Final Report

Stress Dampening Effects of Egg Powder from Fertilized Eggs in the Trier Social Stress Test

Study No. M1-2008

daacro

diagnostic assessment and clinical research organization

Prepared by Johannes Schult

Approved by Juliane Hellhammer

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DAaCRO GmbH & Co. KG, Science Park Trier, Max-Planck-Str. 22,
54296 Trier/Germany, www.daacro.com

Sponsor:

MedEQ as
Farmannsveien 18-22
N-3111 Tønsberg
Phone: +47 (0)33 35 90 60
Fax: +47 (0)33 35 90 69
Email: health@med-eq.com

CRO:

DAaCRO GmbH & Co. KG
CEO Juliane Hellhammer
Max-Planck-Str. 22
D-54296 Trier
Phone: +49 (0)651-9120 494
Fax: + 49 (0)651-9120 564
Email: hellhammer@daacro.de

Project Manager and Biometrician:

Johannes Schult, M.Sc./Dipl.-Psych.
Biostatistician
Max-Planck-Str. 22
D-54296 Trier
Phone: +49 (0)651-9120 494
Fax: + 49 (0)651-9120 564
Email: schult@daacro.de

Study Physician

Monika Rendenbach
University of Trier
Johanniterufer 15
D-54290 Trier / Germany
Phone: +49 (0)651-201 3645
Email: rendenba@uni-trier.de

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List of Abbreviations and Definitions of Terms

Abbreviation or special term	Explanation
ACTH	Adrenocorticotropin hormone
ANOVA	Analysis of variance
BMI	Body mass index
CAR	Cortisol awakening response
CRF	Case report form
DHEA	Dehydroepiandrosteron
GCP	Good Clinical Practice
HPA	Hypothalamic-pituitary-adrenal
ICH	International Conference on Harmonisation
MDBF	Mehrdimensionaler Befindlichkeitsfragebogen
MEMS	Medication Event Monitoring System
min.	Minutes
PSS	Perceived Stress Scale
TICS	Trier Inventory for Chronic Stress
TSST	Trier Social Stress Test
SF-12	Short Form 12 Health Survey Questionnaire
STAI	State Trait Anxiety Inventory
VAS	Visual Analogue Scale
YTE™	Young Tissue Extract

1 Background and Rational for the Study

The aim of this research project is to conduct a study investigating the effects of a nutritive supplement derived from fertilized, partly incubated chicken eggs on stress reactivity (perceived stress and salivary cortisol) and protective factors (DHEA and health outcome).

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 associated with biological activities like novel antimicrobial activities, antiadhesive properties, immunomodulatory, anticancer, and antihypertensive activities, antioxidant properties, protease inhibitors, and nutrient bioavailability (Kovacs-Nolan, Phillips, & Mine, 2005; Mihaescu, Olinescu, & Oancea, 2005).

The egg powder used in the present study, YTE™ (Young Tissue Extract), 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 white 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 (Eskeland, 1997; Eskeland, Thom, & Svendsen, 1997). The substance also has shown to improve cellular testosterone uptake in addition to its effect on cortisol levels (Eskeland, 1997).

The findings that YTE™ can reduce cortisol levels (Eskeland, 1997) raise the question whether the substance can help dampen the physiological stress reaction and the perceived psychological stress in the Trier Social Stress Test (TSST).

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. The hypothalamic-pituitary-adrenal (HPA) axis plays a major role in the response to this kind of stressors with a robust increase of ACTH and cortisol.

An analysis of Dickerson and Kemeny (2004) compared 208 laboratory studies of acute psychological stressors. The analysis showed that the TSST (Kirschbaum, Pirke, & Hellhammer, 1993) is the best standardized and most efficient psychological stress protocol in humans.

With respect to psychological parameters, the TSST leads to a moderate rise in fear. The biological response comprehends an increase of adrenocorticotropin hormone (ACTH), cortisol, prolactin, growth hormone, norepinephrine, epinephrine, heart rate and blood

pressure (e.g., Kirschbaum, et al., 1993). Cortisol is involved in development, metabolism, cognitive and emotional processes, and the immune system. It also exerts influence on the HPA axis itself (Kudielka, Hellhammer, & Kirschbaum, 2007).

2 Study Objectives

2.1 Primary objectives

The primary objective of the 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 Trier Social Stress Test.

2.2 Secondary objectives

The secondary objectives of the study were:

1. to determine beneficial effects of YTE™ by assessment of the cortisol awakening reaction (CAR) as an indicator of chronic stress;
2. to determine whether YTE™ has beneficial effects on health by assessment of a health questionnaire at baseline and after 4 weeks of substance intake;
3. to determine protective effects of YTE™ by assessment of DHEA baseline and pre- and post-TSST.

3 Methods

3.1 Study Dates

Ethic Commission application	10/17/2008
Ethic Commission approval	11/05/2008
First subject enrolled	11/12/2008
First TSST	12/16/2008
Last TSST (last subject completed)	01/20/2009
Laboratory	02/05/2009
Database Lock	02/10/2009
Final study report	02/27/2009

3.2 Overall Study Design

After recruiting and initial screening, the first of two visits to the study site took place. Medical Pre-Examination took place during this visit and baseline questionnaires were administered.

After four weeks of placebo or YTE™ intake and accompanying saliva sample collection, the second visit to the study site took place. The TSST protocol was implemented during this visit. Afterwards, participants were debriefed and paid. The schedule of events is summarized in Table 1.

Time	Step			Visit Study Site	Duration of Visit
Screening Visit	○ Screening, medical check and interview ○ Informed consent ○ Baseline questionnaires ○ Hand out capsules and saliva sampling sets			yes	45 min.
Treatment Period (4 weeks)	Starting on a weekday (not Fridays) after the pre-screening participants start to take their daily dose of the given substance for 28 days.	2 days	2 saliva samples per day: at awakening and +30 min.	no	
		5 days	-		
		2 days	2 saliva samples per day: at awakening and +30 min.		
		5 days	-		
		2 days	2 saliva samples per day: at awakening and +30 min.		
		5 days	-		
		2 days	2 saliva samples per day: at awakening and +30 min.		
		5 days	-		
TSST Visit	○ Capsule bottles are handed back and saliva samples taken over the past four weeks are delivered ○ Administration of the Trier Social Stress Test and questionnaires ○ Debriefing			yes	120 min.

Table 1: Summary of study design

3.2.1 Recruiting and Screening Phase

Participants were recruited on the campus of the local university and via email. Several otherwise interested people did not want to participate because they objected the size of the capsules or to oral substance intake in general. This led to a prolonged recruiting phase.

A first pre-screening and introduction to the study was done by phone or in person. If a person was male, between 20 and 50 years old, non-smoking, and healthy, an appointment for the medical pre-examination was made.

3.2.2 Visit 1: Medical Examination and Baseline Measures

Upon their arrival at the study site, participants were informed about the study procedure, questions were being answered, and their informed consent was obtained. They were also informed about the protection of their personal data, especially health data. An additional privacy statement was signed by those who wanted to participate in the study.

Then participants' health status was examined in a 30 minute medical interview plus a short medical examination. Participants who fulfilled all inclusion criteria and met no exclusion criteria were admitted to further study participation. Participants were randomly assigned to one of the two treatment groups.

The following questionnaires assessing perceived chronic stress, well-being, and trait anxiety were administered during the baseline visit: The Trier Inventory of Chronic Stress (TICS), a German translation of the Perceived Stress Scale (PSS), Mood Questionnaire (Mehrdimensionaler Befindlichkeitsfragebogen , MDBF, long form), the State Trait Anxiety Inventory (trait version: STAI- X2), and the Short Form 12 Health Survey Questionnaire (SF-12).

Study participants then received detailed instructions and a container filled with capsules containing either the test substance or a placebo product. The containers were locked with MEMS TrackCaps, which kept track of the time and date of each opening.

Participants were also given saliva sample collection material for weekly sampling at home and a diary to document their wake-up times on sampling days.

3.2.3 Treatment Period

During the four weeks leading up to the second visit, participants had to take a daily dose of four capsules: the recommended intake was two capsules with breakfast and two capsules with lunch. Four capsules correspond to a dose of 1680 mg/day YTE™/placebo.

For saliva sample collection cotton swaps in suspenders (Salivette®, Sarstedt AG & Co., Nümbrecht, Germany) were used. Participants had to collect saliva on two consecutive days once a week during the month of substance intake. On each of these days, a first sample had to be taken right after awakening and another sample 30 minutes later, prior to breakfast. These pairs of samples were used to determine the cortisol awakening reaction (CAR) for each week, a reliable marker for chronic stress (Kudielka, Bellingrath, & Hellhammer, 2006). Obtained saliva samples were supposed to be stored frozen or at least cooled.

