Stress effects on memory: an update and integration. Neurosci Biobehav Rev 2012;36:1740–9.
[14] Lupien SJ, Gillin CJ, Hauger RL. Working memory is more sensitive than declarative memory to the acute effects of corticosteroids: a dose-response study in humans. Behav Neurosci 1999;113:420–30.
[15] Lupien SJ, Wilkinson CW, Briere S, Menard C, Kin N, Nair NPV. The modulatory effects of corticosteroids on cognition: studies in young human populations. Psychoneuroendocrinology 2002;27:401–16.
[16] Buchanan TW, Tranel D. Stress and emotional memory retrieval: effects of sex and cortisol response. Neurobiol Learn Memo 2008;89:134–41.
[17] Elzinga BM, Roelofs K. Cortisol-induced impairments of working memory require acute sympathetic activation. Behav Neurosci 2005;119:98–103.
[18] Schubert M, Contreras C, Franz N, Hellhamrner J. Milk-based phospholipids increase morning cortisol availability and improve memory in chronically stressed men. Nutr Res 2011;31:413–20.
[19] Parker AG, Gordon J, Thornton A, Byars A, Lubker J, Bartlett M, et al. The effects of IQPLUS Focus on cognitive function, mood and endocrine response before and following acute exercise. J Int Soc Sports Nutr 2011;8:16.
[20] Baumeister J, Barthel T, Geiss KR, Weiss M. Influence of phosphatidylserine on cognitive performance and cortical activity after induced stress. Nutr Neurosci 2008;11:103–10.
[21] Benton D, Donohoe RT, Sillance B, Nabb S. The influence of phosphatidylserine supplementation on mood and heart rate when faced with an acute stressor. Nutr Neurosci 2001;4:169–78.
[22] Hellhammer J, Hero T, Franz N, Contreras C, Schubert M. Omega-3 fatty acids administered in phosphatidylserine improved certain aspects of high chronic stress in men. Nutr Res 2012;32:241–50.
[23] Flett GL, Hewitt PL, Blankstein KR, Mosher SW. Perfectionism, self-actualization, and personal adjustment. J Soc Behav Pers 1991;6:147–60.
[24] Shafran R, Mansell W. Perfectionism and psychopathology: a review of research and treatment. Clin Psychol Rev 2001;21:879–906.
[25] Wirtz PH, Elsenbruch S, Emini L, Rudisuli K, Groessbauer S, Ehlert U. Perfectionism and the cortisol response to psychosocial stress in men. Psychosom Med 2007;69:249–55.
[26] Frost RO, Marten P, Lahart C, Rosenblate R. The dimensions of perfectionism. Cogn Ther Res 1990;14:449–68.
[27] Hsu FC, Garside MJ, Massey AE, McAllister-Williams RH. Effects of a single dose of cortisol on the neural correlates of episodic memory and error processing in healthy volunteers. Psychopharmacology (Berl) 2003;167:431–42.
[28] Schoofs D, Preuss D, Wolf OT. Psychosocial stress induces working memory impairments in an n-back paradigm. Psychoneuroendocrinology 2008;33:643–53.
[29] Schoofs D, Wolf OT, Smeets T. Cold pressor stress impairs performance on working memory tasks requiring executive functions in healthy young men. Behav Neurosci 2009;123:1066–75.
[30] Young AH, Sahakian BJ, Robbins TW, Cowen PJ. The effects of chronic administration of hydrocortisone on cognitive function in normal male volunteers. Psychopharmacology (Berl) 1999;145:260–6.
[31] Butts KA, Floresco SB, Phillips AG. Acute stress impairs set-shifting but not reversal learning. Behav Brain Res 2013;252:222–9.
[32] Plessow F, Fischer R, Kirschbaum C, Goschke T. Inflexibly focused under stress: acute psychosocial stress increases shielding of action goals at the expense of reduced cognitive flexibility with increasing time lag to the stressor. J Cogn Neurosci 2011;23:3218–27.
