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HPA axis responses to laboratory psychosocial stress in healthy elderly adults, younger adults, and children: impact of age and gender

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Abstract

Data from five independent studies were reanalyzed in order to investigate the impact of age and gender on HPA axis responses to an acute psychosocial laboratory stress task. The total sample consisted of 102 healthy subjects with 30 older adults (mean age: 67.3 y), 41 young adults (mean age: 23.5 y), and 31 children (mean age: 12.1 y). All participants were exposed to the Trier Social Stress Test (TSST).

The stress protocol caused highly significant ACTH and total plasma cortisol responses in older and younger male and female adults (all $p<0.0001$) as well as salivary free cortisol responses in all six age and gender groups (all $p<0.0001$). Three-way ANOVAs for repeated measurement were applied to investigate the impact of age and gender on ACTH and cortisol responses. Results showed that the ACTH response to stress was higher in younger adults compared to older adults (main effect: $p=0.009$, interaction: $p=0.06$). Post hoc analyses revealed that there was no age effect in the subgroup of women (p=n.s.), while younger men had higher ACTH responses compared to older men ($p=0.01$). For total plasma cortisol, ANOVA results showed that the pattern of reactivity did not differ between age and gender groups (all interactional effects p=n.s.), although older females had hightened overall cortisol levels compared to the other groups, as proofed in post hoc analyses (all $p<0.05$). For free

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salivary cortisol, a significant main effect of gender (p=0.05) and an almost significant three-way-interaction (p=0.09) emerged. Post hoc analyses showed an elevated overall free salivary cortisol response in elderly men compared to elderly women (p=0.006), while no gender differences emerged in neither young adults nor children (both p=n.s.).

In sum, the stressor induced significant HPA axis responses in all age and gender groups. The observed ACTH response patterns in young and elderly adults may suggest that a heightened hypothalamic drive in young men decreases with age, resulting in similar ACTH responses in elderly men and women. Alternative interpretations are also discussed. The data also supports the idea of a greater adrenal cortex sensitivity to ACTH signals in young females. Free salivary cortisol responses were elevated in elderly men compared to elderly women, an effect which cannot be explained by gender differences in perceived stress responses to the TSST. It can be speculated if corticosteroid binding globulin (CBG) and/or sex steroids are important modulators of these effects.

Keywords: HPA axis; Salivary cortisol; Age; Gender; Stress; TSST; ACTH

1. Introduction

Although it is known from animal as well as human studies that there exist age-related alterations in hypothalamic–pituitary–adrenal (HPA) axis regulation, it still remains an open question whether stress-related HPA axis functioning alters significantly with age.

While in humans there are only little differences in daytime basal ACTH and cortisol levels (Seeman and Robbins, 1994; Gotthardt et al., 1995; Kudielka et al., 1999, 2000), the circadian rhythm seems to advance with age and diurnal amplitudes appear to flatten (Sherman et al., 1985; Van Coevorden et al., 1991; Deuschle et al., 1997). Primarily, cortisol concentrations show age-related changes during night-time at the circadian trough of HPA activity (Van Cauter et al., 1996).

Human studies which apply psychological stress protocols in young and elderly

Nomenclature

Abbreviations

ACTH	adrenocorticotropin
CBG	corticosteroid binding globulin
HPA axis	hypothalamic–pituitary–adrenal axis
sem	standard error of mean
TSST	Trier Social Stress Test
VAS	visual analog scale
y	years

subjects simultaneously are rare. While a study from Gotthardt et al. (1995) report on a significant age effect (with older subjects showing larger cortisol stress responses) two other studies did not show age-related changes in HPA axis functioning neither in men nor in women (Kudielka et al., 1999, 2000). In contrast, a fourth study report that the cortisol responses to provoked stress were higher in premenopausal women compared to postmenopausal women (Lindheim et al., 1992), whereas another study only evoked minor HPA axis stress responses in a laboratory setting (Nicolson et al., 1997).