3.2.4 Visit 2: Investigation of Treatment Effects: the TSST

After 28 days (four weeks) of substance intake, participants visited the study site again and performed the TSST protocol. They returned the collected saliva samples and handed over the pill bottles with the MEMS TrackCaps, which were used to assess compliance.

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 participants had to deliver a free speech. In the second half they had to perform mental arithmetic in front of an audience.

Participants also had to fill out a number of questionnaires during their stay, before, during, and after the stress test:

- the PSS and SF-12 before the stress test;
- the Mood Questionnaire (MDBF) in a short version A (pre-TSST) and a short version B (post-TSST), respectively;
- the State Trait Anxiety Inventory (STAI-X1) assessing state anxiety pre- and post-TSST;
- on a Visual Analogue Scale (VAS) they were asked to rate their degree of perceived stress, anxiety, and insecurity (0–100). This VAS was assessed 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 minutes after the beginning of heart rate measuring subjects were asked to stand up. This serves to avoid confounding orthostatic effects during the TSST measurement. Once the participant returned from the TSST, he remained standing until 10 minutes after the end of the TSST.

The protocol included measures of saliva cortisol (1 pre- and 5 post-measurements at –2 min., +1 min., +10 min., +20 min.,+30 min., and +60 min., respectively) and saliva DHEA (–2 min., +20 min., +60 min.). The overall time sequence is illustrated in Figure 1.

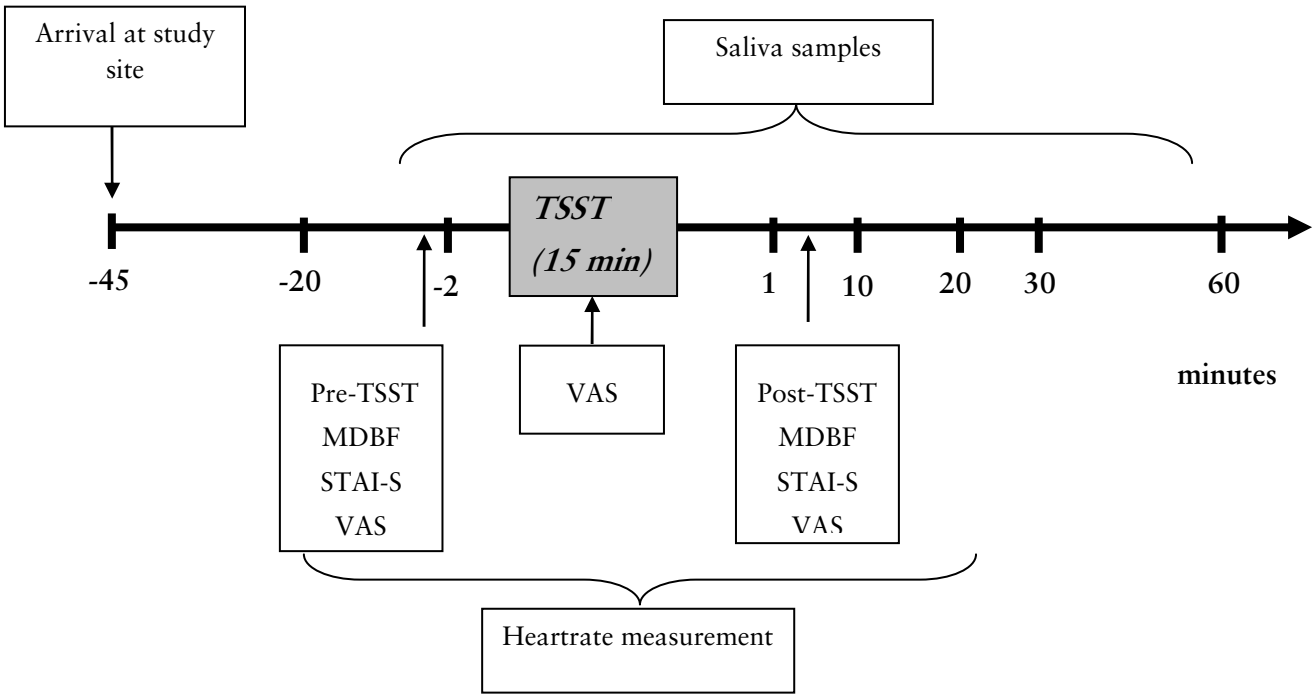

Figure 1: Time line of the TSST protocol

After the post-TSST questionnaires participants stayed for another hour during which additional saliva samples are collected. Then they were debriefed and received their reimbursement.

3.3 Selection of the Study Population

3.3.1 Inclusion Criteria

Healthy, non-smoking men with an age between 20 and 50 years old.

3.3.2 Exclusion Criteria

Persons were excluded if

- they had experienced allergic reactions after consumption of hen's eggs or had a lactose intolerance;
- smoked or were addicted to drugs or alcohol;
- they had any acute or chronic illness (including psychiatric disorders);
- they were on interfering medication or the study physician doubts the truthfulness of their corresponding health information;
- they suffered from an acute illness within the last 14 days;
- they were apparently unsuited as participant (lack of cognitive or verbal skills);
- they had previously participated in the TSST;
- it was expected that they would not complete the study;
- the study physician assessed a lack of good health based on the medical examination.

No persons were included who were doubted to be able to speak for themselves or unable to speak for themselves.

3.4 Treatment

3.4.1 Identity of Investigative Products, Comparators, Doses, and Treatment Regimens

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 and contains proteins and peptides from freeze-dried egg white powder. The YTE™ used in the present study was provided by MedEQ as, Tønsberg, Norway and encapsulated by Laboratoire GEFA, ZA Bas-Rocomps Route de Noyal-sur-Vilaine, Chateaugiron, France. It has a shelf life of at least 36 months.

The placebo product contained the following ingredients: 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 Laboratoire GEFA, ZA Bas-Rocomps Route de Noyal-sur-Vilaine, Chateaugiron, France.

Half of the participants were randomly assigned to the first group, the other half to the second group. The investigator was blind to the groups' identity. An independent study nurse filled the capsules into the bottles, accordingly, wearing plastic gloves. Group identity was kept in an envelope which was locked up.

3.5 Questionnaires

In this study, several questionnaires were assessed. During their baseline visit subjects filled in the following questionnaires to verify that groups do not differ with respect to chronic stress and trait anxiety: TICS and STAI-X2.

3.5.1 Chronic Stress (TICS)

Participants filled in the TICS at their baseline visit before intake of any study compound. The TICS ("Trierer Inventar zum chronischen Stress"; Schulz, Schlotz, & Becker, 2004) assesses the subjective perception of stress load during the last three month. With 57 items, subjects rate how often they experienced different situations characterizing chronic stress. Summarized, the items describe 9 scales:

- Work overload,
- Social overload,
- Pressure to succeed,
- Dissatisfaction with work,
- Excessive demands,
- Lack of acceptation,
- Social tension,
- Social isolation,
- Chronic worrying.

The scales "work overload", "social overload" and "pressure to succeed" describe stress resulting from high demands. The scales "dissatisfaction at work", "excessive demands", "lack of acceptation", "social stress", and "social isolation" measure stress resulting from a lack of fulfillment of needs. Additionally, the questionnaire assesses a "screening scale for chronic stress" consisting of 12 items.

3.5.2 Trait and State Anxiety (STAI)

The State-Trait Anxiety Inventory (STAI; "State-Trait-Angstinventar", Laux, Glanzmann, Schaffner, & Spielberger, 1981) is the German version of the STAI developed by Spielberger and colleagues in 1970. The two scales with 20 items each assess (1) anxiety as a trait (STAI-X2) and (2) anxiety as a state (STAI-X1). Anxiety as a trait characterizes a stable tendency to evaluate situations as threatening and to react with an increase of state anxiety. State anxiety describes an emotional state characterized by tension, worrying, nervousness, agitation, fear of future events and an increased activity of the autonomic nervous system (Laux, et al., 1981). For trait anxiety subjects have to rate various statements on emotions regarding how they feel

in general ("nearly never", "sometimes", "often", "very often"), for state anxiety they have to rate statements how they feel currently ("not at all", "a bit", "quite a lot", "very much so").

Participants completed the trait version STAI-X2 after the medical pre-examination and before intake of any study compound. They filled in the state version STAI-X1 twice, once immediately before and once after the TSST.

3.5.3 Perceived Stress (PSS)

The Perceived Stress Scale (PSS; Cohen, Kamarck, & Mermelstein, 1983) is a widely used psychological instrument for measuring the perception of stress in the recent month. It consists of 14 items.

