Identification of Biomarkers Related to Perceived Stress Scale-10 and the Evaluation of the Survey Components

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Abstract

Perceived stress surveys have been used by health specialists for decades to provide quick assessments of the degree of stress during a specified time, and have even been shown to be predictive of response to stressors or treatment outcomes. While these surveys have become an essential tool to evaluate the psychological state of individuals as a result of traumatic events or health concerns, very little research has related known biomarkers of stress to survey results. Identification of biomarkers associated with perceived stress scale scores, and even specific survey questions, provides important supplemental information for both practitioners and researchers using these surveys, and could offer a means to enhance interpretation of survey responses from a biological perspective. In this study, subjects completed a commonly accepted PSS-10 (Perceived Stress Scale-10) survey and provided corresponding blood samples. To identify biomarkers associated with perceived stress scores, plasma was analyzed for a variety of biomarkers. Adrenocorticotropic Hormone (ACTH) was identified as a key biomarker, with concentrations significantly different between low and high perceived stress subjects. The ten questions of the PSS-10 were further dissected to understand the degree of contribution each question offers to the overall stress assessment. ACTH again, along with cortisol and insulin were observed to be associated with scores of individual questions, highlighting certain questions as the driving forces of the overall score. An understanding of the impact each individual question possesses may be essential to acquiring an accurate assessment of perceived stress.

Keywords: Adrenocorticotropic Hormone (ACTH); Biomarkers of perceived stress, Cortisol; Insulin; Perceived Stress Scale-10 (PSS-10)

Introduction

Dynamic physiological, behavioral, and biochemical changes are anticipated outcomes of stress, triggered by external demands overwhelming an individual's ability to cope [1]. The degree of stress can have a considerable negative or positive impact on performance [2], mood [3], and even health [4]. Evidence suggesting stress levels may prompt or alter biochemical responses [5], and even epigenetic mechanisms [6], offers a link between stress and the progression of mental or physical diseases. Monitoring stress levels of patients may be an important component of treatment or recovery, and stress surveys are one of the frequently used methods to make such assessments. Hospital Anxiety and Depression Scale (HADS) [7], Perceived Stress Scale (PSS) [8], State Trait Anxiety Inventory (STAI) [9], and General Health Questionnaire (GHQ) [10] are several notable stress assessment tools designed to extract and assemble pertinent information indicative of stress.

While stress scales are informative, there are limitations in the various ways questions may be interpreted, emphasizing the importance of appropriately selected and constructed questions [11]. Inevitable and uncontrollable factors, such as the mood and condition of the subject, may additionally influence the manner by which questions are read and answered [12]. As such, further investigation of biomarkers related to perceived stress scales provides insight regarding the accuracy and reliability of perceived stress scales. Well known biomarkers of stress, such as cortisol [13], cytokines [14], and catecholamines [15] have been reported to be correlated to scores of perceived stress [16], and potentially even predictive of varying degrees of stress and stress-related outcomes [17].

The PSS-10 (Perceived Stress Scale-10) is a specific stress survey which provides a means to measure self-evaluated stress [18], and is a widely employed tool that has been used to assess the psychological impact contributing to experienced stress [19]. The questions of the PSS-10 are formed to assess stress levels accumulated in the last month. Therefore, significant biomarkers detected in blood samples are assumed to result from a collection of pre-existing stress instead of a single stressful event. Perceived stress scales serve to quickly appraise the degree of stress under demanding and traumatic conditions [20,21], gauge the effectiveness of treatments aimed to reduce stress [22], and investigate the relationship between the level of stress and behavioral patterns or health disorders [23,24]. In the present study, the PSS-10 survey is examined in whole, but also dissected in order to identify unique biomarkers related to each question individually. Comprised of 10 questions (See PSS-10 questions in table 1), the survey is designed to incorporate both positive and negative approaches of evaluation. Factors such as the ability to maintain control over stressful events, along with the positive and negative emotions involved in coping with stress are addressed in the PSS-10. Questions 4,5,7 and 8 address positive factors anticipated to reduce perceived stress, while questions 1,2,3,6,9 and 10 target negative aspects contributing to stress. An output of numerical scores between zero and 40 allows for simple comparison or differentiation among subjects participating in the same study.
Examination of individual questions of the survey may indicate which types of questions offer significant contribution toward generating the overall PSS-10 score.