Concerning the impact of gender, human stress studies revealed that there are (a) no significant gender differences or (b) higher cortisol responses in young men compared to young women (Collins and Frankenhaeuser, 1978; Frankenhaeuser et al., 1978, 1980; Forsman and Lundberg, 1982; Lundberg, 1983; Polefrone and Manuck, 1987; Stoney et al., 1987; Kirschbaum et al., 1992, 1995). In a recent paper, Kirschbaum and coworkers disclosed that the effect of gender is masked in total plasma cortisol stress responses, while significant gender differences emerge for ACTH and free salivary cortisol (Kirschbaum et al., 1999). The study showed that ACTH responses are elevated in men compared to women, regardless of menstrual cycle phase or use of oral contraceptives. Women in the luteal phase have comparable saliva cortisol stress responses compared to men whereas women in the follicular phase or taking oral contraceptives show significantly lower free cortisol responses. These observations point at the necessity to strictly distinguish between the total cortisol *secretion* and the bioavailable cortisol *levels*. The same gender effect with higher ACTH and free salivary cortisol emerged for elderly subjects, as shown by Kudielka et al. (1998). In contrast, Seeman et al. (1995) reported on a higher cortisol reactivity in elderly women compared to elderly men employing a driving simulation challenge. Recently, these observations were corroborated using a 30-min cognitive challenge paradigm by the same group (Seeman et al., 2001).

Human studies investigating the impact of age and gender on HPA axis responses after psychological stress are still rare and results remained contradictory. Therefore, the present reanalysis aims to contribute to the question of age and gender effects on HPA axis stress responses including healthy male and female elderly adults, young adults, as well as children.

2. Methods

2.1. Subjects

Data for the present reanalysis originally come from five independent studies conducted by Kudielka et al. (1999, 2000); Kirschbaum et al. (1999); Buske-Kirschbaum et al. (1997), and Buske-Kirschbaum et al. (unpublished data). All participants had reported to the laboratory at least twice. At a first appointment, all volunteers underwent a medical examination to identify healthy individuals and patients suffering from specific diseases. Volunteers with psychiatric, endocrine, cardiovascular, other specific chronic diseases or those medicated with psychoactive drugs, β-block-

ers, estrogens (including oral contraceptives), or glucocorticoids were not admitted to the studies. In the present reanalysis, only those subjects were included who were healthy (patient groups were excluded) and received only placebo treatment. Postmenopausal women were free of any hormonal replacement therapy (HRT) and in case of premenopausal women, the stress session was scheduled during the luteal phase of the menstrual cycle to avoid potential confounding effects of different phases of the menstrual cycle, birth control pills, or HRT on stress reactivity patterns. The remaining sample consisted of 102 subjects with 30 elderly adults (15 men+15 women; mean age: 67.3±1.0 y sem; age range: 60–76 y, data from Kudielka et al., 1999, 2000), 41 younger adults (20 men+21 women; mean age: 23.5±0.5 y sem; age range: 19–32 y; data from Kirschbaum et al., 1999), and 31 children (16 boys+15 girls; mean age: 12.1±0.3 y sem; age range: 9–15 y; data from Buske-Kirschbaum et al., 1997 and Buske-Kirschbaum et al., unpublished data). The older subjects were part of a larger project investigating the effects of placebo versus short-term sex steroid treatments (e.g., a two-week estradiol treatment). The younger adults were also part of a larger study investigating the effects of menstrual cycle phase and oral contraceptives on HPA axis stress responses. In these subjects, the psychosocial stress task was administered at the third test session. The children studies focused on group differences in the stress reactivity between healthy volunteers and children with atopic dermatitis or allergic asthma. Adult participants and parents of all children gave written informed consent. The study protocols were approved by the ethics committee of the University of Trier.

2.2. Study protocol

At the second, respectively third appointment, subjects were confronted with the stress test (see below), that means all subjects were familiar with the laboratory setting and the experimenters. All stress sessions took part in the afternoon (3 pm–7 pm). For blood samples, an intravenous catheter was inserted in older and younger adults. The sampling collection begun after a rest period of 45 min. In old and young adults, blood samples were drawn directly before onset of the stressor as well as 1, 10, 20, 30, 45, 60 min thereafter for ACTH and total plasma cortisol assays. Saliva samples were obtained in all 102 subjects using Salivette sampling devices (Sarstedt, Rommelsdorf, Germany) directly before onset of the stress test as well as 1, 10, 20, and 30 min after stress exposure.