3.5.4 Mood (MDBF)

The Mehrdimensionaler Befindlichkeitsfragebogen (MDBF; Steyer, Schwenkmezger, Notz, & Eid, 1997) assesses three bipolar dimensions of actual psychological wellbeing: good-bad mood, wakefulness-tiredness, and calmness-agitation. There are two short forms of the MDBF (A and B), which together form the long form.

3.5.5 Physiological and Psychological Health (SF-12)

The Short Form 12 Health Survey Questionnaire (SF-12; Bullinger & Kirchberger, 1998) was administered. Its health-related items measure quality of life on two main subscales, a physiological sum score and a psychological sum score.

3.5.6 Perception of the TSST (VAS)

Participants were asked to rate their subjective perception of the stress test three times: right before, during, and immediately after the TSST. Each time they received a sheet with three visual analogue scales (VAS) and were asked to mark on bipolar dimension ("low" to "high") how high the perceived stress load was, how much anxiety they perceived and how high perceived insecurity during the TSST was.

3.6 Measurement Devices

3.6.1 Intake Compliance Check

The containers were 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 participants' intake compliance. The caps are depicted in Figure 2.

Figure 2: A container locked with a MEMS TrackCap

3.6.2 Assessment of Heart Rate

For each participant, heart rate was measured continuously over a period of 55 minutes during the TSST visit. Heart rate assessment started 20 minutes prior to the TSST, continued throughout the TSST, and ended 20 minutes after termination of the stress test. Assessment took place with a Polar watch device (S610i and S710i, Polar Electro GmbH, Büttelborn, Germany). The Polar watch recorded data every 5 seconds. Data were transferred with a Polar-electro interface to the Polar Precision Performance SW Program (Polar Version 4.00.020, Polar Electro Oy 2003) on a personal computer. Data were aggregated to mean values for 7 time phases:

- 10 minutes sitting before the TSST,
- 10 minutes standing before the TSST,
- 5 minutes of introduction to the TSST and preparation,
- 5 minutes interview,
- 5 minutes mental arithmetic,
- 10 minutes standing after the TSST, and
- 10 minutes sitting after the TSST.

3.7 Laboratory Analyses

Saliva samples were stored cooled at 4 °C and eventually frozen at -20 °C on the day before they were transferred to the laboratory. There the samples were kept frozen at -20 °C and only temporarily thawed for the analyses.

After thawing, saliva samples were centrifuged at 3000 rpm for 5 minutes, which resulted in a clear supernatant of low viscosity.

3.7.1 Determination of Free Cortisol in Saliva

Free saliva cortisol levels were determined employing 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 to 7.12%, inter-assay variation between 6.69 to 6.88%.

3.7.2 Determination of DHEA in Saliva

DHEA in saliva was determined using an enzyme immunoassay (EIA, Salimetrics, State College, PA, USA) based on the competition principle.

Intra-assay variation of this assay ranges between 5.3 to 5.8%, inter-assay variation between 7.9 to 8.5%.

3.8 Statistical Methods

3.8.1 Determination of Sample Size

For a priori determination of sample size several parameters had to be specified: α-error probability was defined as α = 0.05, β-error probability as β = 0.20, respectively. Sample size is supposed to be large enough to reveal a small to medium effect (effect size f = 0.175). Considering all these parameters, optimum sample size was n = 20 per group, referring to a total sample size of N = 40 for identifying an effect of 17.5% on a significance level of 5% with a probability of 80%.

3.8.2 Data Analysis

Analyses of data took place after the final TSST of the study and laboratory analyses of biological parameters (data collection of all 40 participants). Interim analyses were not conducted.

Data were entered twice and subsequently compared and cleaned using EpiData 3.1 (Lauritsen & Bruus, 2004) for verification of correct input. The project manager screened and corrected ambiguous entries.

Before statistical analyses took place data were processed as following: if values of biological parameters were implausible, respective analyses were repeated. Missing data were defined and accounted in the analyses. Cases with missing data were case wise excluded of analyses. Data analysis was carried out using SPSS 15.0.1 (SPSS Inc., Chicago, USA) and, for the multilevel linear models, MLwiN 2.02 (University of Bristol, England).

In consideration of the high number of analyses and, going along with this, an increasing α-error probability, analyses of endocrine, cardiovascular and psychological parameters for both groups were performed with analyses of variance (ANOVAs) for repeated measures. With this method, possible differences between treatment groups regarding the response to the TSST were investigated.

4 Results

4.1 Study Population

A total of 44 male subjects commenced the screening visit. Two of them were excluded because they were already familiar with the TSST. One was excluded because of lactose intolerance. One person aborted the study shortly after the first visit and before baseline saliva sampling, due to parents' concerns.

The remaining 40 participants finished the study. Descriptive statistics of their age and their body measures are shown in Table 2 and Table 3, respectively.

Group	mean age in years (SD)	*n*
Placebo	22.65 (2.11)	20
Egg powder	23.16 (2.69)	19[a]

Table 2: Descriptive age statistics
[a] One missing date of birth in the case report form.

Group	mean height in m (SD)	mean weight in kg (SD)	mean BMI (SD)	*n*
Placebo	1.81 (0.07)	80.30 (15.24)	24.41 (3.96)	20
Egg powder	1.80 (0.06)	77.25 (9.83)	23.86 (2.69)	20

Table 3: Descriptive body measures statistics

The MEMS compliance check revealed no substantive intake refusals. One participant had a whole week missing. He claimed to have been on vacation and to have had a week's dose with him. Two other participants showed more than one consecutive day of missing openings, repeatedly. Figure 3 shows the distribution of bottle openings for one of the participants as an example.

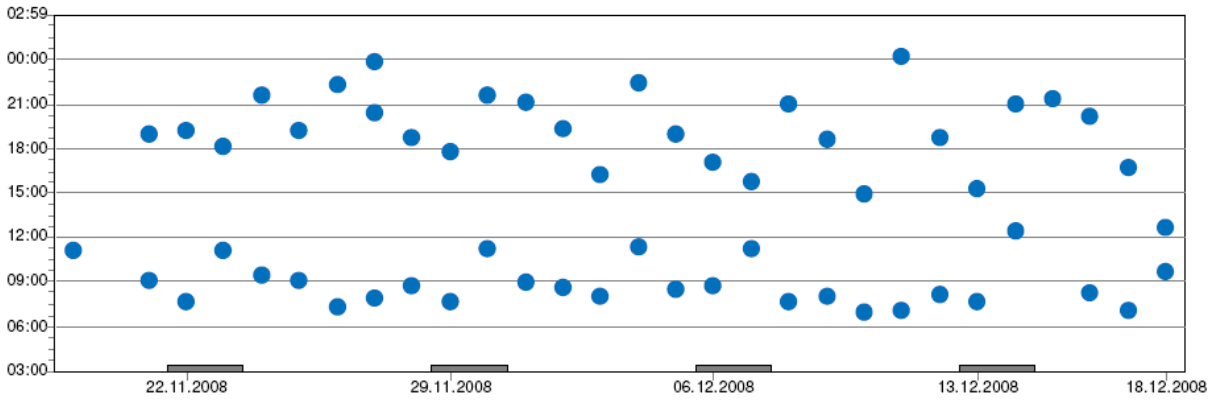

Figure 3: MEMS log data for one participant (VP11). Grey bars denote weekends.

About 1% of the saliva samples (12 out of 1040) contained no or insufficient saliva for cortisol and DHEA determination. One participant's awakening+30 min. cortisol data had to be discarded, because he had misunderstood the instructions and had collected the second samples 30 minutes before bed-time. Heart rate data of a few participants were partially distorted by insufficient humidity or malfunction of the Polar watches. They were subsequently excluded from the analysis.

Participants were asked to report their guessed group membership after the four weeks of capsule intake. Table 4 contains the cross tabulation of guessed and true group membership.

		Guessed group membership			
		Egg powder	**Placebo**	**don't know**	**total**
Experimental group	**Placebo**	3	11	6	20
	Egg powder	7	9	4	20
total		10	20	10	40

Table 4: Cross tabulation of believed and true group membership

4.2 Biological Parameters

4.2.1 Free Cortisol in Saliva

Free cortisol levels were measured in saliva 2 minutes prior to the TSST as well as 1, 10, 20, 30, and 60 minutes after the TSST. The stress test induced a significant increase in cortisol levels in saliva (effect of time: $F_{(5,34)} = 14.13$, $p = 0.00$). 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: $F_{(5,34)} = 0.83$, $p = 0.54$). Figure 4 and Table 5 show the data.

