Psychosocial determinants of diurnal alpha-amylase among healthy Quebec workers

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A B S T R A C T
Salivary alpha-amylase (sAA) is a stress-sensitive biomarker that shows promise as an indirect proxy of sympathetic-adrenal-medullary axis activities that are otherwise difficult to discern non-invasively. This comprehensive study investigated diurnal sAA in association with numerous psychosocial characteristics related to mental health, work stress, and non-work stress. Participants included 395 workers (56.1% women, age: M = 41.3, SD = 10.81) from across 34 distinct workplaces. Diurnal sAA was sampled over two non-consecutive work days at awakening, 30 min after awakening, 14h00, 16h00, and bedtime. Well-validated psychometrics and survey items were used to measure mental health (psychological distress, depression, burnout, work characteristics) (task design, demands, social relations, gratifications), and non-work characteristics (marital/parental status, economic statuses, marital and parental stress, work-family conflicts). Preliminary results revealed that men showed occasionally higher sAA concentrations than women. Multilevel regressions were used to analyze sAA concentrations nested according to levels (i) for each time-point, (ii) between workers, and (iii) across workplaces while covarying for time of awakening, sex, age, cigarette smoking, alcohol consumption, regular physical activity, psychotropic drug use, and body mass index. Main results revealed that psychological demands, support from colleagues, interpersonal conflicts, job recognition and job insecurity appear to be associated with diurnal sAA, while non-work factors did not. Our findings showed a distinct diurnal profile for sAA replicate and expand those of Nater et al. (2007, Psychoneuroendocrinology 32, 392–401), providing further evidence that sAA is associated to subjective psychosocial factors.

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1. Introduction

Biomarkers related to psychosocial stress provide insights into the pathophysiological correlates of diverse human conditions. To date, the biomedical literature on stress-sensitive biomarkers secreted into circulation have centered on (1) the hypothalamic-pituitary-adrenal (HPA)-axis production of the stress hormone cortisol, (2) the sympathetic-adrenal-medullary (SAM)-axis release of catecholamines like adrenaline, and (3) the immune system mobilization of pro- and anti-inflammatory cytokines like interleukin-6 (Nater et al., 2013). Technological advances that led to the biochemical assessment of cortisol via saliva (Kirschbaum and Hellhammer, 1994) promoted non-invasive sampling methods that have revolutionized our understanding of the stress-disease link.

By contrast, the SAM-axis and immune systems are still bound to extraction from urine and/or blood, making their incorporation into field studies less feasible. However, salivary alpha-amylase (sAA) has been found to be a proxy of SAM-axis activities (Rohleder et al., 2004) and shows promise as a stress-sensitive biomarker of health. Alpha-amylase is a primary salivary protein involved in mucosal immune functioning by inhibition of bacteria (Scannapieco, 1994). sAA release is neurologically controlled by acinar cells that are innervated by both the sympathetic and the parasympathetic branches of the autonomic nervous system (Emmelin, 1987). This makes sAA a prime candidate to approximate SAM-axis activities that are unreliably assessed in saliva. Indeed, salivary catecholamine concentrations are several times lower than those obtained from blood, and so do not reflect the acute changes in SAM-axis activities (Kennedy et al., 2001). A growing number of studies reveal that sAA profiles are sensitive to stress exposure (Rohleder et al., 2004; Nater et al., 2005; Nater et al., 2006). Critically, sAA correlates with SAM-axis release of noradrenaline

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suggesting that sAA is indeed a proxy of adrenergic activities.

Diurnal variation of stress biomarkers can be assessed non-invasively via saliva. Salivary flow rate and saliva composition vary according to 24-h circadian rhythms (Dawes, 1974). Early studies showed that sAA displays low concentrations in the morning and high values in the afternoon (Ferguson et al., 1973). In the first comprehensive assessment of diurnal determinants of sAA, Nater et al. (2007) asked healthy German university students (N = 76) to sample saliva 15 times over the course of a single day. Their data confirmed that sAA has a distinct diurnal profile characterized by a pronounced decrease 60 min after awakening and a steady increase of activity thereafter (Nater et al., 2007). Using diary information on momentary stress, mood, food, or physical activity, they further showed that diurnal sAA appears to be relatively independent of self-reported perceived stress and salivary cortisol, but is associated with chronic stress, positive moods, and to age differences (Nater et al., 2007). In the spirit of replication and expansion, this latter finding requires further exploration in a larger and older sample.

Psychiatric research has also endeavoured to identify whether sAA might be useful in the context of psychiatric illnesses like major depressive disorder (MDD). In a case-controlled assessment of afternoon sAA (12h00–16h00), sAA was higher among Japanese individuals with unremitted MDD (n = 28) compared to remitted MDD (n = 43) and healthy controls (n = 103) (Ishitobi et al., 2010). Likewise in another Japanese assessment of afternoon sAA (13h00–17h00), concentrations were higher among female MDD patients (n = 88) than healthy controls (n = 41) prior to an electrical stimulation task (Tanaka et al., 2012). A Polish study showed that depressed individual also show higher morning sAA (8h20–9h00) than age and sex matched controls (Cubala and Landowski, 2014). In a large Dutch cohort study (Veen et al., 2013), MDD patients showed a gradient trend for the highest evening sAA concentrations (22h00 and 23h00) among current MDD (n = 752) followed by remitted MDD patients (n = 611) and lastly healthy controls (n = 329). Recent studies have also endeavoured to link sAA to stress reactivity habituation in panic disorder patients (Petrowski et al., 2015). In sum, psychiatric conditions appear to be associated with elevated diurnal sAA; however, it is unclear whether sAA is associated with other psychiatric symptoms.