[33] Plessow F, Kiesel A, Kirschbaum C. The stressed prefrontal cortex and goal-directed behaviour: acute psychosocial stress impairs the flexible implementation of task goals. Exp Brain Res 2012;216:397–408.
[34] Zigmond AS, Snaith RP. The Hospital Anxiety and Depression Scale. Acta Psychiatr Scand 1983;67:361–70.
[35] Bjelland I, Dahl AA, Haug TT, Neckelmann D. The validity of the Hospital Anxiety and Depression Scale. An updated literature review. J Psychosom Res 2002;52:69–77.
[36] Kirschbaum C, Pirke KM, Hellhammer DH. The Trier Social Stress Test—a tool for investigating psychobiological stress responses in a laboratory setting. Neuropsychobiology 1993;28:76–81.
[37] Schwabe L, Haddad L, Schachinger H. HPA axis activation by a socially evaluated cold-pressor test. Psychoneuroendocrinology 2008;33:890–5.
[38] Boyle NB, Lawton CL, Arkbåge K, West SG, Thorell L, Hofman D, et al. No habituation in salivary cortisol response in healthy adult males following repeated exposure to a combined psychosocial and physical social-evaluative laboratory stressor. Psychoneuroendocrinology 2016;63:119–27.
[39] Mackay C, Cox T, Burrows G, Lazzerini T. Inventory for measurement of self-reported stress and arousal. Br J Soc Clin Psychol 1978;17:283–4.
[40] Cohen S, Kamarck T, Mermelstein R. A global measure of perceived stress. J Health Soc Behav 1983;24:385–96.
[41] Engle RW, Tuholski SW, Laughlin JE, Conway ARA. Working memory, short-term memory, and general fluid intelligence: a latent-variable approach. J Exp Psychol Gen 1999;128:309–31.
[42] Monsell S. Task switching. Trends Cogn Sci 2003;7:134–40.
[43] Rogers RD, Monsell S. Costs of a predictable switch between simple cognitive tasks. J Exp Psychol Gen 1995;124:207–31.

[44] Wylie GR, Javitt DC, Foxe JJ. Task switching: a high-density electrical mapping study. Neuroimage 2003;20:2322–42.
[45] Bartels C, Wegrzyn M, Wiedl A, Ackermann V, Ehrenreich H. Practice effects in healthy adults: a longitudinal study on frequent repetitive cognitive testing. BMC Neurosci 2010;11:118.
[46] Benedict RHB, Zgaljardic DJ. Practice effects during repeated administrations of memory tests with and without alternate forms. J Clin Exp Neuropsychol 1998;20:339–52.
[47] McCaffrey RJ, Westervelt HJ. Issues associated with repeated neuropsychological assessments. Neuropsychol Rev 1995;5:203–21.
[48] McCaffrey RJ, Ortega A, Haase RF. Effects of repeated neuropsychological assessments. Arch Clin Neuropsychol 1993;8:519–24.
[49] Gonzalez-Bono E, Rohleder N, Hellhammer DH, Salvador A, Kirschbaum C. Glucose but not protein or fat load amplifies the cortisol response to psychosocial stress. Horm Behav 2002;41:328–33.
[50] Kirschbaum C, Bono EG, Rohleder N, Gessner C, Pirke KM, Salvador A, et al. Effects of fasting and glucose load on free cortisol responses to stress and nicotine. J Clin Endocrinol Metab 1997;82:1101–5.
[51] Rohleder N, Kirschbaum C. Effects of nutrition on neuro-endocrine stress responses. Curr Opin Clin Nutr Metab Care 2007;10:504–10.
[52] Pruessner JC, Kirschbaum C, Meinlschmid G, Hellhammer DH. Two formulas for computation of the area under the curve represent measures of total hormone concentration versus time-dependent change. Psychoneuroendocrinology 2003;28:916–31.
[53] Tukey JW. Quick and dirty methods in statistics, part II—simple analyses for standard designs. In: Quality control conference papers fifth American convention, New York: American Society of Quality Control; 1951:189–97.
