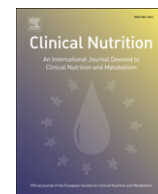




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Original Article

Effects of powdered fertilized eggs on the stress response

Johannes Schult^a, Torsten Hero^{a,b}, Juliane Hellhammer^{a,*}^a DAaCRO, Diagnostic Assessment and Clinical Research Organization, Science Park, D-54296 Trier, Germany^b FPP, Center for Psychobiology and Psychosomatic Research, University of Trier, D-54290 Trier, Germany

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SUMMARY

Background & aims: Effects of nutritional supplements on psychological wellbeing receive increasing attention. This double-blind placebo-controlled study investigated effects of a four week intake of powder of fertilized eggs (Young Tissue Extract; YTE™) in a laboratory protocol (Trier Social Stress Test; TSST).

Methods: Aside the laboratory stress test, we examined differential effects on subjects with high and low levels of chronic stress. Thus, subjects were further divided into two subgroups with scores for chronic stress scores below and above average, respectively.

Results: Compared to placebo, a four week intake of YTE™ did not result in superior effects on general wellbeing. However, beneficial effects of YTE™ were observed in subjects with enhanced levels of chronic stress. When compared to placebo these subjects showed an improvement of both the psychological and endocrine stress response.

Conclusions: Group differences suggest that YTE™ selectively improves adaptation to acute stress by normalizing the endocrine and the subjective stress response. Subjects with less chronic stress also reported less subjective stress but did not show beneficial effects on the endocrine stress response.

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1. Introduction

The avian egg contains a multitude of the proteins, lipids, vitamins, minerals, and growth factors. There are also additional defense factors contained to protect against bacterial and viral infection, and biologically active components, making it more than just a source of nutrients. Proteins and peptides can be derived from the whole egg. Intake of egg components has been reported to be associated with novel antimicrobial activities, antiadhesive properties, immunomodulatory, anticancer, and antihypertensive activities, antioxidant properties, protease inhibitors, and nutrient bioavailability.^{1,2}

The egg powder used in the present study, YTE™ (Young Tissue Extract; Med-Eq as, Tønsberg, Norway), is extracted from fertilized, partially incubated hen eggs. It is obtained through separation of oligopeptides from the total mass and contains proteins and peptides from freeze-dried egg powder. These can pass freely through the digestive barrier. The embryonic peptides work via elevation of 17-ketosteroid levels in the adrenal glands, which improves anabolism through increased synthesis of androgens and

a decrease in cortisol. Double-blind, placebo-controlled studies showed that the substance has a positive effect on libido in healthy humans as well as in patients on anti-depressant medication.^{3,4} It has also shown to improve cellular testosterone uptake in addition to its effect on cortisol levels.³ These findings raise the question whether the substance can help dampen the physiological stress reaction and the perceived psychological stress in an acute stress situation.

Although stress has been described as a non-specific response of the body, it is possible to discern specific endocrine stress responses caused by specific emotional reactions to novel, ambivalent or uncontrollable situations and stimuli. Social stress induces elevated cortisol levels, particularly if the stressor is perceived as uncontrollable, unpredictable, and constitutes a social-evaluative threat due to the judgment of others.⁵ The hypothalamic-pituitary-adrenal (HPA) axis plays a major role in the response to this kind of stressors with a robust increase of adrenocorticotropin hormone (ACTH) and cortisol.

An analysis of Dickerson and Kemeny⁶ showed that the Trier Social Stress Test⁷ (TSST) is the best standardized and most efficient psychological stress protocol in humans which is currently available. With respect to psychological parameters, the TSST leads to a moderate rise in fear. The biological response comprehends an increase of ACTH, cortisol, prolactin, growth hormone, norepinephrine, epinephrine, heart rate and blood pressure.⁷ Cortisol is

* Corresponding author. DAaCRO, Science Park, Max-Planck-Str. 22, D-54296 Trier, Germany. Tel.: +49 0 651 9120 494; fax: +49 0 651 9120 564.

E-mail address: hellhammer@daacro.com (J. Hellhammer).

involved in development, metabolism, cognitive and emotional processes, and the immune system. It also exerts influence on the HPA axis itself.⁸

The primary objective of the present study was to determine the efficacy of egg powder YTE™ in dampening stress reactivity in an acute stressful situation by assessment of cortisol, heartrate and perceived stress in the TSST. The secondary objective of this study was to discriminate effects between subjects which scored relatively high or low on a measure of perceived chronic stress, respectively.