All subjects were confronted with the Trier Social Stress Test (TSST). It has been repeatedly shown that the TSST is a valid and reliable instrument to induce physiological stress responses in children, young as well as elderly adults. Additionally, in a recent metaanalysis of 165 laboratory stress studies, the TSST was found to produce the most robust physiological stress responses as compared with several other stress tasks (see Dickerson and Kemeny, 2002). For adults, this brief psychosocial stress protocol consists of a 3 min preparation period, a 5 min free speech and a 5 min mental arithmethic task in front of an audience (Kirschbaum et al., 1993; Kudielka et al., 1998). The adapted TSST for children (TSST-C) consists of a 5 min preparation period, 5 min public speaking and a 5 min mental arithmetic task. In

the speaking part, children receive the beginning of a story and are told that they should finish telling the story as excitingly as possible in front of the committee (Buske-Kirschbaum et al., 1997). After cessation of the stress task, visual analog scales were filled out by adult participants (see below).

2.3. Psychological assessment

Visual analog scales (VAS) were employed in older and young adults to measure subjective perceptions of the stressor. In elderly subjects, 14 VAS were applied. After cessation of the TSST, participants rated the extent of their personal involvement, how strenuous the task was, how difficult the free speech and the mental arithmetic task was, how new, stressful, uncontrollable, threatening the task was, and whether they anticipated negative consequences of their performance on a scale ranging from 0 to 100. In young adults, six visual analog scales (VAS) were used for subjective ratings of the stressfulness of the stressor. After cessation of the stress situation, participants were required to rate the extent of their personal involvement, how stressful, new, uncontrollable, and unpredictable the task was, and whether they anticipated negative consequences on a scale ranging from 0 to 10. In the two children samples comparable visual analog scales were not applied.

2.4. Blood and saliva sampling, biochemical analyses

ACTH (adrenocorticotropin) was measured with a two-site chemiluminescence assay (Nichols Institute, Bad Nauheim, Germany). Total plasma cortisol was measured by radioimmunoassay (IBL, Hamburg, Germany). Total plasma cortisol was analyzed in all seven blood samples, ACTH levels were assayed in the first four blood samples.

The Salivette sampling device mainly consists of a small cotton swab on which the subjects gently chew for 0.5 to 1 minute. Thereafter, the swab is transferred into a small plastic tube. Samples were stored at −20°C before analysis. The free cortisol concentrations in saliva were measured using a time-resolved immunoassay with fluorometric detection. The procedure is described in detail in Dressenörfer et al. (1992).

Additionally, basal corticosteroid binding globulin (CBG) levels were analyzed in young and older adults at the day of the stress session (RIA, IBL, Hamburg, Germany). Inter- and intraassay coefficients of variance were below 10–12% for all analytes.

2.5. Statistical analyses

Three-way ANOVA procedures (analyses of variance) were used to analyze endocrine responses to the stressor with the independent factors age groups (older adults vs younger adults vs children) and gender (male vs female) and the repeated factor sampling time (ACTH: four samples, total plasma cortisol: seven samples, free salivary cortisol: five samples). All reported results were corrected by the Greenhouse–

Geisser procedure where appropriate, which is indicated by an adjustment of the degree of freedom (Greenhouse and Geisser, 1959; Vasey and Thayer, 1987). In case of significant results in the overall (three-way) ANOVA, post hoc planned comparisons were applied for effects without repeated measurement factor and specific one- and two-way ANOVAs were conducted for effects with repeated measurement factor to further evaluate the observed effects. Finally, differences in pre-stressor (baseline) ACTH and cortisol levels were reported using two-way ANOVAs with the factors age and gender. Correlations between chronological age and endocrine baseline values were computed following Pearson product–moment procedure. For all analytes, the significance level was α=0.05. All results shown are the mean±standard error of mean (sem).