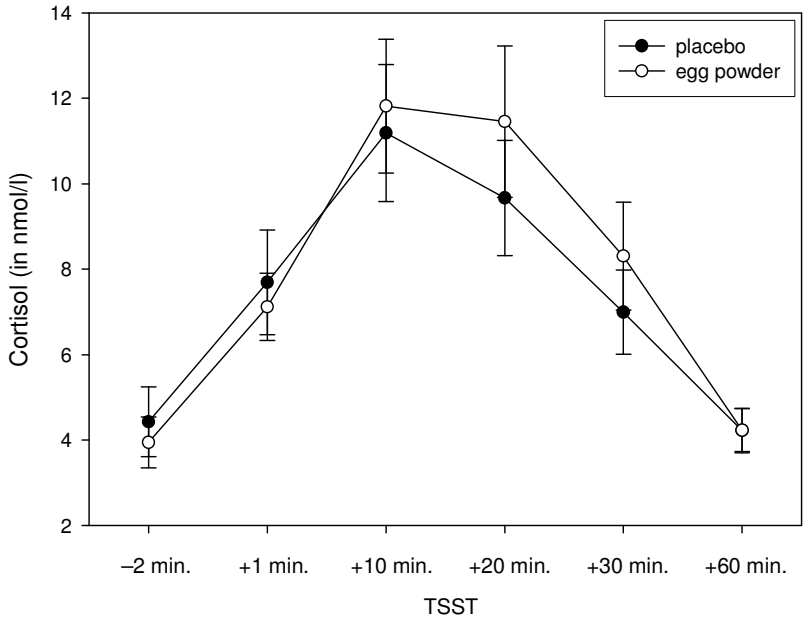

Figure 4: Time course of saliva cortisol secretion in response to the TSST. The graphs show group means with standard error bars.

Group	time relative to TSST						*n*
	–2 min.	+1 min.	+10 min.	+20 min.	+30 min.	+60 min.	
Placebo	4.43 (3.66)	7.69 (5.48)	11.19 (7.17)	9.67 (6.04)	6.99 (4.39)	4.23 (2.32)	20
Egg powder	3.95 (2.68)	7.12 (3.51)	11.82 (7.00)	11.45 (7.93)	8.30 (5.65)	4.23 (2.25)	20

Table 5: Saliva cortisol secretion in response to the TSST. Means and standard deviations.

In order to explore the differential effect of treatment on cortisol levels, similar repeated measurement ANOVAs were run for the 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; this corresponds to the 52nd percentile of the norm sample distribution (Schulz, et al., 2004, p. 55).

As expected, the change across time remains significant (effect of time for high stress: $F_{(5,14)}$ = 13.80, p = 0.00; effect of time for low stress: $F_{(5,14)}$ = 6.33, 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). Interestingly, the average cortisol level of the placebo group is higher at all times in the low stress subsample, whereas the previously reported trend in the high stress goes into the opposite direction. The interaction term is not significant in both subgroup analyses (effect of time x group for high stress: $F_{(5,14)}$ = 1.09, p = 0.41; effect of time x group for low stress: $F_{(5,14)}$ = 0.87, p = 0.53). Figure 5 and Table 6 show the data and illustrates the opposite relationship between the egg powder and placebo groups.

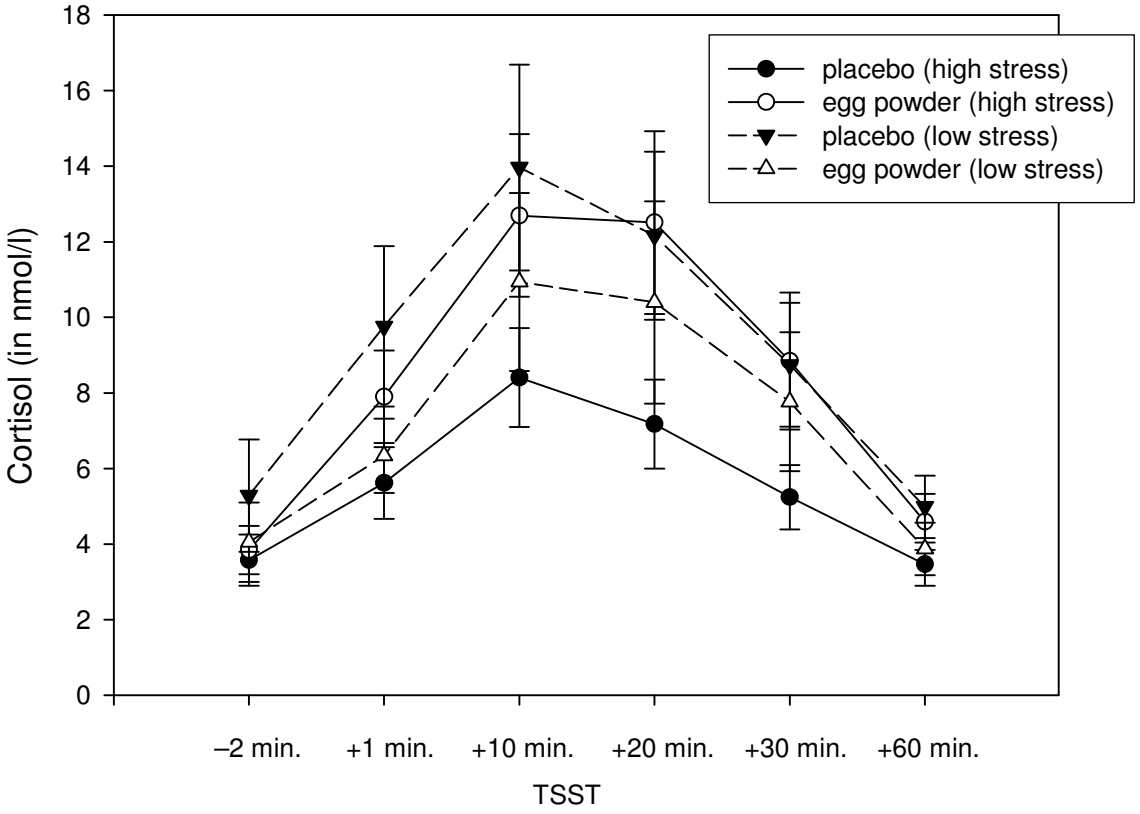

Figure 5: Time course of saliva cortisol secretion in response to the TSST separately for the high stress half and the low stress half of the sample. The graphs show group means with standard error bars.

	time relative to TSST						
Group	**–2 min.**	**+1 min.**	**+10 min.**	**+20 min.**	**+30 min.**	**+60 min.**	***n***
Placebo high stress	3.57 (2.14)	5.62 (2.99)	8.41 (4.15)	7.17 (3.72)	5.24 (2.69)	3.46 (1.80)	10
Egg powder high stress	3.94 (2.02)	7.90 (3.87)	12.70 (6.81)	12.51 (7.64)	8.84 (5.73)	4.59 (2.35)	10
Placebo low stress	5.28 (4.70)	9.76 (6.71)	13.97 (8.62)	12.16 (7.03)	8.75 (5.17)	4.99 (2.62)	10
Egg powder low stress	4.05 (3.32)	6.34 (3.10)	10.94 (7.44)	10.40 (8.47)	7.77 (5.82)	3.87 (2.21)	10

Table 6: Saliva cortisol secretion in response to the TSST by high and the low stress groups. Means and standard deviations.

4.2.2 DHEA in Saliva

Due to the presence of a few very influential outliers in the DHEA data, values beyond two standard deviations were discarded after careful inspection, yielding much smaller standard errors.

DHEA levels were measured in saliva 2 minutes before the TSST, as well as 20 and 60 minutes after the TSST. DHEA levels remained rather stable throughout the stress test (effect of time: $F_{(2,34)} = 1.16$, $p = 0.33$). There are no tangible interaction effects (effect of time x group: $F_{(2,34)} = 0.46$, $p = 0.64$). There is a significant difference between the two groups differ in the overall DHEA levels (effect of group: $F_{(1,35)} = 4.25$, $p < 0.05$), which is depicted in figure 6.

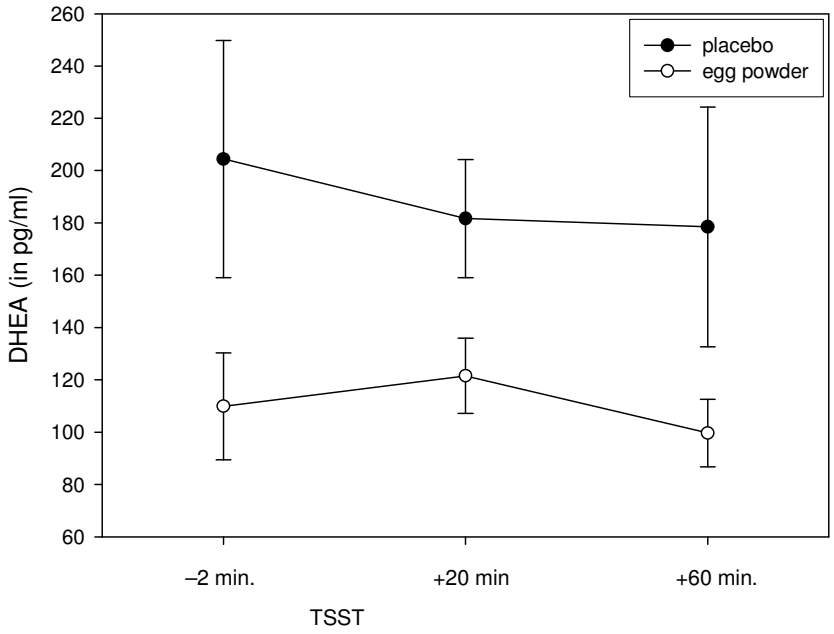

Figure 6: Time course of DHEA in the TSST. The graphs show group means with standard error bars.