2. Methods

2.1. Subjects

Forty (mean age 23 (range 20–29) years) out of 44 screened healthy non-smoking young men completed the study. Two were excluded because they were already familiar with the TSST. One was excluded because of lactose intolerance. One aborted the study after the screening visit and before substance intake started.

Only healthy, non-diabetic, and non-smoking subjects were included to this study. Neither the two treatment groups, nor the four subgroups (experimental group membership and high stress/low stress group) differed in terms of body mass index (BMI), weight, height, age and baseline heart rate (all $p > 0.44$).

2.2. Study procedure

Subjects were informed about the study procedure and gave their informed consent for study participation during the initial screening visit. Their health status was then examined with a medical questionnaire. Subjects were randomly assigned to one of the two treatment groups. Both, subjects and the investigator were blind to the groups' identity.

The following questionnaires were administered during the screening visit: Trier Inventory of Chronic Stress (TICS), Perceived Stress Scale (PSS), Mood Questionnaire ("Mehrdimensionaler Befindlichkeitsfragebogen", MDBF, long form), State Trait Anxiety Inventory (trait version: STAI- X2), and Short Form 12 Health Survey Questionnaire (SF-12).

Subjects then received a container filled with capsules containing either the test product or the placebo. The containers were locked with MEMS (Medication Event Monitoring System) Track-Caps. During the four weeks leading up to the second visit (including the day of the second visit), subjects ingested a daily dose of four capsules (i.e., 1680 mg YTE™/placebo per day): two capsules with breakfast and two with the principal meal.

After 28 days of substance intake, subjects returned to the study site and performed the TSST protocol. The TSST consisted of a resting and anticipation period (45 min), a test period (15 min), and a subsequent resting period (60 min).

During the first half of the test period subjects had to deliver a free speech. In the second half they had to perform mental arithmetic in front of an audience.

Subjects also had to fill out a number of questionnaires during the TSST visit: PSS and SF-12 before the stress test; MDBF short version A (pre-TSST) and short version B (post-TSST), respectively; the State Trait Anxiety Inventory (STAI-X1, state anxiety) pre- and post-TSST; ratings of perceived stress, anxiety, and insecurity on visual analogue scales (VAS) three times (pre-TSST, in the middle of the TSST, and post-TSST).

Heart rate was recorded from –20 min to +20 min in relation to TSST timing by Polar Vantage NV heart rate measurement devices. 10 min after the beginning of heart rate measuring subjects had to stand up. This served to avoid confounding orthostatic effects during the TSST measurement. Once a subject returned from the TSST, he remained standing for further 10 min after the end of the TSST. The protocol included measures of saliva cortisol (at –2 min, +1 min, +10 min, +20 min, +30 min, and +60 min, respectively) relative to the TSST. After the last saliva sample was collected, subjects were debriefed and received their reimbursement. The time line of the TSST protocol is summarized in Fig. 1.

2.3. Treatment

The test substance YTE™ is an egg powder extracted from fertilized, partially incubated hen eggs. It is obtained through separation of oligopeptides from the total mass. The resultant oligopeptide product contains 80–85% protein fractions (peptides), approximately 8% lipids, 5.5% carbohydrates and 3.5% ash in addition to some moisture. The YTE™ used in the present study was produced by Med-Eq as, Tønsberg, Norway and encapsulated by the Laboratoire GEFA, ZA Bas-Rocomps Route de Noyal-sur-Vilaine, Chateaugiron, France.

The placebo product contained rice starch, hydroxypropylmethylcellulose (HPMC), magnesium stearat, colouring agent (yellow iron oxide, black iron oxide, red iron oxide on a lactose carrier) and was produced by the Laboratoire GEFA, ZA Bas-Rocomps Route de Noyal-sur-Vilaine, Chateaugiron, France.

