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Impact of physical fitness on salivary stress markers in sedentary to low-active young to middle-aged men

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ABSTRACT

Introduction: Salivary cortisol (sCort) and alpha-amylase (sAA) are popular measurement tools for investigating hypothalamic-pituitary-adrenal and autonomic functioning, respectively. Despite their widespread use, only little is known about whether lifestyle factors such as regular exercise and physical fitness impact these two salivary stress markers. The lack of an objectively assessed fitness status is a major drawback of nearly all previous studies in this area. Our main objective was therefore to examine the impact of fitness status on sCort and sAA responses to a validated stressor.
Method: Sedentary to low-active men ($N = 115$, 45.7 ± 10.1 years, 26.7 ± 3.4 kg/m^2) were exposed to the Trier Social Stress Test for Groups and provided saliva samples before and repeatedly after the stressor. An ergometer lactate exercise test provided information on physical fitness, that is, power achieved at the individual anaerobic threshold relative to body weight. From age-specific percentiles, a 2- ($n = 65$ low fitness, $n = 50$ high fitness) and a 3-group ($n = 37$ low fitness, $n = 49$ moderate fitness, $n = 29$ high fitness) solution were calculated to elucidate the impact of different fitness levels.
Results: Prior to stress testing, sCort and sAA levels were similar in all groups. While both markers increased significantly due to stress exposure, fitness effects appeared rather small. When applying the 3-group solution, we observed attenuated sCort slopes in high- and moderate-fit men. Although sAA response slopes were pronounced in high-fit men, they failed to reach statistical significance. Mean stress reactivity (area under the curve with respect to ground, baseline, and response maximum) did not differ between groups and no association with fitness level was found.
Discussion: Our findings suggest only a weak effect of objectively assessed physical fitness on biological stress responses with attenuated hypothalamic-pituitary-adrenal but (non-significantly) higher autonomic responses in fitter men. Physical fitness is a factor that should not be neglected in future studies employing salivary stress markers, especially in studies interested in acute stress responses.

1. Introduction

Due to their convenient collection and analysis, salivary biomarkers have easily found their way into neuroendocrine (stress) research. First and foremost, this includes the steroid hormone cortisol (sCort) as a marker of hypothalamic-pituitary-adrenal activity (e.g., Kirschbaum and Hellhammer, 1994) and the enzyme alpha-amylase (sAA) as a marker of autonomic functioning (e.g., Granger et al., 2007; Nater and Rohleder, 2009).

During the past decade, a plethora of review articles have summarized possible confounders of these biomarkers (e.g., Kudielka et al., 2012; Rohleder and Nater, 2009). This development notwithstanding, there is still only little knowledge of how lifestyle factors such as regular exercise and physical fitness might impact basal as well as stress-induced levels of these biomarkers. While an attenuating effect of physical exercise and fitness on social evaluative stress-induced sCort has repeatedly been shown in cross-sectional studies comparing trained and less trained subjects (Klaperski et al., 2013; Rimmele et al., 2009, 2007) as well as in randomized controlled intervention studies (Klaperski et al., 2014), basal levels of sCort seem to be unaffected by fitness status (Gill et al., 2014; Klaperski et al., 2014; Moya-Albiol et al., 2001; Rimmele et al., 2009, 2007; Rudolph and McAuley, 1998; Zschucke et al., 2015). Research on regular exercise/fitness and sAA is still in its early stages. So far, there is one study showing no difference in sAA levels between highly-trained and sedentary subjects after a

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social-evaluative stressor (Zschucke et al., 2015). In line with sCort, comparable basal sAA values have been shown between physically fit and less fit individuals (Gill et al., 2014; Zschucke et al., 2015). However, there is evidence that sAA initially decreases in response to intense training loads in athletes (Diaz et al., 2013b) but returns to baseline with ongoing training (Diaz et al., 2013b). With regard to the effect of physical fitness on other markers of ANS tone, such as cardiovascular reactivity, findings demonstrate higher reactivity scores but also faster recovery in fitter subjects (for meta-analytical reviews see Crews and Landers, 1987; Jackson and Dishman, 2006).

One of the major drawbacks in most of the aforementioned studies is that they did not objectively assess physical fitness status. The vast majority of studies cited above compared athletes who follow intense training schedules with sedentary subjects not regularly engaging in any aerobic activities (e.g., Moya-Albiol et al., 2001; Rimmele et al., 2007) or assign groups according to self-reported exercise obtained via validated questionnaires (Klaperski et al., 2014; Rudolph and McAuley, 1995). While a heightened physical fitness can be considered to be a given in well-trained athletes, the fitness status of low- to moderate-active individuals can hardly be estimated. It is therefore of utmost importance to examine fitness status via validated procedures when investigating regular exercise/physical fitness in relation to neuroendocrine and autonomic functioning. As regular exercise and everyday life activities are hypothesized to be a major confounder in stress research in the investigation of non-athlete samples, the main aim of this study was to examine the impact of physical fitness status on sCort and sAA responses to a validated stressor in young to middle-aged, sedentary to low-active men. sCort responses are hypothesized to be blunted in fitter men, while they should at the same time demonstrate higher initial sAA increases followed by a more rapid recovery, as previously demonstrated for cardiovascular responses.