3. Results

3.1. ACTH (only older and younger adults)

First of all, the applied three-way ANOVA for ACTH resulted in a significant main effect of time ($F(3,183)=56.12$, $p<0.0001$) and age ($F(1,61)=7.35$, $p<0.009$). Furthermore, the main effect of gender ($F(1,61)=3.12$, $p<0.08$) and the two-way interactions 'age by time' ($F(1.2,72.1)=3.44$, $p<0.06$) and 'gender by time' ($F(1.2,72.1)=3.11$, $p<0.08$) approached the level of significance.

In order to clarify whether all different groups had a significant ACTH response, one-way repeated measurement ANOVAs for each of the four groups were conducted separately. The results confirmed a significant ACTH time effect for older men, older women as well as younger men and younger women (all $F>9$, all $p<0.0001$). To further investigate the observed age effects, two-way ANOVAs with the factors age and time were conducted for men and women separately. While no age effect could be found in females (both $F<1$, both p=n.s.), the ACTH response to stress differed between older and younger male adults with younger men showing the higher ACTH response to stress (main effect of age: $F(1,31)=7.55$, $p<0.01$; interaction 'age by time': $F(1.2,36.4)=3.20$, $p<0.08$). Pre-stress (baseline) ACTH levels differed between age groups (main effect of age: $F(1,61)=6.98$, $p<0.01$) and correlated significantly with chronological age ($r=-0.29$, $p=0.02$, explained variance: r^2=8%).

These results show that brief psychosocial stress provoked marked ACTH stress responses in older and younger male and female adults with younger adults, primarily the young males, showing a hightened ACTH stress response to stress (see Fig. 1). Beside stress reactivity, baseline ACTH levels were also higher in younger adults.

3.2. Total plasma cortisol (only older and younger adults)

For total plasma cortisol, the analyses of variance again revealed a highly significant stress effect (main effect of time: $F(6,330)=60.23$, $p<0.0001$) and a significant main effect of age ($F(1,55)=5.28$, $p<0.03$). Additionally, only the two-way interaction 'age by gender' reached significance ($F(1,55)=5.02$, $p<0.03$).

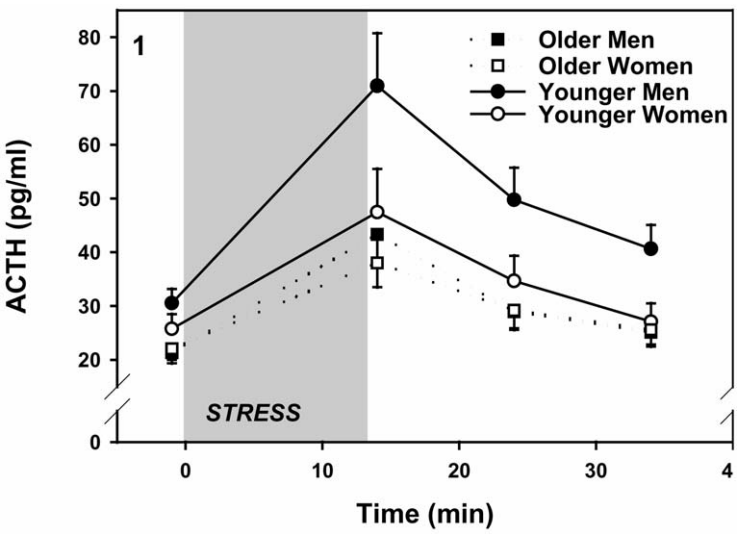

Fig. 1. Mean (±sem) ACTH responses (pg/ml) in elderly and younger men and women before and after stress (TSST). The shaded area indicates the period of stress exposure.

One-way ANOVAs for each age and gender group separately proved that all four groups showed a significant total plasma cortisol stress response (all $F<10$, all $p<0.0001$). To further elucidate the 'age by gender' interaction, post hoc planned comparisons were conducted. The analyses revealed that the overall total plasma cortisol response was hightened in elderly women compared to younger women ($p=0.002$), elderly men ($p=0.04$) and younger men ($p=0.05$). Finally, baseline (pre-stress) total plasma cortisol levels were higher in elderly adults as indicated by a significant main effect of age ($F(1,56)=4.99$, $p<0.03$) and a positive correlation between the baseline levels and chronological age ($r=0.3$, $p=0.02$, explained variance: $r^2=9\%$).