[54] Kirschbaum C, Prussner JC, Stone AA, Federenko I, Gaab J, Lintz D, et al. Persistent high cortisol responses to repeated psychological stress in a subpopulation of healthy-men. Psychosom Med 1995;57:468–74.
[55] Wust S, Federenko IS, van Rossum EF, Koper JW, Hellhammer DH, Wust S, et al. Habituation of cortisol responses to repeated psychosocial stress—further characterization and impact of genetic factors. Psychoneuroendocrinology 2005;30:199–211.
[56] Mason JW, Hartley H, Kotchen TA, Mougey EH, Ricketts PT, Jones LG. Plasma cortisol and norepinephrine responses in anticipation of muscular exercise. Psychosom Med 1973;35:406–14.
[57] Salvador A, Suay F, Gonzalez-Bono E, Serrano MA. Anticipatory cortisol, testosterone and psychological responses to judo competition in young men. Psychoneuroendocrinology 2003;28:364–75.
[58] Sutton JR, Casey JH. Adrenocortical response to competitive athletics in veteran athletes. J Clin Endocrinol Metab 1975;40:135–8.
[59] Kuellenberg D, Taylor LA, Schneider M, Massing U. Health effects of dietary phospholipids. Lipids Health Dis 2012;11:3.
[60] Pepeu G, Pepeu IM, Amaducci L. A review of phosphatidylserine pharmacological and clinical effects. Is phosphatidylserine a drug for the ageing brain? Pharmacol Res 1996;33:73–80.
[61] Bruni A, Bellini F, Mietto L, Monastra G, Viola G, Toffano G. Target-cells for serine phospholipids. In: Freysz L, Hawthorne JN, Toffano G, eds. Neurochemical aspects of phospholipid metabolism, Padova: Liviana Press/Springer Verlag; 1989:211–7.
[62] Park EJ, Suh M, Ramanujam K, Steiner K, Begg D, Clandinin MT. Diet-induced changes in membrane gangliosides in rat intestinal mucosa, plasma and brain. J Pediatr Gastroenterol Nutr 2005;40:487–95.
[63] Liu H, Radlowski EC, Conrad MS, Li Y, Dilger RN, Johnson RW. Early supplementation of phospholipids and gangliosides affects brain and cognitive development in neonatal piglets. J Nutr 2014;144:1903–9.
[64] Het S, Wolf OT. Mood changes in response to psychosocial stress in healthy young women: effects of pretreatment with cortisol. Behav Neurosci 2007;121:11–20.
[65] Schlotz W, Kumsta R, Layes I, Entringer S, Jones A, Wust S. Covariance between psychological and endocrine responses to pharmacological challenge and psychosocial stress: a question of timing. Psychosom Med 2008;70:787–96.
[66] Richter Y, Herzog Y, Lifshitz Y, Hayun R, Zchut S. The effect of soybean-derived phosphatidylserine on cognitive performance in elderly with subjective memory complaints: a pilot study. Clin Interv Aging 2013;8:557–63.
[67] Sapolsky RM, Romero LM, Munck AU. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. Endocr Rev 2000;21:55–89.
[68] Krakoff LR. Glucocorticoid excess syndromes causing hypertension. Cardiol Clin 1998;6:537–45.
[69] Sambhi MP, Weil MH, Udhoji VN. Acute pharmacodynamic effects of glucocorticoids—cardiac output and related hemodynamic changes in normal subjects and patients in shock. Circulation 1965;31:523.
[70] Gibson A. The influence of endocrine hormones on the autonomic nervous-system. J Auton Pharmacol 1981;1:331–58.
[71] Kennedy B, Ziegler MG. Cardiac epinephrine synthesis—regulation by a glucocorticoid. Circulation 1991;84:891–5.
[72] Kudielka BM, Hellhammer D, Wust S. Why do we respond so differently? Reviewing determinants of human salivary cortisol responses to challenge. Psychoneuroendocrinology 2009;34:2–18.
[73] Luethi M, Meier B, Sandi C. Stress effects on working memory, explicit memory, and implicit memory for neutral and emotional stimuli in healthy men. Front Behav Neurosci 2009;3:11.