Table 1: 10 questions of the PSS-10 survey supplied to the subjects. The 10 questions from the PSS-10 survey provided by Cohen et al., are presented in this table. The italicized questions (Q4,5,7 and 8) are positively correlated to PSS-10 results. The intent of this study is not to ascertain biomarkers after a controlled exposure to stressors, but to identify and examine biomarkers related to self-perceived stress. Over 20 endogenous biomarkers, including cortical, insulin, adrenocorticotropic hormone, testosterone, epinephrine, and cytokines were selected for this study. A full list of the biomarkers is detailed in the discussion section. This selection of biomarkers focuses on general indicators related to stress and the response to stress (i.e., cortisol [25], ACTH [26], epinephrine and norepinephrine [27]), as well as key regulators of basic cellular activities (i.e., glucose, insulin, glutathione, adenosine triphosphate, chloride). Considerably low or high levels of these biomarkers may describe the degree of stress [28].

Results

Biomarkers of overall PSS-10 scores

Analysis of overall PSS-10 scores in relation to biomarkers found in samples of plasma, highlighted ACTH as a potential indicator of perceived stress levels, as levels of plasma ACTH were significantly decreased in subjects with lower overall PSS-10 scores. Mean ACTH concentrations of subjects in low and high perceived stress score groups were 18.4 ± 1.3 pg/mL (N=6) and 27.3 ± 3.2 pg/mL (N=9), respectively, p < 0.05 (Figure 1A).

Biomarkers of PSS-10 Question 2 (Q2) scores

Additional examination of each individual question comprising the PSS-10 revealed perceived stress scores of select questions were considerably related to levels of certain plasma biomarkers. In specific, plasma cortisol was identified to be distinct between the group of subjects with higher scores for Question 2 (Q2) and the group with lower scores. Mean cortisol concentrations of subjects in low and high perceived stress groups were 61.3 ± 8.8 pg/mL (N=9) and 35.8 ± 6.4 pg/mL (N=6), respectively, p < 0.05 (Figure 2).
Discussion

As informative as these stress surveys may be, there is always a possibility of misrepresentation or misinterpretation. Identifying plasma biomarkers that are significantly different between subjects of low and high perceived stress may be a suitable and quantitative alternative, or supplement, to assessing perceived stress. In this study, various stress-related biomarkers (ACTH, cortisol, insulin, glucose, leptin, testosterone, cytokines [IL-1β, IL10, IL6, GM-CSF, IL5, IFNy, TNFa, IL2, and IL4], epinephrine, norepinephrine, adenosine triphosphate, aspartate aminotransferase, creatine kinase, chloride, and glutathione) were measured. Of the biomarkers measured, ACTH was identified to exhibit significantly distinct concentrations in plasma of subjects belonging in low and high perceived stress groups (Figure 1A). ACTH levels generally increase upon exposure to stressors [29,30] and have been linked to perceived stress [31]. In accordance with these previous findings associating ACTH with stress, subjects with higher perceived stress had significantly elevated plasma ACTH levels in this study. Although ACTH was the only statistically significant biomarker related to overall PSS-10 scores, further analysis of each individual question uncovered several other biomarkers.

