Supporting Healthy Individuals Dealing with Acute Psychosocial Stress: Investigations of a Fixed Herbal Drug Combination and a Social Support Stress Management

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Declaration of Independence

The submitted studies in partial fulfilment of the requirements of the degree of Doctor of Philosophy were written in collaboration with the mentioned co-authors. Neither the author, co-authors nor any other persons published the studies elsewhere. All citations are indicated and only the tools cited were used.

For the purpose of the cumulative dissertation, the following manuscripts have been submitted for publication in various journals. Copies of the manuscripts are attached in the appendices A, B, and C.

Study I, Publication I:

Meier, S., Haschke, M., Zahner, C., Kruttschnitt, E., Drewe, J., Liakoni, E., Hammann, F., & Gaab, J. (2018). Effects of a fixed herbal drug combination (Ze 185) to an experimental acute stress setting in healthy men–An explorative randomized placebo-controlled double blind study. *Phytomedicine, 39, 85-92.* doi.org/10.1016/j.phymed.2017.12.005

Study I, Publication II:

Meier, S., La Marca, R., Haschke, M., Zahner, C., Kruttschnitt, E., Drewe, J., Liakoni, E., Hammann, F., Kossowsky, J., Heimgartner, N., & Gaab, J. (2018). *Autonomic nervous system stress response under the intake of the fixed herbal drug combination Ze 185: a placebo-controlled randomized trial with healthy men.* Manuscript submitted for publication.

Study II, Publication III:

Heimgartner, N., Meier, S., Hochuli, S., Ponti, S., Arpagaus, S., Kappeler, F., & Gaab, J. (2018). *Randomized controlled evaluation of the psychophysiological effects of social support stress management in healthy women.* Manuscript submitted for publication.

With my signature, I testify that all statements are true and complete.

Basel, 06th of February 2018

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Abstract

Stress responses can be functional adaptions to help individuals cope with hassles by changing physiological processes during stressful episodes. In contrast, inadequate or excessive or too brief or prolonged stress responses can be harmful to the organism as stress has been shown to negatively impact on mental and physical health in the long term. Exposure to a stressor alters numerous biological functions with the hypothalamic-pituitary-adrenal axis and the autonomous nervous system accounting to the major human biological stress system. Research on the effects of pharmacological as well as psychological interventions often used the standardized psychosocial stress test named Trier Social Stress Test to reliably induce acute stress in a laboratory setting in the major human stress systems.

The emphasis of the current dissertation was to investigate two different interventions aiming to reduce physiological and emotional stress responses to the Trier Social Stress Test. Therefore, the thesis had two objectives: one was to examine the effects of the fixed herbal drug combination Ze 185 in men on stress responses of the hypothalamic-pituitary-adrenal axis and self-reported anxiety (Study I, Publication I) and of the autonomous nervous system (Study I, Publication II). The second goal was to investigate the effects of a social support stress management on stress responses in women assessing parameters of the hypothalamic-pituitary-adrenal axis, self-reported anxiety, and the autonomous nervous system (Study II, Publication III). For this reason, basic research approaches with healthy participants undergoing the Trier Social Stress Test and psychophysiological methods were chosen and applied in two randomized controlled studies.

The first study revealed that the participants receiving the fixed herbal drug reported reduced anxiety in comparison with the placebo (p = .03) and no treatment groups (p = .05) in response to the stress test while there were no significant differences in the biological stress response of the hypothalamic-pituitary-adrenal axis assessed with cortisol (p = .97; Study I, Publication I). In line with this, the parameters of the autonomous nervous system namely salivary alpha amylase (p = .51), heart rate (p = .17), heart rate variability (p = .56), skin conductance level (p = .18), and skin temperature (p = 0.65) did not differ significantly in the three study groups (Study I, Publication II). The second study revealed no significant differences in cortisol (p = .78), heart rate (p = .49), and heart rate variability (p = .53) levels in response to the stress test between the two conditions while participants in the social support stress management showed condition showed a significantly attenuated integrated self-reported anxiety response (p = .03) in comparison to those in the control condition.

In the second study participants in the social stress management and the waitlist control condition did not differ in their cortisol (p = .78), heart rate (p = .49), and heart rate variability (p = .53) reactions over time in response to the Trier Social Stress Test. Participants in the social support stress management showed a significantly attenuated integrated state anxiety response (p = .03) in comparison to those in the control condition.

Both the fixed herbal drug combination and our social support stress management interventions attenuated the subjective emotional stress response in healthy participants and maintained the biological reactions in response to the standardized psychosocial stress test. However, as stress is a part of our daily lives, it is important to investigate and develop interventions supporting individuals to cope with stress to increase well-being during challenging episodes.

1. Theoretical Background

1.1. Stress and biology of human stress responses

Stress is conceptualized as the occurrence of life events that are interpreted by the person as undesirable and that represent a strain on the person's adaptive capability causing an interruption of the person's habitual functioning. Stress reflects those factors that interfere with the system's physiological and psychological equilibrium (Ingram & Luxton, 2005). Furthermore, stress enables humans to adjust to challenging situations whereby the organism activates compensator responses that functionally correspond to the stressor (von Dawans, Fischbacher, Kirschbaum, Fehr, & Heinrichs, 2012). Thus, stress responses can be functional and healthy adaptions to help individuals cope with daily hassles by changing physiological processes during stressful episodes (McEwen, 1998; Sterling, 2004). In contrast, inadequate or excessive or too brief or prolonged stress responses as well as insufficient adaption can be harmful to the organism (Cacioppo, Tassinary, & Berntson, 2007).

Various health and illness models emphasis stress as a possible contributing factor in the development of diseases (e.g., Ingram & Luxton, 2005; Wade & Halligan, 2017). For example, the diathesis-stress model considers the combination of biological predispositions and environmental stressors in the development of psychological disorders. Thereby the model claims that if the combination of predispositions and the stress levels exceed a certain threshold, the individual experiences health-related impairments (Monroe & Simons, 1991). In line with that, several meta-analyses found negative impact on mental and physical health of stress (Booth et al., 2015; Toussaint, Shields, Dorn, & Slavich, 2016; Yu, Chiu, Lin, Wang, & Chen, 2007). One of them demonstrated for example that perceived psychosocial stress was associated with increased risk for experiencing a stroke (Booth et al., 2015). The American Psychological Association's (APA) Stress in America survey revealed that 20% of respondents stated extreme stress in their daily lives and 31% reported that their stress had increased in the past year. Moreover, 80% of respondents stated at least one physical and/or emotional symptom of stress over the past month (American Psychological Association, 2017). Furthermore, estimates indicate that between 75% and 90% of visits to primary care physicians are stress-related (Head & Kelly, 2009).

Exposure to an acute stressor alters numerous biological functions of human organisms and leads to the release of chemicals, which can help cope with the stressor (Allen, Kennedy, Cryan, Dinan, & Clarke, 2014). The two principal components of the adaptional stress response are the cortiocotropin-releasing hormone located in the hypothalamus and the locus cerluleus-norepinephrine (LC-NE)/sympathetic system in the brain stem (Cacioppo et al., 2007; Chrousos

& Gold, 1992). The hypothalamic-pituitary-adrenal (HPA) axis and parts of the autonomous nervous system (ANS) are the afferent limbs of the stress system, whose central function is to maintain stress-related equilibrium (Cacioppo et al., 2007).

Looking at the HPA stress response, the paraventricular nucleus of the hypothalamus releases corticotropin-releasing factor which leads to the release of adrenocorticotropic hormone from the pituitary gland followed by the secretion of cortisol from the adrenal gland. A significant increase in salivary cortisol following exposure to acute psychosocial stressor is well established (Allen et al., 2014; Cacioppo et al., 2007). Dysregulation of the HPA axis is associated to adverse outcomes on somatic as well as to a wide range of outcomes on mental well-being (Chrousos, 2009; Heim, Ehlert, & Hellhammer, 2000; Tirabassi, Muscogiuri, Colao, & Balercia, 2015).

The autonomous nervous system (ANS) is divided into two branches namely parasympathetic and the sympathetic nervous system, which establish patterns of parasympathetic and sympathetic activation across the various physiological systems (Cacioppo et al., 2007). Fibers of the sympathetic nervous system release the neurotransmitter norepinephrine at the adrenal medulla and in consequence epinephrine, which both mediate different adaptive physiological processes to perfectly prepare the body for the fight-or-flight reaction. The adaption to acute stress on the cardiovascular system is reflected for instance as increased heart rate (HR) and blood pressure, as well as decreased heart rate variability (Chrousos & Gold, 1992). Another parameter of the ANS whose levels are increased under high autonomic activation is the enzyme salivary alpha amylase (sAA; Nater & Rohleder, 2009). Also, electro dermal activity as a measure of activity of sweat glands are innervated by the ANS (Allen et al., 2014; Jezova, Makatsori, Duncko, Moncek, & Jakubek, 2004; Rohrmann, Hennig, & Netter, 1999). Additionally, changes in skin temperature (SKT) are controlled by the ANS though vasoconstriction and vasodilation of peripheral blood vessels (Ahmed, Begum, Funk, Xiong, & von Scheele, 2011; Karthikeyan, Murugappan, & Yaacob, 2012).

One of the emotional responses to stress is represented by elevated anxiety levels. Thereby, the individual's predisposition to respond and the stressful situation must be congruent in order to evoke increases in state anxiety (Endler & Kocovski, 2001). One of the many definitions of the ambiguous construct anxiety is noted by (Lewis, 1969) as "an emotional state, with the subjectively experienced quality of fear as a closely related emotion" (p. 77). Together with the definition of Spielberger (1966) anxiety can be understood as an unpleasant emotion characterized by physiological arousal and perceived feelings of apprehension, dread, and tension (Endler & Kocovski, 2001).

Taken together, when experiencing acute stress, the human organism activates compensator responses in altering numerous biological functions regulated by the HPA and ANS to functionally correspond to the stressor. Levels of salivary cortisol as a measure of the HPA axis and sAA, HR, and electro dermal activity as measures of ANS activation increase under stress while heart rate variability and distal SKT decrease (Allen et al., 2014; Castaldo et al., 2015; Vinkers et al., 2013). Furthermore, elevated subjective state anxiety levels as one of the emotional responses were found in acute stress situations (e.g., Britton, Shahar, Szepsenwol, & Jacobs, 2012; Rosenkranz et al., 2013).

1.2. Trier Social Stress Test

The Trier Social Stress Test (TSST; Kirschbaum, Pirke, & Hellhammer, 1993) is an acute psychosocial stress protocol used in research to experimentally induce HPA axis, ANS, and emotional stress responses in a laboratory setting (Allen et al., 2014). The protocol provokes stress responses with combining high levels of social-evaluative threat and uncontrollability using a public speaking and a mental arithmetic task. The TSST is one of the most popular used stress tests and has been applied in a broad field of research. Research included for example investigation of synthetic drugs (e.g. benzodiazepine; Fries, Hellhammer, & Hellhammer, 2006), sports (e.g. an endurance training; Klaperski, von Dawans, Heinrichs, & Fuchs, 2014), psychological interventions with an intraindividual focus (e.g. cognitive behavioral stress management training; Gaab et al., 2003; Hammerfald et al., 2006) as well as with an interindividual perspective (e.g. social interaction; Ditzen et al., 2007). The TSST has repeatedly been found to induce a reliable and profound psychophysiological stress responses in 70–80% of tested subjects with cortisol being most frequently assessed (Allen et al., 2014; Kirschbaum et al., 1993).

The procedure of the TSST follows a standardized protocol suggested by Kirschbaum and colleagues (1993): Upon arrival, the participant rests in a quiet room A in order to help establish clear baseline measurements. After the resting period, the participant is taken to the TSST room B, where a selection committee consisting of one male and one female confederates is already sitting at a table with a video camera and a voice-recorder connected to a microphone visible for the participant. There, the task which the participant would have to perform subsequently is introduced during two minutes. The investigator asks the participant to take over the role of a job applicant who is invited for a personal interview with the staff managers (committee). After the introduction of the subsequent tasks the participant is guided back to room A for the preparation phase lasting for eight minutes. Then, the participant gives his or her speech in

front of the committee and followed by serially subtraction task for five minutes. Once completing the TSST exposure participant are guided into another room for recovery.

1.3. Research on stress interventions with herbal medicinal products

One approach to deal with stress reactions is the use of pharmacological treatments. Several synthetic drugs were investigated using the TSST to observe human stress responses under pharmacological treatment. For example, an antiepileptic drug that decreases glutamate release (Makatsori et al., 2004), an opioid partial agonist (Bershad, Jaffe, Childs, & de Wit, 2015), and benzodiazepine (Fries et al., 2006) were found to inhibit cortisol stress responses in comparison to placebo. Thereby there were no significant differences in HR and anxiety in the study with benzodiazepine (Fries et al., 2006). Despite their observed effectiveness to reduce stress responses, some pharmacological drugs can be associated with unwanted side effects and the risk of addiction and depend on compliance (e.g., Anthierens et al., 2010).

Complementary and alternative medicine (CAM) for the treatment of psychological and physical problems is gaining more popularity (Roessler et al., 2007). For some individuals herbal medicinal products (belonging to phytotherapy) are appealing, considering their low risk of side effects and risks, at least in comparison with conventional pharmacological medicine which may be expressed in a higher compliance (Falch, Eltbogen, & Meier, 2013; Lynch & Berry, 2007). Herbal medicinal products differ from synthetic drugs in that they contain as active substance a plant preparation instead of a chemically precisely defined individual substance. Rational phytopharmaceutical drugs use plants and parts of plants for therapeutic purpose meeting the requirements for quality, safety and efficacy for herbal medicinal products. This plant preparation (e.g. an extract) is composed of many different substances, so called multicomponent mixtures. Thus, in phytotherapy, the extract as a whole is the active ingredient. As mentioned, they underlie the requirements of the Drug Law regarding quality, efficacy, and safety for evidence-based products (Wagner & Wiesenauer, 2003). One advantage of herbal medicinal products is their broad spectrum of pharmacological targets: as herbal multicomponent mixtures, they interact with several biochemical structures instead of having a single effect on one biochemical mechanism. Although the onset of action may often need more time, the curative treatment is possibly more thorough and comprehensive (Falch et al., 2013).

Considering the empirical status of herbal medicinal products for the treatment of stress and stress-reactivity, a number of randomized placebo-controlled trials indicate the potential of herbal drugs. A randomized-controlled study demonstrated reduced HR responses in participants taking tablets of valerian (LI 156; Sedonium, containing 300 mg ethanolic dried valerian

root extract; drug/extract ratio 3-6:1; Cropley, Cave, Ellis, & Middleton, 2002). In addition, a randomized-placebo controlled study with a combination of *Melissae officinalis* L. and *Valeriana officinalis* L (each tablet containing 120 mg of *Valeriana officinalis* extract; drug/extract ratio 4,5:1 and 80 mg of *Melissa officinalis* extract; drug/extract ratio 5:1; Songha Night™) reduced anxiety in healthy individuals undergoing cognitive tasks with the 600 mg dose of the combination (Kennedy, Little, Haskell, & Scholey, 2006). Also, treatment with another combination remedy (Sandrin® containing 320 mg of the quantified dry extract WS 1014 from valerian root; drug/extract ratio 3–6: 1; solvent ethanol 62% (m/m)) and 160 mg of the quantified lemon balm dry extract WS 1303 (drug/extract ratio 4–6:1; solvent ethanol 30% (m/m)) reduced anxiety in preschool children with concentration difficulties reported by parents (Gromball, Beschorner, Wantzen, Paulsen, & Burkart, 2014). Moreover, reduced stress-related symptoms were found in participants with anxiety disorder taking capsules with a standardized, hydroalcoholic *Melissa officinalis* L. leaf extract with the 600 mg dose of the combination (300 mg; Cases, Ibarra, Feuillere, Roller, & Sukkar, 2011). A *Passiflora incarnata* L. extract (424 mg; drug/extract ratio 5:1-7:1; extraction solvent: ethanol 50%) increased stress resistance after 12 weeks of treatment in patients with nervous restlessness (Gibbert, Kreimendahl, Lebert, Rychlik, & Trompetter, 2017). Previous research with the fixed herbal drug combination Ze 185, containing extracts of *Petasites hybridus* (L.) P. Gaertn, *Valerianae officinalis* L., *Passiflora incarnata* L., and *Melissa officinalis* L., decreased anxiety levels in healthy participants after cognitive tasks in a randomized-placebo controlled study (Steiner & Opwis, 2000), as well as in patients with psychosomatic complaints (Schellenberg, Sauer, & Brattström, 2004). Herbal preparations containing *Passiflora incarnata* L., *Melissa officinalis* L. as well as *Valeriana officinalis* L. have been traditionally used for the relief of mild symptoms of mental stress, assessed by the Committee on Herbal Medicinal Products (HMPC) of the European Medicines Agency (HMPC 2013, 2014, 2016). On the basis of the empirical evidence so far as well as considering their acceptability and apparent safety, herbal medicinal products warrant further scientific examination.

1.4. Research on stress interventions and social support

Another approach to deal with stress is the use of social support which was investigated in previous research (Burton, Bonanno, & Hatzenbuehler, 2014; Ditzen et al., 2008; Heinrichs, Baumgartner, Kirschbaum, & Ehlert, 2003). Positive social interactions are crucial for both physiological and psychological well-being and health (Holt-Lunstad, Smith, & Layton, 2010; Jenkins & Elliott, 2004). A meta-analysis including 148 studies investigated the influence of

social relationships on risk for mortality and detected a 50% increased likelihood of survival for participants with stronger social relationships (Holt-Lunstad et al., 2010). In addition, a study with long-term nursing stuff reported that support from supervisors, coworkers, spouses friends or family members was associated with less emotional exhaustion and higher levels of personal accomplishment (Woodhead, Northrop, & Edelstein, 2016). Amongst the many conceptualizations of social support, Wills (1991) defined it as the perception or experience that one is loved and cared for by others, esteemed and valued, and part of a social network of mutual assistance and obligations (Taylor, 2011). Thus, social support can involve helping another person to understand a stressful event better, provision of specific aid or goods, as well as providing warmth and reassurement of the other person's value. To conclude, social support involves the perception of available resources should they be needed (Taylor, 2011).

Presented in a review, Christenfeld and Gerin (2000) described several studies in which social support was associated with reduced cardiovascular reactivity under stress. Investigating stress responses induced by the TSST, several studies reported suppressed cortisol stress responses in participants having positive social interactions before stress exposure (Burton et al., 2014; Ditzen et al., 2008; Heinrichs et al., 2003). Previous studies with an interindividual perspective included e.g. healthy male participants receiving social support from the participant's best friend (Heinrichs et al., 2003), from the partner (Ditzen et al., 2008), and individuals receiving family as well as peer support (Burton et al., 2014). Interestingly, greater levels of family support were associated with diminished cortisol stress reactivity while peer support was not associated with the neuroendocrine response (Burton et al., 2014). Furthermore, social support from a same-gender stranger during the TSST preparation phase was associated with equivalent cortisol and cardiovascular reactions in participants who received no support in a healthy sample (Robles, 2007). Moreover, a study with healthy individuals including partner as well as stranger support revealed different results for men and women. While men in the partner-supported group experienced reduced cortisol peak responses compared to those in the stranger and no support groups, women in the partner-supported group showed higher cortisol responses than those in the stranger support group (Kirschbaum, Klauer, Filipp, & Hellhammer, 1995). In a more recent study, women that were verbally supported by their partners before the TSST showed elevated cortisol and HR stress responses. In contrast, healthy women experiencing positive physical partner contact prior the TSST exhibited significantly lower cortisol and HR responses compared to women who received verbal support from their partners or no social interaction (Ditzen et al., 2007). Furthermore, in healthy women completing a mental arithmetic task, support from a male friend was associated with attenuated blood pressure reactivity while

support from a female friend or a male stranger was associated with augmented blood pressure reactivity (Phillips, Gallagher, & Carroll, 2009). Although not applying the TSST, a previous study evaluated the effects of an intervention employing the buffering effect of social support and revealed no effects on emotional as well as cardiovascular reactivity to a psychosocial stressor (Anthony & O'Brien, 2002).