2.4. Measures

The TICS⁹ assesses the subjective perception of stress load during the last three months. It comprehends 57 items out of which

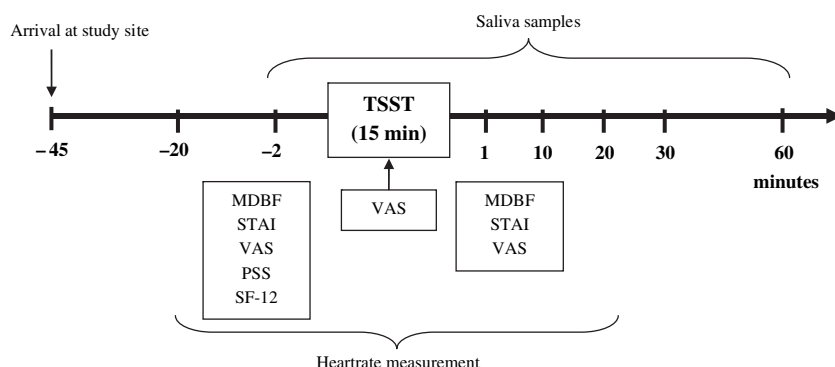


Fig. 1. Time line of the TSST protocol.

12 form a screening scale for chronic stress. The STAI¹⁰ has two scales with 20 items each, which assess state and trait anxiety, respectively. The PSS¹¹ is a psychological instrument for measuring the perception of stress in the past month. A German translation was used. It consists of 14 items. The MDBF¹² uses three bipolar dimensions of present psychological wellbeing: good-bad mood, wakefulness-tiredness, and calmness-agitation. There are two short forms (A and B), which together form the long form. The SF-12¹³ is a health-related measure of quality of life, discriminating physical and psychological components. Subjects further rated their subjective perception of the stress test three times on VAS of 10 cm length, on which they marked on bipolar dimension (“low” to “high”) the perceived stress load, anxiety and insecurity.

YTETM capsules were provided in containers locked with MEMS TrackCaps (AARDEX Ltd., Zug, Switzerland), which record the time and date of each opening. This system was implemented to enforce and to check for subjects' compliance.

Heart rate assessment took place with a Polar watch device (S610i and S710i, Polar Electro GmbH, Büttelborn, Germany) that recorded data every 5 s. Data were aggregated to mean values for 7 time phases: sitting (10 min), standing (10 min), introduction to the TSST and preparation (5 min), interview (5 min), mental arithmetic (5 min), standing (10 min), and sitting (10 min).

2.5. Laboratory analyses

For saliva sample collection cotton swabs in suspenders (Salivette®, Sarstedt AG & Co., Nümbrecht, Germany) were used. Saliva samples were stored at 4 °C and frozen at –20 °C before they were transferred to the laboratory. After thawing, saliva samples were centrifuged at 3000 rounds per minute for 5 min, which resulted in a clear supernatant of low viscosity. Free saliva cortisol levels were determined using an optical density immunoassay (Coated Well EIA, Salimetrics, State College, PA, USA) based on the competition principle. Intra-assay variation of this assay ranges between 3.88 and 7.12%, inter-assay variation between 6.69 and 6.88%.

2.6. Data analysis

Analyses of data took place after data collection and laboratory analyses of biological parameters. Interim analyses were not conducted.

If values of biological parameters were implausible, respective sample analyses were repeated before the statistical analysis. Cases with missing data were case wise excluded of analyses. Data analysis was carried out using SPSS 15.0.1.

Analyses of endocrine, cardiovascular and psychological parameters for both groups were performed with analyses of variance (ANOVAs) for repeated measurements. In case of significant results in the Mauchly test for sphericity, a Greenhouse-Geisser correction of degrees of freedom was conducted. Change parameters were compared with *t*-tests.

2.7. Ethics approval

The study was performed in accordance with the principles of the declaration of Helsinki and was approved by the International Medical & Dental Ethics Commission (IMDEC), Freiburg, Germany.

3. Results

3.1. Development during the intake period

Comparing the groups' PSS scores at baseline with those at the TSST day yielded no significant results (effect of time: $F_{(1,38)} = 0.40$,

$p = 0.53$, effect of group: $F_{(1,38)} = 0.71$, $p = 0.41$, effect time x group: $F_{(1,38)} = 0.26$, $p = 0.61$). The scores of the two SF-12 scales didn't differ significantly between groups, between baseline and TSST day, and between groups across time (physiological well-being: effect of time: $F_{(1,35)} = 1.17$, $p = 0.29$, effect of group: $F_{(1,35)} = 0.23$, $p = 0.64$, effect time x group: $F_{(1,35)} = 0.41$, $p = 0.53$; psychological well-being: effect of time: $F_{(1,35)} = 0.02$, $p = 0.89$, effect of group: $F_{(1,35)} = 0.00$, $p = 0.95$, effect time x group: $F_{(1,35)} = 0.03$, $p = 0.86$).