The purpose of dissecting the survey and analyzing each question separately was to understand how and to what extent each question contributes to the overall stress assessment. Upon analysis of the individual questions, ACTH, cortisol, and insulin were found to be associated with several questions. The group of subjects that scored higher for Q5 had significantly greater levels of plasma ACTH (Figure 1B). ACTH concentrations were related to overall PSS-10 scores, a similar outcome between ACTH levels and individual question scores was also expected. These results suggest how a participant answers Q5 may be particularly important to assessing overall perceived stress. Along with Q5, three other questions (Q2, Q7 and Q9), offered insight regarding the connection between the survey results and related biomarkers. Q2 scores were related to plasma cortisol (Figure 2), while Q7 and Q9 scores were associated with plasma insulin levels (Figures 3A & B). Cortisol and insulin were only significantly significant when analyzed against individual questions, suggesting the importance of analyzing the results of individual question in conjunction with overall scores.

As a proposed hallmark of stress, cortisol is often measured to determine the degree of stress after demanding and taxing events [25]. A general increase in cortisol levels is expected in those that are more stressed [32], but there is also great variability in cortisol secretion [33]. In this study, the group of subjects that scored higher for Q2 (i.e., exhibited more perceived stress) had a lower average plasma cortisol than the group that had lower scores. The data suggest that cortisol levels may be lower in subjects that perceive higher stress since this survey question assesses an accumulation of perceived stress over a prolonged period of one month, instead of after one specific incident. In a previous study, cortisol secretion was suppressed in subjects who had experienced stress over an extended duration of time [34].

Insulin was an unexpected biomarker as it is not a central stress-related hormone, but nonetheless, has been linked to stress [35]. In this study, subjects with lower PSS-10 scores had higher insulin levels, which suggest subjects with lower insulin levels actually experienced more stress. In fact, stress hormones are able to strongly regulate, and in some cases, inhibit the release of insulin [36]. A more apparent relationship between the survey results and measured insulin levels concerns when the subject had last eaten. An increase in plasma insulin concentration normally occurs after meals [37], and a correlation between the time of last food intake and insulin levels is expected to be negatively related. In agreement with the anticipated results, the plasma insulin levels in these subjects were significantly (p < 0.05) and negatively correlated (r = -0.6524) to the time since they had last eaten. Subjects that had recently eaten, scored lower on the overall PSS-10 (i.e., lower Perceived Stress), suggesting factors such as insulin concentration may affect how stress surveys are completed. While insulin is related to both Q7 and Q9 (one positive and one negative, respectively), it is unclear how positive and negative questions are distinctly related to a shared biomarker. In this study, we can only identify which questions and biomarkers are related, but to fully understand how biomarkers are related to individual questions, additional research is necessary. There were a few limitations to this study. First, the sample size was not ideal, even though recruitment was attempted several times. Due to the small sample size, there were several questions (Q3,Q4,Q8,Q10) that were not analyzed because there weren’t definite low score and high score groups. For instance, for question 4 and 10, there was only one subject that belonged in the high score group, making it impossible to make any comparisons. With a bigger sample size, it may have been possible to analyze more individual questions and identify more biomarkers that may have been missed. The other biomarkers in our selection included glucose, leptin, testosterone, cytokines (IL-1β, IL10, IL6, GM-CSF, IL5, IFNy, TNFa, IL2, and IL4), epinephrine, norepinephrine, adenosine triphosphate, aspartate aminotransferase, creatine kinase, chloride, and glutathione. While these biomarkers were not significant in our analysis, many of them are strongly related to stress. Due to the limitations of a small sample, it is feasible that anticipated biomarkers were statistically eliminated. Nonetheless, this study demonstrates a significance in identifying biomarkers to assess perceived stress, as well as a benefit in analyzing individual components of the survey. A bigger sample size and a wider selection of biomarkers will be necessary for future studies.

The findings of this study enabled us to identify key biomarkers associated with perceived stress and relate them to results determined from a commonly used and accepted survey. Analysis of these pertinent biomarkers then uncovered select questions that may be essential to the interpretation of perceived stress. An improved
understanding of the perceived stress scale, from a biological perspective, may aid in the general assessment of stress and stress related outcomes.