Occupational health psychology has also endeavoured to understand how work stress relates to sAA. In a German study of 215 nurses from different hospitals (Wingenfeld et al., 2010), diurnal sAA was collected at four time-points (7h00, 11h30, 17h30, and 20h00). While female nurses showed more pronounced increases in sAA over the day, psychiatric symptoms related to depression, anxiety, work stress, and burnout were not related to sAA. Among Japanese nurses (N = 25) from one hospital who provided samples 12 times over 6 days, morning and evening sAA was higher among night shifts than day shifts (Morita et al., 2014).

The effect of shift work is also important in further understanding diurnal sAA. A study of Canadian paramedics (N = 21) assessed five time-points (+30 min after awakening, 6h00, 12h00, 18h00, and bedtime) on two work days and one rest day (Wong et al., 2012). Dispatchers showed lower daily sAA production than ambulance paramedics, while rotating shift-workers exhibited a flatter sAA diurnal slope than daytime-only workers. Despite mixed directionality, these findings suggest that diurnal sAA is related to workplace stress in non-clinical populations. However, other important workplace stressors have been omitted by sAA studies. For example, stressors associated with task design (skill utilization, decision authority), demands (physical, psychological, contractual), social relations (social support, interpersonal conflicts, harassment), and gratifications (job recognition, career perspective, job insecurity) have been routinely linked to mental health symptoms (Marchand et al., 2015). Yet, whether these factors relate to sAA are, to the best of our knowledge, unknown.

Psychosocial contexts outside of the workplace are also related to sAA. For example, the stress and strain of caregiving (Savla et al., 2013), interparental aggression (Gordis et al., 2010), and acculturation (Snodgrass et al., 2012) are related to distinct diurnal patterns of sAA. This may suggest that the pervasive effects of adversity can be captured using measures of sAA. Notwithstanding, other non-work factors that are important in the stress-disease literature have often been neglected in sAA studies. These include factors like marital status, parenting, socioeconomic status, strained marital and parental relationships as well as social support outside the workplace. These stressors have also been routinely related to problems associated with psychological distress, depression, and burnout (Marchand et al., 2015).

Overall, previous sAA studies exhibit some limitations that the current study endeavours to compliment. Psychoneuroendocrine studies of sAA generally have small sample sizes, varying sampling designs, and diverse protocols that make comparisons difficult in light of the multi-directional findings reported in this growing literature. In the context of workplace stress, studies that include workers generally focus on specific employment types (e.g., nurses, teachers, managers) within specific companies that ultimately limit generalizability both nationally and internationally. Given the vast individual differences in psychosocial contexts, it is also essential that sAA be investigated while controlling for numerous factors related to health behaviors, mental health, as well as work and non-work characteristics.

In the current comprehensive study, we examined diurnal variations in sAA concentrations over the course of two work-days in a sample of 395 workers from across 34 distinct workplaces. Our research objectives were to evaluate how sub-clinical psychiatric symptoms (e.g., psychological distress, depression, burnout),
work characteristics (e.g., task design, demands, social relations, gratifications), and non-work characteristics (e.g., marital, parental, economic statuses, marital and parental stress, work-family conflicts) correlate to sAA time-points throughout the diurnal course while controlling for key covariates.

2. Methods

2.1. Participants

Data came from the SALVEO study conducted in Canada to evaluate the contribution of work, family, individual characteristics, and social networks vis-à-vis workers’ mental health problems (Marchand et al., 2015). This study also aimed to understand how work, non-work, and individual stressors ‘get under the skin and skull’ to promote variations in the stress hormone cortisol and how this can explain mental symptoms in workers. For the physiological part of this study, data were collected throughout 2009–2012 using a total sample comprised of 34 Canadian workplaces randomly selected from a list of over 500 companies insured by a large insurance company. For each workplace, a random sample of employees was first selected to answer a questionnaire battery that included 300 questions pertaining to the individual, work conditions, occupation, family, and community characteristics. The original sample was composed of 1301 workers with an average response rate of 66.7% (range 55.3–95.5%).

From among these respondents, our goal was to recruit a sample of 10–15 workers per workplace to participate in the second phase of the research project whereby saliva samples were collected for assessment of sAA concentrations. Previous reports by our group have confirmed that this selection process can be utilized successfully (Marchand et al., 2014a; Marchand et al., 2014b).
Table 2
Contribution of mental health, work, and non-work factors in predicting alpha-amylase concentrations.