Food intake of participants was not recorded during the study. When asked about their experience during the intake period, subjects did not report on changes in food consumption. Subjects accepted and tolerated the supplement well.

3.1.1. Free cortisol in saliva

The stress test induced a significant increase in cortisol levels in saliva (effect of time: $F_{(1,7,65,1)} = 35.80$, $p = 0.00$). The two groups did not differ in the overall saliva cortisol levels (effect of group: $F_{(1,38)} = 0.11$, $p = 0.74$), nor in the course of saliva cortisol secretion (effect of time x group: $F_{(1,7,65,1)} = 0.89$, $p = 0.40$).

In order to explore differential treatment effects on cortisol levels, similar repeated measurement ANOVAs were run for half of the sample with TICS screening scale scores above and below the median, respectively. In our study, the sample median of the screening scale for chronic stress was 16 which corresponds to the 52nd percentile of the norm sample distribution.⁹

As expected, the change across time remains significant (effect of time for high stress: $F_{(1,7,30,1)} = 22.46$, $p = 0.00$; effect of time for low stress: $F_{(1,7,30,3)} = 15.48$, $p = 0.00$). For the high stress group, there is a trend for the overall saliva cortisol levels to be higher in the egg powder group (effect of group: $F_{(1,18)} = 3.27$, $p = 0.09$). For the low stress group, there is no significant treatment effect (effect of group: $F_{(1,18)} = 0.82$, $p = 0.38$). The interaction term is not significant in both subgroup analyses (effect of time x group for high stress: $F_{(1,7,30,1)} = 2.61$, $p = 0.10$; effect of time x group for low stress: $F_{(1,7,30,3)} = 0.40$, $p = 0.64$). Differential cortisol levels are shown in Fig. 2.

Splitting the sample into more than two groups depending on their chronic stress leads to very small (e.g., $n = 3$) and unbalanced groups. As a consequence, these results are not pursued.

3.1.2. Heart rate

The TSST induced a significant increase of heart rate (effect of time: $F_{(2,3,73,5)} = 48.25$, $p = 0.00$). However, the two experimental

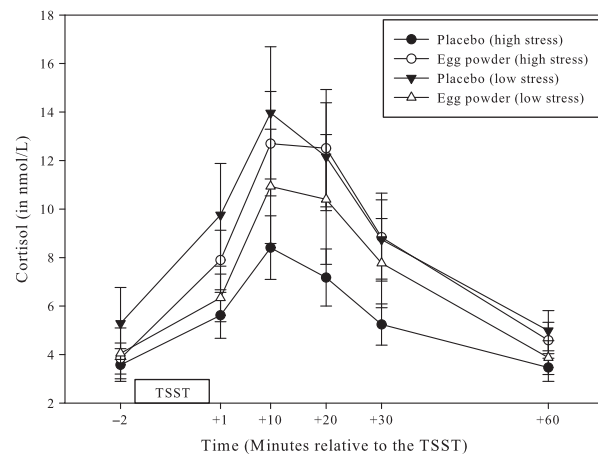


Fig. 2. Time course of saliva cortisol secretion in response to the TSST separately for the high stress half and the low stress half of the sample ($n = 40$). The graph shows group means with standard error bars.

groups showed no overall differences in heart rate (effect of group: $F_{(1,32)} = 0.07$, $p = 0.80$), nor differences in the time course of heart rate changes (effect time x group: $F_{(2,3,73.5)} = 0.24$, $p = 0.82$).

Additional heart rate analyses were run for the half of the sample with TICS screening scale scores above and below the median, respectively. The change across time remains significant (effect of time for high stress: $F_{(2,4,40.0)} = 20.23$, $p = 0.00$; effect of time for low stress: $F_{(2,0,25.8)} = 31.28$, $p = 0.00$). There is no significant group effect in either subsample (effect of group for high stress: $F_{(1,17)} = 1.10$, $p = 0.31$; effect of group for low stress: $F_{(1,13)} = 0.43$, $p = 0.53$), and also no significant interaction term (effect of time x group for high stress: $F_{(2,4,40.0)} = 0.76$, $p = 0.50$; effect of time x group for low stress: $F_{(2,0,25.8)} = 0.23$, $p = 0.80$). Fig. 3 shows the change of heart rates across time.