Materials and Methods

Subjects

Students from the Air Force Reserve Officers’ Training Corps at West Virginia University were recruited to participate in this study. A total of 17 healthy subjects (15 male, 1 female), between the ages of 18-22, were enrolled. One male subject was excluded from analysis due to insufficient blood sample. Additionally, a female subject was excluded because we were only able to recruit one female. The protocol for this study was approved by the Institutional Review Board at West Virginia University and USAMRMC Office of Research Protections. All subjects provided written and informed consent to be willing test subjects prior to proceeding with the study. A detailed profile (Age, body fat percentage, exercise, tobacco and alcohol use, caffeine and food intake, and medication use) of each subject is included in table 2.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age</th>
<th>Body fat %</th>
<th>Exercise (days per week)</th>
<th>Tobacco</th>
<th>Alcohol (how often)</th>
<th>Caffeinated beverage (when, how much)</th>
<th>Time since last meal (hours)</th>
<th>Medication</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>22</td>
<td>13.5</td>
<td>6–7</td>
<td>No</td>
<td>Yes (weekends)</td>
<td>Lunch, 12 oz Coke</td>
<td>2.5</td>
<td>NA</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>10.2</td>
<td>4–5</td>
<td>Yes</td>
<td>Yes (1-2 times a week)</td>
<td>6 days ago, 1 can</td>
<td>0.5</td>
<td>Ibuprofen, Sudafed, Mucinex (once every 4 days)</td>
</tr>
<tr>
<td>3</td>
<td>19</td>
<td>20.9</td>
<td>2–3</td>
<td>No</td>
<td>No</td>
<td>2 days ago, 1 soda can</td>
<td>1.0</td>
<td>NA</td>
</tr>
<tr>
<td>4</td>
<td>19</td>
<td>6.1</td>
<td>2–3</td>
<td>Yes</td>
<td>Yes (once a month)</td>
<td>Months ago</td>
<td>4.0</td>
<td>NA</td>
</tr>
<tr>
<td>5</td>
<td>19</td>
<td>14.4</td>
<td>4–5</td>
<td>No</td>
<td>No</td>
<td>NA</td>
<td>0.75</td>
<td>NA</td>
</tr>
<tr>
<td>6</td>
<td>19</td>
<td>6.2</td>
<td>6–7</td>
<td>No</td>
<td>Yes (once every couple weeks)</td>
<td>A week ago, cup of coffee</td>
<td>2.0</td>
<td>NA</td>
</tr>
<tr>
<td>7</td>
<td>20</td>
<td>15.9</td>
<td>6–7</td>
<td>Yes</td>
<td>Yes (occasionally)</td>
<td>10:30 AM, cup of coffee</td>
<td>1.0</td>
<td>NA</td>
</tr>
<tr>
<td>8</td>
<td>19</td>
<td>12.5</td>
<td>6–7</td>
<td>Yes</td>
<td>Yes (2-4 times a month)</td>
<td>Morning, 6 oz coffee</td>
<td>1.5</td>
<td>Vitamin C supplement, Ibuprofen, Melatonin</td>
</tr>
<tr>
<td>9</td>
<td>18</td>
<td>17.9</td>
<td>2–3</td>
<td>No</td>
<td>Yes (Rarely)</td>
<td>Earlier that day, cup of tea</td>
<td>5.0</td>
<td>NA</td>
</tr>
<tr>
<td>10</td>
<td>20</td>
<td>13.7</td>
<td>6–7</td>
<td>No</td>
<td>No</td>
<td>Month ago, cup of coffee</td>
<td>2.0</td>
<td>NA</td>
</tr>
<tr>
<td>11</td>
<td>19</td>
<td>17.2</td>
<td>4–5</td>
<td>No</td>
<td>No</td>
<td>1-2 weeks ago, cup of coffee</td>
<td>5.5</td>
<td>NA</td>
</tr>
<tr>
<td>12</td>
<td>18</td>
<td>22.9</td>
<td>2–3</td>
<td>Yes</td>
<td>Yes (Once a week)</td>
<td>10:40 AM, 2 glasses of tea</td>
<td>0.67</td>
<td>Multivitamin, Advil</td>
</tr>
<tr>
<td>13</td>
<td>19</td>
<td>15.8</td>
<td>4–5</td>
<td>No</td>
<td>No</td>
<td>1 hour, 4 fl. Oz</td>
<td>1.0</td>
<td>NA</td>
</tr>
<tr>
<td>14</td>
<td>19</td>
<td>14</td>
<td>6–7</td>
<td>No</td>
<td>Yes (not often)</td>
<td>Morning, 20 fl. Oz coke</td>
<td>1.0</td>
<td>NA</td>
</tr>
<tr>
<td>15</td>
<td>21</td>
<td>21</td>
<td>4–5</td>
<td>Yes</td>
<td>Yes (5 nights a week)</td>
<td>30 min, 1 can of coke</td>
<td>2.0</td>
<td>NA</td>
</tr>
</tbody>
</table>