2. Aims of the thesis

The thesis had the main goal to investigate two different interventions aiming to reduce emotional and physiological stress responses to an acute psychosocial stress test in healthy individuals. Therefore, the thesis had two objectives: one was to examine the effects of the fixed herbal drug combination Ze 185 on human emotional and physiological stress responses in men. The second goal was to investigate the effects of a social support stress management on emotional and physiological stress responses in women. For this reason, basic research approaches with healthy participants and psychophysiological methods were chosen and applied in two experimental studies both applying the TSST. Psychophysiological methods are able to enhance our understanding of basic psychophysiological processes being part of the human stress responses. Furthermore, they provide a systematic approach to empirically investigate the effects of specific treatments aimed at reducing human stress responses.

Two studies described in this thesis were developed to answer following research questions. The results of Study I are presented in two publications with Publication I reporting results on salivary cortisol and state anxiety and Publication II reporting results on ANS parameters.

(1) What effects does the fixed herbal drug combination Ze 185 have in healthy participants undergoing the TSST in regards of emotional and physiological stress responses compared to placebo and no treatment?

Study I. Herbal medicinal products are appealing, with low incidence of adverse drug reactions and a well benefit-risk ratio (Lynch & Berry, 2007). Considering the empirical status of herbal medicinal products for the treatment of stress and stress-reactivity, a number of randomized placebo-controlled trials indicate the potential of this approach. However, research on the effects of the fixed herbal combination Ze 185 with assessment of the major biological stress systems, namely the HPA axis and the ANS using a standardized acute psychosocial stressor,

is missing. Thus, the goal of Study I was to investigate emotional stress responses with self-reported state anxiety and physiological stress responses with assessments of salivary cortisol, sAA, HR, heart rate variability, skin conductance level, and SKT in healthy participants under the intake of Ze 185.

(2) What effects does a social support stress management have in healthy women undergoing an acute psychosocial stress test in regards of emotional and physiological stress responses?

Study II. Previous research has confirmed beneficial effects of positive social interactions on stress responses (e.g., Burton et al., 2014; Ditzen et al., 2008). One study observed the effects of a short intervention employing the buffering effect of social support, albeit with no effects on an array of psychological parameters as well as cardiovascular reactivity to a psychosocial stressor (Anthony & O'Brien, 2002). Hence, the goal in Study II was to conceptualize, implement and evaluate the effects of a social support stress management intended to employ and improve social support skills and to reinforce the stress buffering effect of social support on emotional and psychophysiological stress responses in women.

3. Methods

3.1. Effects of a fixed herbal drug combination (Ze 185) to an experimental acute stress setting in healthy men – an explorative randomized placebo-controlled double blind study (Study I, Publication I)

Participants. Healthy male participants aged between 18 and 45 years from the general Swiss population were recruited via online advertisements on www.markt.unibas.ch between January 2015 and June 2016. The screening procedures were conducted at the University Hospital of Basel by a medical team. Exclusion criteria were the presence of somatic or psychiatric disorders, or any other clinically relevant diseases, as well as the intake of medication, smoking, previous participation in a TSST, and being currently in any psychotherapy.

Procedure. Participants were randomly assigned to the fixed herbal combination Ze 185 ($n = 24$), placebo ($n = 24$), and no treatment ($n = 24$). Participants as well as the study team were blind regarding group allocation. During the first three days of the study participation all participants completed online questionnaires at home and the two medication groups took three tablets per day. The Ze 185 and placebo tablets were identical in presentation, color, and shape.

On the fourth day all participants underwent the TSST at the division of Clinical Psychology and Psychotherapy of the University of Basel. Thereby, salivary cortisol, self-reported anxiety, sAA, HR, heart rate variability, electro dermal activity, and SKT parameters were assessed before, during and after the stress test. The end of the study visit was conducted three to five days later at the University Hospital of Basel.

Outcome measures. On the fourth day at the division of Clinical Psychology and Psychotherapy, the saliva cortisol levels were assessed eleven times in total, four times before the TSST and seven afterwards. The first assessment was intended to help the participants getting used to the procedure and was not included in the analyses. The mean value of the second and third saliva collection was used as baseline assessments. The first post TSST saliva collection was conducted right after the TSST completion and the last one 90 minutes after the TSST. Saliva samples were collected using polypropylene saliva tubes (Sarstedt, Nümbrecht, Germany). Salivary free cortisol was assayed using the Salimetrics immunoassay method (EIA kits; Salimetrics LLC, USA). All samples were analyzed by the Swiss-Analysis AG (Tägerwilen, Switzerland). The assay kits had an intra-assay variation of 3 to 7% and inter-assay variation of 3 to 11%. The minimum detectable level of cortisol concentration was 0.007 µg/dL.

As emotional stress response, self-reported state anxiety was assessed with the State-Trait Anxiety Inventory (STAI; Laux, Glanzmann, Schaffner, & Spielberger, 1981) four times in total. One assessment took place before the TSST as baseline measurement (45 minutes prior to the TSST) and three assessments after the stress test (directly after TSST completion; ten minutes post TSST completion; 30 minutes post TSST completion) The STAI-state part consists of 20 items rated on a four-point scale with 1 = ”not at all” and 4 = “very much” and has acceptable internal consistency and test-retest reliability (Lee, Park, & Moon, 2004).

Statistical analyses. Analyses were performed with the IBM SPSS Version 21 (IBM Corperation, 2012). The shown analyses are based on the Intention to Treat (ITT) dataset. Data were tested for normal distribution using Kolmogorov–Smirnov test. Cortisol, state anxiety, and baseline variables were log-transformed to approximate normal distribution. For better readability, the original values were used for Tables and Figures. Baseline characteristics of the study groups were compared with one-way analyses of variance or Chi-square tests. Analysis of variance for repeated measures were computed to analyze cortisol and state anxiety responses between the study groups. Post hoc analyses were examined with separate analyses of variance. Additionally, area under the curve with respect to increase (AUC_I) and area under the curve with respect to ground (AUC_G) were calculated for cortisol data using the trapezoidal method (Pruessner, Kirschbaum, Meinlschmid, & Hellhammer, 2003). Post hoc analyses were

examined with separate analyses of variance.

3.2. Autonomic nervous system stress response under the intake of the fixed herbal drug combination Ze 185: a placebo-controlled randomized trial with healthy men (Study I, Publication II)

Participants and Procedure. For the description of study participants and data analysis kindly refer to the description under 3.1.

Outcome measures. On the fourth day at the division of Clinical Psychology and Psychotherapy, eleven saliva samples were collected leading to two sAA values before the TSST and seven after the TSST (until 90 minutes post TSST). The first saliva collection was not included in the analyses as the intention was for the participants getting used to the procedure. Baseline sAA values were calculated as the mean values of the second and third assessment. Completely defrosted saliva samples were vortexed and subsequently centrifuged at 1500 x g (3000rpm) for 15 minutes. The centrifuged saliva samples were diluted 1:200 by first diluting samples 1:10 with the alpha amylase diluent provided followed by a 1:20 dilution of the 1:10 diluent sample resulting in a final dilution of 1:200. Intra-assay and inter-assay coefficients of variation were less than 7.3%. and 5.9%, respectively. All samples were analyzed by Swiss-Analysis AG (Tägerwilen, Switzerland).

Furthermore, HR, heart rate variability, electro dermal activity, and SKT were continuously recorded during the whole visit. Heart rate variability was assessed with the square root of the mean of the sum of the squares of differences between adjacent normal-to-normal intervals (RMSSD). Electro dermal activity was assessed with skin conductance level (SCL). All ANS parameters were assessed with the wireless physiological recording system BioNomadix® (Biopac Systems, Inc., Santa Barbara, CA 93117) allowing subjects to move freely. For each parameter, the same five minutes intervals were measured at seven time points: at baseline (-35 minutes before TSST completion), preparation directly after the TSST introduction (-17 minutes before TSST completion), TSST speech (first five minutes of the TSST), TSST arithmetic task (second five minutes of the TSST), recovery 1 (five minutes after TSST completion), recovery 2 (20 minutes after TSST completion), and recovery 3 (25 minutes after TSST completion). After data collection, HR and RMSSD parameters were derived with the VivoSense® software (VivoNoetics, US). SCL and SKT was derived with the AcqKnowledge® software (Biopac Systems Inc.)

Statistical analyses. Statistical analyses were performed with the IBM SPSS Version 21 (IBM Corperation, 2012). The shown analyses are based on the Intention to Treat (ITT)

dataset. Data were tested for normal distribution using Shapiro-Wilk test. sAA variables were square-root-transformed and HR, RMSSD, SCL, and SKT variables were log transformed to approximate normal distribution. For better readability, the original values were used for Tables and Figures. Analysis of variance for repeated measures were computed to compare sAA, HR, RMSSD, SCL, and SKT data between the three study groups. For effect sizes, partial eta square were reported, where appropriate, according to the following conventions: 0.01 = small, 0.06 = medium, 0.13 = large (J. Cohen, 1988). Regarding electrophysiological measures, artifacts, loosening electrodes and technical problems resulted in a loss of data from single subjects, resulting in different *n*.

3.3. Randomized controlled evaluation of the psychophysiological effects of social support stress management in healthy women (Study II, Publication III)

Participants. Healthy female participants from the general Swiss population were recruited in lectures and via online advertisements at the University of Basel and the university of Applied Sciences and Arts of Northwestern Switzerland. Some researchers suggested that females' behavioral stress response is next to the typical fight-or-flight stress response additionally marked by a pattern of "tend and befriend" (Taylor et al., 2000). Thus, we assumed women to readily accept the rational of the social support stress management, which would facilitate the implementation of the intervention. Additionally, participants had to be aged between 18 and 60 years. Exclusion criteria were any current or chronic somatic diseases or psychiatric disorders, current medication, being in psychological treatment, previous participation in studies using the TSST, and smoking more than five cigarettes a day.

Procedure. A total of 53 participants fulfilled inclusion criteria and were randomly assigned to the intervention (n = 28*)* and waitlist control condition (n = 25*)*. Due to withdrawal before the intervention (intervention condition n = 2, waitlist control condition n = 4), insufficient intervention attendance (intervention condition n = 5), not completing post-training assessment (intervention condition n = 1, waitlist control condition n = 1) and refusing participation in the TSST (intervention condition n = 0, waitlist control condition n = *3*) the sample completing the TSST consisted of n = *37:* n = 20 in the intervention and n = 17 in the waitlist control condition. After the screening procedure, participants in the intervention condition received a two-week social support stress management in groups of five to seven participants (week 2 and 3). The control condition was a waiting list condition with participants receiving the intervention after all the measurements were completed (week 8). All participants from both conditions underwent the TSST (week 5 to 7). Self-report questionnaires were completed online

at baseline (week 1), post-training (week 4) and follow-up (week 8). At the TSST visit (week 5 to 7) at the division of Clinical Psychology and Psychotherapy of the University of Basel, salivary cortisol levels, HR, RMSSD, and self-reported anxiety were assessed.

Outcome measures. Nine saliva samples were collected using Salivette collection devices (Sarstedt, Sevelen, Switzerland): three before the TSST and five after the TSST (time points ranging from right after the TSST to 50 minutes post TSST). Biochemical analyses were conducted in the biochemical laboratory of the Clinical Psychology and Psychotherapy department at the University of Zurich, Switzerland. A highly sensitive liquid chromatography–tandem mass spectrometry (LC–MS/MS) method was used (Perogamvros et al., 2009).

HR and RMSSD were continuously assessed during the whole TSST visit using the wireless physiological recording system BioNomadix® (Biopac Systems, Inc., Santa Barbara, CA 93117) and derived with the VivoSense® software (VivoNoetics, US). For HR and RMSSD, the same five minutes intervals were measured at seven time points: baseline (-30 minutes before TSST completion), preparation directly after the TSST introduction (-17 minutes before TSST completion), TSST speech (first half of the TSST), TSST arithmetic task (second half of the TSST), recovery 1 (five minutes after TSST completion), recovery 2 (15 minutes after TSST completion), and recovery 3 (25 minutes after TSST completion).

Additionally, emotional stress responses were assessed with self-reported state anxiety using the STAI (Laux et al., 1981) before the TSST (-45 minutes), after the introduction to the TSST (-20 minutes), immediately after the TSST (0 minutes) and in the recovery phase (50 minutes). For the description of the STAI questionnaire kindly refer to 3.1.

Next to the evaluation of the social support stress management in the TSST, we also observed psychometric parameters as well as feasibility and applicability of the social support stress management in the longer-term with self-reported questionnaires (e.g. Perceived Stress Scale; S. Cohen, Kamarck, & Mermelstein, 1983; Working Alliance Inventory; Wilmers et al., 2008). Participants filled in those questionnaires online several weeks before and after completion of the TSST (baseline week 1, post-training week 4 and follow-up week 8). This thesis focuses on effects under acute psychosocial stress and therefore data is not shown here.

Statistical analyses. Statistical analyses were performed with the IBM SPSS Statistics Version 23 (IBM Corperation, 2015). The shown analyses are based on the sample who completed the TSST visit (n = 37: n = 20 in the intervention and n = 17 in the waitlist control condition). Due to technical problems during the TSST visit there is a loss of data from single subjects, resulting in different n (state anxiety n = 35; cortisol n = 37; HR and RMSSD n = 34). Two subjects had missing HR values at baseline, which were replaced with the arithmetical

mean of the recovery values. Three subjects showed missing values in one or two recovery assessments which were replaced with the arithmetical mean of the available recovery values. State anxiety, salivary cortisol, HR, and RMSSD were tested for normal distribution and homogeneity of variance using the Kolmogorov-Smirnov test before statistical procedures were applied. When normal distribution was violated, calculations were repeated with log transformed data. For better readability, the original values were used for Tables and Figures. Analysis of covariance for repeated measures were computed to analyze state anxiety, cortisol, HR, and RMSSD responses between the study groups with age as covariate. Additionally, body mass index (BMI) was included as covariate in the analyses of the physiological parameters. For state anxiety and cortisol AUC_I were calculated using the trapezoidal method (Pruessner et al., 2003) as an indicator for the integrated stress response in the TSST. AUC_I values were compared with univariate analyses of covariance with age and for cortisol analysis together with BMI included as covariables. For effect sizes, partial eta square were reported, where appropriate, according to the following conventions: 0.01 = small, 0.06 = medium, 0.13 = large (J. Cohen, 1988).

4. Summary of the results

4.1. Effects of a fixed herbal drug combination (Ze 185) to an experimental acute stress setting in healthy men – an explorative randomized placebo-controlled double blind study (Study I, Publication I)

Out of the 72 randomized participants two dropped out after randomization but before receiving study medication. The remaining 70 participants had a mean age of 26.07 years (SD = 5.17), a mean BMI of 24.41 kg/m^2 (SD = 2.88) and with 96.3% the majority was Caucasian. The study groups did not differ with respect to any of the assessed demographic variables in age ($F(2, 67) = 1.08$, $p = .35$), BMI ($F(2, 67) = 0.05$, $p = .96$), distribution of ethnicity ($\chi^2(4, N = 70) = 4.02$, $p = .40$).

The TSST induced significant and substantial increases in salivary cortisol levels ($F(8, 60) = 40.28$, $p < .001$). Cortisol levels among all participants did not differ between the three groups at baseline ($F(2, 67) = 0.05$, $p = .95$) and groups did not differ significantly in their cortisol responses over time ($F(16, 122) = 0.43$, $p = .97$) nor did integrated cortisol responses (AUC_I: $F(2, 67) = 0.17$, $p = .84$; AUC_G: $F(2, 67) = 0.002$, $p = .99$). Groups did not differ significantly in state anxiety baseline scores ($F(2, 67) = 0.50$, $p = .61$) and the TSST resulted in a significant state anxiety response ($F(3, 64) = 25.89$, $p < .001$). Groups differed however

significantly in the state anxiety response over time ($F(6, 130) = 2.41$, $p = .03$) and post hoc analyses revealed a significant time by group interaction effect between placebo and Ze 185 ($F(3, 41) = 3.33$, $p = .03$), as well as between no treatment and Ze 185 ($F(3, 43) = 2.77$, $p = .05$) while response differences between no treatment and placebo were not significant ($F(3, 42) = 1.42$, $p = .25$).

4.2. Autonomic nervous system stress response under the intake of the fixed herbal drug combination Ze 185: a placebo-controlled randomized trial with healthy men (Study I, Publication II)

Kindly refer to 4.1. for a description of the participant's demographic information. The TSST induced a significant increase in sAA levels ($F(8, 60) = 28.17$, $p < .001$, partial eta square = .79). sAA levels did not differ between the three groups (Ze 185 $n = 23$, placebo $n = 23$, no treatment $n = 24$) at baseline ($F(2, 67) = 0.05$, $p = .95$) as well as over time ($F(16, 122) = 0.95$, $p = .51$). There were no significant differences in HR values at baseline ($F(2, 52) = 2.26$, $p = .11$) between the groups (Ze 185 $n = 17$, placebo $n = 18$, no treatment $n = 20$). The TSST induced significant and substantial increases in HR levels ($F(6, 47) = 55.17$, $p < .001$, partial eta square = .88). HR levels did not differ between the three groups over time ($F(12, 96) = 1.42$, $p = .17$). The TSST induced significant and substantial decreases in RMSSD levels ($F(6, 47) = 6.95$, $p < .001$, partial eta square = .47). RMSSD levels did not differ between the three groups (Ze 185 $n = 17$, placebo $n = 18$, no treatment $n = 20$) at baseline ($F(2, 52) = 0.54$, $p = .58$) and over time ($F(12, 96) = 0.90$, $p = .56$). The TSST induced significant and substantial changes in SCL ($F(6, 44) = 27.64$, $p < .001$, partial eta square = .79). SCL did not differ between the three groups (Ze 185 $n = 20$, placebo $n = 14$, no treatment $n = 18$) at baseline ($F(2, 49) = 0.54$, $p = .59$) and over time ($F(12, 90) = 1.39$, $p = .18$). The TSST induced significant changes in SKT ($F(6, 57) = 104.74$, $p < .001$, partial eta square = 0.92). The SKT did not differ between the three groups at baseline ($F(2, 65) = 0.87$, $p = .42$) and there were no significant time by group interaction effects ($F(12, 116) = 0.80$, $p = .65$).

4.3. Randomized controlled evaluation of the psychophysiological effects of social support stress management in healthy women (Study II, Publication III)

Participants completing the TSST ($n = 37$) had a mean age of 29.51 years ($SD = 12.50$) and a mean BMI of 21.23 kg/m^2 ($SD = 2.68$). There was no significant difference in BMI between the two groups ($t(23.84) = -.11$, $p = .914$). There was a significant group difference in age with subjects in the control group being significantly older than subjects in the intervention condition

(t(19.15) = -3.57, p = .002); intervention condition M = 23.35, SD = 5.03, waitlist control condition M = 36.76, SD = 14.77).

With regard to state anxiety (n = 35), there were no significant baseline differences between the two conditions (intervention condition M = 38.15, SD = 8.50; waitlist control condition M = 35.53, SD = 5.16; t(35) = 1.11, p = .28). With regard to state anxiety responses to the TSST, participants in the intervention condition showed a trend to an attenuated response over time (n = 35; F(2.87, 91.81) = 2.59, p = .06, partial eta square = .08) as well as a significant lower integrated response in comparison to waitlist control condition (AUC_I: intervention condition M = 153.21, SD = 515.26; waitlist control condition M = 592.63, SD = 910.22; F(1, 32) = 5.51, p = .03, partial eta square = .15).

Cortisol levels among all participants did not differ between the two conditions (n = 37) at baseline (intervention condition M = 4.68, SD = 3.43; waitlist control condition M = 4.74, SD = 2.98, t(35) = -0.06, p = .95). There was no significant difference between groups in their cortisol response over time (F(2.02, 66.65) = 0.25, p = .78). Similarly, integrated cortisol stress responses did not differ significantly between conditions (AUC_I: intervention condition M = 53.51, SD = 198.18; waitlist control condition M = 38.89, SD = 171.01, F(1, 33) = 0.11, p = .74).

Conditions did not differ in baseline HR (n = 34; intervention condition M = 74.51, SD = 10.14; waitlist control condition M = 71.12, SD = 11.19; t(32) = 0.93, p = .36) and baseline RMSSD (n = 34; intervention condition M = 53.10, SD = 23.88; waitlist control condition M = 42.53, SD = 23.01; t(32) = 1.31, p = .20). Also, participants in both conditions did not differ significantly in their HR (F(1.69, 50.63) = 0.67, p = .49) nor in RMSSD responses to the TSST (F(1.99, 59.56) = 0.65, p = .53).