<table>
<thead>
<tr>
<th></th>
<th>Main effect $\chi^2 (df=1)$</th>
<th>$p$</th>
<th>By occasion $\chi^2 (df=5)$</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mental health</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Psychological distress</td>
<td>1.00</td>
<td>0.32</td>
<td>12.48</td>
<td>0.03</td>
</tr>
<tr>
<td>Depression</td>
<td>0.08</td>
<td>0.77</td>
<td>4.22</td>
<td>0.49</td>
</tr>
<tr>
<td>Emotional exhaustion</td>
<td>0.10</td>
<td>0.75</td>
<td>4.85</td>
<td>0.43</td>
</tr>
<tr>
<td>Work</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skill utilisation</td>
<td>0.01</td>
<td>0.92</td>
<td>4.23</td>
<td>0.52</td>
</tr>
<tr>
<td>Decision authority</td>
<td>0.00</td>
<td>0.95</td>
<td>2.03</td>
<td>0.84</td>
</tr>
<tr>
<td>Physical demands</td>
<td>0.74</td>
<td>0.39</td>
<td>7.40</td>
<td>0.19</td>
</tr>
<tr>
<td>Psychological demands</td>
<td>3.89</td>
<td>0.05</td>
<td>16.56</td>
<td>0.01</td>
</tr>
<tr>
<td>Working hours</td>
<td>0.95</td>
<td>0.33</td>
<td>8.07</td>
<td>0.15</td>
</tr>
<tr>
<td>Work schedule (irregular)</td>
<td>0.21</td>
<td>0.64</td>
<td>3.55</td>
<td>0.62</td>
</tr>
<tr>
<td>Social support-colleagues</td>
<td>0.02</td>
<td>0.90</td>
<td>33.40</td>
<td>0.00</td>
</tr>
<tr>
<td>Social support-supervisor</td>
<td>1.49</td>
<td>0.22</td>
<td>4.47</td>
<td>0.48</td>
</tr>
<tr>
<td>Abusive supervision</td>
<td>0.17</td>
<td>0.68</td>
<td>7.43</td>
<td>0.19</td>
</tr>
<tr>
<td>Interpersonal conflicts</td>
<td>8.49</td>
<td>0.00</td>
<td>16.23</td>
<td>0.01</td>
</tr>
<tr>
<td>Harassment</td>
<td>1.16</td>
<td>0.28</td>
<td>2.81</td>
<td>0.85</td>
</tr>
<tr>
<td>Job recognition</td>
<td>0.12</td>
<td>0.72</td>
<td>12.10</td>
<td>0.03</td>
</tr>
<tr>
<td>Career perspective</td>
<td>0.80</td>
<td>0.37</td>
<td>1.20</td>
<td>0.94</td>
</tr>
<tr>
<td>Job insecurity</td>
<td>2.78</td>
<td>0.10</td>
<td>11.25</td>
<td>0.05</td>
</tr>
<tr>
<td>Non-work</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Marital status (coupled)</td>
<td>0.06</td>
<td>0.81</td>
<td>1.44</td>
<td>0.92</td>
</tr>
<tr>
<td>Presence of minor children</td>
<td>2.90</td>
<td>0.09</td>
<td>4.62</td>
<td>0.46</td>
</tr>
<tr>
<td>Household income</td>
<td>0.05</td>
<td>0.82</td>
<td>8.31</td>
<td>0.14</td>
</tr>
<tr>
<td>Marital strains</td>
<td>1.01</td>
<td>0.32</td>
<td>3.94</td>
<td>0.56</td>
</tr>
<tr>
<td>Parental strains</td>
<td>0.52</td>
<td>0.47</td>
<td>2.66</td>
<td>0.75</td>
</tr>
<tr>
<td>Family-work conflicts</td>
<td>1.06</td>
<td>0.30</td>
<td>2.05</td>
<td>0.84</td>
</tr>
<tr>
<td>Work-family conflicts</td>
<td>0.66</td>
<td>0.76</td>
<td>7.51</td>
<td>0.19</td>
</tr>
<tr>
<td>Social support outside work</td>
<td>0.04</td>
<td>0.84</td>
<td>3.09</td>
<td>0.69</td>
</tr>
</tbody>
</table>

Note: Adjusted for self-reported awakening time, sex, age, smoking, alcohol intake, physical activity, psychotropic drug use, and body mass index.

In so doing, a random subsample of 1043 workers was re-invited for the current biomarker sub-study, of which 401 workers agreed to participate (11.8 workers per workplace on average), for a total response rate of 38.4%. Response rates between women (40.8%) and men (36.1%) were not statistically significant ($\chi^2 = 2.50, df=1$, $p = 0.114$). Women therefore represented 56.1% of workers and the mean age of the entire sample was 41.3 years (SD = 10.81, range: 19–69).

Participants signed an informed consent form and were given detailed instructions. The study protocol for the first and second phase of this research project was approved by the Ethics Committee of the University of Montreal, McGill University, Laval University, Bishop’s University, and Concordia University.

2.2. Saliva sampling and alpha-amylase determination

Consenting workers were instructed to provide five saliva samples per day at the following occasions: (1) awakening, (2) 30 min after awakening, (3) 14h00, (4) 16h00, (5) bedtime. Previous studies have validated that these sampling times are reliable time-points from which to assess diurnal cortisol secretory patterns (Lupien et al., 1998) that have been previously reported elsewhere using the current sample (Marchand et al., 2014a; Marchand et al., 2014b). Likewise, our previous studies have applied non-consecutive days as a collection criterion to avoid confounding on especially stressful days that might spillover onto the next day. In this manner, sampling was repeated for two non-consecutive working days over the course of one week in order to also provide ecological validity representative of differences that could occur in work and non-work contexts (Kunz-Ebrecht et al., 2004).

Thirty minutes before providing saliva samples, participants were instructed to not eat a major meal, smoke cigarettes, drink caffeinated beverages (e.g., tea, coffee, soda), drink fruit juices, consume dairy products (e.g., yoghurt, milk, cheese), and additionally asked to rinse their mouths with water to eliminate any lodged food deposits. They were further instructed to not brush their teeth, floss, or engage in strenuous physical activity two hours prior to sampling. Compliance to the collection schedule was assessed using a logbook in which participants documented the time of sampling for each sample.

Saliva was collected using the pure-spit method whereby a small quantity of saliva is guided by a straw into sterilized 5 mL 57 × 15.3 mm screw cap tubes (Sarstedt®, Item No. 62.558.201). Participants stored saliva samples in their home freezer until a research assistant retrieved the participants’ samples at their workplace one week later. Samples were frozen at −20°C in a portable freezer and then stored in an industrial −20°C freezer until sAA determination. It is important to note that while we have previously reported cortisol findings using this sample (Marchand et al., 2014a; Marchand et al., 2014b), we assayed sAA first before assaying cortisol from the same saliva tubes.

Alpha-amylase was assayed using a kinetic enzyme assay (Saliometrics State college, PA, United States of America). The sAA assay uses a substrate containing 2-Chloro-p-nitrophenol (CNP) that is linked with maltotriose. The link between these two elements is broken by the action of alpha-amylase, allowing free CNP to circulate. The free CNP is a yellow color, which is directly proportional to the quantity of alpha-amylase in the sample and can be measured by a spectrophotometer at 450 nm.