Table 2: Profile descriptions of all subjects.

Specifications of each subject involved in the study are assembled in this table. Age, body fat percentage, exercise, tobacco and alcohol use, caffeine and food intake, and medication use are presented in the table.

Perceived Stress Scale (PSS-10)

The subjects were directed to answer 10 questions pertaining to stress appraisal upon arrival to the research site. Overall perceived stress scores based on the questions of the survey and individual scores for each question were determined per published direction [18]. All questions were provided with possible answer choices of a 5-level Likert type scale (never, almost never, sometimes, fairly often, very often). Each answer was assigned a number rating (0 - 4) and summed up for all 10 questions to acquire the overall score. The number ratings for positively stated questions (items 4,5,7 & 8) were reversed.

Plasma sample preparation and analysis

Approximately one hour after the subjects completed the PSS-10 (between 8 and 8:30 PM), blood samples were acquired. A butterfly needle was used to draw blood samples into BD Vacutainer plasma preparation tubes (BD, Franklin Lakes, NJ) or BD Vacutainer plastic blood collection tubes coated with K2EDTA (BD, Franklin Lakes, NJ). Immediately after collection of the blood, the tubes were inverted 10 times, followed by centrifugation at 2500 RPM for 12 minutes to form the top plasma layer. The samples were stored in ice until they were transported to a BSL-2 lab at West Virginia University. To avoid freeze/thaw cycles, 200 μL aliquots of the plasma layer were removed from the blood tube and stored in cryogenic vials at -80ºC. An ELISA kit from ALPCO (Salem, NH, Catalog # 21-ACTHU-E01) was purchased to quantify ACTH. The intra-assay and inter-assay Coefficients of Variance (CV) for ACTH were less than 7% and 8%, respectively. Insulin was measured using a custom designed Milliplex kit obtained from EMD Millipore (Billerica, MA, Catalog # HMMHAG34K). The intra-assay and inter-assay CV for insulin were less than 16% and 25% respectively. Cortisol was assayed with an EIA kit from Cayman Chemical (Ann Arbor, MI, Catalog # 500360). The intra-assay and inter-assay CV for cortisol were less than 14% and 26%, respectively.

Statistical analysis

Maximum and minimum scores for the overall survey were 40 and zero, respectively, and four and zero for each individual question.
Subjects with overall scores between zero and ten were grouped as low stress subjects and those with overall scores above ten were identified as high-stressed subjects. Subjects with scores of 0 and 1 on individual questions were grouped as low stressed and those with scores above 2 were assigned to the high stressed group. Levels of relevant biomarkers found in initial samples of plasma were compared between the low stress and high stress groups via Welch-corrected t-tests using Prism V5 (Graphpad Software, San Diego, CA). Outliers were removed accordingly by the use of a Grubbs’ test.

References