A similar difference appears already in the baseline samples. The comparison of both groups' average baseline DHEA levels is not significant ($t_{(36)} = 1.27$, $p = 0.21$), though. Table 7 lists the descriptive statistics of the average baseline DHEA levels.

Group	mean DHEA in pg/ml (SD)	*n*
Placebo	605.73 (81.73)	18
Egg powder	465.10 (74.62)	20

Table 7: Average DHEA levels at baseline.

4.2.3 Heart Rate

Heart rate was measured continuously starting 20 minutes prior to the beginning of the TSST, throughout the TSST and 20 minutes after the TSST. Data were aggregated describing different

study phases: first, 10 minutes while subjects were sitting, then 10 minutes before the TSST while subjects were standing, 5 minutes of introduction to and preparation for the TSST, 5 minutes of interview during the TSST, 5 minutes of mental arithmetic during the TSST, 10 minutes after the TSST while subjects were standing, and finally 10 more minutes of sitting.

Analyses show that the TSST induced a significant increase of heart rate (effect of time: $F_{(6,27)}$ = 33.27, p = 0.00). However, the two experimental groups neither showed overall differences in heart rate (effect of group: $F_{(2,60)}$ = 0.06, p = 0.94) nor in the time course of heart rate changes (effect time x group: $F_{(4,27)}$ = 0.07, p = 0.80). Data are shown in Figure 7 and Table 8.

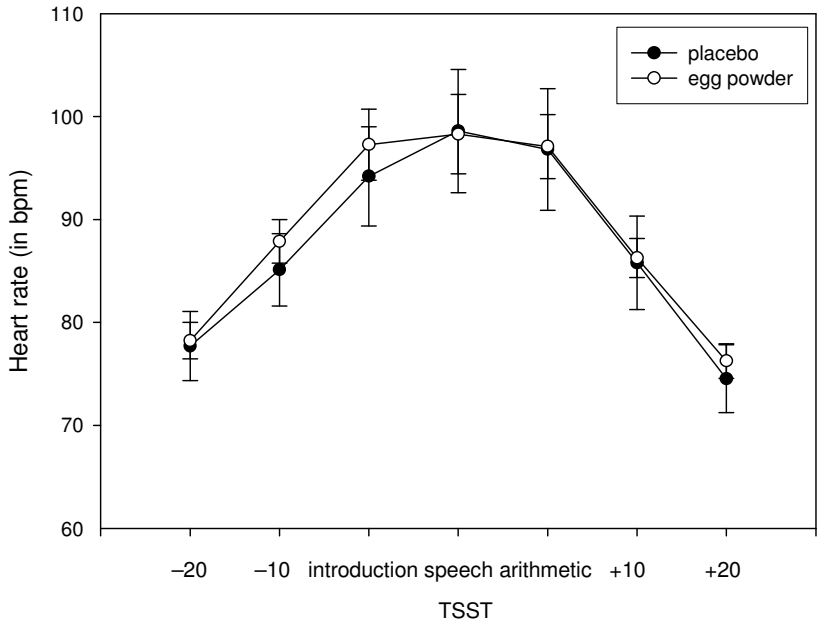

Figure 7: Time course of heart rate in response to the TSST. The graphs show group means with standard error bars.

Group	TSST segment							*n*
	–20 min. seat	–10 min. stand	intro-duction	speech	arithmetic	+10 min. stand	+20 min. stand	
Placebo	77.72 (13.84)	85.13 (14.47)	94.20 (19.85)	98.60 (24.68)	96.81 (24.39)	85.79 (18.71)	74.54 (13.61)	17[a]
Egg powder	78.25 (7.22)	87.88 (8.69)	97.28 (14.24)	98.30 (15.93)	97.09 (12.77)	86.27 (7.78)	76.26 (6.92)	17[a]

Table 8: Heart rate during the TSST. Means and standard deviations.
[a] A few cases were left out of the analysis due to partial gaps in the heart rate recording.

As with the cortisol data, 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: $F_{(6,12)} = 14.94$, $p = 0.00$; effect of time for low stress: $F_{(6,8)} = 22.10$, $p = 0.00$). 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: $F_{(1,13)} = 0.43$, $p = 0.53$). The interaction term approaches significance in the low stress group (effect of time x group: $F_{(6,8)} = 2.89$, $p = 0.08$), but not in the high stress group (effect of time x group for high stress: $F_{(6,12)} = 1.20$, $p = 0.37$). Figure 8 and Table 9 show the data and reveal a differential pattern that matches the cortisol data described above.

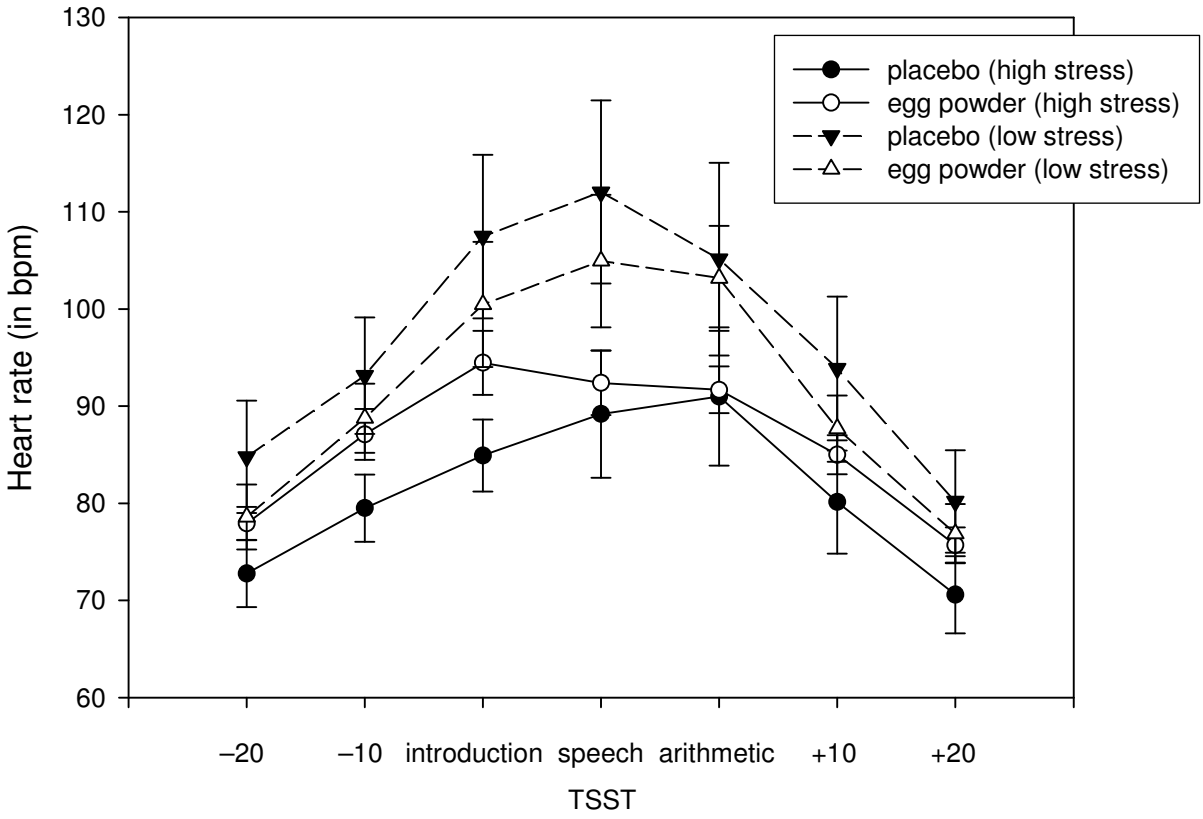

Figure 8: Time course of heart rates separately for the high stress half and the low stress half of the sample. The graphs show group means with standard error bars.