The saliva is brought to room temperature and then centrifuged at 1500 × g (3000 rpm) for 15 min. The saliva is then diluted to 1:200 with assay diluent while the substrate is being heated to 37°C. 8 µl of controls and samples are reverse pipetted into wells one strip at a time, followed by 320 µl of heated substrate. The plate is immediately placed into a plate reader also set to 37°C, where an optical density (OD) read is set for exactly 1-min and 3-min. The change in optical density between the two set time points is used to calculate the alpha amylase activity in the sample and is converted into a
### Table 3
Results of multilevel regression modelling of alpha-amylase concentrations in ln (U/ml) for psychological distress psychological demands, support form colleagues, interpersonal conflicts, job recognition and job insecurity (unstandardized coefficients).

<table>
<thead>
<tr>
<th></th>
<th>Psychological distress and sAA</th>
<th>Psychological demands and sAA</th>
<th>Support-colleagues And sAA</th>
<th>Interpersonal conflicts And sAA</th>
<th>Job recognition And sAA</th>
<th>Job insecurity And sAA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Constant</strong></td>
<td>3.687** 0.338</td>
<td>3.792** 0.434</td>
<td>4.444** 0.487</td>
<td>4.280** 0.379</td>
<td>3.955** 0.479</td>
<td>3.554** 0.356</td>
</tr>
<tr>
<td><strong>Awakening time</strong></td>
<td>0.001 0.027</td>
<td>0.001 0.027</td>
<td>0.000 0.027</td>
<td>0.000 0.027</td>
<td>0.001 0.027</td>
<td>0.003 0.027</td>
</tr>
<tr>
<td><strong>Workday-2</strong></td>
<td>0.008 0.024</td>
<td>0.009 0.024</td>
<td>0.009 0.024</td>
<td>0.007 0.024</td>
<td>0.009 0.024</td>
<td>0.009 0.024</td>
</tr>
<tr>
<td><strong>Occasion-2 (+30 min awake)</strong></td>
<td>−0.377 0.049</td>
<td>0.378 0.233</td>
<td>−1.065 0.247</td>
<td>−0.637 0.143</td>
<td>−0.729 0.244</td>
<td>−0.075 0.117</td>
</tr>
<tr>
<td><strong>Occasion-3 (2:00 PM)</strong></td>
<td>0.470 0.049</td>
<td>0.630 0.235</td>
<td>−0.607 0.247</td>
<td>0.222 0.144</td>
<td>−0.018 0.245</td>
<td>0.802 0.119</td>
</tr>
<tr>
<td><strong>Occasion-4 (4:00 PM)</strong></td>
<td>0.454 0.049</td>
<td>0.697 0.235</td>
<td>−0.703 0.247</td>
<td>0.204 0.144</td>
<td>−0.157 0.245</td>
<td>0.786 0.118</td>
</tr>
<tr>
<td><strong>Occasion-5 (bedtime)</strong></td>
<td>0.247 0.049</td>
<td>0.695 0.234</td>
<td>−0.637 0.247</td>
<td>0.116 0.144</td>
<td>−0.390 0.245</td>
<td>0.560 0.119</td>
</tr>
<tr>
<td><strong>Predictor (awakening)</strong></td>
<td>−0.041 0.019</td>
<td>−0.011 0.013</td>
<td>−0.066 0.025</td>
<td>−0.097 0.025</td>
<td>−0.023 0.020</td>
<td>−0.003 0.038</td>
</tr>
<tr>
<td><strong>Predictor (+30 min Awake)</strong></td>
<td>0.012 0.015</td>
<td>−0.031 0.010</td>
<td>0.056 0.019</td>
<td>0.041 0.020</td>
<td>0.024 0.015</td>
<td>−0.073 0.030</td>
</tr>
<tr>
<td><strong>Predictor (14h00)</strong></td>
<td>0.039 0.015</td>
<td>−0.003 0.010</td>
<td>0.091 0.019</td>
<td>0.048 0.020</td>
<td>0.036 0.015</td>
<td>−0.066 0.030</td>
</tr>
<tr>
<td><strong>Predictor (16h00)</strong></td>
<td>0.032 0.015</td>
<td>−0.007 0.010</td>
<td>0.096 0.019</td>
<td>0.046 0.020</td>
<td>0.042 0.015</td>
<td>−0.070 0.030</td>
</tr>
<tr>
<td><strong>Predictor (bedtime)</strong></td>
<td>0.040 0.015</td>
<td>−0.015 0.010</td>
<td>0.076 0.019</td>
<td>0.031 0.020</td>
<td>0.045 0.015</td>
<td>−0.060 0.030</td>
</tr>
<tr>
<td><strong>Sex (women)</strong></td>
<td>−0.142 0.094</td>
<td>−0.145 0.093</td>
<td>−0.152 0.093</td>
<td>−0.159 0.093</td>
<td>−0.155 0.094</td>
<td>−0.153 0.093</td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td>0.016 0.004</td>
<td>0.016 0.004</td>
<td>0.016 0.004</td>
<td>0.016 0.004</td>
<td>0.016 0.004</td>
<td>0.017 0.004</td>
</tr>
<tr>
<td><strong>Smoking</strong></td>
<td>−0.011 0.010</td>
<td>−0.011 0.010</td>
<td>−0.012 0.011</td>
<td>−0.008 0.010</td>
<td>−0.011 0.011</td>
<td>−0.011 0.010</td>
</tr>
<tr>
<td><strong>Alcohol</strong></td>
<td>−0.017 0.008</td>
<td>−0.016 0.008</td>
<td>−0.017 0.008</td>
<td>−0.018 0.008</td>
<td>−0.017 0.008</td>
<td>−0.017 0.008</td>
</tr>
<tr>
<td><strong>Physical activities</strong></td>
<td>0.005 0.022</td>
<td>0.009 0.021</td>
<td>0.008 0.021</td>
<td>0.002 0.021</td>
<td>0.007 0.021</td>
<td>0.007 0.021</td>
</tr>
<tr>
<td><strong>Psychotropic medication</strong></td>
<td>0.179 0.138</td>
<td>0.181 0.138</td>
<td>0.166 0.138</td>
<td>0.174 0.136</td>
<td>0.166 0.138</td>
<td>0.179 0.138</td>
</tr>
<tr>
<td><strong>BMI</strong></td>
<td>−0.005 0.006</td>
<td>−0.004 0.006</td>
<td>−0.005 0.006</td>
<td>−0.004 0.006</td>
<td>−0.005 0.006</td>
<td>−0.005 0.006</td>
</tr>
<tr>
<td><strong>Random part and fit</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\sigma^2$ (workplaces)</td>
<td>0.026 0.020</td>
<td>0.022 0.019</td>
<td>0.024 0.196</td>
<td>0.028 0.020</td>
<td>0.024 0.020</td>
<td>0.025 0.020</td>
</tr>
<tr>
<td>$\sigma^2$ (workers)</td>
<td>0.602** 0.049</td>
<td>0.603** 0.049</td>
<td>0.608** 0.049</td>
<td>0.588** 0.048</td>
<td>0.607** 0.049</td>
<td>0.602** 0.049</td>
</tr>
<tr>
<td>$\sigma^2$ (samples)</td>
<td>0.544** 0.013</td>
<td>0.545** 0.013</td>
<td>0.542** 0.013</td>
<td>0.544** 0.013</td>
<td>0.545** 0.013</td>
<td>0.546** 0.013</td>
</tr>
<tr>
<td>Model $\chi^2$ (df)</td>
<td>5297.43 (18)</td>
<td>5338.10 (18)</td>
<td>5354.77 (18)</td>
<td>5301.20 (18)</td>
<td>5333.54 (18)</td>
<td>5332.72 (18)</td>
</tr>
</tbody>
</table>

