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The effects of psychological distress on salivary secretory immunity

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Abstract

Stress-induced impairments of mucosal immunity may increase susceptibility to infectious diseases. The present study investigated the association of perceived stress, depressive symptoms, and loneliness with salivary levels of secretory immunoglobulin A (S-IgA), the subclasses S-IgA1, S-IgA2, and their transporter molecule Secretory Component (SC). S-IgA/SC, IgA1/SC and IgA2/SC ratios were calculated to assess the differential effects of stress on immunoglobulin transport versus availability.

This study involved 113 university students, in part selected on high scores on the UCLA Loneliness Scale and/or the Beck Depression Inventory. Stress levels were assessed using the Perceived Stress Scale. Unstimulated saliva was collected and analyzed for total S-IgA and its subclasses, as well as SC and total salivary protein. Multiple linear regression analyses, adjusted for gender, age, health behaviors, and concentration effects (total protein) revealed that higher perceived stress was associated with lower levels of IgA1 but not IgA2. Perceived stress, loneliness and depressive symptoms were all associated with lower IgA1/SC ratios. Surprisingly, higher SC levels were associated with loneliness and depressive symptoms, indicative of enhanced transport activity, which explained a lower IgA1/SC ratio (loneliness and depression) and IgA2/SC ratio (depression).

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This is the first study to investigate the effects of protracted psychological stress across S-IgA subclasses and its transporter SC. Psychological stress was negatively associated with secretory immunity, specifically IgA1. The lower immunoglobulin/transporter ratio that was associated with higher loneliness and depression suggested a relative immunoglobulin depletion, whereby availability was not keeping up with enhanced transport demand.

Keywords

Immunoglobulin A; Stress; Depression; Loneliness; Saliva; Secretory Component; Immunoglobulin subclass; IgA1; IgA2

1. Introduction

Human and animal studies have provided convincing evidence that psychological stress may increase susceptibility to infection and infectious diseases (Engeland and Marucha, 2009; Moreira et al., 2008; Ojard et al., 2015; Pedersen et al., 2010). Approximately 95% of all infections start at mucosal surfaces such as the lining of the mouth, the respiratory and gastrointestinal tracts, and the eyes (Bosch et al., 2002; Castro-Sanchez and Martin-Villa, 2013; Mayer and Walker, 2005; Sato and Kiyono, 2012). These vulnerable soft tissues are protected by various antimicrobial proteins secreted by local exocrine glands, which constitute a first line of immunological defense (Mayer and Walker, 2005). A key immunological component in these secretions is secretory Immunoglobulin A (S-IgA) (Macpherson et al., 2008; Mantis and Forbes, 2010; Mantis et al., 2011). Studies have shown that S-IgA concentrations predict susceptibility to respiratory, oral, and aural infections (Lee et al., 2010; Nakamura et al., 2006; Tiollier et al., 2005). Hence, total S-IgA is considered an immunologically meaningful measure of mucosal host resistance (Macpherson et al., 2008).

The release of S-IgA is under strong neuroendocrine control, and acute stress studies have shown robust effects on S-IgA whereby its concentration typically increases (Bosch et al., 2002; Takatsuji et al., 2008; Trueba et al., 2012). This likely occurs due to increased release of antibody from B-lymphocytes and/or increased transport of IgA across the epithelium into saliva (Bosch et al., 2002). Evidence from chronic stress studies is less robust, although the balance of evidence indicates that protracted stress (e.g., caregiving) is associated with decreased levels of S-IgA (Bosch et al., 2002; Teeuw et al., 2004). For example, a study conducted by Phillips and colleagues involving two cohorts (total $N = 1282$), found that the experience of major stressful life events was associated with lower salivary IgA levels (Phillips et al., 2006).