Group	TSST segment							*n*
	–20 min. seat	–10 min. stand	intro-duction	speech	arithmetic	+10 min. stand	+20 min. stand	
Placebo high stress	72.77 (10.89)	79.52 (10.94)	84.92 (11.69)	89.19 (20.73)	90.99 (22.51)	80.13 (16.76)	70.59 (12.53)	10
Egg powder high stress	77.84 (5.04)	87.10 (7.81)	94.46 (9.88)	92.40 (9.92)	91.69 (7.24)	85.00 (6.01)	75.70 (5.41)	9
Placebo low stress	84.80 (15.28)	93.15 (15.86)	107.46 (22.28)	112.04 (24.93)	105.13 (26.24)	93.87 (19.55)	80.18 (13.98)	7
Egg powder low stress	78.59 (9.49)	88.75 (10.06)	100.46 (18.16)	104.93 (19.29)	103.17 (15.27)	87.69 (9.63)	76.89 (8.66)	8

Table 9: Heart rates during the TSST by high and the low stress groups. Means and standard deviations.

4.3 Questionnaires pre-/post-TSST

4.3.1 STAI: State Anxiety

The STAI-X1 questionnaire was administered twice on the TSST day: once shortly before the TSST, once shortly after the TSST. The intervention yielded an significant higher post-test score (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: $F_{(1,37)} = 1.41$, $p = 0.24$). Data are shown in Figure 9.

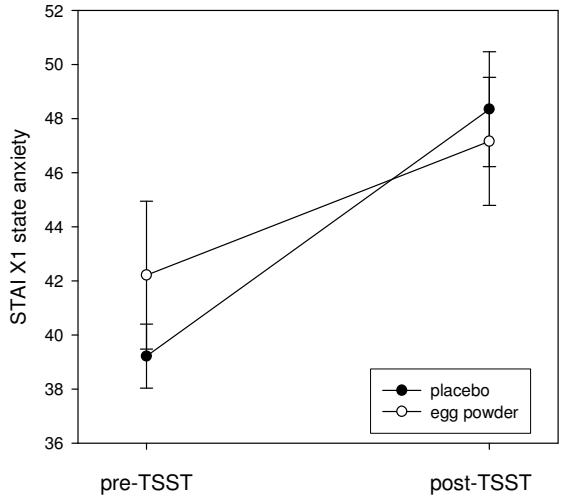

Figure 9: Time course of state anxiety in response to the TSST. The STAI-X1 scores before and after the TSST are displayed. The graphs show group means with standard error bars.

The increase of state anxiety 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 increase of 2.4 (eggpowder) and 12.5 (placebo) scale points, respectively ($F_{(1,18)}$ = 3.98, p = 0.06); a one-side t-test achieved significance ($t_{(18)}$ = 2.00, p = 0.03). For the low stress subsample, there was no significant effect of group ($F_{(1,17)}$ = 0.18, p = 0.68).

4.3.2 MDBF: Mood, Wakefulness, Calmness

The effect of the TSST was similar for all three MDBF subscales. Their data are shown in Figure 10.

Mood ratings decreased significantly (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 = 0.96; effect time x group: $F_{(1,37)}$ = 0.53, p = 0.47).

Wakefulness/tiredness ratings decreased 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).

Calmness/agitation ratings decreased significantly (effect of time: $F_{(1,37)}$ = 12.18, p = 0.00), too, but there were also no significant group or 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).

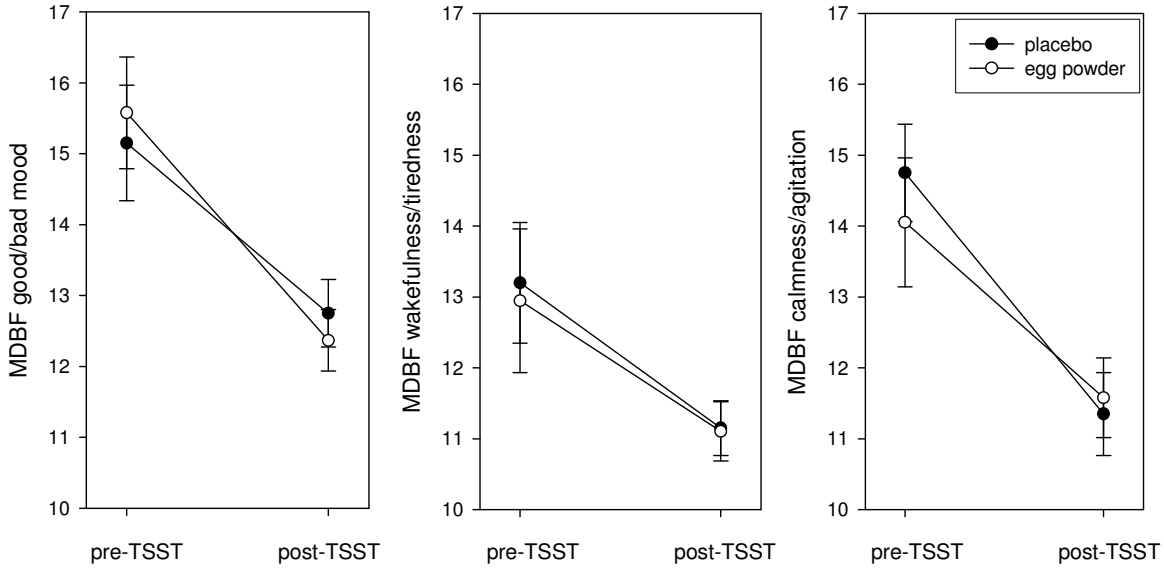

Figure 10: Time course of the three MDBF subscales in response to the TSST. Mood ratings (left), wakefulness (middle), and calmness (right) are displayed. The grapsh show group means with standard error bars.

4.3.3 Visual Analogue Scales

Participants rated their perception of the stress test on visual analogue scales three times: before, during, and after the TSST. They rated how high their "stress perception" (VAS 1), "anxiousness" (VAS 2) and their "insecurity" (VAS 3) during the TSST was. There were no initial differences in the rating of the TSST 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 on all scales in both groups (effect of time: $F_{(2,37)} = 29.61$, $p = 0.00$). The time course of VAS stress rating approached significance (effect time x group: $F_{(2,37)} = 2.75$, $p = 0.08$). There was no significant difference between the two experimental groups in stress experience (effect of group: $F_{(1,38)} = 0.02$, $p = 0.90$). Data are shown in Figure 11.

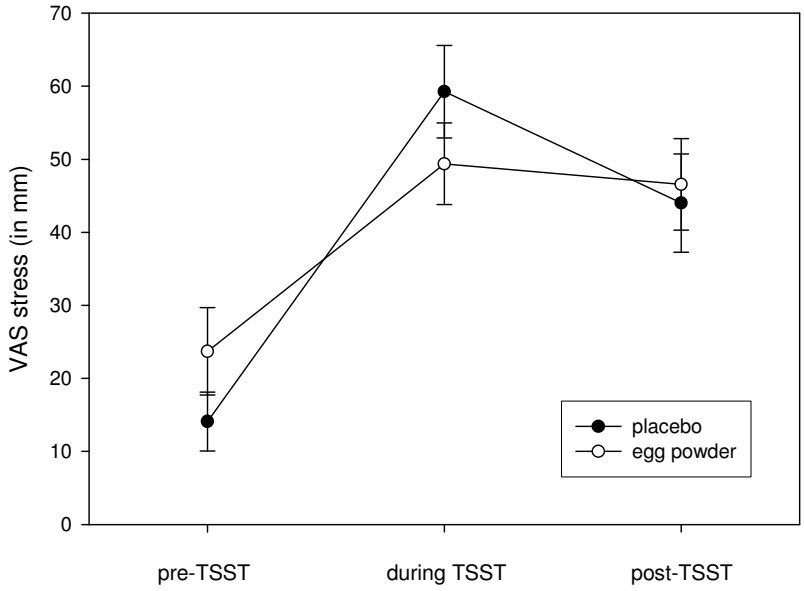

Figure 11: Time course of subjective stress experience in response to the TSST. The graph indicates the mean VAS ratings before, during, and immediately after the TSST. The graphs show group means with standard error bars.

Ratings on the other two visual analogue scales, anxiety and uncertainty, increased significantly in both groups (effect of time for anxiety: $F_{(2,37)} = 15.70$, $p = 0.00$; effect of time for insecurity: $F_{(2,37)} = 28.43$, $p = 0.00$). However, there were neither time x group, nor group effects approaching or achieving significance (effect time x group for anxiety: $F_{(2,37)} = 0.17$, $p = 0.85$; effect time x group for insecurity: $F_{(2,37)} = 0.54$, $p = 0.59$; effect of group for anxiety: $F_{(1,38)} = 0.00$, $p = 0.95$; effect of group for insecurity: $F_{(1,38)} = 0.28$, $p = 0.60$). Data for both scales are shown in Figure 12.