*Note: *p < 0.05, **p < 0.01.*
U/ml. Samples that show a reading of more than 400 U/ml or are too low to read and are automatically re-run at a different dilution. Absolute range of assay is 3.1 to 423 U/ml.

2.3. Psychosocial measures

2.3.1. Mental health

Psychological distress was measured with the General Health Questionnaire short-form 12-items (McDowell and Newell, 1996). Each item (e.g. unable to concentrate on whatever you're doing, feeling constantly under strain) was measured on a 4-point scale (1 = better than usual, 4 = much worse than usual). As recommended (McDowell and Newell, 1996), items were binary recoded (1–2 = 0, 3–4 = 12) before being summed (α = 0.85).

Depression was measured with the Beck Depression Inventory 21-items (Beck et al., 1996). Each item (e.g. feeling sad, suicidal thoughts) was measured on a 4-point scale with different ordinal classifications according to the symptom severity evaluated. Sum scores were then used (α = 0.91).

Emotional exhaustion was assessed with five items from the Maslach Burnout Inventory (MBI) general survey (Schaufeli et al., 1996). Only the emotional exhaustion sub-scale (α = 0.90) was retained because it is widely viewed as the most representative burnout construct (Shirom, 2003). While the additional sub-scales of cynicism and professional efficacy can also be used, a recent study using the current sample showed that emotional exhaustion is indeed the sub-scale most correlated with workers’ diurnal stress hormones (Marchand et al., 2014b).

2.3.2. Work factors

Skill utilisation, decision authority, psychological demands, and social support from colleagues and the supervisor(s) were derived from the Job Content Questionnaire (Karasek et al., 1985). Responses were based on a 4-point Likert scale (strongly disagree–agree). Skill utilisation consisted of six items (ex: my job requires that I learn new things, α = 0.80). Decision authority contained three items (ex: my job allows me to make a lot of decisions on my own; α = 0.79). Psychological demands were measured by nine items (ex: my job requires working very fast; α = 0.73). Social support from colleagues was measured with four items (ex: the people I work with are helpful in getting the job done; α = 0.83), and four items for the support from the supervisor (ex: my supervisor is helpful in getting the job done; α = 0.89).

Physical demands, recognition, career perspectives and job insecurity were derived from the Effort-Reward Imbalance questionnaire (Siegrist and Peter, 1996; Siegrist et al., 2004). Responses were based on a 4-point Likert scale (strongly disagree–agree). Physical demands were based on a single item (my job is physically demanding). Recognition contained six items (ex: I receive the respect I deserve from my superiors; α = 0.82), career perspectives 4 items (ex: my job promotion prospects are poor, reverse coding; α = 0.69), and job insecurity 2 items (ex: I have experienced or I expect to experience an undesirable change in my work situation; α = 0.65).

Abusive supervision was measured with 15 items from Tepper abusive supervision questionnaire (Tepper, 2000) (ex: tells me my thoughts or feelings are stupid; α = 0.91). Responses are based on a 5-point scale (1 = I cannot remember him/her ever using this behavior with me, 6 = he/she uses this behavior very often with me).

Interpersonal conflicts during the previous 12 months contained five items from Harvey and colleagues questionnaire (ex: have you had an argument with someone; α = 0.80). Response are based on a 4 point scale (1 = never, 4 = very often) (Harvey et al., 2006).

Workplace harassment was measured using three 4-point Likert indicators (1 = never, 4 = very often) from the Quebec Health-Social Survey. Over the course of the previous year, the respondent was asked to indicate whether he or she had been subjected to physical violence or intimidation and/or been the object of unwelcome remarks or actions of a sexual nature in the workplace.