5. Discussion

The first objective of this thesis was to assess the effects of the fixed herbal drug combination Ze 185 on emotional and physiological responses to acute psychosocial stress in healthy men (Study I). Given that individuals demonstrate stress-relieving responses in trials with herbal medicinal products and the low risk of side effects and risks compared to conventional medicinal drugs, it is important to evaluate the potential of the fixed herbal drug combination in a three-arm trial using a standardized acute psychosocial stress test. In regard of the second objective of this thesis, previous research demonstrated promising results showing benefits of social support in stressful situations. Therefore, it is important to implement a social support

stress management under acute psychosocial stress. Thus, a further goal of this thesis was to evaluate the effects of a social support stress management intended to employ and improve social support skills in women in terms of biological and emotional responses to a standardized acute psychosocial stress test (Study II).

Results of the randomized placebo-controlled study with Ze 185 (Study I) showed that the fixed herbal drug combination significantly attenuated subjective state anxiety responses after acute psychosocial stress in healthy men compared to the placebo and no treatment groups, without affecting the parameters of the biological stress response system. Results of the effects of the social support stress management (Study II) revealed no significant different biological stress responses to the standardized psychosocial stress test, albeit the intervention condition showed a significantly attenuated state anxiety response in comparison to the control condition.

5.1. Effects of a fixed herbal drug combination (Ze 185) to an experimental acute stress setting in healthy men – an explorative randomized placebo-controlled double blind study (Study I, Article I)

In line with our findings, other randomized-placebo controlled trials investigating herbal interventions have reported decreases in anxiety in healthy subjects (Kennedy et al., 2006; Lee et al., 2004). Participants who experienced stress by completing cognitive tasks reported diminished anxiety levels after the intake of *Melissae officinalis* and *Valeriana officinalis* (Kennedy et al., 2006). Investigated in a naturally setting, *Ondamatanggamibang*, a Korean traditional herbal remedy, reduced anxiety in medical students experiencing naturally occurring stress due to academic examination (Lee et al., 2004). Previous research with Ze 185 did not include inducement of acute psychosocial stress in healthy participants. Nevertheless, reduced anxiety was found under the intake of Ze 185 in participants experiencing stress induced by completing cognitive tasks (Steiner & Opwis, 2000). Although not observing acute stress situation, the treatment with Ze 185 reduced general self-reported anxiety in patients with psychosomatic complaints (Schellenberg et al., 2004) and with somatoform disorders (Melzer, Schrader, Brattström, Schellenberg, & Saller, 2009). Noteworthy, several psychological and pharmacological interventions had no effect on self-reported anxiety in response to stress, including a psychological intervention designed to shift goal orientation from self-promotion to helping others (Abelson et al., 2014) and 1 mg of alprazolam (Fries et al., 2006).

Similar to our results, a number of trials demonstrated a dissociation of emotional and biological stress responses, i.e. reduced anxiety responses in face of no decrease in cortisol responses (self-compassion training; Arch et al., 2014; mindfulness meditation; Creswell,

Pacilio, Lindsay, & Brown, 2014; compassion meditation; Pace et al., 2009; brief attentional training; Pilgrim, Ellenbogen, & Paquin, 2014; aromatherapy; Takeda, Tsujita, Kaya, Takemura, & Oku, 2008). Noteworthy, the adaptive function of increased cortisol levels has been highlighted in a recent study. The authors report that elevated cortisol responses in the TSST were associated with fewer errors in threat-related decision making in police officers (Akinola & Mendes, 2012). Moreover, higher cortisol levels were associated with subsequent increased activeness, and relaxation, and reductions in perceived stress in everyday life (Hoyt, Zeiders, Ehrlich, & Adam, 2016). One possible explanation for the dissociation of subjective and objective stress responses could be the assumption of an important functionality of cortisol stress responses, which may serve to support positive and/or protect against negative emotional responses.

Additionally in line with previous trials of this fixed herbal drug combination (Schellenberg et al., 2004; Steiner & Opwis, 2000), herbal interventions, such as Ze 185, appear to be specifically effective in reducing subjective anxiety and anxiety responses to stress without affecting the HPA axis stress response system. The mechanism of action of Ze 185 is not fully understood. However, for valerian, passion flower and lemon balm anxiolytic effects have been suggested in several preclinical studies. A possible mechanism for certain constituents from valerian could be the modulation of the Gamma-Aminobutyric acid $(GABA)_A$ receptor (e.g. valerenic acid; Becker, Felgentreff, Schröder, Meier, & Brattström, 2014). In addition, a glutamatergic mechanism has been suggested (Del Valle-Mojica et al., 2011). Regarding passion flower and lemon balm, anxiolytic effects have been suggested to be mediated also via the GABAergic system (Grundmann, Wang, McGregor, & Butterweck, 2008; Ibarra, Feuillere, Roller, Lesburgere, & Beracochea, 2010).

5.2. Autonomic nervous system stress response under the intake of the fixed herbal drug combination Ze 185: a placebo-controlled randomized trial with healthy men

Men (Study I, Article II)

Previous studies on herbal medicinal products predominantly considered HPA activity with assessments of cortisol levels (e.g. al'Absi et al., 2013; Lee et al., 2004). Despite sAA is a popular biomarker of ANS in stress research in the context of psychological interventions (e.g. Thoma et al., 2013) and synthetically drugs (e.g. van Veen et al., 2009), research on herbal medicinal products has made limited use of sAA. However, our results of an increase in sAA and a decrease in distal SKT in participants being confronted to the TSST are in line with previous research (Allen et al., 2014; Het, Schoofs, Rohleder, & Wolf, 2012; Vinkers et al., 2013).

Furthermore, studies observing the effects of herbal medicinal products on cardiovascular parameters produced mixed results and thus were less convincing. In line with our results, a randomized placebo controlled study investigating HR in response to a dentist surgery demonstrated no significant differences between participants taking either 100 mg valerian or placebo capsules (Pinheiro, Alcântara, de Moraes, & de Andrade, 2014). In contrast, another randomized controlled study reported reduced HR responses within subjects in response to a mental stress task after taking tablets containing valerian with the 600 mg dose of the combination (LI 156; Sedonium, containing 300 mg ethanolic dried valerian root extract; drug/extract ratio 3-6:1; Cropley et al., 2002). However, none of those studies applied psychosocial stress and only one study (Pinheiro et al., 2014) included a placebo control group.

Our results demonstrate that participants exhibited a significant stress response in the ANS parameters. The results of the ANS parameters are in line with those of the other major biological stress system (e.g. HPA axis) assessed with salivary cortisol (Study I, Publication II). The findings suggest that the response of the ANS to acute psychosocial stress is maintained with Ze 185 and similar compared to placebo and no treatment. Considering the effects on self-reported emotional parameters and their high acceptability and low risks (e.g. Meier et al., 2018; Melzer et al., 2009), further studies on the effects of herbal medicinal products are warranted to investigate who might benefit from these products and for which conditions.

5.3. Randomized controlled evaluation of the psychophysiological effects of social support stress management in healthy women (Study II, Article III)

In line with previous studies, our findings show that the TSST elicited an increase in state anxiety, cortisol, HR and a decrease in RMSSD (Allen et al., 2014). Moreover, previous research on psychological interventions with intraindividual focus found reduced self-reported anxiety stress responses in the TSST as well. Hoge et al. (2013) have demonstrated that participants experienced a larger reduction in anxiety responses after they underwent a mindfulness-based stress reduction program than an attention control group to repeated TSST confrontation. Likewise, participants in a mindfulness-based cognitive therapy group showed significantly decreased anxiety responses to the TSST when compared to pre-intervention levels whereas there were no differences in the control condition (Britton et al., 2012).

Moreover and similar to our results, a number of studies with interventions with an intraindividual perspective reported reduced anxiety responses in face of unaltered cortisol responses (e.g. self-compassion training; Arch et al., 2014; mindfulness meditation; Creswell et al., 2014). However, opposed to this, several randomized-controlled trials with an

intraindividual (e.g. cognitive behavioral stress management training; Gaab et al., 2003; Hammerfald et al., 2006) as well as interindividual focus (Burton et al., 2014; Ditzen et al., 2008; Heinrichs et al., 2003; Kirschbaum et al., 1995; Robles, 2007) significantly attenuated cortisol stress responses in subjects completing the TSST. Those studies with interindividual perspectives that reported suppressed cortisol responses to the TSST included men receiving support from partners compared with unsupported and stranger-supported men (Kirschbaum et al., 1995) and individuals receiving support from family members although peer support was not associated with the neuroendocrine response (Burton et al., 2014). Noteworthy, next to the diminished cortisol response there were no significant differences in anxiety stress responses in men receiving support from the best friend (Heinrichs et al., 2003), from partner in comparison to no social support (Ditzen et al., 2008) as well as in women with positive physical partner contact in comparison to verbal social support and no social interaction (Ditzen et al., 2007).

Regarding cardiovascular parameters, a study with an interindividual focus revealed similar cardiovascular reactions in participants who received social support from a same-gender confederate before the TSST and no support in a healthy sample (Robles, 2007). Moreover, there were no significant differences in HR in women receiving support from a friend, a supportive or neutral confederate during a speech (Christenfeld et al., 1997). In contrast, physical contact from the partner resulted in significantly reduced HR increase in response to the TSST while verbal social support was not associated with reduced HR stress responses in women (Ditzen et al., 2007). Although applying a different social stressor than the TSST but in line with our results, a group-based social support intervention showed no effects on cardiovascular stress responses and in contrast to our results, no effects on emotional stress responses (Anthony & O'Brien, 2002).

The protocol of the TSST (Kirschbaum et al., 1993) could contribute to the explanation of our results of unaltered biological stress responses. The procedure of the TSST prevents any possibility to use or obtain social support in the stressful situation. The two confederates forming the TSST committee are instructed to interact in a neutral way with the participants and not to give any positive verbal or non-verbal feedback or signs of communication other than that specified in the manual (Kirschbaum et al., 1993). Consequently, all efforts of participants to interact with the committee members in possible supportive ways are unsuccessful. Furthermore, otherwise stress-reducing social skills are not possible to be applied. Although not investigating a social support management, studies observing direct positive social interactions before or during a psychosocial stress test indicated a reduction of endocrine and cardiovascular reactivity to the stressor (Christenfeld et al., 1997; Ditzen et al., 2007; Kirschbaum et al., 1995;

Lepore, 1995; Uchino & Garvey, 1997).

5.4. Limitations

Study I (Publication I and II) has the following limitations: Only males were included as female menstrual cycle potentially could contribute to differences in HPA axis responses to psychosocial stress (Kirschbaum, Kudielka, Gaab, Schommer, & Hellhammer, 1999). Furthermore, only healthy participants were included as medication intake could contribute as well to differences in HPA axis responses (Granger, Hibel, Fortunato, & Kapelewski, 2009). Considering the clinical potential of Ze 185, future studies should include females as well as a clinical population with chronically stressed individuals or patients with stress-related disorders to establish the clinical value of Ze 185. It is important to note that the TSST is a laboratory stress setting. This allowed us to control some of the factors that are associated with changes in salivary cortisol secretion or in ANS parameters such as the consumption of caffeine (Lovallo, Farag, Vincent, Thomas, & Wilson, 2006) and alcohol (Zimmermann et al., 2004), smoking (Direk, Newson, Hofman, Kirschbaum, & Tiemeier, 2011), chewing (Scholey et al., 2009), high-intensity exercise (Jacks, Sowash, Anning, Mcgloughlin, & Andres, 2002), and circadian rhythm of salivary cortisol secretion (Kudielka, Schommer, Hellhammer, & Kirschbaum, 2004). However, future studies should investigate stress occurring in more naturalistic settings closer to everyday life.

Study II (Publication III) has the following limitations: There was a significant difference in age between the two study group. In consequence, age was included as a control variable in all calculations. Furthermore, the sample size was rather small, limiting the statistical power of the study to detect small group differences. Also, only healthy women were included in the study. There is evidence for men also showing an increase in prosocial behavior during stress (von Dawans et al., 2012) which challenges the assumption that women are more likely to mobilize social support when confronted with stress (Taylor et al., 2000). It is also important to note that the participants in our study reported high levels of perceived social support at baseline. The TSST is a laboratory stress setting with explicitly no possibility applying positive social interactions. Furthermore, the completion of the TSST took place several weeks after the social support stress management (2-5 weeks).

5.5. Conclusions and implications for future research

Both the fixed herbal drug combination as well as the social support stress management attenuated the subjective emotional stress response the former in healthy men and the latter in healthy women, without altering the biological reactions in response to the standardized

psychosocial stress test.

The results from Study I revealed that the fixed herbal drug combination significantly attenuated the self-reported state anxiety response in healthy men, without affecting the HPA axis and ANS stress responses. Given that a circumscribed biological stress response is to be considered as an adaptive mechanism, Ze 185 reduces state anxiety response without affecting assumingly adaptive biological stress responses to stress. In our study participants took a total of eleven film coated tablets over four days. Former studies demonstrating the effectiveness of Ze 185 used treatment doses of three tablets a day for up to ten days (Steiner & Opwis, 2000), 20 days (Schellenberg et al., 2004), and 14 days with an onset of action on day 4 for anxiety symptoms (Melzer et al., 2009). Considering those results, future studies should observe the effects of longer treatment durations and dosages on the HPA axis and ANS stress responses. Additionally, future studies should include females as well as a clinical population with chronically stressed individuals or patients with psychiatric disorders. Furthermore, although the TSST is to be considered a valid and effective stress test, future investigation should test the effects of Ze 185 on stress responses in naturalistic environments. The mechanisms through which Ze 185 affects the emotional stress responses are not yet understood. Future studies including functional and structural neuroimaging could provide understanding on how Ze 185 impacts emotional stress responses in humans. As such, stress-specific sustained increases in the amygdala, striatum, hypothalamus, midbrain, right insula, and right dorsolateral prefrontal cortex regions supported the stress processing and reactivity circuit (Sinha, Lacadie, Constable, & Seo, 2016). Considering medicinal plant extracts being part of Ze 185, several studies suggested mechanisms mediated by the $GABA_A$, $5\text{-}HT_{1A}$, and the adenosine A1 receptors (Becker et al., 2014; Bodesheim & Hölzl, 1997; Schumacher et al., 2002). Taking into account that Ze 185 is a fixed herbal drug combination, its clinical efficacy can only be explained by a complex polypharmacological mechanism which still needs to be elucidated.

Participants in our social support stress management did not differ in their cortisol, HR, and heart rate variability stress responses, albeit the intervention condition showed a significantly attenuated integrated state anxiety response in comparison to the waitlist control condition. Considering the stress-buffering effect of the social stress management together with previous studies on social support, our results show the potential of social support in the context of dealing with stress (Ditzen et al., 2008). However, our results suggest that a testing situation with opportunities for positive interactions would be suitable in future studies to evaluate social stress management. Moreover, future research should include individuals reporting low levels of supportive social support as well as with clinical conditions. Furthermore, other stressors

closer to real life like measuring stress during exam periods could be appropriate way to reflect possible effects of the social support stress management. To boost a transfer into daily life it might be important to lengthen the duration of the intervention at a less intense rate and place higher emphasis on tasks which can be trained with friends and family in the intervals between the sessions. Although research has clearly shown that social support has a notable impact on morbidity and mortality, it rests fairly unclear how naturally occurring social support can be improved and if this is possible in a long-term way by training skills useful for receiving and offering social support.

6. References

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Appendix A

Study I, Publication I (accepted version attached):

Meier, S., Haschke, M., Zahner, C., Kruttschnitt, E., Drewe, J., Liakoni, E., Hammann, F., & Gaab, J. (2018). Effects of a fixed herbal drug combination (Ze 185) to an experimental acute stress setting in healthy men–An explorative randomized placebo-controlled double blind study. *Phytomedicine, 39, 85-92.*
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EFFECTS OF A FIXED HERBAL DRUG COMBINATION (ZE 185) TO AN EXPERIMENTAL ACUTE STRESS SETTING IN HEALTHY MEN – AN EXPLORATIVE RANDOMIZED PLACEBO-CONTROLLED DOUBLE BLIND STUDY

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Abstract

Background: Considering the negative effects of stress on health, there is a growing interest in stress-reducing interventions. The present study examines the effects of a fixed combination of valerian, passion flower, lemon balm, and butterbur extracts (Ze 185) on biological and affective responses to a standardized psychosocial stress paradigm.

Purpose: The aim of the present study was to investigate the efficacy of Ze 185 on cortisol and anxiety stress responses to acute psychosocial stress in healthy subjects.

Study Design: This study was a randomized, placebo-controlled, double blind study with 3 parallel groups.

Methods: 72 healthy male participants were randomized to 3 groups Ze 185, placebo or no treatment during 4 days prior to a standardized psychosocial stress paradigm. Principle outcomes were salivary cortisol and self-reported anxiety responses to stress assessed at the fourth day.

Results: The stress paradigm induced significant and large cortisol and self-reported anxiety responses. Groups did not differ significantly in their salivary cortisol response to stress, but participants in the Ze 185 condition showed significantly attenuated responses in self-reported anxiety in comparison to placebo ($F(3, 41) = 3.33$, $p = .03$) and no treatment ($F(3, 43) = 2.77$, $p = .05$).

Conclusion: The results show that Ze 185 significantly attenuated the subjective emotional stress response during an acute stress situation, without affecting biological stress responses. Given that a circumscribed biological stress response is to be considered as an adaptive mechanism, Ze 185 reduces self-reported anxiety response to stress without affecting assumingly adaptive biological stress responses.

Keywords: *Valeriana officinalis, Passiflora incarnata, Petasites hybridus, Melissa officinalis*, cortisol, anxiety

Abbreviations:

Adverse Event (AE), Area Under the Curve with respect to increase (AUC_I) and ground (AUC_G), Body Mass Index (BMI), Committee on Herbal Medicinal Products (HMPC), Drug Extract Ratio (DER), European Pharmacopoeia (Ph. Eur.), Flame Ionization Detector (FID), Gamma-Aminobutyric acid (GABA), Gas Chromatography (GC), High Performance Liquid Chromatography (HPLC), Hypothalamic-Pituitary-Adrenal (HPA), Intention To Treat (ITT), Per Protocol (PP), Standard Deviation (SD), State Anxiety of the State-Trait Anxiety Inventory (STAI-state), Trait anxiety of the State-Trait Anxiety Inventory (STAI-trait), Trier Social Stress Test (TSST)

Introduction

While physiological and psychological responses in the face of adversities are both functional and necessary, biological, emotional as well as behavioral stress responses have been shown to negatively impact on mental and physical health in the long term (McEwen, 2012). Thus, approaches to mitigate stress-related health impairments become a matter of public health. Here, several randomized-controlled trials have shown that stress management trainings significantly attenuated acute cortisol stress responses in healthy subjects (Hammerfald et al., 2006; Storch et al., 2007). Also, several pharmacological substances have been shown to attenuate saliva cortisol responses to acute stress in healthy participants such as benzodiazepine (Fries et al., 2006) and opioid agonists (Bershad et al., 2015).

Considering the empirical status of herbal medicinal products for the treatment of stress and stress-reactivity, a number of randomized placebo-controlled trials indicate the potential of this approach in acute stress settings. In a randomized placebo-controlled study 600 mg of an extract combination of *Melissa officinalis* L. leafs and *Valeriana officinalis* L. roots reduced negative effects of the acute stress on ratings of anxiety in healthy individuals undergoing cognitive tasks (Kennedy et al., 2006). Similarly, the fixed herbal drug combination Ze 185, containing extracts of *Valeriana officinalis* L. radix, *Passiflora incarnata* L. herba, *Petasites hybridus* (L.) P. Gaertn roots and *Melissa officinalis* L. folium, decreased anxiety levels in healthy participants in response to cognitive tasks in a randomized-placebo controlled study (Steiner and Opwis, 2000). Herbal preparations containing *Passiflora incarnata* L., *Melissa officinalis* L. as well as *Valeriana officinalis* L. have been traditionally used for the relief of mild symptoms of mental stress, assessed by the Committee on Herbal Medicinal Products (HMPC) of the European Medicines Agency (HMPC, 2013, 2014, 2016).