3.2. State anxiety

Subjects had significantly higher post-test STAI-state anxiety scores (effect of time: $F_{(1,37)} = 15.95$, $p = 0.00$). There was no significant main effect of experimental group and no interaction effect (effect of group: $F_{(1,37)} = 0.13$, $p = 0.72$; effect time x group: $F_{(1,37)} = 1.41$, $p = 0.24$).

The increase of state anxiety was also tested for group differences. There was no significant group effect for the whole sample ($F_{(1,37)} = 1.41$, $p = 0.24$). The high stress subsample shows an average increase of 2.4 (egg powder) and 12.5 (placebo) scale points, respectively ($F_{(1,18)} = 3.98$, $p = 0.06$), with a one-sided t -test achieving significance ($t_{(18)} = 2.00$, $p = 0.03$). This result is illustrated in Fig. 4. For the low stress subsample, there was no significant effect of group ($F_{(1,17)} = 0.18$, $p = 0.68$).

3.3. Mood, wakefulness, calmness

The effect of the TSST was similar for all three MDBF subscales. Mood ratings changed significantly towards bad mood (effect of time: $F_{(1,37)} = 25.56$, $p = 0.00$), but there were no significant group or interaction effects (effect of group: $F_{(1,37)} = 0.00$, $p = 0.98$; effect time x group: $F_{(1,37)} = 0.53$, $p = 0.47$). Tiredness ratings increased significantly (effect of time: $F_{(1,37)} = 6.57$, $p = 0.02$), but again there were no significant group or interaction effects (effect of group: $F_{(1,37)} = 0.05$, $p = 0.83$; effect time x group: $F_{(1,37)} = 0.02$, $p = 0.89$). Agitation ratings also increased significantly (effect of time: $F_{(1,37)} = 12.18$, $p = 0.00$), but there were also no significant group or

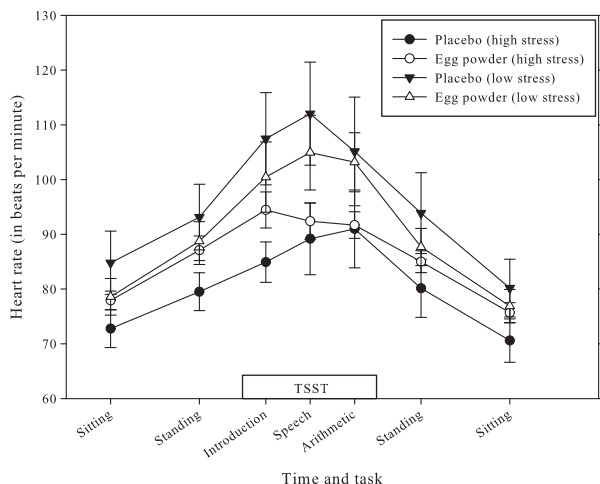


Fig. 3. Time course of heart rates separately for the highly stressed and low stressed subjects ($n = 34$). The graph shows group means with standard error bars.

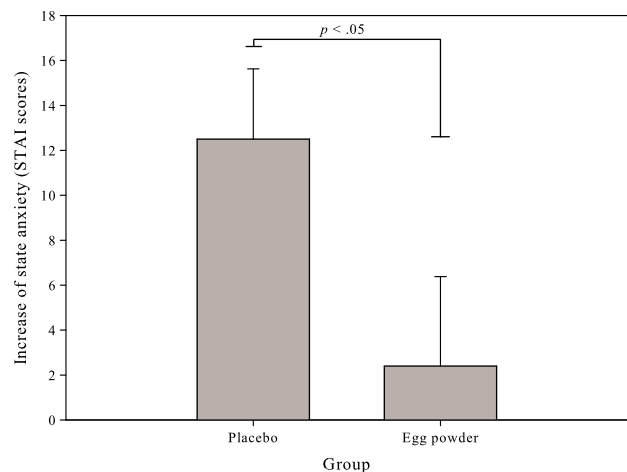


Fig. 4. Increase of state anxiety (STAI) in response to the TSST for the high stress subsample ($n = 20$). The graph shows group means with standard error bars and one-sided t -test.

interaction effects (effect of group: $F_{(1,37)} = 0.21$, $p = 0.65$; effect time x group: $F_{(1,37)} = 0.30$, $p = 0.59$).