The results show that the exposure to brief psychosocial stress led to highly significant total plasma cortisol stress responses in younger and older male and female adults. Furthermore, older females had higher overall total cortisol levels, although the pattern of reactivity did not differ between age and gender groups as indicated by the lack of interactional effects with the factor time (see Fig. 2).

3.3. Salivary free cortisol (older adults, younger adults and children)

For salivary free cortisol, the three-way ANOVA procedure resulted in significant main effects of time ($F(4,364)=50.29$, $p<0.0001$) and gender ($F(1,91)=3.95$, $p<0.05$). Furthermore, the three-way interaction 'age by gender by time' approached the level of significance ($F(3.2,145.8)=2.13$, $p<0.09$).

One-way ANOVAs for the different age and gender groups separately proved that all six groups showed a significant salivary free cortisol stress reaction (all $F>6$, all

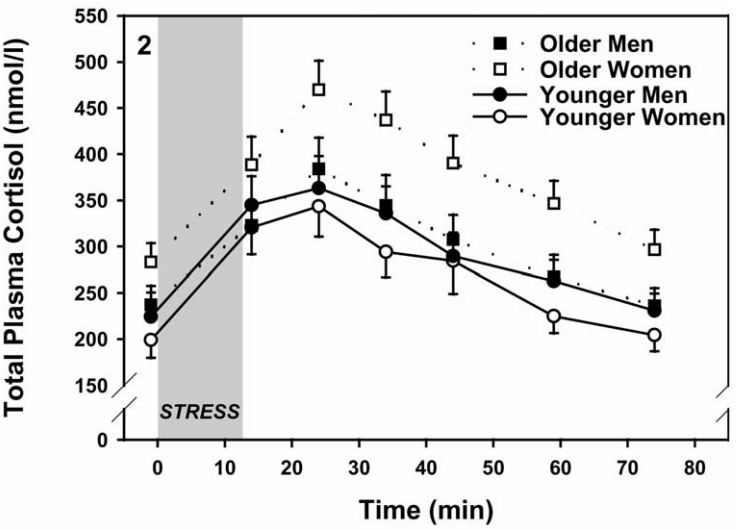

Fig. 2. Mean (±sem) total plasma cortisol responses (nmol/l) in elderly and younger men and women before and after stress (TSST). The shaded area indicates the period of stress exposure.

$p>0.0004$). In order to investigate the observed gender effect in more detail, post hoc planned comparisons were conducted. In the group of elderly adults, men showed a significantly elevated overall free salivary cortisol response ($p=0.006$), while no gender differences were observed in either young adults or children (both p=n.s.). Baseline (pre-stress) free salivary cortisol levels differed between age and gender groups as indicated by significant main effects of age ($F(2,91)=7.44$, $p<0.001$) and gender ($F(1,91)=4.36$, $p<0.04$). The free salivary cortisol baseline levels also correlated positively with chronological age ($r=0.3$, $p=0.001$, explained variance: $r^2=9\%$).

These results show that the stress task provoked highly significant salivary free cortisol stress responses in male and female older and younger adults as well as children. Furthermore, older men showed a significantly increased free salivary cortisol stress response (see Fig. 3).

3.4. Corticosteroid binding globulin (CBG)

CBG levels (Table 1) were higher in younger adults compared to older adults (main effect of age: $F(1,63)=10.39$, $p<0.002$; interaction 'age by gender': $F(1,63)=6.07$, $p<0.02$). Post hoc planned comparisons showed that CBG levels were higher in older women compared to older men ($p=0.03$), but no gender differences emerged in younger adults (p=n.s.).