However, data regarding the effects of the herbal extracts used in Ze 185 on psychophysiological responses including the assessment of the activation of one of the major biological stress systems, namely the hypothalamic-pituitary-adrenal (HPA) axis with neuroendocrine cortisol stress responses, under acute psychosocial stress is missing. A previous study investigated physiological and psychological stress responses in healthy participants completing cognitive tasks under the intake of Ze 185 and

placebo (Steiner and Opwis, 2000). In other studies, the Ze 185 was investigated as treatment for psychosomatic complaints in comparison of oxazepam (Schellenberg et al., 2004) and for somatoform disorders (Melzer et al., 2009). The aim of the present study was therefore to investigate the efficacy of Ze 185, a fixed herbal drug combination, regarding neuroendocrine and psychological responses to acute psychosocial stress in healthy subjects. Considering the previous research with Ze 185 (Schellenberg et al., 2004; Steiner and Opwis, 2000; Melzer et al., 2009) we assume that Ze 185 will reduce the emotional responses to the stress tests, with possible attenuation of neuroendocrine responses in consequence.

Material and methods

To observe the effects on neuroendocrine responses, the primary outcome was salivary cortisol secretion as the HPA axis is one of the major biological stress response systems. Previous research shows that the Trier Social Stress Test (TSST; see below; Kirschbaum et al., 1993) -protocol is a reliable tool to experimentally evoke HPA axis stress responses (Allen et al., 2014). As secondary outcome, we chose self-reported sate anxiety as a measure of psychological stress response. Furthermore, safety and compliance of Ze 185 and placebo were assessed with laboratory tests, physical examination, assessment of adverse events and drug accountability.

Study medication

Active ingredients of the herbal medicinal product Ze 185, a fixed herbal drug combination, were 90 mg of a 90% (*w/w*) ethanolic extract of *Petasites hybridus* (L.) P. Gaertn., B. Mey. et Scherb. (Drug Extract Ratio; DER 7–14:1); 90 mg of a 45% (*w/w*) methanolic extract of *Valeriana officinalis* L. roots (DER 4–6:1); 90 mg of a 50% (*w/w*) ethanolic extract of *Passiflora incarnata* L. herb (DER 3–6:1); 60 mg of a 20% (*w/w*) ethanolic extract of *Melissa officinalis* L. leaves (DER 2.5–3.9:1) per film-coated tablet. The extract has been registered in Switzerland since 1970. The herbal medicinal products containing Ze 185 are indicated for the following complaints: nervousness, tension, restless-

ness and exam nerves. These can lead to the following symptoms, amongst others: spasmodic gastrointestinal complaints, increased irritability and occasional trouble falling asleep and sleeping through the night. High performance liquid chromatography (HPLC) fingerprints of one extract batch of each extract of Ze 185 are shown in Fig. 1-4. For the study, the commercially available formulation of Zeller Entspannung film coated tablets was used. Placebo was identical in presentation, color, and shape.

Participants

85 participants were screened, of which 13 met one or more of the exclusion criteria. The remaining 72 participants were randomized to namely Ze 185 (n = 24), placebo (n = 24), and no treatment (n = 24) groups (see Fig. 5). We included a no treatment group to be able to detect a possible placebo effect (i.e. difference between placebo and no treatment) in the placebo response (i.e. absolute effects of placebo administration). Inclusion criteria for the study were age between 18 and 45 years, male gender, and written informed consent. Previous research on the influence of age on cortisol responses is inconsistent (Agrigoroaei et al., 2013; Rohleder et al., 2002). However, Allen et al. (2014) point out the consideration that the testing environment of the standardized stress test (see below) itself might be more stressful for older participants compared to a younger population. Previous research has shown a broad moderating effect of gender on cortisol responses as well as has suggested a different behavioral stress response pattern in men and women (Allen et al., 2014; Taylor et al., 2000). Smoking, presence of somatic or psychiatric disorders, or any other clinically relevant diseases like hepatic, renal, cardiac respiratory disorders, alcohol or drug abuse, as well as previous participation in a TSST and in any psychotherapy were counted among the main exclusion criteria together with the use of defined concomitant medication. To check for exclusion criteria the screening procedures included anamnesis of medical and psychiatric history and urinalysis for cotinine and drug use. All participants gave written informed consent. Randomized participants were financially compensated for their participation in the study. The study was approved by the local Ethics Committee and Swiss Agency for Therapeutic Products (Swissmedic,

trial number: ZE185-4-2014-02) and registered on clinicaltrials.gov (ID: NCT02189239) and in the supplementary federal database (Swiss National Clinical Trials Portal; ID: SNCTP000001075). The study followed the guidelines of the Declaration of Helsinki and Tokyo for humans.

Procedure

At the screening visit, electrocardiogram, physical examinations, blood samples for hematological and clinical chemistry parameters, and the vital signs were assessed for safety analyses and to detect any clinically relevant abnormalities. All those procedures were conducted by a physician, and his team at the University Hospital of Basel. Participants completed 3 visits (baseline: day 1, stress test: day 4, and end of study). At baseline (day 1) eligible participants were randomized to one of the study groups. Randomization was provided centrally with randomly permuted blocks of variable length with equal numbers of assignments to the 3 arms. The random code was supplied by an external provider using a validated random program. Participants were instructed to orally administer 3 film-coated tablets per day for the following 3 days, i.e. one tablet every morning, midday, and evening on days 1, 2 and 3. On day 4, all participants underwent a standardized psychosocial stress test. On this day, participants took 2 film-coated tablets, i.e. in the morning and midday. The end of study visit was scheduled 3 to 5 days later and included a physical examination and laboratory tests. Adverse Events (AEs) were assessed throughout the study from giving informed consent signature until last visit and were evaluated by a medical doctor.

Stress test

On day 4 all participants were subjected to the TSST at the division of Psychology and Psychotherapy of the University Basel. The stress paradigm has repeatedly been found to induce profound psychobiological stress responses in 70–80% of subjects (Kudielka et al., 2007). After 50 min upon arrival including cortisol baseline assessment, participants were taken to the testing room and introduced to the TSST. The TSST procedure involves giving a free speech and a serially subtraction in front of a committee

consisting of one male and one female as well as a video camera and microphone, both tasks lasting 5 min each. Once completing the TSST further measurements for the next 90 min were assessed.

Overall, 11 salivary cortisol samples were collected (-50, -35, -20, -10 min pre TSST and 0, 10, 20, 30, 45, 60, 90 min post TSST; see Fig. 6). Saliva cortisol was collected using polypropylene saliva tubes (Sarstedt, Nümbrecht, Germany). Salivary free cortisol was assayed using the Salimetrics immunoassay method (EIA kits; Salimetrics LLC, USA). All samples were analyzed by the Swiss-Analysis AG (Tägerwilen, Switzerland). The assay kits had an intra-assay variation of 3 to 7 % and inter-assay variation of 3 to 11%. The minimum detectable level of cortisol concentration was 0.007 μ g/dl.

Subjective anxiety responses were assessed with the State-Trait Anxiety Inventory (STAI; Laux et al., 1981). The STAI-state part consists of 20 items rated on a 4-point scale with 1 = "not at all" and 4 = "very much" and has acceptable internal consistency and test-retest reliability (Lee et al., 2004). Participants completed the STAI-state before the TSST procedure (STAI-state 1; -45 min to TSST completion), right after completing the TSST (STAI-state 2), 30 min (STAI-state 3) as well as 90 min (STAI-state 4) after the TSST in the recovery room (see Fig. 6). Furthermore, the STAI-state as well as STAI-trait (Laux et al., 1981) were completed at day 1 for baseline assessment. At the end of study visit participants in the Ze 185 and placebo groups were asked by the study nurse about their assumption regarding which treatment they received. Their answers were noted.

Statistical analysis

The clinical trial data was collected in an electronic data capture system, named secuTrial®. An electronic case report form was implemented by the clinical trial unit at the University Hospital Basel. Analyses were performed with the IBM SPSS version 21 statistical package. The shown analyses are based on the Intention to Treat (ITT) dataset. At the end of the study, one participant reported the intake of a medication defined as prohibited during participation of the study. Therefore, all analyses were additionally run on the Per Protocol (PP) dataset without this participant to exclude a possible bias of study results due to the concomitant medication. However, all analyses with the PP dataset led to similar

results as with the ITT dataset (data not shown). Data were tested for normal distribution using Kolmogorov–Smirnov test. Cortisol, STAI-state values, and baseline variables were log-transformed to approximate normal distribution. For better readability, the original values were used for Tables and Figures. Baseline characteristics of the study groups were compared with one-way analyses of variance or chi-square tests. Analysis of variance for repeated measures were computed to analyze cortisol and anxiety responses between the study groups. Additionally, area under the curve with respect to increase (AUC_I) and area under the curve with respect to ground (AUC_G) were calculated for cortisol data using the trapezoidal method (Pruessner et al., 2003). Post hoc analyses were examined with separate analyses of variance. According to the guidelines provided by Miller and colleagues (2013) we identified cortisol responders and non-responders. Non-responders were classified as cortisol baseline to peak increase lower than 1 nmol/l (Miller et al., 2013). Differences in the distribution of AEs in the study groups as well as accurate and incorrect treatment expectations between the Ze 185 and placebo groups were tested with separate chi-square tests. The endpoint used for the sample size estimation was cortisol AUC_G. Sample size was estimated to be able to detect a difference in AUC_G of 580 nmol/l between the groups treated with Ze 185 and placebo. Based on 0.8 power to detect a significant difference of this effect size and a 5 % significance level, 63 participants were required. In total 72 participants were enrolled considering an overall drop-out rate of 12%.

Results

Participants

72 male healthy participants were randomly assigned to the Ze 185 group (n = 24), placebo group (n = 24), and no treatment group (n = 24). 2 participants dropped out after randomization but before receiving study medication. The remaining 70 participants (ITT dataset) were between 18-44 years of age, had a mean body mass index (BMI) of 24.4 kg/m^2 and the majority was Caucasian (see Table 1). The study groups did not differ with respect to any of the assessed demographic variables in age ($F(2, 67) = 1.08$, $p = .35$), BMI ($F(2, 67) = 0.05$, $p = .96$), distribution of ethnicity ($\chi^2(4, N = 70) = 4.02$, $p = .40$), state anxiety on day 1 ($F(2, 67) = 0.09$, $p = .92$), and trait anxiety on day 1 ($F(2, 67) = 1.81$, $p = .17$).

Cortisol and affective stress responses

The TSST induced significant and substantial increases in salivary cortisol levels ($F(8, 60) = 40.28$, $p < .001$). Our analyses revealed 10 participants classified as non-responders. Groups did not differ in the distribution of cortisol responders and non-responders ($\chi^2(2, N = 70) = 0.27$, $p = .87$). Cortisol levels among all participants did not differ between the 3 groups at baseline ($F(2, 67) = 0.05$, $p = .95$) and groups did not differ significantly in their cortisol responses over time ($F(16, 122) = 0.43$, $p = .97$) nor integrated cortisol responses (AUC_I: $F(2, 67) = 0.17$, $p = .84$; AUC_G: $F(2, 67) = 0.002$, $p = .99$; see Fig. 7).

Groups did not differ significantly in STAI-state baseline scores on day 4 ($F(2, 67) = 0.50$, $p = .61$) and the TSST resulted in a significant STAI-state response ($F(3, 64) = 25.89$, $p < .001$). Groups differed however significantly in the STAI-state response over time ($F(6, 130) = 2.41$, $p = .03$) and post hoc analyses revealed a significant time by group interaction effect between placebo and Ze 185 ($F(3, 41) = 3.33$, $p = .03$), as well as between no treatment and Ze 185 ($F(3, 43) = 2.77$, $p = .05$) while response differences between no treatment and placebo were not significant ($F(3, 42) = 1.42$, $p = .25$; see Fig. 8).

Adverse events

In total, 11 participants (Ze 185 n = 3; placebo n = 5; no treatment n = 3) reported 16 non-serious AEs (Ze 185 n = 3; placebo n = 9; no treatment n = 4) with no differences in their distribution between groups ($\chi^2(2, n = 70) = 0.94$, $p = .63$; n = 3 in Ze 185, n = 5 in placebo and n = 3 in no treatment group). All AEs were of mild intensity. 3 AEs in the Ze 185 group (headache, intermittent abdominal pain and flatulence) were recovered until the end of the study. The causality to the study medication was judged as possible by the physician according to World Health Organization criteria.

Treatment expectation

Groups significantly differed in correctly guessing drug assignment ($\chi^2(1, N = 45) = 10.02$, $p = .002$), with more accurate ratings in the placebo group than in the Ze 185 group (in the Ze 185 group: 36.4% "verum group", 63.3% "placebo group"; in the placebo group: 17.4% "verum group", 82.6% "placebo group").

Drug adherence

One participant in the Ze 185 group took one tablet too much and thus self-administered 12 instead of 11 film coated tablets of Ze 185. Drug intake of all participants completing the TSST was within the predefined range of 80-120% (9-13 tablets).

Discussion

The present double-blind randomized placebo-controlled study investigated the effect of the herbal medicinal product Ze 185 on cortisol and self-reported state anxiety stress responses to acute experimentally induced stress in healthy men.

In comparison to placebo and no treatment, participants receiving Ze 185 showed significantly attenuated subjective anxiety response to stress, marked by a lower response as well as a more rapid recovery in comparison to placebo or no treatment. Groups did not differ significantly in salivary cortisol stress responses. Ze 185 was safe and well tolerated. No serious AEs and only 3 non-serious AEs were reported in the Ze 185 group. Noteworthy, nearly two-third of participants in the Ze 185 group assumed they were randomized to the placebo group, while over 80% of participants in the placebo condition correctly presumed to have received placebo. Groups did not differ in the distribution of cortisol responders and non-responders and also, the rate of responders goes in line with previous reported response rates (Kudielka et al., 2007).

In line with our results, several randomized placebo-controlled trials investigating herbal medicinal interventions (e.g. Kennedy et al., 2006; Lee et al., 2004) have reported decreased levels of anxiety in healthy subjects undergoing cognitive tasks (Kennedy et al., 2006) and an academic examination (Lee et al., 2004). Thus and also in line with previous studies with Ze 185 (Melzer et al., 2009; Schellenberg et al., 2004; Steiner and Opwis, 2000), specific herbal medicinal interventions appear to be effective in reducing subjective anxiety and anxiety responses to acute stress. Although the mechanism of action of Ze 185 is not fully understood, for valerian, passion flower and lemon balm anxiolytic effects have been suggested in several preclinical studies. The modulation of the Gamma-Aminobutyric acid $(GABA)_A$ receptor provides a plausible mechanism for certain constituents from valerian (e.g. valerenic acid; Becker et al., 2014). Further, a glutamatergic mechanism has been suggested (Del Valle-Mojica et al., 2011). In addition, the lignans 8-hydroxypinoresinol and other olivil derivatives have been shown to potently bind to $5\text{-}HT_{1A}$ or adenosine A1 receptors, both representing pharmacological targets mediating anxiolytic effects (Bodesheim and Hölzl, 1997; Schumacher et al., 2002). As for passion flower and lemon balm, anxiolytic effects have been proposed to be mediated also via the GABAergic

system (Grundmann et al., 2008; Ibarra et al., 2010).

Similar to our results, a number of trials reported a dissociation of subjective and objective stress responses, i.e. reduced anxiety responses in face of unaltered cortisol responses (e.g. mindfulness meditation: Creswell et al., 2014). Noteworthy, the adaptive function of increased cortisol levels has recently received new support. For example, higher cortisol responses in the TSST are associated with fewer errors in threat-related decision making in police officers (Akinola and Mendes, 2012). Thus, there is reason to assume an important functionality of cortisol stress responses, which may serve to support positive and/or protect against negative emotional responses.

The present study has the following limitations. Only healthy males were included as female menstrual cycle stress potentially could contribute to differences in HPA axis responses to psychosocial stress. Considering the clinical potential of Ze 185, future studies should include females as well as a clinical population with chronically stressed individuals or patients with psychiatric disorders. In this regard, it is important to note that we assessed anxiety response to experimentally induced acute psychosocial stress, which should not be equated to clinical conditions. Additionally, as we observed short term outcome of acute stress, we want to emphasize that we do not suggest adaptogenic effects. Furthermore, although the TSST is to be considered a valid and effective stress test, future investigation should test the effects of Ze 185 on stress responses in naturalistic environments. We controlled several factors that are associated with changes in salivary cortisol secretion such as circadian rhythm, the consumption of alcohol, smoking, chewing and high-intensity exercise (Kudielka et al., 2007). In our study participants took a total of 11 film coated tablets over 4 days. In previous studies demonstrating the effectiveness of Ze 185 the medication was taken 3 times a day for up to 10 days (Steiner and Opwis, 2000), 20 days (Schellenberg et al., 2004), and 14 days with an onset of action on day 4 for anxiety (Melzer et al., 2009).

The mechanisms through which Ze 185 affects the emotional stress responses are not yet understood. Future studies including functional and structural neuroimaging could provide understanding on how Ze 185 impacts affective stress responses in humans. As such, specific patterns of neural activation in brain circuits underlying a stress response have been demonstrated. Stress-specific sustained

increases in the amygdala, striatum, hypothalamus, midbrain, right insula, and right dorsolateral prefrontal cortex regions supported the stress processing and reactivity circuit (Sinha et al., 2016). Several studies investigating separately the medicinal plant extracts being part of Ze 185 suggest mechanisms mediated by the $GABA_A$, $5\text{-}HT_{1A}$ and the adenosine A1 receptors (Becker et al., 2014; Bodesheim and Hölzl, 1997; Schumacher et al., 2002). As Ze 185 is a fixed herbal drug combination its clinical efficacy can only be explained by a complex polypharmacological mechanism which still needs to be elucidated.

In summary, our results show that Ze 185 significantly attenuates the subjective emotional stress response during acute stress situation in healthy men, without affecting physiological parameters. Given that a circumscribed biological stress response is to be considered as an adaptive mechanism, the attenuation of the subjective anxiety response may reflect the possibility of Ze 185 being an effective intervention to reduce subjective stress while maintaining the adaptive biological stress responses. However, the underlying mechanisms leading to decreased subjective anxiety levels remain unclear. Further research is needed to elucidate the mechanisms of action of Ze 185 with regard to emotional stress responses.

Author contributions

All authors declare that Max Zeller Söhne AG, Switzerland, provided the herbal medicinal product Ze 185 and funding for the implementation of the study procedures. CZ, EK, and JD are employees of Max Zeller Söhne AG. The authors were autonomous in data analyses, study report and manuscript preparation. The sponsor had no influence on the performance, outcome, and evaluation of the study and no input into data analyses. SM and JG, designed the study, were coauthor of study protocol, analyzed data, prepared the manuscript; MH, EL, and FH, performed screening and end of study visit, revised the manuscript; CZ, EK, and JD, provided study medication, were coauthor of study protocol and prepared the manuscript regarding plant specific information.

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Conflict of interest

All authors declare that Max Zeller Söhne AG, Switzerland, provided funding for the execution of the study and data assessment procedure. The authors were autonomous in data analyses and study report and declare no conflict of interest.

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Table and Figure legends

Table 1

Group means (SD) and distribution for demographics characteristics at baseline (n = 70; ITT-dataset).

	Ze 185 (n = 23)	Placebo (n = 23)	No treatment (n = 24)	Total sample (n = 70)
Age (years)	24.70 (3.28)	26.74 (5.55)	26.75 (6.13)	26.07 (5.17)
BMI (kg/m^2)	24.40 (3.27)	24.59 (3.15)	24.25 (2.27)	24.41 (2.88)
Caucasian	95.7% (n = 22)	100.0% (n = 23)	87.5% (n = 21)	94.3% (n = 66)
Afro-American	4.3% (n = 1)	0.0% (n = 0)	8.3% (n = 2)	4.3% (n = 3)
Other ethnicity	0.0% (n = 0)	0.0% (n = 0)	4.2% (n = 1)	1.4% (n = 1)
Cortisol (nmol/l)	5.94 (2.67)	6.28 (4.01)	6.61 (4.88)	6.28 (3.93)
STAI-state	35.17 (7.18)	35.78 (7.53)	36.25 (8.13)	35.74 (7.53)
STAI-trait	34.43 (5.90)	34.43 (7.77)	38.00 (7.70)	35.66 (7.28)

Note: : SD = standard deviation; BMI = body mass index; STAI-state = state anxiety of the State-Trait Anxiety Inventory; STAI-trait = trait anxiety of the State-Trait Anxiety Inventory.

Fig. 1. HPLC fingerprint of valerian dry extract batch 140218 performed with reversed phase HPLC (HPLC 1100 Series Agilent); stationary phase: C-18 column (5 μm); mobile phase: gradient using acetonitrile and 5 g/ l solution of phosphoric acid in water; ultraviolet-detection at 220 nm (according to Ph. Eur. 8, 07/2014:1897).