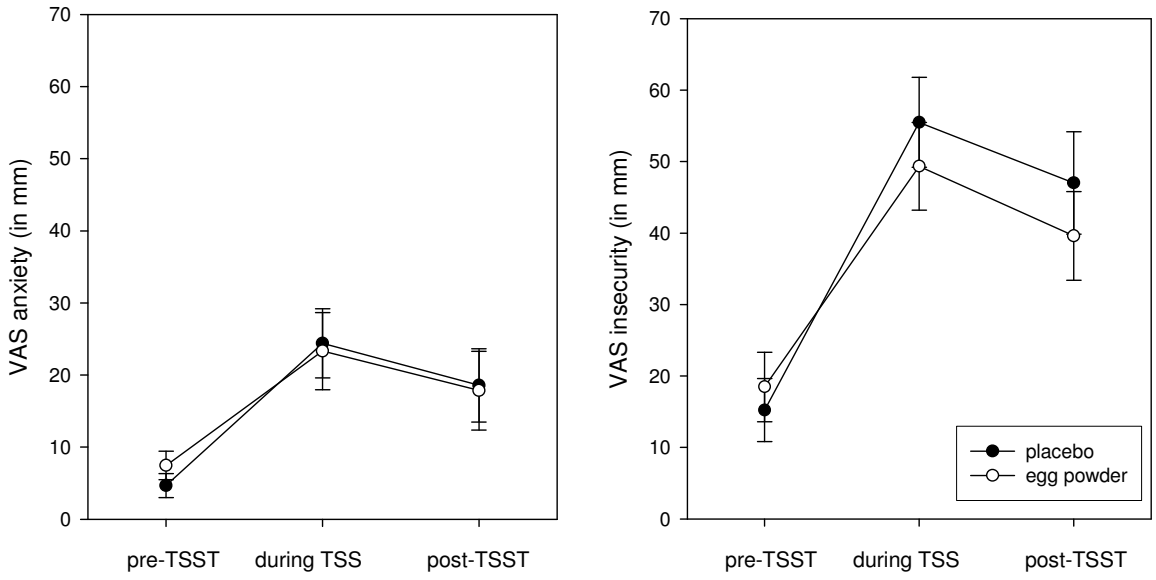

Figure 12: Time course of experienced anxiety (left) and insecurity (right) in response to the TSST. The graphs indicate the VAS ratings before, during, and immediately after the TSST. The graphs show group means with standard error bars.

Additionally, the maximum and the 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-side t-test of this effect achieves significance ($t_{(38)} = 1.83$, $p = 0.04$). 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: $F_{(1,38)} = 0.58$, $p = 0.45$). Comparing the groups' mean increase in VAS ratings yielded no significant results either (effect of group for stress: $F_{(1,38)} = 1.88$, $p = 0.18$, effect of group for anxiety: $F_{(1,38)} = 0.31$, $p = 0.58$, effect of group for insecurity: $F_{(1,38)} = 1.11$, $p = 0.30$). Data are listed in Table 10 and Table 11.

Group	VAS stress (SD)	VAS anxiety (SD)	VAS insecurity (SD)	*n*
Placebo	50.18 (26.57)	21.14 (22.42)	43.39 (26.74)	20
Egg powder	33.26 (31.83)	18.56 (24.83)	35.90 (35.14)	20

Table 10: Mean maximal increase of VAS ratings during the TSST (in mm).

Group	VAS stress (SD)	VAS anxiety (SD)	VAS insecurity (SD)	*n*
Placebo	37.52 (26.88)	16.81 (21.41)	36.01 (27.36)	20
Egg powder	24.26 (33.93)	13.13 (20.42)	26.00 (32.59)	20

Table 11: Mean average increase of VAS ratings during the TSST (in mm).

4.4 Development During the Intake Period

4.4.1 Free Cortisol in Saliva

Free cortisol levels were measured in saliva on two subsequent days per week for a total of five weeks. Each sampling day began with a saliva sample immediately after awakening and another saliva sample 30 minutes later. The total area under the curve (AUCt) was calculated as a measure of the cortsol awakening response (CAR) using the formula by (Pruessner, Kirschbaum, Meinlschmid, & Hellhammer, 2003)

$$AUCt = \sum_{i=1}^{n-1} \frac{\left(m_{(i+1)} + m_i\right) \cdot t_i}{2}$$

with t_i denoting the time span between measurements, m_i the individual measurement, and n the total amount of measures. The resulting values have the unit (nmol/l)*minute.

There were no significant changes in cortisol levels in saliva across the five weeks; neither did the two groups differ (effect of time: $F_{(4,33)} = 1.86$, $p = 0.14$; effect of group: $F_{(1,36)} = 0.90$, $p = 0.35$; effect of time x group: $F_{(4,33)} = 0.24$, $p = 0.92$). Figure 13 shows the average AUCt across weeks for both groups.

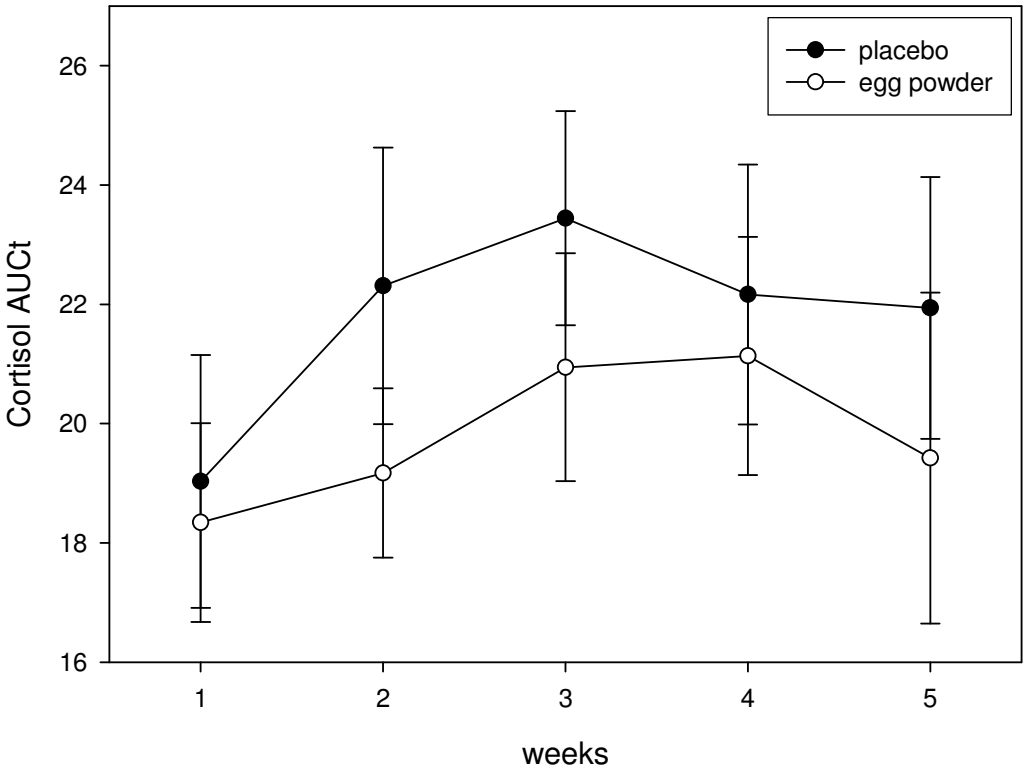

Figure 13: Mean AUCt values across weeks with standard error bars. Week 1 denotes the two days baseline before intake start.

The time of awakening turned out to be a confounding factor in the CAR measures with later wake-up times leading to less steep increases. Multilevel linear models were run for both, AUCt

and cortisol increase, to account for the tendency of the participants in the egg powder group to get up later than the placebo group (effect of group: $F_{(1,36)} = 4.06$, $p = 0{,}051$). In both cases, the group differences, as well as the interaction terms fail to approach or achieve significance.

Figure 14 shows the AUCt data of the five weeks for high stress and low stress subsamples. In the low stress half, the egg powder group remains rather on the same level and is significantly lower than the placebo group (effect of group: $F_{(1,18)} = 5.16$, $p = 0.04$).