3.5. Visual analog scales (VAS)

In elderly subjects, analyses of the VAS revealed no differences in subjective responses to the stressor between men and women (all $F<0.3$, all p=n.s.). In younger

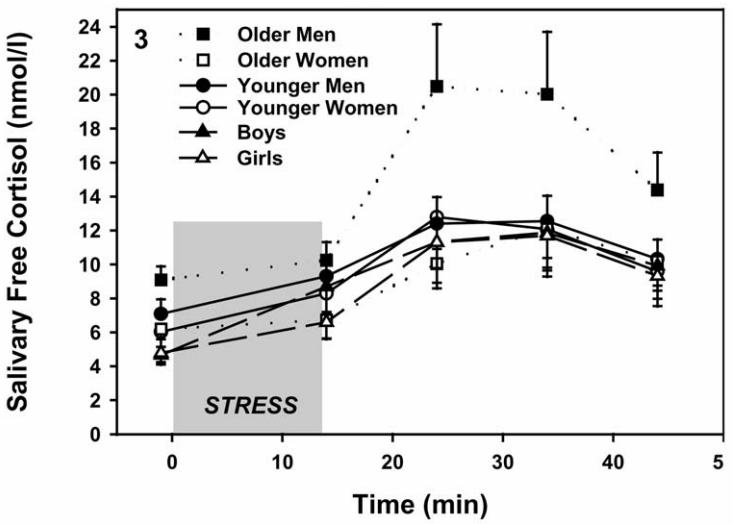

Fig. 3. Mean (±sem) free salivary cortisol (nmol/l) responses in elderly and younger men and women as well as boys and girls before and after stress (TSST). The shaded area indicates the period of stress exposure.

Table 1
CBG levels at the day of the stress session in younger and older men and women, mean±sem

	Younger men	Younger women	Older men	Older women	*P*
CBG (μg/ml)	42.4±1.58	40.0±0.76	33.6±1.89	38.8±1.97	$p<0.002$[a] $p<0.02$[b] p=n.s.[c] $p=0.03$[d]

[a] Main effect age.
[b] Interaction age by gender.
[c] Post hoc: younger men vs younger women.
[d] Post hoc: older men vs older women.

adults, the perceived stressfulness (VAS 1) was significantly higher in women compared to men, exclusively ($F(1,38)=6.25$, $p<0.02$). All other VAS did not show gender differences (all $F<0.6$, all p=n.s.). However, after adjustment of the nominal α-level for six comparisons following Bonferroni (adjusted $\alpha=0.008$), this result is no longer statistically significant.

4. Discussion

The present data show that the psychosocial stress protocol TSST (Trier Social Stress Test) induced significant HPA axis responses in male and female elderly adults, younger adults, as well as children. Therefore, the TSST as described by Kirschbaum, Pirke, and Hellhammer a decade ago (1993) can be considered as a valid psychosocial stress protocol in laboratory settings in a wide range of age groups in both sexes. This observation is strongly supported by a recently conducted independent meta–analytical review of 165 stress studies from different laboratories by Dickerson and Kemeny (2002). They concluded that the TSST-protocol is one of the best standardized tools to evoke HPA axis stress responses in a laboratory setting.

Furthermore, the bioavailable free cortisol response patterns in older adults, younger adults, and children did not differ significantly in terms of age, although a gender effect indicated that the free salivary cortisol response was elevated in elderly men. Also for total plasma cortisol, the response patterns did not differ between age and gender groups. However, total plasma cortisol concentrations were generally hightened in elderly women (see below). For ACTH, the response was higher in older adults, primarily due to an elevated response in younger men.

In the past, only a few other studies have investigated cortisol responses to standardized psychosocial stress protocols in different age and gender groups. Particularly in children, controlled stress studies are rare. The few data available, including responses to surgical stress, psychosocial laboratory stress, and CRF-provocation seem to point at similar stress-related cortisol responses in younger and older children with no apparent sex differences (Lundberg, 1983; Dahl et al., 1992; Khilnani et al., 1993; Buske-Kirschbaum et al., 1997). Further studies on this field are needed to draw final conclusions.