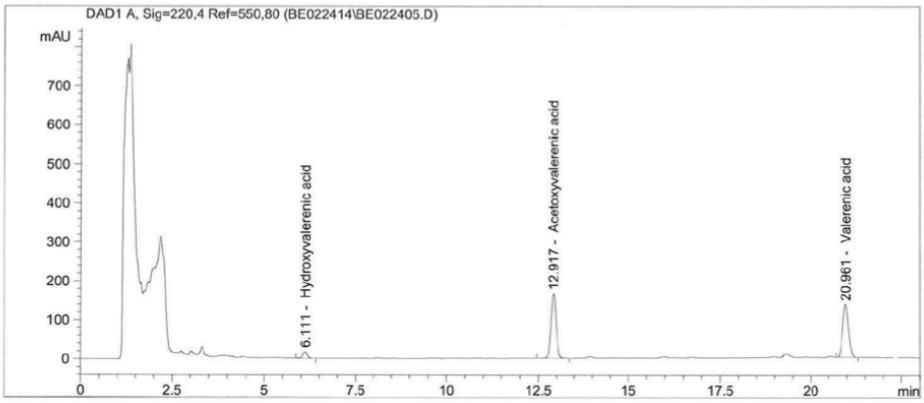


Fig. 2 . HPLC fingerprint of melissa dry extract batch 121283 performed with reversed phase HPLC (HPLC 1100 series Agilent); stationary phase: C-18 column (5 μm); mobile phase: gradient using (A) phosphoric acid, acetonitrile, water (1:19:80 *V/V/V*) and (B) phosphoric acid, methanol, acetonitrile (1:40:59 *V/V/V*); ultraviolet-detection at 330 nm (according to Ph. Eur. 6, 01/2010:2524).

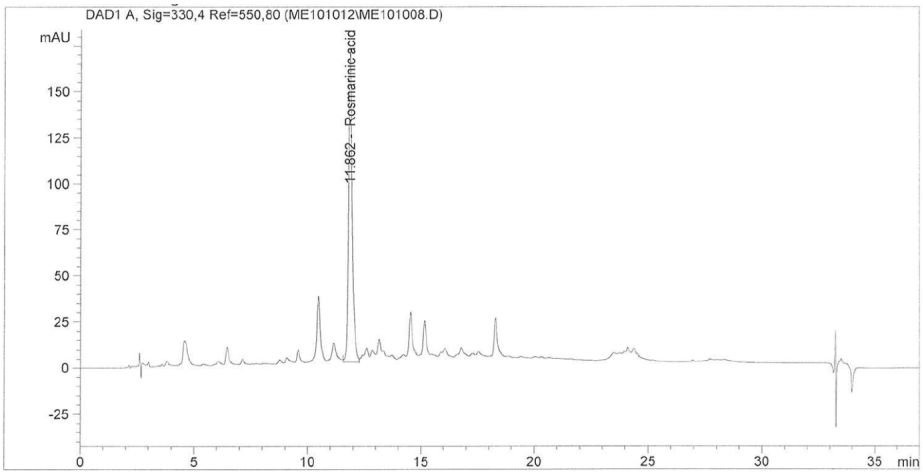


Fig. 3. HPLC fingerprint of passion flower dry extract batch 130810 performed with reversed phase HPLC (HPLC 1100 series Agilent); stationary phase: C-18 column (3 μm) containing a C-18 pre-column; mobile phase: gradient using 0.05 M phosphoric acid in water and acetonitrile; ultraviolet-detection at 336 nm.

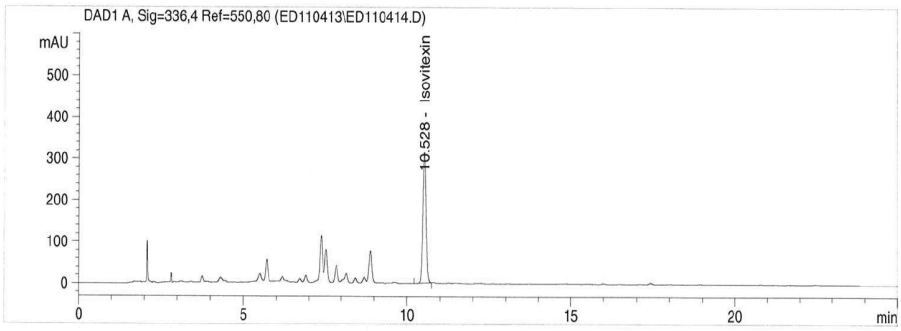


Fig. 4. GC fingerprint in petasites root dry extract batch 131661 performed with gas chromatography (GC Agilent 6890N Series); stationary phase: DB-1 column (25 m, Ø 0.32 mm, 0.52 μm); mobile phase: hydrogen gas; gradient: temperature gradient from 180 °C to 300 °C; detection: FID.

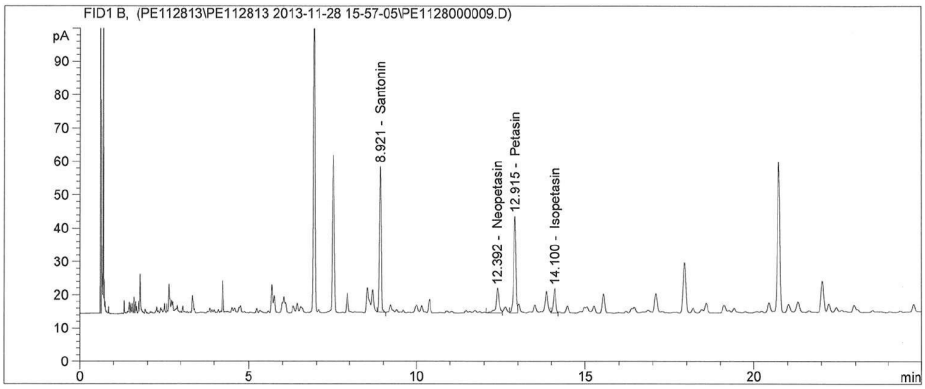


Fig. 5. Flow chart of participants from initial assessment for eligibility sample to salivary cortisol analytic sample.

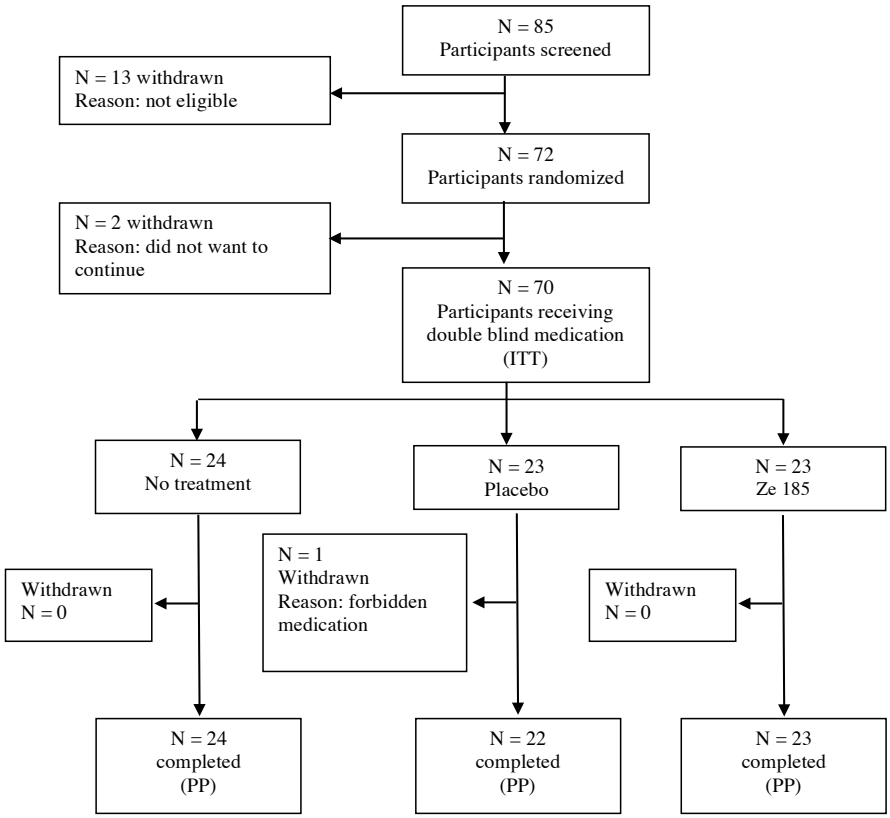


Fig. 6. Study design of day 4 with state anxiety of the State-Trait Anxiety Inventory (STAI-state) as well as saliva measuring points in relation to time to completion of the Trier Social Stress Test (TSST).

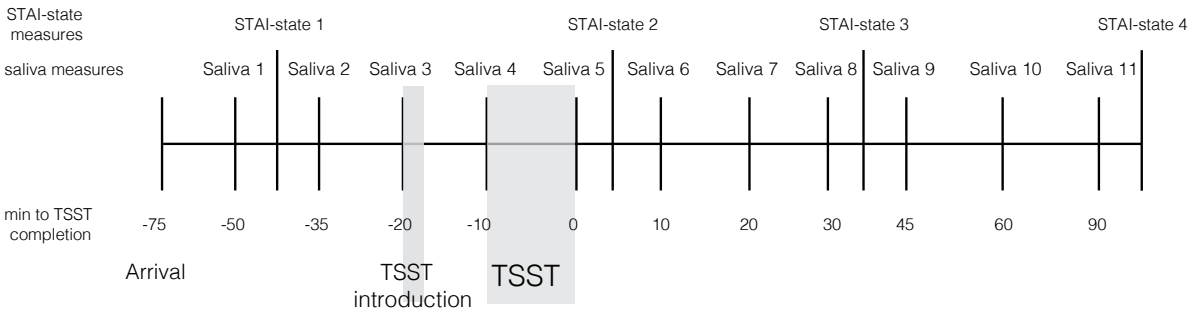


Fig. 7. Absolute salivary cortisol responses (mean/standard error of the mean) of the 3 study groups in the Trier Social Stress Test (TSST) at day 4. Intro = introduction to the TSST.

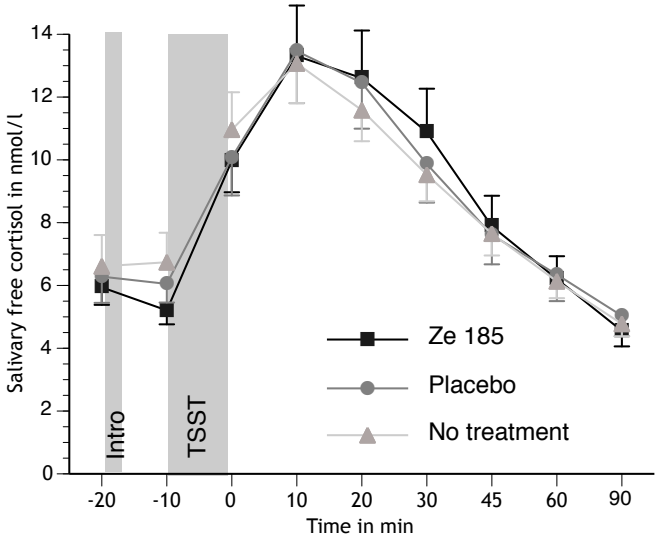
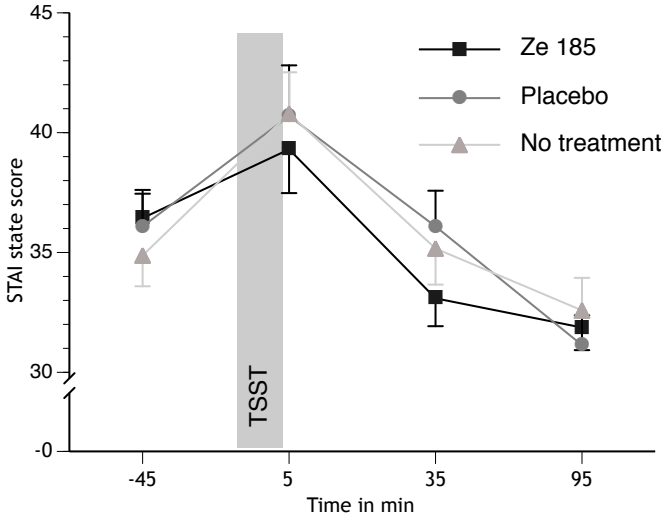


Fig. 8. Absolute state anxiety of the State-Trait Anxiety Inventory (STAI-state) responses (mean/standard error of the mean) of the 3 study groups in the Trier Social Stress Test (TSST) at day 4.



Appendix B

Study I, Publication II:

Meier, S., La Marca, R., Haschke, M., Zahner, C., Kruttschnitt, E., Drewe, J., Liakoni, E., Hammann, F., Kossowsky, J., Heimgartner, N., & Gaab, J. (2018). *Autonomic nervous system stress response under the intake of the fixed herbal drug combination Ze 185: a placebo-controlled randomized trial with healthy men.* Manuscript submitted for publication.

Autonomic nervous system stress response under the intake of the fixed herbal drug combination Ze 185: a placebo-controlled randomized trial with healthy men

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Keywords: Trier Social Stress Test, phytotherapy, heart rate variability, psychophysiology, salivary alpha-amylase, skin conductance level

Abstract

Background and Objectives: Stress-related dysregulation of the autonomic nervous system has been shown to be associated with disease processes and clinical outcomes. As we previously reported a significant reduction of anxiety responses in face of normal cortisol stress responses in healthy men undergoing a standardized psychosocial stressor and on treatment with Ze 185, a fixed herbal drug combination, we here set out to assess whether Ze 185 has an effect on autonomic nervous system responses to acute psychosocial stress.

Design: A randomized, placebo-controlled, double blind study with three parallel groups. Seventy-two healthy men were randomly assigned to three groups receiving Ze 185, placebo, or no treatment.

Methods: Participants underwent a standardized psychosocial stress test while heart rate, heart rate variability, salivary alpha-amylase, skin conductance level and skin temperature were repeatedly assessed. The study was approved by the local Ethics Committee and Swiss Agency for Therapeutic Products (Swissmedic, trial number: ZE185-4-2014-02) and registered on clinicaltrials.gov (ID: NCT02189239) and in the supplementary federal database (Swiss National Clinical Trials Portal; ID: SNCTP000001075).

Results: Significant stress responses (all $p<.05$) were evident on all physiological parameters. The stress responses of any of the assessed physiological parameters between the three study groups did not differ significantly (all $p>.05$).

Conclusion: Our results indicate that autonomic nervous system stress responses remained unaffected by Ze 185, while it attenuated subjective anxiety responses (as previously reported). In line with the previous findings, Ze 185 attenuates the affective stress response without affecting the physiological stress response.

Introduction

Stress has been shown to induce activity alterations of the autonomic nervous system (ANS; (Allen, Kennedy, Cryan, Dinan, & Clarke, 2014). Stress and stress-related dysregulations of ANS activity have been shown to be associated with disease processes and clinical outcome (Booth et al., 2015; Suh et al., 2013; Xhyheri, Manfrini, Mazzolini, Pizzi, & Bugiardini, 2012). Clinical trials addressing autonomic dysfunctions indicate benefits of beta-blockers, at least in part due to heart rate lowering (Caetano & Alves, 2015; London, 2008). However, the use of beta-blockers is a controversially discussed as e.g. beta-blockers increased risk for new-onset diabetes in patients with hypertension (Bangalore, Parkar, Grossman, & Messerli, 2007; Caetano & Alves, 2015; London, 2008). To counter or prevent stress-associated detrimental ANS processes, several studies also examined the effects of non-pharmacological interventions on ANS parameters and functioning in healthy subjects. Here, randomized-controlled trials have shown a buffering effect on the ANS stress response. For example, cardiovascular stress responses to psychosocial stress was dampened in subjects undergoing psychological and behavioral interventions (Kemeny et al., 2012; Klaperski, von Dawans, Heinrichs, & Fuchs, 2014) and cardiovascular as well as salivary alpha-amylase (sAA) stress reactivity were diminished in subjects participating in a self-compassion intervention (Arch et al., 2014). Furthermore, physical partner contact prior to acute stress led to reduced heart rate responses (Ditzen et al., 2007) and listening to relaxing music resulted in a faster sAA recovery after stress (Thoma et al., 2013).

Herbal preparations containing *Passiflora incarnata* L., *Melissa officinalis* L. as well as *Valeriana officinalis* L. have been traditionally used for the relief of mild symptoms of mental stress, assessed by the Committee on Herbal Medicinal Products (HMPC) of the European Medicines Agency (HMPC, 2013, 2014, 2016). Reduced stress-related symptoms (e.g. anxiety manifestations, insomnia, psychosomatic symptoms) were found in participants with anxiety disorder taking capsules with a standardized, hydroalcoholic *Melissa officinalis* L. leaf extract with the 600 mg dose of the combination (300 mg; Cases, Ibarra, Feuillere, Roller, & Sukkar, 2011) and better stress resistance was experienced after 12 weeks of treatment with *Passiflora incarnate* L. in patients with nervous restlessness (424 mg, drug/extract ratio 5:1-7:1, extraction solvent: ethanol 50%; Gibbert, Kreimendahl, Lebert, Rychlik, & Trompetter, 2017). With regard to stress reactivity, randomized placebo-controlled trials reported promising results. A fixed herbal drug combination of *Valeriana officinalis* L. radix, *Passiflora incarnata* L. herba, *Petasites hybridus* (L.) P. Gaertn roots and *Melissa officinalis* L. folium decreased electro dermal activity in patients with psychosomatic

complaints (Schellenberg, Sauer, & Brattström, 2004). A randomized-controlled study found reduced heart rate responses in participants taking tablets of valerian (LI 156, Sedonium, containing 300mg ethanolic dried valerian root extract, drug/extract ratio 3-6:1; Cropley, Cave, Ellis, & Middleton, 2002).

However, researchers in the field of herbal medicinal products have made limited use of ANS stress indicators under rigorously controlled conditions. We therefore set out to assess the effects of the fixed herbal drug combination Ze 185 on several ANS biomarkers in healthy men undergoing a standardized psychosocial stressor in a three-armed trial including placebo and no treatment. In a first publication, we reported results of cortisol and anxiety stress responses (Meier et al., 2018), here we report the stress responses of the secondary outcomes.

Methods

Participants

Healthy non-smoking men between the age of 18 and 45 years were invited to the University Hospital Basel to attend a screening procedure to test study eligibility. Participants were recruited at the division of Clinical Pharmacology and Toxicology of the University Hospital Basel using a recruitment notice on the Internet platform of the University of Basel. After the participants were provided with complete written and oral description of the study, written informed consent was obtained. Throughout this study, all data was identified only by an identification number to guarantee anonymity of the participants. The screening procedures included laboratory and urinalysis for cotinine and drug tests, an electrocardiogram, physical examinations and the check of vital signs. Individuals with previous participation in the employed psychosocial stress paradigm (see below), undergoing psychotherapy, and who had participated in another clinical trial in the preceding four weeks were excluded. A total of 85 participants were screened, of which 13 met one or more of the exclusion criteria. The remaining 72 participants were randomized to Ze 185 (n=24), placebo (n=24), and no treatment (n=24) groups.

Procedures

After the screening visit the participants completed three visits (baseline, stress test, and end of study). At baseline, participants completed the baseline questionnaires for assessing state and trait anxiety with the State-Trait Anxiety Inventory (STAI-state and STAI-trait; Spielberger, Laux, Glanzmann, & Schaffner, 1981). Randomization was delivered centrally

with randomly permuted blocks of variable length with equal numbers of assignments to the three arms, and the random code was supplied by an external provider using a validated random program. Participants in the Ze 185 and the placebo group received film coated tablets. Active ingredients of Ze 185 were 90 mg of an ethanolic extract of *Petasites hybridus* (L.) P. Gaertn. Roots, 90 mg of a methanolic extract of *Valeriana officinalis* L. roots, 90 mg of an ethanolic extract of *Passiflora incarnata* L. herb, and 60 mg of an ethanolic extract of *Melissa officinalis* L. leaves per film-coated tablet. Placebo was identical in presentation, color and shape. Participants were instructed to orally administer three film-coated tablets per day: one tablet every morning, midday, and evening for three days. On day 4, participants took one film-coated tablet in the morning and one at midday before undergoing a standardized psychosocial stress test (Trier Social Stress Test; TSST; see below; Kirschbaum, Pirke, & Hellhammer, 1993). During this stress examination, eleven saliva samples were collected (-50, -35, -20, -10 minutes pre TSST, and 0, 10, 20, 30, 45, 60, 90 minutes post TSST). All ANS parameters were assessed with the wireless physiological recording system BioNomadix® (Biopac Systems, Inc., Santa Barbara, CA 93117). For each parameter, 5-minute intervals were measured at seven time points: baseline (-35 minutes before TSST completion), preparation directly after the TSST introduction (-17 minutes before TSST completion), TSST speech (first half of the TSST), TSST arithmetic task (second half of the TSST), recovery 1 (5-10 minutes after TSST completion), recovery 2 (20-25 minutes after TSST completion), and recovery 3 (25-30 minutes after TSST completion). The end of study visit was scheduled 3 to 5 days later to conduct physical examination and laboratory tests. Adverse events were recorded throughout the study.