3.4. Visual analogue scales

There were no initial differences in the VAS ratings between the two experimental groups before the test; effect of group for stress: $F_{(1,38)} = 1.77$, $p = 0.19$, effect of group for anxiety: $F_{(1,38)} = 1.18$, $p = 0.29$, effect of group for insecurity: $F_{(1,38)} = 0.24$, $p = 0.63$.

The TSST induced a significant increase in stress experience in both groups (effect of time: $F_{(1,7,65.9)} = 25.56$, $p = 0.00$). The time x group effect did not achieve significance ($F_{(1,7,65.9)} = 1.84$, $p = 0.17$). There was no significant difference between the two experimental groups (effect of group: $F_{(1,38)} = 0.02$, $p = 0.90$). Ratings on the other two visual analogue scales, anxiety and uncertainty, also increased significantly in both groups (effect of time for anxiety: $F_{(1,6,58.9)} = 17.21$, $p = 0.00$; effect of time for insecurity: $F_{(1,6,60.8)} = 33.34$, $p = 0.00$). However, there were neither time x group, nor group effects approaching or achieving significance (effect time x group for anxiety: $F_{(1,6,58.9)} = 0.24$, $p = 0.73$; effect time x group for insecurity: $F_{(1,6,60.8)} = 0.83$, $p = 0.42$; effect of group for anxiety: $F_{(1,38)} = 0.00$, $p = 0.95$; effect of group for insecurity: $F_{(1,38)} = 0.28$, $p = 0.60$).

Additionally, maximum and mean increase for each scale were also tested for group differences. The comparison of maximum increase in reported subjective stress showed a trend towards significance (effect of group: $F_{(1,38)} = 3.330$, $p = 0.08$). A one-sided t -test of this effect is significant ($t_{(38)} = 1.83$, $p = 0.04$). Fig. 5 shows the differential increase in VAS stress ratings. The differences for anxiety and insecurity were less pronounced (effect of group for anxiety: $F_{(1,38)} = 0.12$, $p = 0.73$, effect of group for insecurity: $F_{(1,38)} = 0.58$, $p = 0.45$).

4. Discussion

The present study investigated whether the intake of YTE™, an extract from fertilized, partially incubated hen eggs, dampens the stress response to an acute stressful situation.

The TSST, a standardized psychosocial stress test, successfully induced significant changes in cortisol and heart rate, as well as in several psychological variables such as perceived stress, state anxiety, mood, and calmness.

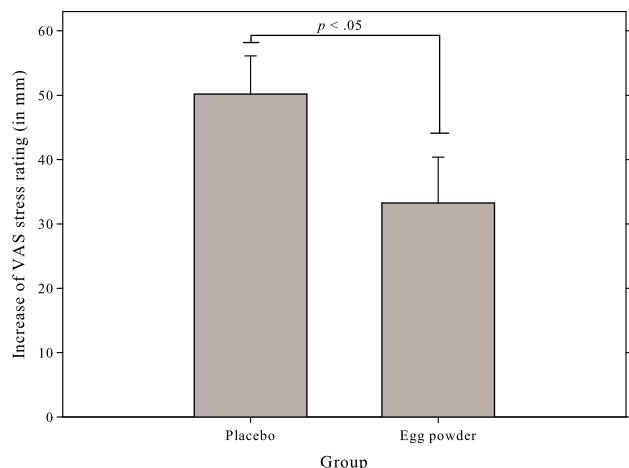


Fig. 5. Maximum increase of stress rating (VAS) in response to the TSST for the whole sample ($n = 40$). The graph shows group means with standard error bars and one-sided t -test.