Concerning older age, Seeman and Robbins (1994) discuss whether the resilience of HPA axis functioning is reduced in older human beings, showing for example higher stimulation peaks and a prolonged recovery phase after stress. The present data does not support the idea of a generally hyperactive HPA axis regulation after acute psychological stress with advanced age (Sapolsky et al., 1986). However, alternative explanations for the observed results could be raised, like age-related compensatory vasopressinergic effects or a new receptor balance, as proposed by de Kloet and coworkers (1991, 1998). It has also to be taken into consideration that pharmacological stimulation tests (e.g., CRF, metyrapone pretreatment followed by exogenous glucocorticoids) in contrast to psychological stress repeatedly resulted in elevated ACTH and cortisol responses and reduced feedback sensitivity in elderly subjects (Dodt et al., 1991; Heuser et al., 1994; Born et al., 1995; Kudielka et al., 1999; Wilkinson et al., 2001).

Furthermore, the present data revealed that ACTH stress responses were elevated in young men compared to young women. Older men and women showed similar ACTH responses, which were comparable to the ACTH response pattern in younger women. This supports the idea of an enhanced hypothalamic drive in young adult men (Roelfsema et al., 1993; Kirschbaum et al., 1999) and suggests an age-related decrease of the hypothalamic drive in men, resulting in similar ACTH responses in

elderly men and women. Although, alternative explanations cannot be excluded. For example, the observed effect could also be based on age-related changes in pituitary sensitivity to CRF signals in men. Also, a low sensitivity of the adrenal to ACTH might necessitate higher (compensatory) ACTH responses to achieve a 'normal' response of the active end product cortisol. Or a decrease in ACTH in aging (men) may be the results of increased free cortisol, which decreases ACTH responses. Total plasma cortisol levels were hightened in elderly women compared to elderly men, whereas total plasma cortisol patterns did not differ between younger men and women. These observations corroborate the idea that young women have a greater adrenal cortex sensitivity to ACTH signals than young men (Roelfsema et al., 1993; Horrocks et al., 1990), because a smaller ACTH reaction in women resulted in comparable total plasma cortisol responses in men and women. Although free cortisol responses did not differ significantly between the three age groups, there was a marked gender difference in the group of elderly subjects only. Elderly men showed significant larger free salivary cortisol levels than elderly women. Subjective stress responses, like perceived stressfulness of the TSST, cannot explain this observation, because no gender differences in any of the 14 VAS could be found between old men and women with the same effect in younger subjects. In another sample of elderly subjects, we even reported that women gave higher ratings of the subjective stressfulness of the TSST, an effect which did not correlate with the endocrine response patterns (Kudielka et al., 1998).

It is known, that endocrine stress reactivity could depend on baseline levels. So, one might expect that heightened endocrine baseline/pre-stressor levels flattened the extent of the superimposed stress reaction, for example, providing less 'space' for a stress effect. However, in the present data set, the study groups with high baseline levels also showed high stress reactivity, indicating that the elevated baseline levels probably did not weaken the response pattern in a crucial manner. Additionally, the positive correlations between cortisol baseline levels and chronological age might be interpreted as a sign for a hyperactive basal HPA axis regulation with advanced age. Then, one might question why the ACTH baseline levels correlated negatively with age. However, a very cautious interpretation of these findings is warranted because the data comes from five different independent studies with slightly different study protocols (for example, elderly subjects received placebo treatment, younger adults had several appointments, children were tested applying the revised TSST for children).

It can be speculated whether some of the observed differences in HPA axis reactivity could be explained by different levels of corticosteroid binding globulin (CBG) in males and females. In the present reanalysis, CBG levels were available for younger and older adults. CBG levels were significantly higher in older women compared to older men, while no gender differences emerged in younger adults. Therefore, primarily the elevated total plasma cortisol levels in older women and possibly, at least in part, the higher free salivary free cortisol responses in older men are attributable to the observed differences in CBG levels. Besides CBG, sex steroids seem to be important modulators of the HPA stress response. We recently observed that women in the luteal phase of the menstrual cycle showed as high free cortisol