Stress test

On day 4, all participants underwent the TSST (Kirschbaum et al., 1993), a psychosocial stress test, considered as the gold standard test to induce reliable psychobiological stress responses (Bali & Jaggi, 2015). Participants were instructed not to undergo excessive physical activity for 24 hours prior to the visit, not to consume caffeine and alcohol for 12 hours prior to the visit as well as not to eat a major meal and drink anything other than water 60 minutes prior to the visit. Stress tests were scheduled on Mondays or Fridays in the afternoon, with the stress test starting between 2pm and 4:30pm. Upon arrival at day 4 at the division of Clinical Psychology and Psychotherapy of the University Basel, participants were seated in a quiet room for instructions. After 30 minutes, which included a saliva baseline assessment, participants were taken to the testing room and introduced to the TSST. A fake selection

committee consisting of one male and one female were already sitting at a table with a video camera and a laptop cable-connected to a microphone visible for the participants in the testing room. The TSST was briefly explained to participants, which were then guided back to the first room for the preparation phase. After preparing for the TSST the participants were guided to the testing room to give a free speech in front of the committee during the next 5 minutes. Then, the participants were asked to conduct a serial subtraction for the next 5 minutes. In case of failure, subjects were instructed to restart. After the TSST exposure, participants were guided into a third room for further measurements over the next 90 minutes.

Measures

Saliva samples: salivary alpha-amylase

The sympathetic nervous system activity can be tracked using the enzyme sAA (Allen et al., 2014). Several studies clearly show that sAA increases in states of stress or arousal (Grisham, King, Makkar, & Felmingham, 2015; Nater & Rohleder, 2009). Saliva samples were stored at -20 °C until analysis. Frozen saliva samples will precipitate mucins. Completely defrosted saliva samples were vortexed and subsequently centrifuged at 1500 x g (3000rpm) for 15 minutes. The centrifuged saliva samples were diluted 1:200 by first diluting samples 1:10 with the alpha-amylase diluent provided followed by a 1:20 dilution of the 1:10 diluent sample resulting in a final dilution of 1:200. The Salimetrics® α-Amylase Kinetic Enzyme Assay Kit is specifically designed and validated for the kinetic measurement of sAA activity. The principle of this enzymatic test is based on a chromagenic, 2-chloro-p-nitrophenol linked with maltotriose. The enzymatic action of alpha-amylase on this substrate yields 2-chloro-p-nitrophenol, which can be spectrophotochemically measured at 405 nm. The amount of alpha-amylase activity (U/ml) present in the sample is directly proportional to the increase in absorbance at 405 nm. All reagents and kit components were prepared according to the manufacturer´s instructions. Plate reader and heater were set to incubate at 37°C. Intra- and inter-assay coefficients of variation were less than 7.3%. and 5.9%, respectively. All samples were analyzed by Swiss-Analysis AG (Tägerwilen, Switzerland).

Electrophysiological measures

Electrocardiographic activity was continuously recorded at 1000 Hz with three disposable, pre-gelled Ag/AgCI electrodes attached to the skin at the beginning of the visit with the BioNomadix® system of wearable wireless devices (Biopac Systems Inc.). The ECG data was filtered with a FIR bandpass filter from 0.5 Hz to 35 Hz with 8000 Coefficients with the

AcqKnowlege® software (Biopac Systems Inc.). All data were visually inspected and artifacts edited by linear interpolation. After data collection, heart rate (HR) and heart rate variability (HRV) parameters were derived with the VivoSense® software (VivoNoetics, US). For HRV, a time domain measure, namely the square root of the mean of the sum of the squares of differences between adjacent normal-to-normal intervals (RMSSD), was determined. This parameter is one of the most commonly used measures derived from interval differences (Task Force of the European Society of Cardiology, 1996) and indicates the activity of the vagus nerve.

Two electrodes pre-gelled with isotonic gel and Ag/AgCl contact (EL507, Biopac Inc.) were placed at the volar surfaces of distal phalanges of two fingers of the non-dominant hand to record electro dermal activity (EDA). The volar phalanges of the fingers show greater responsivity and seat gland activity than other spots of the hand (Boucsein et al., 2012). EDA was continuously recorded at 500 Hz with the BioNomadix® system (Biopac, Inc.). The EDA data was filtered with a FIR low pass filter of 1 Hz with the AcqKnowledge® software (Biopac Systems Inc.). The same 5-minute intervals as for the ECG data were chosen to build the EDA intervals (baseline, preparation, TSST 1, TSST 2, recovery 1, recovery 2, and recovery 3). All data were visually inspected and artifacts edited by linear interpolation. To investigate EDA, skin conductance level (SCL) was obtained with the AcqKnowledge System (Biopac, Inc.). SCL is a measure of the long-term tonic state of electro dermal activity of the skin. SCL is widely used as index of sympathetic nervous system arousal (Boucsein et al., 2012).

Skin surface temperature was continuously recorded at 500 Hz with the BioNomadix® system. The stainless-steel banjo sensor was attached on the volar surfaces of distal phalanges of the middle finger of the non-dominant hand. The skin temperature (SKT) data was resampled to 15.625 samples per second with the AcqKnowledge software (Biopac Systems Inc). All data were visually inspected and artifacts edited by linear interpolation. With the AcqKnowledge® software, the mean values of SKT for the intervals were obtained (Biopac Systems Inc).

Data analyses

The clinical trial data was collected in the electronic data capture system secuTrial®. The electronic case report form was implemented by the clinical trial unit at the University Hospital Basel. Statistical analyses were performed with the IBM SPSS version 21 statistical package. The shown analyses are based on the Intention to Treat (ITT) dataset. Since one participant in the placebo group was excluded due to medication defined as exclusion criteria, all analyses

were also run on the Per Protocol (PP) dataset. However, all analyses with the PP dataset led to similar results as with the ITT dataset (data not shown). Data were tested for normal distribution using Shapiro-Wilk test. sAA variables were square-root-transformed and HR, RMSSD, SCL, and SKT variables were log transformed to approximate normal distribution. For better readability, the original values were used for Figures. Baseline characteristics of the study groups were compared with one-way analyses of variance or chi-square tests. Analysis of variance for repeated measures were computed to compare sAA, HR, RMSSD, SCL, and SKT data between the three study groups. For effect sizes, partial eta^2 were reported, where appropriate, according to the following conventions: 0.01=small, 0.06=medium, 0.13=large (Cohen, 1988). With regard to electrophysiological measures, artifacts, loosening electrodes and technical problems resulted in a loss of data from single subjects, resulting in different *n*.

Results

Participants

Seventy-two male healthy participants were randomly assigned to the Ze 185 group (n=24), placebo group (n=24), and no treatment group (n=24). Two participants dropped out after randomization, but before receiving study medication (see Figure 1). The remaining 70 participants (ITT dataset) were between 18-44 years of age (M=26.07, SD=5.17 years), had a mean body mass index (BMI) of 24.4 kg/m^2 and the majority was Caucasian. The study groups did not differ with respect to any of the assessed demographic variables, i.e. age (F(2, 67)=1.08, p=.35), BMI (F(2, 67)=0.05, p=.96), distribution of ethnicity (χ^2(4, N=70)=4.02, p=.40), state anxiety on day 1 (F(2, 67)=0.09, p=.92), and trait anxiety on day 1 (F(2, 67)=1.81, p=.17).

Salivary alpha-amylase

The TSST induced a significant increase in sAA levels (F(8, 60)=28.17, p<.001, partial eta^2= .79). sAA levels did not differ between the three groups (Ze 185 n=23, placebo n=23, no treatment n=24) at baseline (F(2, 67)=0.05, p=.95) as well as over time (F(16, 122)=0.95, p=.51; see Figure 2).

Cardiovascular stress responses

The TSST induced significant and substantial increases in HR levels (F(6, 47)=55.17, p<.001, partial eta^2= .88). HR levels did not differ between the three groups (Ze 185 n=17, placebo

n=18, no treatment *n*=20) at baseline ($F(2, 52)=2.26$, $p=.11$) as well as over time ($F(12, 96)=1.42$, $p=.17$; see Figure 3).

The TSST induced significant and substantial decreases in RMSSD levels ($F(6, 47)=6.95$, $p<.001$, partial $eta^2= .47$). RMSSD levels did not differ between the three groups (Ze 185 *n*=17, placebo *n*=18, no treatment *n*=20) at baseline ($F(2, 52)=0.54$, $p=.58$) as well as over time ($F(12, 96)=0.90$, $p=.56$).

Skin conductance level

The TSST induced significant and substantial changes in SCL ($F(6, 44)=27.64$, $p<.001$, partial $eta^2=.79$). SCL did not differ between the three groups (Ze 185 *n*=20, placebo *n*=14, no treatment *n*=18) at baseline ($F(2, 49)=0.54$, $p=.59$) as well as over time ($F(12, 90)=1.39$, $p=.18$).

Skin temperature

The TSST induced significant changes shown as decreases in SKT ($F(6, 57)=104.74$, $p<.001$, partial $eta^2=0.92$). The SKT did not differ between the three groups at baseline ($F(2, 65)=0.87$, $p=0.42$). There were no significant time by group interaction effects ($F(12, 116)=0.80$, $p=0.65$).

Discussion

The aim of the present work was to compare the effects of the fixed herbal drug Ze 185 and placebo on ANS stress responses compared to a no treatment group in participants undergoing an acute psychosocial stress situation. All participants experienced significant and strong stress responses, but there were no significant differences in sAA, HR, HRV, SCL, and SKT stress responses between the three conditions.

Several studies aimed at investigating the effects of herbal products as used in the current study (i.e. valerian, passion flower, butterbur, lemon balm) in the context of stress. Although these studies were not without promise, i.e. reporting reduced stress-related symptoms in participants with anxiety disorder taking capsules with *Melissa officinalis* L. leaf extract with the 600 mg dose of the combination (300 mg; Cases et al., 2011) or better stress resistance after 12 weeks of treatment with a standardized extract of *Passiflora incarnate* L. in patients with nervous restlessness (424 mg, drug/extract ratio 5:1-7:1, extraction solvent: ethanol 50%; Gibbert et al., 2017), studies examining the effects of herbal products on cardiovascular parameters produced mixed results and thus were less convincing. One

randomized placebo controlled study examining HR in response to a dentist surgery found no significant differences between participants taking either 100 mg valerian or placebo capsules (Pinheiro, Alcântara, de Moraes, & de Andrade, 2014), while another randomized controlled study found declined HR responses within subjects in response to a mental stress task after taking 2 times daily 300 mg tablets containing valerian (LI 156, Sedonium, containing 300 mg ethanolic dried valerian root extract, drug/extract ratio 3-6:1; Cropley et al., 2002). Nevertheless, the comparison to our results is limited as none of the studies induced acute psychosocial stress in a laboratory setting and only one of the mentioned studies included a placebo group (Pinheiro et al., 2014), while others did not encompass any comparison group (Cases et al., 2011; Gibbert et al., 2017).

In line with our results, other studies found an increase in sAA in participants being confronted to the TSST (Allen et al., 2014; Het, Schoofs, Rohleder, & Wolf, 2012). Although sAA is a popular and promising biomarker of ANS in stress research in the context of psychological approaches (e.g. Thoma et al., 2013) as well as synthetically drugs (e.g. van Veen et al., 2009), previous studies on herbal medicine mainly focused on hypothalamic-pituitary-adrenal activity (e.g. al'Absi et al., 2013; Lee, Park, & Moon, 2004).

The participants in our study experienced a decrease in distal SKT in response to the acute stress. This stress response in SKT is in line with a study applying the TSST which found reduced SKT measured at fingertips (Vinkers et al., 2013). Changes in SKT are mediated by the sympathetic nervous system. A decrease in diameter in the peripheral blood vessels and, as a consequence, decreased blood flow results in reduced SKT (Ahmed, Begum, Funk, Xiong, & von Scheele, 2011). SKT is often used in biofeedback techniques as a simple way of identifying stress changes (Karthikeyan, Murugappan, & Yaacob, 2012). However, it is important to note that SKT measurement may not perfectly reflect peripheral vasomotion as this parameter may be affected by factors as evaporation, muscle tone, and core temperature (Shusterman & Barnea, 2005).

We have previously reported that Ze 185 (Meier et al., 2018) led to a reduced anxiety response to acute psychosocial stress, with no effect on the cortisol stress response. In line with these findings, stress responses of the parameters of the ANS remained unaffected by the administration of Ze 185. Whether this is a result of the used dosage or duration of treatment can only be speculated and needs to be addressed by future studies. Previous studies with Ze 185 used longer treatment periods of 20 days (Schellenberg et al., 2004), two weeks (Melzer, Schrader, Brattström, Schellenberg, & Saller, 2009), and up to 10 days (Steiner & Opwis, 2000).

Although the mechanism of action of Ze 185 is not fully understood, the effects of valerian as well as lemon balm have been shown to be mediated through modulation of GABA receptors (Awad, Muhammad, Durst, Trudeau, & Arnason, 2009; Becker, Felgentreff, Schröder, Meier, & Brattström, 2014; Ibarra, Feuillere, Roller, Lesburgere, & Beracochea, 2010). The potential mechanisms through which Ze 185 could affect ANS stress responses are yet to be elaborated. As such, specific patterns of neural activation in brain circuits underlying a stress response have been demonstrated, such as the amygdala, striatum, hypothalamus, midbrain, right insula, and right dorsolateral prefrontal cortex regions (Sinha, Lacadie, Constable, & Seo, 2016). Our results are based on a laboratory setting applying a standardized stress test with healthy men. Future studies should as well include female participants to investigate possible gender effects. Also, it would be interesting to investigate real-life stressors over a longer period (e.g. examination phase) rather than experimentally induced acute psychosocial stress. Furthermore, the physiological effects of Ze 185 in clinical populations should be taken into account. Hereby, the investigation of patients with stress-related clinical conditions would be especially interesting.

In summary, our results show that the stress responses of the ANS parameters i.e. sAA, HR, HRV, SCL, and SKT, remained unaffected of Ze 185 in healthy men compared to placebo and no treatment. This finding goes in line with previous reported cortisol stress responses (Meier et al., 2018). Taken these findings together, the responses of the two major biological stress systems namely the hypothalamic-pituitary-adrenal axis and the ANS are maintained under the intake of Ze 185 while subjective anxiety is reduced. Given that a circumscribed biological stress response is to be considered as an adaptive mechanism and considering the effects on subjective affective parameters (e.g., Cases et al., 2011; Melzer et al., 2009; Schellenberg et al., 2004; Steiner & Opwis, 2000) next to the high acceptability and low risks (Lynch & Berry, 2007), further studies on the effects of herbal medicinal products are warranted to understand who might benefit from these products and for which conditions.

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Conflict of interests

All authors declare that Max Zeller Söhne AG, Switzerland, provided the herbal medicinal product Ze 185 and funding for the implementation of the study procedures. CZ, EK, and JD are employees of Max Zeller Söhne AG, and JG and RLM have received fees for talks organized by the company. The authors were autonomous in data analyses, study report and manuscript preparation. The sponsor had no influence on the performance, outcome, and evaluation of the study.

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Figures

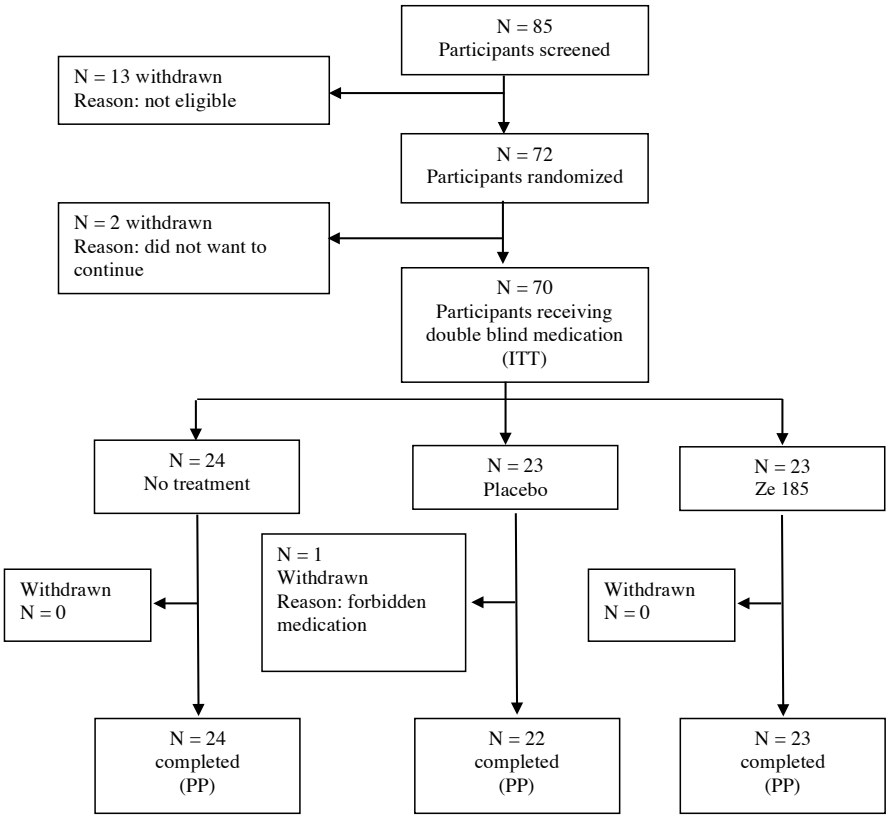

Figure 1. Flow chart of participants from initial assessment for eligibility sample to TSST completion sample.

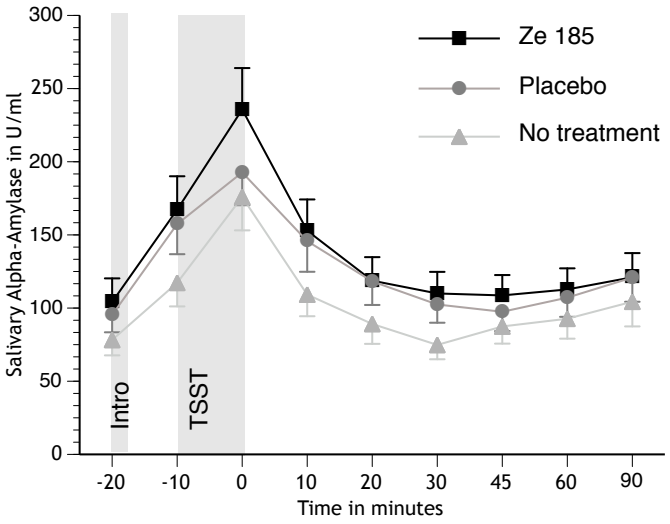

Figure 2. Changes in salivary alpha-amylase over time by groups.

Note: Intro= Introduction to the TTST, TSST= Trier Social Stress Test.

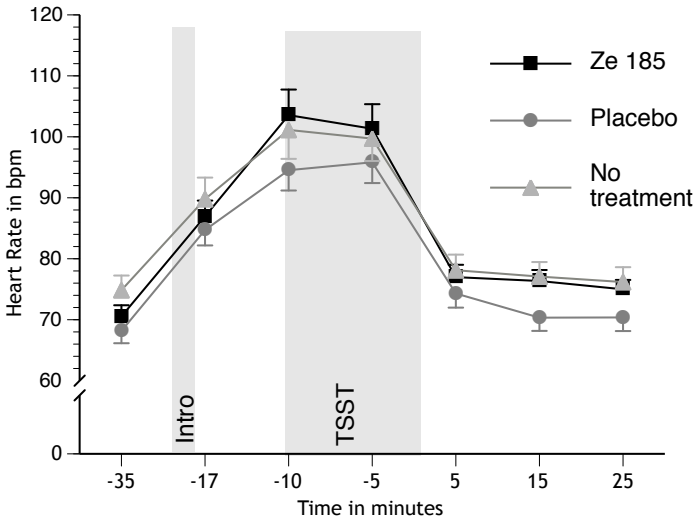

Figure 3. Changes in heart rate over time by groups in beats per minute.