2.3.3. Non-work factors

Marital status was coded 1 for people married or living in a civil union and 0 for others. Parental status measured the presence (yes/no) of minor children in the household. Household income was determined using a 10-point ordinal scale (1 = less than $20,000, 12 = $120,000 and more). Marital strains was assessed with four binary items (false–true, no–yes) taken from Wheaton (ex: your partner doesn’t understand you; α = 0.70) (Wheaton, 1994). Parental strains had three items (false/true) taken from Wheaton (ex: a child's behaviour is a source of serious concern to you; α = 0.60) (Wheaton, 1994).

Work-family conflict was measured with the Gutek et al. (1991) instrument with responses based on a 5-point scale (strongly disagree–strongly agree) that distinguish both directions of the conflict: (1) work-to-family spillover (four items, ex: my work takes up time that I’d like to spend with family/friends; α = 0.79) and (2) family-to-work spillover (4 items, ex: I'm often too tired at work because of the things I have to do at home; α = 0.74).

Social support outside the workplace was based on four items (no–yes) from the Statistics Canada National Population Health Survey asking respondents if they had a confidant, someone to count on in a crisis situation, someone to count on when making personal decisions, someone who makes them feel loved and cared for. The scale was dichotomised as low (0 = 0–3) and high (1 = 4) social support in order to correct for high asymmetry.

2.4. Covariates

Based on previous reports identifying key confounders related to diurnal cortisol (Chida and Hamer, 2008) and sAA (Nater et al., 2007), our statistical analyses were similarly adjusted for the following covariates: self-reported time of awakening, sex, age, cigarette smoking, alcohol consumption, regular physical activity, psychotropic drug use, and body mass index.

Sex was coded as binary (0 = men, 1 = women) and age was measured in continuous years. Smoking was recorded continuously as the number of cigarettes smoked per day. For alcohol, respondents indicated the number of drinks they had on each of the seven days during the week preceding the questionnaire administration. Alcohol intake was measured by summing the number of days consumed daily (standard Canadian drink of 13.6 grams of alcohol equivalents for beer, wine, and spirits) over the preceding week. Physical activity over the last three months was measured as the frequency of physical activities performed for more than 20 min. Respondents indicated this frequency on a 7 point Likert-type scale (1 = never, 7 = four times and more a week). Prescribed psychotropic drugs over the last month were dichotomously coded (0 = non-user and 1 = user) for at least one of the following: tranquilizers, antidepressants, codeine-demerol-morphine, and sleeping pills. Body mass index (BMI) was computed as weight (kg) divided by height squared (m)².

2.5. Statistical analysis

Comparison of respondents (n = 401) and non-respondents (n = 642) was first carried out to evaluate possible biases. Compared to non-respondents, respondents had a higher level of skill utilisation (p < 0.01), but lower levels of physical demands (p < 0.01), abusive supervision (p < 0.01), interpersonal conflicts (p < 0.05), harassment (p < 0.01), smoking (p < 0.01), alcohol intake (p < 0.05), and BMI (p < 0.01).

Compliance to the salivary collection protocol was evaluated using logbook entries and assessing minutes deviation from
scheduled times as previously reported (Marchand et al., 2014a; Marchand et al., 2014b). Specifically, analyses ascertained adherence to saliva collection scheduling using a liberal criterion set within a 30 min deviation. Note that this cannot be done for the first awakening and the last bedtime samples. The proportion of compliant participants, that is, those who respected saliva scheduling within 30 min or less, are as follows: +30 min after awakening (98.5%), 14h00 (72.6%), and 16h00 (64.8%). Total adherence was calculated as complete conformity to all three sampling time-points, which was the case for 60.9% of participants. Overall, total adherence was not associated with diurnal sAA variation (p > 0.931).

In assessing missing data for questionnaires and a total of 4010 saliva samples, 3806 samples were analyzable in conjunction to complete information on mental health, work, and non-work variables. The final sample of workers remaining was N = 395.

Our main analysis applied multilevel regression models (Goldstein, 1995) to analyze sAA concentrations nested in the following levels: (i) sAA measurements at each occasion within a day at level-1, (ii) workers at level-2, and finally (iii) workplaces at level-3. In this manner, variations within a day were embedded within each unit of the second level, followed by variations between participants that were then embedded within each unit of the third level, and finally the variation between workplaces. This approach allows the full range of data to be taken into account when estimating cortisol variations across each level of the hierarchical data structure that furthermore inherently incorporates individual and contextual variability within each sequential level of analysis.

In addition, the regression models adjusted for self-reported time of awakening, followed by four time of the day dummy coded variables measuring cortisol concentration at occasion-2 (30 min after awakening), at occasion-3 (2:00 PM), at occasion-4 (4:00 PM), and at occasion-5 (bedtime), as well as two dummy coded variables indexing cortisol concentrations for workday-2 compared to the workday-1. Statistical analyses were also controlled for sex, age, smoking, alcohol, physical activities, psychotropic drug use, and BMI.

The model parameters were estimated by the restricted iterative generalized least-squares method (RIGLS) using MLwiN Statistical Software version 2.26. To reduce the asymmetrical distribution and to improve the convergence of the estimation algorithm, sAA concentrations in ug/dl were log-transformed (natural logarithm). The main effect model was first estimated, followed by a series of interaction tests between mental health, work, and non-work measures and time of the day. Finally, interactions with sex were estimated to assess potential sex moderating effects. The significance of individual regression coefficients was evaluated using a bilateral Z test, and the probability of rejection of the null hypothesis was set at p < 0.05. The joint contribution of the variables was assessed by means of a likelihood ratio test that followed a χ² distribution with the degrees of freedom equal to the number of additional parameters in the model. Interactions were tested using χ² with rejection of the null hypothesis at p < 0.05.