2. Methods

The present analysis is based on data from a larger study conducted at the University of Freiburg, Germany. For a detailed description of recruitment and study procedures, see Klaperski et al. (2014). Overall, 142 men recruited at banking and insurance companies entered the study procedures. From this initial sample, five withdrew their consent immediately after the testing procedure and we excluded a further 22 men from the following statistical analyses because they were not sedentary or low-active (>120 min/week of sport activities), had a BMI >35 kg/m^2, reported smoking >5 cigarettes/day, did not comply with instructions such as not to drink alcohol on the testing day, failed to provide sufficient amounts of saliva, or aborted fitness testing. The final sample comprised 115 men (45.7 ± 10.1 years, 19–64 years; 26.7 ± 3.4 kg/m^2, 17.7–34.7 kg/m^2). Of note, albeit relying on partially overlapping data (115 vs. 98 subjects), none of the cortisol analyses presented herein have been reported in our previous publication (Klaperski et al., 2014). The study protocol was approved by the local Ethics Committee of the University of Freiburg, and written informed consent was provided by all participants prior to study entry.

Data collection took place in two waves from February to June 2012 and August to December 2012. After examination of eligibility and exclusion criteria (a health history checklist asking for chronic and current physical and psychiatric disorders, medication intake, habitual alcohol and nicotine consumption, as well as eating, drinking, feeling ill and physical exercise 24 h prior testing), all participants were subjected to a social-evaluative stress task followed by a fitness test. After a resting period of 30–45 min, the men were exposed to the *Trier Social Stress Test for Groups* (von Dawans et al., 2011) in groups of three. In short, the subjects had to give a 3 min mock job interview followed by 3 × 30 s of mental arithmetic (alternating between participants) in front of two judges (male and female).

Each participant provided six *saliva samples* (Salivettes® Blue cap, Sarstedt, Nümbrecht, Germany) throughout the experiment (relative to stressor onset: −3, +15, +25, +40, +55, +75 min). Participants were asked to gently move the swab around in their mouth, but to not chew for 1 min to absorb whole saliva. In all samples, we assessed sCort using commercially available enzyme-linked immunosorbent assays (IBL International, Hamburg, Germany) at Dresden Lab-Service GmbH (Germany). As sAA shows immediate increases after autonomic stimulation and recovers rapidly, we analyzed only the first four measurement time points using a kinetic colorimetric test with reagents from Roche (Roche Diagnostics, Mannheim, Germany) at the Biochemical Laboratory of the Department of Clinical Biopsychology, University of Marburg.

A *standardized fitness test* (graded exercise on a bicycle ergometer, initial workload 60 watts, increased by 25 watts every 3 min until exhaustion) followed the stress task procedures (the test itself lasted 6 to 33 min, depending on the participants' fitness levels; the total time the participants spent at the fitness test facilities was about an hour). Blood lactate was examined in capillary blood before each load increase. As an indicator of physical fitness, we calculated individual *power* achieved at the *anaerobic threshold relative to body weight* (p[IAT]/kg) (Roecker et al., 1998). For further analysis, we determined age-specific percentiles of the p[IAT]/kg according to reference ranges of the "Radlabor"—a professional performance testing company.

To address any significant chronic stress and anxiety levels in our sample, participants filled in the Screening Scale for the Assessment of Chronic Stress (SSCS; Schulz et al., 2004) and the trait version of the State-Trait-Anxiety-Inventory (STAI-T; Spielberger et al., 1983).

Exploratory *statistical analyses* (SPSS, Version 22) revealed 5 outliers (>3 standard deviations above the mean; 4 sCort, 1 sAA), who were replaced with the next highest non-outlier number.[1] Absolute sCort and sAA values have been natural log-transformed to account for violation of the assumption of normal distribution as indicated by a significant Kolmogorov–Smirnov test. From age-specific percentiles of the p[IAT]/kg, we created two new variables: a dichotomous variable at the 50th age-specific percentile to compare low and high fitness individuals (65 vs. 50 subjects) and a 3-group solution at the 33rd and 66th age-specific percentile to compare low, moderate, and high fitness individuals (37 vs. 49 vs. 29 subjects). For group comparisons regarding descriptive data, questionnaires, and fitness parameters univariate ANOVAs have been employed. Post hoc group comparisons were performed by means of Gabriel's procedure as sample sizes were slightly different. To assess the impact of physical fitness on neuroendocrine and autonomic stress responses, we conducted repeated measures ANOVAs for each salivary marker using a 6/4 (time) × 2/3 (fitness group) design. Furthermore, we conducted univariate ANOVAs to compare mean stress reactivity calculated as "area under the individual response curve with respect to the ground" (AUC_G), "area under the individual response curve with respect to the increase" (AUC_I), and "area under the individual response curve with respect to response maximum" (AUC_R, for sCort for the time period between the 1st and 3rd sample, for sAA for the time period between the 1st and 2nd sample). Associations between AUC's and p[IAT]/kg were examined with Pearson's correlation. Importantly, there were highly significant differences with regard to

[1] It is suggested to transform instead of remove outliers (Field, 2013). Replacement with the largest/smallest non-outlier, so called winsorising, seems to be preferable to replacement with the group mean (Cousineau and Chartier, 2010). Interestingly, this procedure did not alter our main results.