Note: Intro= Introduction to the TTST, TSST= Trier Social Stress Test.

Appendix C

Study II, Publication III:

Heimgartner, N., Meier, S., Hochuli, S., Ponti, S., Arpagaus, S., Kappeler, F., & Gaab, J. (2018). *Randomized controlled evaluation of the psychophysiological effects of social support stress management in healthy women.* Manuscript submitted for publication.

RANDOMIZED CONTROLLED EVALUATION OF THE PSYCHOPHYSIOLOGICAL EFFECTS OF SOCIAL SUPPORT STRESS MANAGEMENT IN HEALTHY WOMEN

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Keywords: social support, stress management, Trier Social Stress Test, perceived stress, subjective anxiety, cortisol, heart rate, heart rate variability

Abstract

Background and Objectives: Enduring exposure to stress has a negative impact on mental and somatic health. Considering the high and increasing prevalence of stress, approaches to mitigate stress-related symptomatic as well as physiological consequences become a matter of public health. Since supportive social interactions contribute substantially to mental and physical health, we set out to develop and evaluate a social support stress management intervention in healthy women.

Methods: In total, 52 healthy women were randomly assigned to a social support stress management or a waitlist control condition. All participants completed self-report questionnaires of perceived stress and social support at baseline and follow-up eight weeks later. Also, all participants underwent a standardized psychosocial stress test where physiological and emotional stress responses were assessed by repeated measurements of anxiety, cortisol, heart rate, and heart rate variability.

Results: The social support stress management was perceived as feasible and highly acceptable to participants. The intervention significantly reduced perceived stress in comparison to the control condition, but perceived stress levels returned to baseline at follow-up. Availability of social resources, levels of trait anxiety, relaxation and preoccupation did not differ significantly between the two conditions at any time. The standardized psychosocial stress test provoked a significant stress response, but conditions did not differ in any of the assessed physiological stress responses. However, participants in the social support stress management showed a significantly attenuated state anxiety response in comparison to those in the control condition.

Conclusion: Our results indicated that our social support stress management intervention had a significant, albeit transient impact on perceived stress. The intervention had no effect on physiological responses to acute psychosocial stress, even though anxiety responses to stress were attenuated. Thus, the social support stress management had the expected – and stress-buffering – effects on subjective parameters, but only as long as the social support was provided in terms of the intervention. Future research on social support stress management is of potential and should focus on the sustainablity of its effects as well as examine subjects reporting low levels of supportive social support and/or with clinical conditions.

Introduction

While the ability to respond both physiologically and psychologically in the face of adversity is functional, enduring exposure to stress has a negative impact on mental and somatic health, partly mediated through its effects on the hypothalamic pituitary adrenal (HPA) axis as well as on sympathetic nervous system (SAM; McEwen, 2017). Accordingly and for example, stress has been associated with the incident of upper respiratory infections (Pedersen, Zachariae, & Bovbjerg, 2010), exacerbation in autoimmune diseases (Mohr, Hart, Julian, Cox, & Pelletier, 2004; Porcelli et al., 2016), increased risk for coronary heart disease (Chida & Steptoe, 2010; Kivimäki et al., 2006), development of functional abdominal pain disorders (Korterink, Diederen, Benninga, & Tabbers, 2015), slower wound healing (Walburn, Vedhara, Hankins, Rixon, & Weinman, 2009) and depressive symptoms (Theorell et al., 2015). Considering that the high and increasing prevalence of stress – a survey in 2017 showed that 20% of respondents stated extreme stress in their daily lives and the percentage of experiencing at least one symptom of stress increased (American Psychological Association, 2017) – approaches to mitigate stress-related biological processes become a matter of public health.

Here, a social perspective is warranted and of potential. In their seminal meta-analyis of 208 laboratory studies, Dickerson and Kemeny (2004) identified uncontrollable social evaluation as the most potent stressor in terms of magnitude of the cortisol reaction and time to recovery. But while social interactions have the potential to be potent stressors when treathening one's social esteem, acceptance or status (Dickerson, Gruenewald, & Kemeny, 2009), social interactions can also contribute substantially to mental and physical health when perceived supportive. For example, Holt-Lunstad, Smith, and Layton (2010) reported a 50% increased likelihood of survival for elderly individuals with adequate socials relationships compared to those with poor or insufficient social relationships over an average of 7.5 years. These protective effects of social support can be explained through both modeling or encouraging healthy behavioural and psychological processes (DiMatteo, 2004) as well as buffering behavioural or physiological responses to acute or chronic stressors (e.g. Ditzen et al., 2007; Eisenberger, Taylor, Gable, Hilmert, & Lieberman, 2007; Nausheen, Gidron, Gregg, Tissarchondou, & Peveler, 2007; Thorsteinsson & James, 1999).

However, interventions intended to manage stress have yet to explicitly encorporated a social perspective as they are predomently based on a intraindividual understanding of stress, i.e. with a focus on individual stress-related cognitions and behaviours (Gaab et al., 2003; Gloster et al., 2017; Hammerfald et al., 2006; Nyklíček, Mommersteeg, Van Beugen, Ramakers, & Van Boxtel, 2013; Storch, Gaab, Küttel, Stüssi, & Fend, 2007). To the contrary

and to the best of our knowledge, only one study evaluated the effects of an intervention employing the buffering effect of social support, albeit with no effects on an array of psychological parameters and symptoms as well as cardiovascular reactivity to a psychoscial stressor (Anthony & O'Brien, 2002).

Thus, considering its protective and buffering effects, it seems promising to make use of social support in stress management interventions. Therefore, we set out to conceptualize, implement and evaluate the effects of a intervention intended to employ and improve social support skills and to reinforce the stress buffering effect of social support on psychoneuroendocrine stress responses.

Methods

Design

A randomized controlled trial with an intervention and a waitlist control condition was conducted at the University of Basel, Switzerland. The study was carried out in accordance with the Declaration of Helsinki principles and the local ethic committee (Ethikkommission Nordwest- und Zentralschweiz) approved the study protocol (reference number EKNZ 2015-005). All participants gave written informed consent.

Participants

The sample consisted of healthy women aged between 18 and 60. We included only women based on the assumption of gender differences in stress responses (Taylor et al., 2000), i.e. that women predominantly engage in a tend and befriend response to stress. Thus, we expected women to readily accept the rational of our intervention, which would facilitate the implementation of our social support stress management. Exclusion criteria were any current or chronic somatic diseases or psychiatric disorders assessed by self-report, current medication, being in psychological or psychiatric treatment, insufficient German language skills, previous participation in studies using the Trier Social Stress Test (TSST; Kirschbaum, Pirke, & Hellhammer, 1993) and smoking more than five cigarettes a day.

Procedure

Participants were recruited in lectures and via online advertisement at the University of Basel

and the University of Applied Sciences and Arts of Northwestern Switzerland. Interested women completed an online screening questionnaire. A total of 53 participants fulfilled inclusion criteria and were randomly allocated to the intervention or the waitlist control condition. Participants in the intervention condition received a two-week social support stress management (SSSM) in groups of 5-8 participants (week 2 and 3). In the waitlist control condition, participants received the intervention after study completion (after week 8). Self-report questionnaires were completed online at baseline (week 1), post-intervention (week 4) and follow-up (week 8). Between the post and the follow-up assessment (week 5 to 7) all participants underwent a psychosocial stress test with assessement of psychological, neuroendocrine and cardiovascular stress responses.

Intervention

The SSSM was conceptualized to address the importance of social support for health and well-being and to improve interpersonal skills to give and receive social support. Besides providing information on social support effects and its possible mechanisms, the SSSM focused on modeling supportive, non-judgemental communication between therapists and participants and on carrying out exercises in small groups to improve interpersonal communication skills. With this, the aim was to enhance the quality of interpersonal interactions and to allow personal and interpersonal exploration. Participants were encouraged to practice these skills in real life, i.e. between sessions, and to actively provide as well as ask for social support, since bi-directional support seems to be more effective in terms of well-being than just receiving support (Maton, 1988). The intervention was based on a manual (for details, see Appendix 1) and adherence to the manual was supervised by a certified psychotherapist (JG).

The intervention consisted of 16 training hours divided into six sessions with the first and the final session lasting four hours and the other four sessions two hours each. Each session focused on a different aspect of social support (see Appendix 1). All sessions took place within a two-week period. Two graduate students in clinical psychology (SH, SP) and a PhD student (NH) with substantial training and experience in psychotherapy conducted the SSSM in the intervention condition. In the control condition, the intervention was conducted by four graduate students (SH, SP, SA, FK) and a PhD student (NH). Graduate students were constantly supervised by the PhD student and all therapists were supervised weekly by a certified psychotherapist (JG).

Stress test

The TSST (Kirschbaum et al., 1993) was conducted to induce psychosocial stress. The TSST consists of a simulated job interview followed by a mental arithmetic task (five minutes each) in front of an audience of two confederates. The TSST has repeatedly been found to induce profound endocrine and cardiovascular responses in 70–80% of the subjects tested (Kirschbaum et al., 1993). Psychological, neuroendocrine, and physiological data were collected before, during and after the test (for details see below). To account for circadian rhythm in cortisol secretion (Kirschbaum & Hellhammer, 1989), all TSSTs took place between 2 p.m. and 6 p.m.

Measures

Demographic data including age and body mass index (BMI) were assessed online together with the inclusion criteria before condition randomization took place. At baseline (week 1), post-intervention (week 4) and at follow-up (week 8) the following questionnaires were administered online:

- The German version of the Perceived Stress Scale (PSS; Cohen, Kamarck, & Mermelstein, 1983), consisting of 14 items rated on a five-point scale, was used to assess the degree to which situations in the last few days have been appraised as stressful.
- The German version of the State Trait Anxiety Inventory (STAI; Laux, Glanzmann, Schaffner, & Spielberger, 1982) was used to assess trait anxiety. The STAI trait form consists of 20 statements each to be rated on a four-point scale with a total score of 20-37 interpreted as little or no anxiety, 38-44 as moderate anxiety and a score of 45-80 as extreme anxiety.
- The German change-sensitive symptom list (ASS-SYM; Krampen, 2006) was employed to assess experience of relaxation, well-being, discomfort and preoccupation. The ASS-SYM has six subscales (physical and psychological exhaustion, nervousness and inner tension, psychophysiological deregulation, performance and behavioral problems, burden of pain, problems with self-determination and -control) with eight items each, rated on a four-point scale. Additionally a sum scale is calculated to reflect the general level of symptoms and problems.
- The perceived and anticipated availability of social resources was assessed with the short version of the German Social Support Questionnaire (F-SozU K-22; Fydrich, Sommer, & Brähler, 2007). The FSozU K-22 includes 22 statements about the perceived

and anticipated availability of social resources to be rated on a five-point likert scale. It covers the dimensions emotional and practical support and social integration.

At post-intervention assessment, two questionnaires were administered to the intervention condition to evaluate perceived group climate and therapeutic alliance:

- The German version of the Group Climate Questionnaire - Short Form (GCQ-S; Tschuschke, Hess, & MacKenzie, 1991) was employed to assess perceived engagement (i.e. group cohesion, cognitive understanding, self-disclosure and empathy) and conflict (i.e. anger, detachment, confrontation and mistrust) with four items each.
- The German version of the Working Alliance Inventory - short revised (WAI-SR; Wilmers et al., 2008) was employed to asses the therapeutic alliance, i.e. agreement on tasks, agreement on goals and development of an affective bond. The twelve items of the WAI-SR were rated on a five-point scale from 1 to 5. The wording of the questionnaire was adapted to fit our intervention (e.g. Johnson, Burlingame, Olsen, Davies, & Gleave, 2005).

Furthermore, to assess physiological and emotional responses during and to the TSST, the following parameters were assessed:

- To assess state anxiety before and after the TSST, the German version of the State Trait Anxiety Inventory (STAI; Laux et al., 1982) was administered before the TSST (-45 minutes), after the introduction to the TSST (-20 minutes), immediately after the TSST (0 minutes) and in the recovery phase (50 minutes post TSST).
- To assess salivary free cortisol levels, nine saliva samples were collected using Salivette collection devices (Sarstedt, Sevelen, Switzerland) at -45, -35, -25, -10, 0, 10, 20, 35 and 50 minutes pre respectively post TSST. Sampling time lasted approximately one minute during which subjects chewed on the cotton swabs as regularly as possible. Salivettes were stored at –20°C until biochemical analysis took place. After thawing, biochemical analyses were conducted in the bio-chemical laboratory of the Clinical Psychology and Psychotherapy department at the University of Zurich, Switzerland, by means of a highly sensitive liquid chromatography–tandem mass spectrometry (LC–MS/MS) method (Perogamvros et al., 2009). Since the use of oral contraceptives and the menstrual cycle phase has been shown to influence the activity of the HPA-axis and therefore the endocrine response to stress (Kirschbaum, Kudielka, Gaab, Schommer, & Hellhammer, 1999), participants reported the use of oral contraceptives, menstrual cycle length and the first day of their last menses. Based on these informations, menstrual

cycle phase, i.e. follicular or the luteal phase, on the day of the TSST was estimated. The follicular phase was defined as the period between the first day and 14 days before the end of the menstrual cycle, while the luteal phase was defined as the last 14 days of the cycle (Bouma, Riese, Ormel, Verhulst, & Oldehinkel, 2009).

- Electrocardiography (ECG) was recorded continuously at 1000 Hz during one hour and 40 minutes using the wireless physiological recording system BioNomadix® (Biopac Systems, Inc., Goleta, CA). Recorded ECG data were filtered using the software AcqKnowledge (Biopac Systems, Inc., Goleta, CA) with a FIR bandpass filter from 0.5 Hz to 35 Hz with 8000 coefficients. The resulting heart period series were visually examined for artifacts and corrected when necessary using the VivoSense® software (Vivonoetics, Inc., San Diego, CA). To assess changes in heart rate over the course of the experiment seven mean heart rate slots of five minutes each were derived. Slots represented the mean heart rate at baseline (-30 to -25 minutes), in the preparation phase of the TSST, during the free speech in the TSST, during the mental arithmetic task in the TSST and in the recovery phase with three slots starting five, 15 and 25 minutes after the TSST ended. Furthermore, heart rate variability was calculated as RMSSD, the square root of the mean of the sum of the squares of differences between adjacent normal-to-normal intervals. This parameter is one of the most commonly used measure derived from interval differences (Berntson et al., 1997). Artefact corrections in one subject were over 10% and this data had therefore to be excluded from further analysis.

Statistical Analysis

All calculations were carried out using the statistic software IBM® SPSS® Statistics Version 23. Data were tested for normal distribution and homogeneity of variance using the Kolmogorov-Smirnov test before statistical procedures were applied. When normal distribution was violated, calculations were repeated with log transformed data. For better readability, the original values were used for Tables and Figures. Two sample t-test were calculated to compare demographic characteristics and baseline values. Associations of age with psychometric or physiological measures were calculated as Pearson correlation coefficients. Descriptive statistics are presented for the GCQ and WAI measures. For the comparision of psychological variables over the course of the study, ANCOVAs for repeated measures were computed with age and baseline values as covariates and condition as between-subjects variable. All analyses were performed according to the principle of intention to treat (ITT), with last observations

carried forward when follow-up data were missing. All analyses were also run on subjects with complete datasets only, but results did not differ from ITT data (unless stated otherwise). For the analyses of stress responses to the TSST, ANCOVAS for repeated measures were computed to analyze changes of state anxiety, cortisol and ECG stress response with condition as between-subjects variable and controlling for age and BMI. For effect sizes, parameters partial eta square were reported, where appropriate, according to the following conventions: 0.01 = small, 0.06 = medium, 0.13 = large (Cohen, 1988). Due to technical problems there was a loss of data from single subjects, resulting in different *N* (state anxiety *N* = 35; cortisol *N* = 37; HR and RMSSD *N* = 34). Two subjects had missing heart rate values at baseline, which were replaced with the arithmetical mean of the recovery values. Three subjects showed missing values in one or two recovery assessments which were replaced with the arithmetical mean of the available recovery values. Ten subjects had artifact corrections between 3 and 10%. Heart rate and RMSSD analyses were repeated with these ten subjects excluded, but results did not differ (data are not shown).

All reported results were corrected by the Greenhouse-Geisser procedure when the sphericity assumption was violated. For state anxiety and cortisol responses areas under the response curve were calculated with respect to increase (AUCi) using the trapezoidal method as an indicator for the integrated response in the TSST (Pruessner, Kirschbaum, Meinlschmid, & Hellhammer, 2003). Distribution between conditions in use of hormonal contraceptives, menstrual cycle phase and frequency of non-responders were tested with Chi-square tests of independence. For all analyses, significance level was set at $\alpha = 5\%$.

Results

Sample characteristics and condition comparison at baseline

In total, 53 subjects were randomly assigned to the intervention (*N* = 28) and control condition (*N* = 25). Due to withdrawal, insufficient intervention attendance, i.e. six h or less, and missing assessments the ITT sample with last observation carried forward consisted of *N* = 20 subjects in the intervention condition and *N* = 20 subjects in the control condition (for details see Figure 1). *N* = 18 subjects in the intervention and *N* = 16 subjects in the control condition completed all assessments including the follow-up assessment. Three subjects of the ITT sample refused to participate in the TSST, resulting in cortisol samples of *N* = 20 in the intervention and *N* = 17 in the control condition.

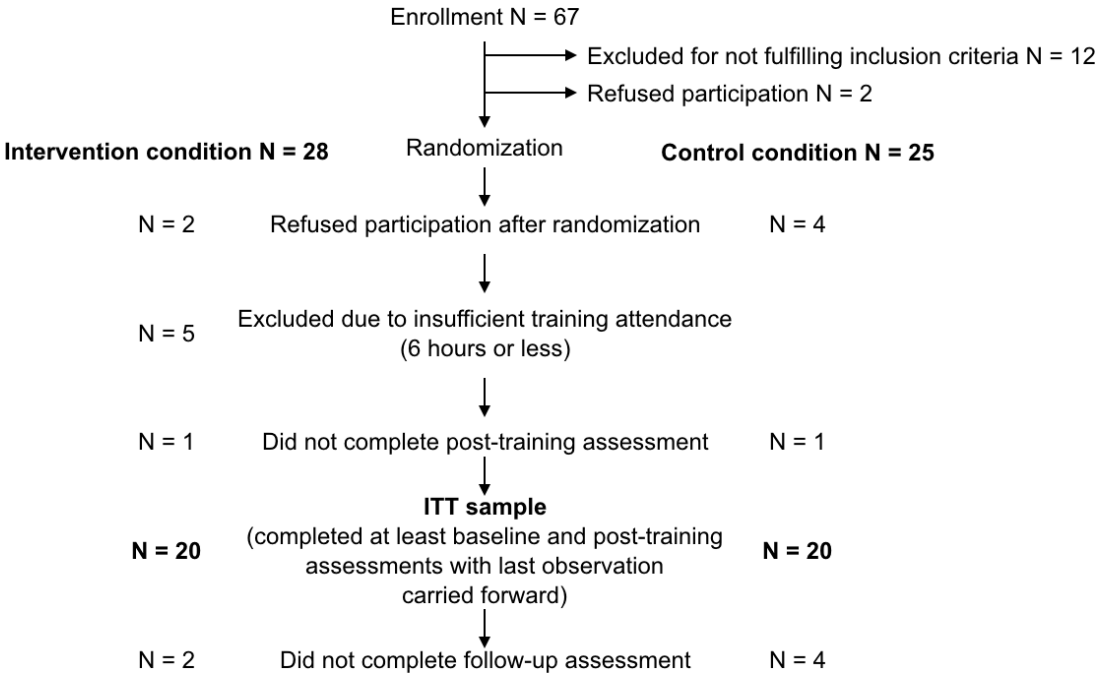

Figure 1. Recruitment and participants flow through the study.