An overall group comparison of cortisol levels during the TSST did not yield significant results. Interestingly, however, differences became visible once groups were divided into subjects with rather high levels of chronic stress and rather low levels of chronic stress (median split of TICS screening scale at baseline). Chronic stress permanently mobilizes the stress response network in the brain and results in a compensatory down regulation at the respective receptor sites. For example, chronic work stress has been reported to be associated with a dampening of the HPA axis response to the TSST.¹⁴ In a recent review, Hellhammer et al.¹⁵ summarized the current knowledge on the differential role of parvocellular arginine vasopressin (AVP) and corticotrophin releasing factor (CRF) in adaptation to chronic stress. They refer to the interplay between CRF and CRF/AVP neurons, which seem to differentially respond to chronic stress: "In animal studies, chronic stress results in a shift towards an increased AVP/CRF ratio in parvocellular neurons (...). Findings in humans are currently not consistent but a certain HPAA hyporeactivity in chronically stressed individuals seems to be likely" (p. 165). CRF/AVP neurons activate ACTH and other peptides, as well as noradrenergic and autonomic responses, which all vary with chronic stress. This complex interplay has been considered to be one reason behind the missing covariance between psychological measures of stress and salivary cortisol levels.¹⁵ It seems that a permanent activation of CRF/AVP neurons desensitizes post-synaptic receptor sites on both corticotrophic cells of the anterior pituitary and noradrenergic neurons of the locus caeruleus, thus resulting in a blunted endocrine and autonomic response to acute stress. Interestingly, several studies observed an elevation of previously low cortisol levels after stress management in patients with posttraumatic stress disorders,¹⁶ fibromyalgia,¹⁷ burnout,¹⁸ foster care children,¹⁹ and children with conduct disorders.²⁰

As expected, high stress subjects of the placebo group showed the tendency towards a blunted cortisol response in the TSST whereas low stress subjects showed a normal increase to this challenge test. Cortisol means of subjects with a high impact of chronic stress almost reached levels of low stressed subjects indicating that they benefit in terms of YTE™ raising their cortisol levels up to a normal range in an acute stressful situation. Group differences suggest that the egg powder actively improves adaptation to acute stress by enhancing the endocrine and reducing the subjective stress response, thus counteracting effects of chronic stress. Subjects with less chronic stress do not show any beneficial effects.

Heart rate as an indicator of the autonomic nervous system showed less pronounced results but points into a similar direction

as the endocrine data: whereas an overall treatment group comparison showed no significant group differences, a similar pattern emerged when looking at the high stress subgroup. Again, YTE™ raises subjects' heart rate in the TSST when compared to the high stress placebo-treated group.

In sum, these findings suggest that YTE™ restores the ability of chronically stressed subjects to adapt to acute stress. Since the brain has no own energy stores, it organizes its own glucose supply via the endocrine and the autonomic stress response. Particularly under enhanced demands (e.g., stress conditions), these mechanisms serve the brain by enhancing the synthesis and release of glucose and to support the allocation of glucose from the muscles to the brain.²¹

Notably, these effects could only be observed under stimulated conditions, whereas the circadian levels (CAR) remained unaffected. In addition, the findings from Eskeland³ suggest that such effects cannot be observed under physical stress (e.g., muscle activity). Rather, cortisol levels seem to drop under these conditions after intake of YTE™. This supports the view that YTE™ has no unspecific effects on the pituitary-adrenal axis but rather differentially improves adaptation to mental and physical stress, depending on the nature of the stressor.

This hypothesis lends further support from the observation that YTE™ dampened subjects' perceived stress assessed by VAS scales. The maximum increase during the stress test protocol was smaller for the egg powder group compared to the placebo. Analyzing the data set separately for subjects with high and low stress chronic stress ratings, this result remains similar for both subgroups. This suggests that all subjects may benefit from YTE™ with respect to their perceived stress in an acute stressful situation.

In addition, egg powder intake is also associated with less increase of TSST-induced state anxiety at least in the high stress subsample. The treatment appears to facilitate stressed subjects' coping with the test situation.

The absence of changes in perceived stress and health-related quality of life across the four weeks of intake suggests that there is no effect of egg powder intake on these more general concepts.

The data of our exploratory analyses in chronically stressed subjects are encouraging, because they suggest that people may only profit both psychologically and physiologically from YTE™ once they are chronically stressed. This, however, needs to be confirmed in selected samples of chronically stressed subjects. In addition, such studies may control for effects of age and gender as well as eating habits.

Conflict of Interest Statement

DAaCRO, Germany performed this study for Med-Eq, Norway. Med-Eq provided all the funding for this study.

Acknowledgements

Statement of authorship: JH conceived of the study and co-wrote the manuscript. TH supervised the data analysis and co-wrote the manuscript. JS carried out the studies and data analysis and wrote the manuscript. All authors read and approved the final manuscript.

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