stress responses compared to men while women in the follicular phase or taking oral contraceptives had blunted free cortisol responses (Kirschbaum et al., 1999). ACTH stress responses were elevated in young adult men compared to women, regardless of menstrual cycle phase or use of oral contraceptives. This observation appears to fit to the present data. While the young women during the luteal phase of the menstrual cycle did not differ from young men, older men showed significantly higher free salivary cortisol responses than postmenopausal women. Postmenopausal women, like women in the follicular phase of the menstrual cycle, have very low estrogen and progesterone levels. Whereas many animal studies can be cited which directly investigated the impact of estrogens on HPA axis regulation, only few experimental studies have been conducted in humans. In animals, estrogens excert an potentiating effect (Kitay, 1961, 1963; Viau and Meaney, 1991; Burgess and Handa, 1992; Carey et al., 1995; Handa and McGivern, 1999) while in humans results are much more contradictory. For example, in young men, a 48-hour estradiol application resulted in elevated cortisol responsivity (Kirschbaum et al., 1996), whereas a two-week estradiol treatment in postmenopausal women did not alter TSST-induced HPA axis responses (Kudielka et al., 1999). However, feedback sensitivity seemed to be increased in postmenopausal women after a two-week estradiol substitution. Lindheim et al. (1992) reported that postmenopausal women showed a stress-induced HPA axis response before estradiol treatment but not after a six-week sex hormone replacement, although it can be speculated if this effect is merely based on habituation effects to the repeatedly applied stress procedure. Del Rio et al. (1998) could not show any estradiol effects on HPA axis responsivity in a cross-over design, applying a relatively mild stressor. From other studies no clear conclusions can be drawn due to small sample sizes and methodological problems (Collins et al., 1982; Liu et al., 1987).

The data from Kirschbaum et al. (1999), young men vs women, Kudielka et al. (1998), elderly men vs women, as well as the present results seem to be in contrast with two studies from Seeman and coworkers, who reported higher HPA axis responses in older women compared to men (Seeman et al., 1995, 2001). A closer look reveals that in the first study (Seeman et al., 1995) no significant gender effects are shown in mean cortisol responses in terms of (a) maximal increase, (b) area under the curve, and (c) repeated measures ANOVA, but solely in simultaneously elevated ACTH- and cortisol responses above the respective sample median. In the second study (Seeman et al., 2001), the reported effect of elevated cortisol responses in older women compared to older men as well as younger men and younger women is based on only two subjects in the group of elderly female responders (non-responders were excluded by authors).

Nevertheless, it cannot be ruled out that different stress protocols causes stressor-specific HPA axis responses. For instance, a recently conducted study by Petrie et al. (1999) measured the endocrine effects after lumbar puncture stress and report on higher and prolonged HPA axis responsiveness in elderly females. Likewise, pharmacological provocation, including the application of different doses of physostigmine, CRF, or metyrapone plus exogenous glucocorticoids, resulted in a significantly or slightly elevated HPA axis responsivity and decreased feedback sensitivity in older

female participants (Greenspan et al., 1993; Heuser et al., 1994; Born et al., 1995; Wilkinson et al., 1997; Luisi et al., 1998). Therefore, further studies using different standardized and validated stress protocols are warranted. The observation that acute psychological stressors on the one hand (like the TSST or real-life college exams) and pharmacological stimulation tests on the other hand (like CRF-injections) seem to result in different gender-specific patterns of HPA axis responsivity points at the necessity to clarify what the applied tests exactly measure and which levels of the HPA axis are activated. While most HPA axis stimulation tests primarily act at the pituitary or adrenal level, psychological stressors certainly require processing at higher brain levels. It has also to be taken into consideration that different doses of a pharmacological trigger change the focus of the chosen test, for example testing HPA axis reactivity *or* its maximum capacity. Reported gender differences could possibly be attributed to differences in the applied HPA axis stimulation procedures.

In sum, the present analyses based on 102 healthy subjects between 9 and 76 years showed that the TSST induces significant HPA axis responses in all age groups in both sexes. The data show no gender differences in free cortisol reponses in children and younger adults, but larger free cortisol responses in elderly men compared to elderly women. This effect does not appear to be attributable to subjective responses to the TSST. The observed ACTH and total plasma cortisol response patterns in younger and older adults suggest that a heightened hypothalamic drive in younger men decreases with age, resulting in similar ACTH responses in elderly men and women and that younger adult females have a greater adrenal cortex sensitivity to ACTH signals. It can be speculated that corticosteroid binding globulin (CBG) and/or sex steroids, like estrogens, could be important modulators of these effects.

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