Conditions differed in age (intervention condition $M = 24.75$, $SD = 7.59$; control condition $M = 35.40$, $SD = 14.07$, $t(29.190) = -2.98$, $p = 0.006$), but not in BMI (intervention condition $M = 21.31$, $SD = 1.88$; control condition $M = 21.17$, $SD = 3.19$, $t(38) = 0.18$, $p = 0.86$) and questionnaires at baseline (see Table 1). Number of students did not differ between conditions (intervention condition N = 13/65%, control condition 12/60%, $\chi^2(1) = 0.11$, $p = 0.74$). In the TSST, conditions did not differ in baseline cortisol (intervention condition $M = 4.68$, $SD = 3.43$; control condition $M = 4.74$, $SD = 2.98$, $t(35) = -0.06$, $p = 0.95$), heart rate (intervention condition $M = 74.51$, $SD = 10.14$; control condition $M = 71.12$, $SD = 11.19$, $t(32) = 0.93$, $p = 0.36$), RMSSD (intervention condition $M = 53.10$, $SD = 23.88$; control condition $M = 42.53$, $SD = 23.01$, $t(32) = 1.31$, $p = 0.20$) and state anxiety level (STAI state; intervention condition $M = 38.15$, $SD = 8.50$; control condition $M = 35.53$, $SD = 5.16$, $t(35) = 1.11$, $p = 0.28$). Age was negatively correlated with perceived stress (PSS) at baseline ($r(38) = -0.36$, $p = 0.02$) and availability of social support (FSozU K-22; $r(38) = -0.39$, $p = 0.01$) and positively with RMSSD at baseline ($N = 34$; $r(32) = -0.54$, $p = 0.001$). There were no significant correlations for age or BMI with cortisol (age $r(35) = -0.13$, $p = 0.45$; BMI $r(35) = -0.01$, p=0.95) and age with heart rate ($r(32) = -0.07$, $p = 0.71$), but a trend for a positive correlation for BMI with heart rate ($r(32) = 0.30$, $p = 0.09$) and a negative with RMSSD at baseline ($r(32) = -0.32$, $p = 0.07$). Use of oral

contraceptives (intervention condition N = 10/50%; control condition N = 4/24%, $\chi^2(1) = 2.74$, $p = 0.10$) as well as number of women in the follicular phase (intervention condition 8/40%; control condition N = 6/35%, $\chi^2(1) = 0.09$, $p = 0.77$) did not differ between conditions.

Participation, attendance and evaluation of the intervention

Twenty-eight women were allocated to the intervention condition, two refused participation after randomization and five had to be excluded from analysis due to insufficient intervention attendance of six hours or less. The mean intervention attendance of the 21 subjects with sufficient intervention attendance was 12.38 hours ($SD = 2.59$).

Results of the GCQ-S indiated high levels of engagement ($M = 4.93$, $SD = 0.62$) and very little interpersonal conflict ($M = 0.49$, $SD = 0.63$). Furthermore, the overall alliance (WAI-SR) with therapists ($M = 3.40$, $SD = 0.58$) and participants in the SSSM condition ($M = 3.41$, $SD = 0.54$) was good and comparable. On the three subscales, development of an affective bond was rated high ($M = 4.05$, $SD = 0.58$ for therapists, $M = 3.98$, $SD = 0.34$ for participants), ratings for agreement on tasks and agreement on goals were one point lower on the likert scale (task $M = 3.08$, $SD = 0.87$ for therapists, $M = 3.18$, $SD = 0.84$ for participants; goal $M = 3.09$, $SD = 0.70$ for therapists, $M = 3.08$, $SD = 0.81$ for participants).

Comparisons of psychological variables over the course of the study

The intervention led to significant, albeit transient reductions in perceived stress (PSS) over the course of the study ($F(2,72) = 4.09$, $p = 0.02$, partial eta square = 0.10), with a reduction of post-intervention PSS scores in the intervention condition and a return to baseline levels at follow-up and no changes in the control condition (see Table 1). Conditions did not differ in any other psychological variables over the course of the study (STAI trait: $F(2, 72) = 0.45$, $p = 0.64$; ASS-SYM: $F(2, 72) = 0.66$, $p = 0.52$; FSozU K-22: $F(2, 72) = 2.23$, $p = 0.12$; see Table 1).

Table 1. Condition differences in psychological variables over the course of the study

	Intervention condition (*N* = 20)			**Control condition (*N* = 20)**		
	Baseline	**Post**	**Follow-up**	**Baseline**	**Post**	**Follow-up**
PSS	23.85 (6.45)	19.35 (5.51)	22.95 (7.51)	19.80 (8.43)	20.95 (9.30)	19.25 (7.74)
STAI trait	37.65 (7.65)	36.95 (8.07)	37.75 (9.22)	35.40 (9.18)	36.35 (10.19)	35.60 (9.29)
ASS-SYM	37.00 (17.50)	32.75 (16.40)	37.35 (20.62)	31.35 (19.25)	30.35 (20.68)	31.05 (20.78)
FSozU K-22	4.55 (0.32)	4.57 (0.33)	4.62 (0.23)	4.41 (0.50)	4.34 (0.55)	4.33 (0.58)

Stress responses

Participants completing the TSST (n = 37) had a mean age of 29.51 years (SD = 12.50) and a mean BMI of 21.23 kg/m^2 (SD = 2.68). There was no significant difference in BMI between the two groups (t(23.84) = -.11, p = .914). However, there was a significant group difference in age with subjects in the control group being significantly older than subjects in the intervention condition (t(19.15) = -3.57, p = .002); intervention condition M = 23.35, SD = 5.03, waitlist control condition M = 36.76, SD = 14.77).

With regard to STAI state anxiety responses to the TSST, participants in the the intervention condition showed a clear trend to an attenuated response over time (N = 35, F(2.87, 91.81) = 2.59, p = 0.06, partial eta square = 0.08; see Figure 2) as well as a significant lower integrated response in comparison to controls (AUCi: intervention condition M = 153.21, SD = 515.26, control condition M = 592.63, SD = 910.22; F(1, 32) = 5.51, p = 0.03, partial eta square = 0.15). However, cortisol responses over times as well as integrated cortisol responses did not differ signifcantly between conditions (F(2.02, 66.65) = 0.25, p = 0.78; see Figure 2; AUCi: intervention condition M = 53.51, SD = 198.18, control condition M = 38.89, SD = 171.01; F(1, 33) = 0.11, p = 0.74). Conditions did not differ in the prevalence of cortisol non-responders (AUCi $\leq$ 0: control condition N = 7, 41%; intervention condition N = 7, 35%; χ^2(1) = 0.15, p = 0.70). Also, participants in both conditions did not differ significantly in their heart rate (F(1.69, 50.63) = 0.67, p = 0.49) nor in RMSSD responses to the TSST (F(1.99, 59.56) = 0.65, p = 0.53; see Figure 3).

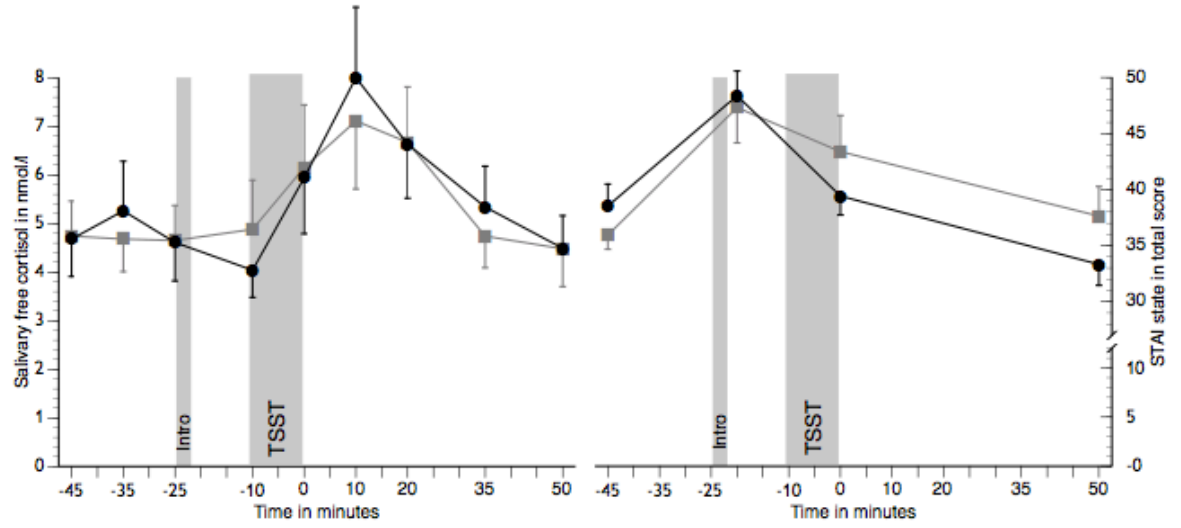

Figure 2. Cortisol and state anxiety responses in the Trier Social Stress Test (TSST) between conditions (means and standard error means). Intervention condition = black circle, control condition = grey square.

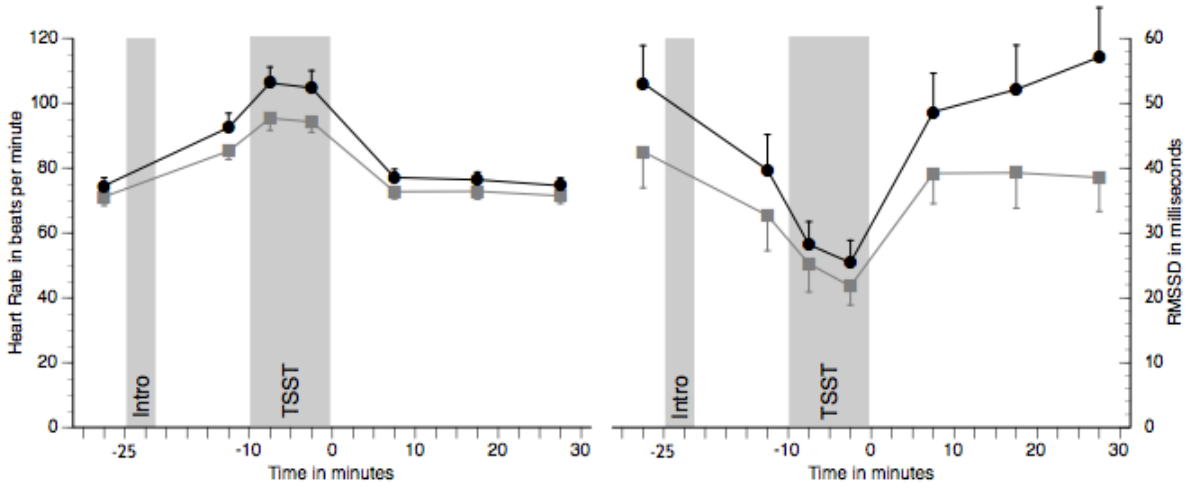

Figure 3. Heart rate and Root Mean Square of Successive Differences (RMSSD) response in the Trier Social Stress Test (TSST) between conditions (means and standard error means). Intervention condition = black circle, control condition = grey square.

Discussion

The goal of the study was to implement and evaluate a social support stress management intervention intended to employ and improve social support skills and to reinforce the stress buffering effect of social support on psychological parameters and physiological stress responses in healthy women. The intervention was favourably rated by participants and led to a significant – albeit transient – reduction in perceived stress. Although some improvements in

the availability of social resources, levels of trait anxiety, relaxation and preoccupation were reported after the intervention, none of them were significant when compared to the control condition. Also, conditions did not differ in their cortisol, heart rate and heart rate variability responses to the standardized psychosocial stress test, but participants in the intervention condition showed a significantly attenuated state anxiety response in comparison to those in the control condition.

The newly conceptualized social support stress management intervention was perceived as practicable in terms of length and effort as well as generally rated very positive, with high engagement and low conflict rating, at least when compared with group psychotherapy (Bormann & Strauß, 2007; Hecke, Brand, Rietz, & Schultz-Venrath, 2016). Also, ratings of bond with psychologists and participants in the intervention condition were high and comparable to those found in other studies (Munder, Wilmers, Leonhart, Linster, & Barth, 2010). However, ratings of agreement on tasks and goals were rather low, possibly due to the focus of the intervention laying on supportive interaction and not on defining individual tasks and goals.

The intervention led to increased levels of perceived social support whereas participants of the control condition showed a decrease in this measure, but this observation was rather moderate and not significant. This differs from trials testing other interventions to strengthen social support, which found trends (Anthony & O'Brien, 2002) or significant improvement in social support measures (Brand, Lakey, & Berman, 1995; Martin, Reece, Lauder, & McClelland, 2011). However, although this studies shared some similarities with our study, such as inclusion of healthy participants and the aim to improve and practice social skills, these studies only included individuals scoring low on social support measures and also included male participants. In contrast, our sample had already high baseline ratings in perceived social support, with scores 12% above those reported in a normative sample of healthy subjects (Fydrich et al., 2007). Thus, the lack of any effect on social support measures in our participants is possibly a consequence of already high social support levels at baseline.

With regard to perceived stress, the social support stress management led to a significant reduction of perceived stress of medium to large effect size, but which also returned to baseline levels at follow-up. These findings partly correspond to previous evaluations of stress management trainings, which similarly reported reduced levels of perceived stress after the training, but were based on different theoretical premises (Gaab et al., 2003; Storch et al., 2007). However, not all interventions based on intraindividual stress management could find an effect of the intervention on perceived stress (Gloster et al., 2017) and also trainings intended to

improve social support so far failed to show an effect on daily stress or perceived work stress (Anthony & O'Brien, 2002; Sallis, Trevorrow, Johnson, Hovell, & Kaplan, 1987). Assumably, our intervention helped participants to reduce stress by giving the opportunity to interact with each other in a supportive way during the intervention, but this did not lead to lasting changes in behaviour and transfer into daily life. A comparision with other interventions improving social support skills is difficult, as most testing did not inlcude a follow-up. Of the 13 studies rewied by Hogan, Linden and Najarian (2002) on social support skills group trainings, only four of 13 studies inlcuded a follow-up, of which three reported effects to be maintained at follow-up three or six months later (Monti, Curran, Corriveau, DeLancey, & Hagerman, 1980; Stravynski, Belisle, Marcouiller, Lavallée, & Eue, 1994; van Dam-Baggen & Kraaimaat, 1986). It has to be taken into account that the studies showing long-term effects were conducted on a population with psychiatric disorders and having considerable deficits in social skills. To the best of our knowledge only one study found positive effects of a social support training maintained at the follow-up tem weeks later in non-clinical subjects, which, however, scored low on social support measures (Martin et al., 2011). It is possible that individuals with considerable deficits in social skills and social support would profit from similar trainings and be able to transfer trained skills into their everyday life.

The TSST elicited an increase in state anxiety, cortisol, heart rate and a decrease in RMSSD. But while heart rate responses to the TSST were comparable to those found in other studies (Ditzen et al., 2007; Kudielka, Buske-Kirschbaum, Hellhammer, & Kirschbaum, 2004), cortisol responses have to be considered as below the norm (i.e. with increases of 50% to 150%; Kudielka, Hellhammer, & Wüst, 2009) and 14 subjects of our sample were cortisol non-responders. This is possibly due to the inclusion of women on oral contraceptives and in the follicular phase of the menstrual cycle (Kirschbaum et al., 1999) and that our participants, of which more than half were students, were used to hold presentations or speak in public (Engert et al., 2013).

When comparing the intervention to the control condition, there were no differences in their physiological reaction to the TSST, indicating no effect of the intervention on acute stress responses. While some of previous stress management trainings with a clear intraindividual focus found effects on the cortisol response to acute stress (Gaab et al., 2003; Hammerfald et al., 2006; Storch et al., 2007), others did not (Gloster et al., 2017) as well as trainings based on social support failed to find such effects (Anthony & O'Brien, 2002; Sallis et al., 1987). With regard to our intervention, this finding is possible due to the protocol of the TSST, which clearly prevents any possibility to use or obtain social support, as the audience of confederates is

instructed to interact in a neutral way with the participants and not to give any positive verbal or non-verbal feedback or signs of communication other than that specified in the manual (Kirschbaum et al., 1993). All efforts of participants to interact with members of the audience in possible supportive way were therefore unsucessful and otherwise stress-reducing social skills could not be applied. To test the potential of similar social support stress managements to reduce psychophysiological responses in stressful situations, the latter should strive for ecological validity and thus offer opportunities for positive interactions, as many studies indicate that positive social interactions before or during a psychosocial stress test can reduce endocrine and cardiovascular reactivity to the stressor (Christenfeld et al., 1997; Ditzen et al., 2007; Kirschbaum, Klauer, Filipp, & Hellhammer, 1995; Lepore, 1995; Lepore, Allen, & Evans, 1993; Uchino & Garvey, 1997).

The following limitations have to be considered when interpreting the results of our study. First, there was a significant difference in age between the two conditions, which was accounted for by including age as a control variable in all calculations. Second, the sample size was rather small, limiting the statistical power to detect small condition differences, but adequate to detect the effects sizes previously reported. Third, the central assumption of women being more likely to mobilize social support in times of stress and investing more in their social relationships than men (Taylor, 2011) has recently been challenged as there is evidence for men also showing an increase in prosocial behaviour during stress (von Dawans, Fischbacher, Kirschbaum, Fehr, & Heinrichs, 2012). It would thus be interesting to investigate the effects of similar interventions in men and to compare them with effects in women.

In summary, our social support stress management in healthy women was successful in terms of feasibility and applicability and had short-term effects on subjectively reported perceived stress. Further evaluation on subjects reporting low levels of supportive social interactions could elucidate if the intervention can sustainably improve social support. Although research has clearly shown that social support has a notable impact on morbidity and mortality, it rests fairly unclear if these effects can be harnessed in a more systematic way.

Conflict of interests

All authors declare no conflict of interest.

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Appendix 1: Abrigded manual of the social support stress management

General comments to therapists

The goal of the intervention is to impart interpersonal skills to deal with stress and give participants opportunities to train these skills. Most likely these skills are not completely new and at least partially already available. Important is to stress their importance, to implement and try them out in a safe space under professional supervision. The therapists model supportive interactions by communicating openly, empathic and validating to each other and to the participants. Problems are handled in a solution-focused way with emphasis on the question what was or could be helpful. Topics will be first discussed on the basis of personal experience and then in the context of scientific studies and models. At the end of each session, participants share their experiences of the session, verbalize what they take home from the session and what they could try out in the next few days.

Summary of the six sessions

The goal of the first session (four hours) is to introduce and discuss the aim of the intervention and to train basic communication skills. In the first module (60 minutes), participants discuss what puts them under stress and what helps them to cope with stress. Therapists seek to put the results of the discussion in context of findings on social evaluative threat and the social dimension of stress (Dickerson & Kemeny, 2004), on social support (Holt-Lunstad et al., 2010) and the assumption of gender-specific coping (Taylor et al., 2000). In the second module (90 minutes), participants and therapists play a board game involving personal questions. The goal of the game is to create a relaxed atmosphere where participants and therapists can get to know each other. In the third module, participants are invited to discuss and practice determinants of good communication, such as empathy, authenticity and unconditional appreciation (90 minutes).

The goal of the second session (two hours) is to improve the perception and communication of one's own feelings (Gendlin, 1982) as a basis for supportive communication. For this purpose, participants are introduced to simple exercises to monitor somatic representations of emotional experiences, which are first demonstrated to the whole group under the guidance of a therapist and then exercised in small groups.

The goal of the third session (two hours) is to focus on one's own social network and the different forms of social support that are enacted in this network, especially when under

stress (Cohen & Wills, 1985). Participants discuss different forms of support and create an illustration of their network. They discuss how their social ties are affected by stress and how and with whom they can increase beneficial social interactions.

The goal of the forth session (two hours) is to address self-disclosure and being vulnerable in social interactions in the delicate balance between feeling threatened by anticipated and real social rejection and the relieving experience to share problematic experience with others. These topics are discussed on the basis of personal experiences and with references to Learys sociometer theory (Leary, Tambor, Terdal, & Downs, 1995).

The goal of the fifth session (two hours) is to differentiate between different types and consequences of stress and how to utilize social support when under stress. In the first module (60 minutes), participants and therapists discuss differences between acute and chronic as well as functional and health-impacting stress. In the second module (60 minutes), the use of imagined persons as social support in times when no-one is directly available is discussed and experiences with this are shared with the group.

The goal of the sixth session (four hours) is, beside the presentation of one new aspect of social support, the review and repetition of contents of the intervention. In the first module (60 minutes), the relevance and importance of physical contact as a form of social support is discussed on the basis of personal experience and scientific research (Ditzen et al., 2007). In the second module (30 minutes), participants discuss what was important for them and possible changes they made during the intervention in small groups. In the closing module (90 minutes), the game from the first session is played again, this time with questions focused on experiences made during the intervention. A feedback round concludes the intervention (60 minutes).