3. Results

Table 1 reports raw sAA concentrations and each psychosocial determinant used in our study sample. Compared to men, women showed lower sAA concentrations 30 min after awakening and at 14h00. Women had higher level of psychological distress, depressive symptoms, and emotional exhaustion symptoms. They also self-reported lower skill utilisation, decision authority, physical demands, working hours, and irregular work schedule. Compared to men, women used more psychotropic drugs, they smoked less, had a lower number of alcoholic drinks per week and lower BMI.

![Fig. 1](link-to-figure) illustrates the distribution of raw sAA concentrations between men and women averaged across sampling occasions. For both sexes, sAA declined from awakening to 30 min after awakening, and then increased until 14h00. After 14h00, sAA declined again until bedtime. Overall, men seem to have higher sAA concentrations than women, but the difference is most notable 30 min after awakening (p < 0.05), 14h00 (p < 0.05), and marginally at 16h00.

Table 2 presents the results of multilevel modelling testing for main effects and interactions by sampling occasion. Only psychological demands and interpersonal conflicts showed a significant main effect on sAA. Sampling occasions interacted significantly with (1) psychological distress, (2) psychological demands, (3) support from colleagues, (4) interpersonal conflicts, (5) job recognition, and (6) job insecurity that are directionally described next in turn. Table 3 presents parameter estimates of multilevel modelling testing for significant interactions of Table 2.

First, psychological distress was negatively associated with sAA concentrations at awakening, but otherwise positively associated with sAA concentrations from 14h00, 16h00, and bedtime. Second, psychological demands were only negatively associated with sAA concentrations 30 min after awakening. Third, support from colleagues was negatively associated with sAA at awakening, but then positively associated with sAA concentrations +30 min after awakening, 14h00, 16h00, and bedtime. Fourth, interpersonal conflicts were negatively associated with awakening sAA concentrations, and positively associated with sAA concentrations +30 min after awakening, 14h00, and 16h00. Fifth, job recognition was positively associated with sAA concentrations at 14h00, 16h00, and bedtime. And sixth, job insecurity was negatively associated with sAA concentrations +30 min after awakening, 14h00, 16h00, and bedtime.

The last series of models estimated the extent to which sex interacts with mental health symptoms, work and non-work factors and time of the day. No significant interaction effects were found.

4. Discussion

The aim of this study was to identify which factors related to workers’ mental health, workplace, and non-work life would show the strongest correlations to diurnal sAA. We replicate and expand the findings of Nater et al. (2007) who showed a distinct diurnal profile of sAA in young university sample. We can confirm that this sAA pattern is characterized by sharp decreases +30 min after awakening followed by rises over the course of the day with only a small decrease thereafter before bedtime. Unlike Nater et al. (2007), peak levels are attained closer to 14h00 rather than at around 16h30. Critically, this study identifies additional psychosocial determinants of sAA in work and non-work contexts. Given the relative novelty of investigating sAA as a stress-sensitive biomarker, we offer the following discussion in the hopes of helping to guide future research.

Despite evidence to the contrary (Yamaguchi et al., 2006), the consensus among human studies is that sAA shows a unique diurnal profile (Rantanen and Meurman, 2000). Interestingly, this profile is a mirror image of the diurnal profile observed for salivary cortisol. Upon awakening, the cortisol awakening response represents a normal surge in cortisol levels reaching maximal concentrations approximately 30 min after awakening (Pruessner et al., 1997). This surge is followed by gradually declining cortisol concentrations throughout the day as pulsatile secretion decreases in amplitude and frequency with the nadir usually occurring around midnight (Clow et al., 2010). sAA shows an opposite pattern using the time-points we often use to assess diurnal cortisol. This oppositional trajectory between salivary cortisol and sAA might represent a circadian compensatory drive related to allostatic mechanisms (Juster et al., 2011; Karatsoreos and McEwen, 2014) that must be
assessed further using multiple biomarkers. Moreover, the inconsistent diurnal profiles of SAM-axis products (Linsell et al., 1985; Scholl et al., 1997) is not clearly correlated with sAA, representing an additional layer of complexity to be elucidated.

Sex differences revealed that men had occasionally higher sAA than women (see Fig. 1). By contrast, sex did not interact with mental health, work, and non-work factors nor at specific diurnal time-points in adjusted models for work and non-work stressors as well as numerous covariates. Previous studies have similarly shown mixed sex differences in sAA concentrations. While Nater et al. (2007) found no sex differences, men’s sAA tended to show a somewhat smaller linear increase and curvature over the diurnal course due to age. Indeed, age is an important factor that modulates sAA (Strahler et al., 2010a; Strahler et al., 2010b). By contrast, in a well-powered sample of nurses (N = 215), Wingenfeld et al. (2010) showed that women manifested higher sAA in the evening than did men. These authors contend that mixed sex differences in the literature may be due to restricted sample size. Given that our sample size is 5 times and 2 times larger than the studies respectively conducted by Nater’s and Wingenfeld’s groups, we provide some additional evidence to guide this debate.

As far as mental health is concerned, only psychological distress was related to sAA concentrations. Higher levels of psychological distress was associated with lower sAA concentrations at awakening, but high values at 14h00, 16h00, and bedtime. This is a sign that distressed workers distinct sAA profiles over the day consistent with the literature. Similar results were obtained on the same dataset using salivary cortisol. Specifically using the same sample, distressed workers had higher cortisol secretions at awakening, but lower values at 14h00, 16h00 and bedtime (Marchand et al., 2014a). Therefore, this convergence demonstrates that subjective distress is associated to both sAA and cortisol secretory patterns. Before we can arrive at any conclusions as to whether researchers should collect one or the other, it will be important to identify whether sAA predicts diseases associated with SAM-axis dysregulations.

Several work characteristics were associated to diurnal sAA concentrations in our study. Specifically, psychological demands, social support from colleagues, interpersonal conflicts, job recognition, and job insecurity were significantly associated with sAA at specific moments during the day. All these work factors showed negative associations with morning sAA concentrations and positive associations with afternoon and evening sAA concentrations. This is not uncharacteristic of the occupational health literature that is equally riddled with multi-directional findings. As such, our data must be interpreted as preliminary exploration that will need to be confirmed. Nevertheless, the directionality of these findings provide some insights into how workplace characteristics modulate sAA secretory patterns.