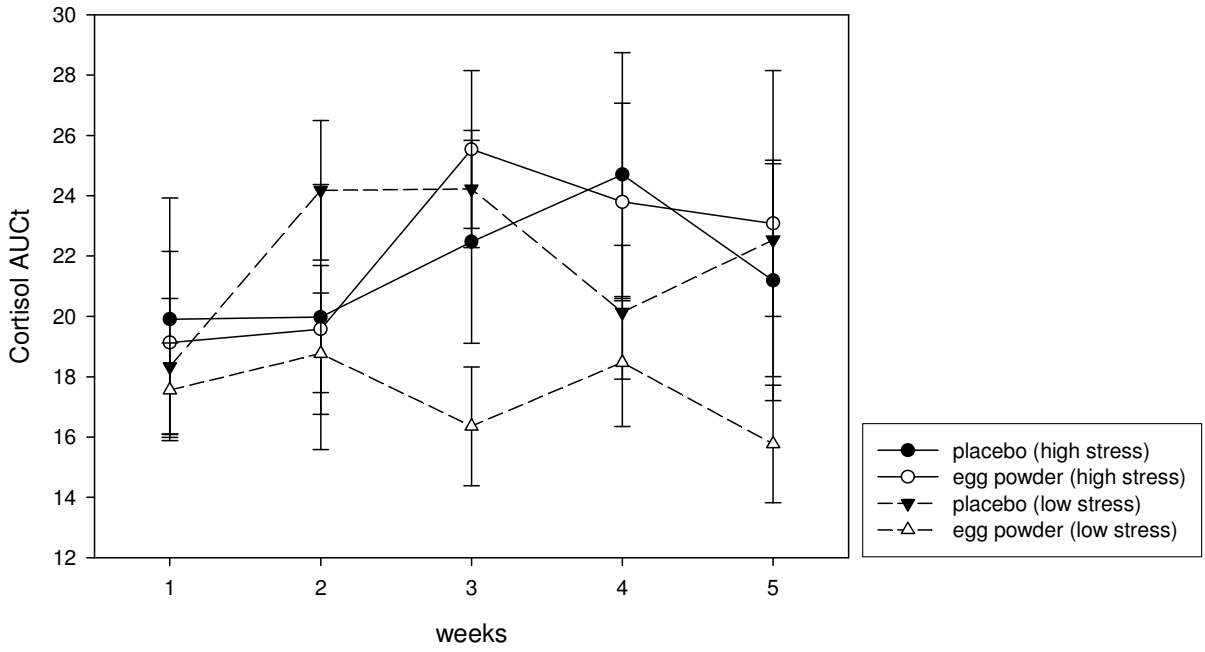

Figure 14: Mean AUCt values across weeks with standard error bars for the high and the low stress subsample.

4.4.2 Questionnaires

Comparing the groups' PSS scores at baseline with those at the TSST day yielded no significant results (effect of time: $F_{(1,38)} = 0.53$, $p = 0.18$, effect of group: $F_{(1,38)} = 0.71$, $p = 0.41$, effect time x group: $F_{(1,38)} = 0.61$, $p = 0.30$). Data are shown in Figure 15.

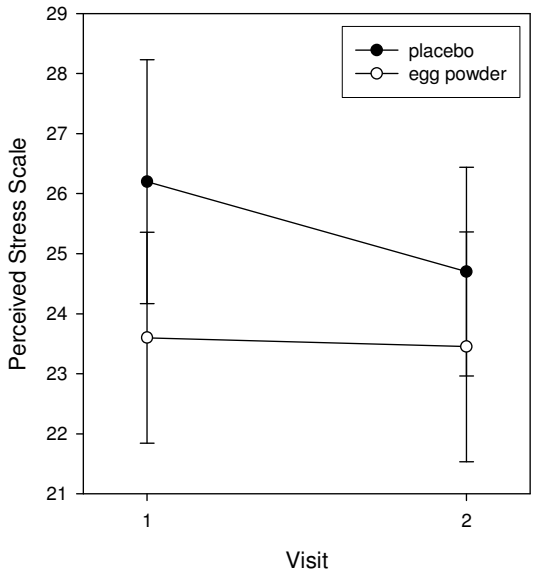

Figure 15: Comparison of PSS scores at baseline and at the TSST day. The graphs show group means with standard error bars.

The scores of the two SF-12 scales didn't differ significantly between groups, between baseline and TSST day, and between groups across time. PSS scores at baseline with those at the TSST day yielded no significant results (physiological well-being: effect of time: $F_{(1,35)} = 1.17$, $p = 0.29$, effect of group: $F_{(1,35)} = 0.22$, $p = 0.64$, 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: $F_{(1,35)} = 0.03$, $p = 0.86$). Data are shown in Figure 16.

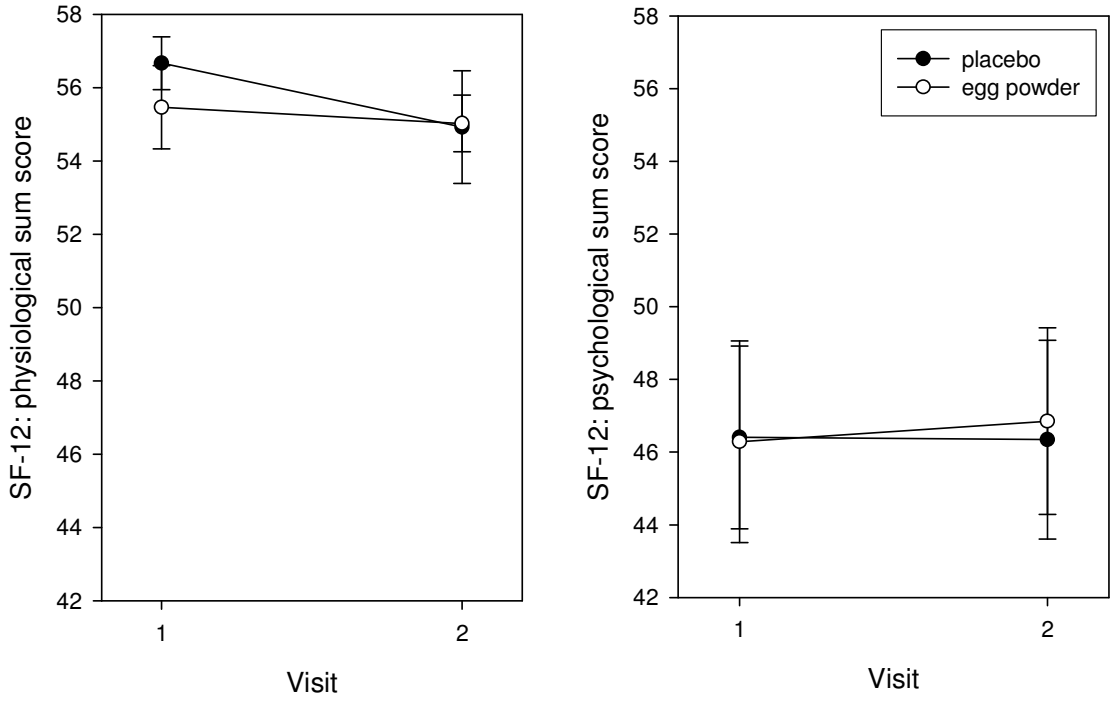

Figure 16: Comparison of SF-12 scores at baseline and at the TSST day. The physiological well-being is plotted in the left part; the psychological well-being is shown in the right part. The graphs show group means with standard error bars.

5 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. Furthermore, several psychological variables changed in response to the TSST, such as perceived stress, state anxiety, mood, and calmness.

An overall group comparison of cortisol levels during the TSST did not yield significant results. Similar results were achieved during the 4 week treatment intake as assessed by the cortisol awakening response (CAR). The trajectories during the intake period yielded no consistent pattern and subsequently no significant effect of YTE™.

Interestingly, results occur when groups are divided into subjects with rather high levels of chronic stress and rather low levels of chronic stress, respectively (median split of TICS 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. As expected, high stressed subjects of the placebo group showed a blunted cortisol response in the TSST whereas low stressed subjects show a normal increase to this challenge. Cortisol means of subjects with a high impact of chronic stress almost reach 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. Subjects with less chronic stress do not show any beneficial effects.

Heart rate as indicator of the autonomic nervous system shows less pronounced results but points to a similar direction as the endocrine data: whereas an overall treatment group comparison showed no significant group differences, results emerge when looking at the high stressed subgroup. Again, YTE™ raises participants' heart rate in the TSST when compared to the high stress placebo treated group.

The trajectories of the CARs revealed a lot of intra- and inter-individual variation, but no clear group effects. The CARs of the egg powder group's low stress half remained rather stable compared to their placebo counterpart. This may indicate that for these persons the hypothesized attenuation of cortisol is actually happening to the degree, that the egg powder helps people without chronic stress attaining a more balanced physiology.

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 (Fehm, Kern, & Peters, 2006).

Notably, these effects could only be observed under stimulated conditions, whereas the circadian levels (CAR) remain unaffected. In addition, the findings from Eskeland (1997) 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™ clearly dampened participants' 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 the high and low stressed subjects, this result remains similar for both subgroups. This indicates that all subjects benefit of YTE™ with respect to their perceived stress in an acute stressful situation.

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 participants' 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.

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