Our study is inconsistent with a similar investigation using overlapping workplace and psychosocial factors among 174 German middle managers – or “sandwich-position” – working in an industrial production line (Limm et al., 2010). This comprehensive study divided workers according to high and low self-perceived stress reactivity and requested saliva sampling upon awakening, +30-min and +60-min thereafter as well as set times at 8h00, 11h00, 15h00, and 20h00; however, no significant findings were detected. The differences between studies could be explained by the categorical or continuous manner in which psychometric information is associated with sAA. Given that our studies are both cross-sectional snapshots, we cannot address the prospective significance of our findings that remain purely correlational in nature.

Lastly, our findings are in stark contrast to the absence of associations with non-work characteristics. More broadly in the work stress literature, it is increasingly acknowledged that non-work sources of stress influence psychological distress like living in a couple, experiencing marital and parental strains, and work-family conflicts (Marchand et al., 2015). As will be described in greater detail below, this lack of association with non-work related factors may be explained by the fact that diurnal sAA was sampled during the work week and largely within workplace contexts. Future research assessing weekend patterns or demarcating differences between work and home concentrations of sAA could potentially unmask hidden effects or interactions of non-work characteristics.

4.1. Strengths and limitations

Several methodological considerations warrant discussion. This study is comprehensive in our extensive intra-individual assessment of sAA over several days while controlling for numerous potential confounders at multiple levels of analyses nested across time, individuals, and workplaces. It is also noteworthy that our error bars (Fig. 1) are quite small by comparison to previous studies with less power. Despite this strength and our respectable sample size, it is possible that the focus on working days rendered our findings to be more aligned with workplace characteristics by virtue of our study design. Indeed, the only non-work characteristic identified was a marginal trend among sAA concentrations and parenting a minor child. As described above, this is not to say that non-work characteristics are not associated with sAA. Moreover, the internal consistency of non-work and work psychometric characteristics are not different. It is possible that our sampling time-points over-representing working days biased our ability to detect non-work modulation of sAA. Therefore, we cannot presume that workplace characteristics are the only factors associated with sAA.

This study was well controlled for using covariates expected to be meaningful. Based largely on the psychoneuroendocrine literature on salivary cortisol, we decided to include a broad array of potential confounders that included self-reported time of awakening, sex, age, smoking, alcohol intake, physical activity, psychotropic drug use, and body mass index. It is noteworthy that none of these covariates were significant in our multi-level regressions. This may suggest that the confounders of the HPA-axis are not the same as those influencing salivary secretion of sAA. This will of course need to be confirmed or not. As standard guidelines are being established for circadian profiles of other stress biomarkers (e.g., cortisol awakening response (Stalder et al., 2015)), it will become essential to do the same for sAA. Informed by such gold standards, a major limitation of our study is the lack of electronic monitoring of saliva sampling that ensure participant compliance in psychoneuroendocrine field research (Kudielka, 2012). Another limitations is the absence of sex hormone data that can otherwise modulate stress biomarkers (Juster et al., 2016).

The first comprehensive study assessing the determinants of diurnal sAA proposed that confounding factors might additionally include (1) essentially anything that activates the SAM-axis like physical exercise and psychosocial stress, (2) things that affect salivary production like eating and drinking; and (3) those that modulate other stress systems like sex, age, sleep, reproductive factors, and time of awakening (Nater et al., 2007). Given that sAA is still a relative new kid on the stress block, it will be essential that future studies identify key factors that mediate and/or moderate its functioning. Despite general limitations due to small sample sizes in other studies, it has been argued that one of the advantages of sAA is that it is “relatively robust” (Wingenfeld et al., 2010) against the confounding effects of constitutional differences.

Relatedly in our default “cortisol-centric” perspective, the diurnal time-points selected were based on meaningful diurnal time-points identified in the salivary cortisol literature. Indeed, the diurnal course of sAA does not appear to follow the same trajectory as salivary cortisol. Researchers must continue to search for alternative time-points that capture clinically significant activities akin to other stress biomarkers (e.g., cortisol awakening response). More-
over, it will be essential to further understand how sAA correlates to other inter-connected biological systems.

5. Conclusions

To reiterate, the inability to reliably measure SAM-axis activities in saliva means that it is limited to collection via invasive blood sampling or systemic output through urine (Nater et al., 2013). We provide a replication of a seminal report in this field (Nater et al., 2007) and show that several work stress characteristics are correlated with diurnal sAA. Similar to salivary cortisol, we cannot advance without taking basal functions into consideration even if our research interests center on pharmacological challenge or stress reactivity paradigms. By carefully controlling for numerous confounders in a large sample of diverse workers, we contribute to the growing literature linking diurnal sAA to distressing psychosocial contexts.

In conclusion, psychological demands, support from colleagues, interpersonal conflicts, job recognition, and job insecurity appear to be associated with diurnal sAA. Linking subjective evaluation of work stressors with physiological profiles clearly helps to identify what are the most important workplace stressors employers need to consider in any workplace intervention devoted to the improvement and prevention of workers’ disabilities. Future research will need to further elucidate the mechanisms whereby sAA interacts with other established stress-sensitive biomarkers in the context of acute and chronic circumstances.

Contributors

Alain Marchand, Pierre Durand and Sonia Lupien designed the study and wrote the protocol. Robert-Paul Juster managed the literature. Alain Marchand undertook the statistical analysis. Alain Marchand and Robert-Paul Juster wrote the first draft of the manuscript. All authors contributed to and have approved the final manuscript.

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Conflict of interest statement

None declared.

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