There are several ways in which knowledge about the effects of chronic stress on S-IgA can be enhanced. For example, it remains unresolved if stress-induced alterations in S-IgA concentrations are primarily determined by effects on immunoglobulin production (by B-lymphocytes), or by altered transport of immunoglobulin to mucosal surfaces. To clarify, S-IgA concentrations are determined through a 2-phase process. First, B lymphocytes, which are present in the glandular tissues, produce and release IgA. This IgA is then transported through the glandular cell, via the transporter molecule Secretory Component (SC), into fluids such as saliva. It is the IgA-SC complex that forms S-IgA (Mora and von Andrian,

2008; Norderhaug et al., 1999; Sun et al., 2004). The transporter SC can also be secreted into mucosal secretions unbound to IgA, and salivary S-IgA and SC are partially independent indicators of B-cell IgA production and glandular transport capacity, respectively. By separately measuring S-IgA and SC, as well as their ratio, it is therefore possible to determine if the effects of stress are due to lower availability of IgA (i.e., decreased release from B-lymphocytes) or due to reduced glandular transport capacity.

A further limitation of the literature is that chronic stress studies have assessed total S-IgA only; however, this is a summary measure of two distinct subclasses, denoted S-IgA1 and S-IgA2 (Woof and Russell, 2011). Differentiating between these subclasses may be relevant because decreased salivary S-IgA1 levels, but not S-IgA2 levels, are associated with an increased risk of upper respiratory tract infections (Gleeson et al., 1999; Moreira et al., 2008; Nakamura et al., 2006). Interestingly, both acute stress and exercise have been found to selectively increase the salivary concentrations of IgA1 but not IgA2 (Bosch et al., 2001; Gleeson et al., 1999). These findings suggest that the secretion of the two subclasses is under differential control, and might therefore be differentially affected by protracted forms of psychological stress.

In light of the preceding discussion, the aim of the current investigation was to determine via ELISAs the salivary levels of S-IgA, IgA1, IgA2, SC, and the S-IgA/SC, IgA1/SC and IgA2/SC ratios in a sufficiently powered cohort selected for high and low levels of depressive symptoms and loneliness (Bosch et al., 2007). It was hypothesized that being more lonely, depressed, or stressed would result in decreased levels of S-IgA in saliva. Further, on the basis of acute stress studies (Bosch et al., 2001; Gleeson et al., 1999) it was anticipated that these effects would be stronger for S-IgA1 than S-IgA2. Finally, we quantified the S-IgA/SC ratio to examine the differential effects of these psychosocial factors on IgA production versus transport.

2. Methods

2.1 Participants

This present study was based on a sub-sample of a larger cohort (Bosch et al., 2007), a portion of which were selected to have particularly high scores on depression or loneliness measures. The Beck Depression Inventory short form (BDI-sf) and the Revised UCLA Loneliness Scale (UCLA-R) were administered to 1630 undergraduate Ohio State University students. This was done to increase the range of depression and loneliness scores in our study sample. Participants who scored in the upper or lower quintile on one or both of these questionnaires were invited to participate. These cut-offs were determined a priori on the basis of previous research in a similar population (Hawkley et al., 2005). For the UCLA-R, the inclusion criterion was a score of ≤28 or ≥46; for the BDI-sf, the inclusion criterion was a score of ≤1 or ≥8 (for further details see Bosch et al., 2007).

Based on the above criteria and available saliva samples, 113 undergraduate volunteers were included in this study (61 male, 52 female, mean age 20.7±2.9, range 17–33). Most participants were Caucasian (N=86, 76%). Exclusion criteria included the use of prescribed oral medication, reporting medical problems (e.g., inflammatory, endocrine) that have

known effects on immune or salivary gland function, oral health problems that required prompt treatment, and self-reported signs of infectious disease (e.g., cold) within 2 weeks before assessment. In addition, individuals receiving treatment/medication for systemic diseases, including diabetes, were excluded from our sample. Given the low age of participants (highest age 33 years) no periodontitis was observed in these individuals. Participants provided informed consent and received financial compensation for their time and effort. The study protocol was approved by the Institutional Review Board (IRB) of OSU where the study was conducted and by the IRB of the University of Illinois at Chicago (UIC) where samples were analyzed.

2.2 Procedures

Participants were scheduled between 10:00 am and 11:30 am to minimize the influence of circadian variation. In preparation for the study, participants were instructed not to engage in strenuous physical exercise, and to refrain from using alcohol or non-prescription drugs 24 hours before the experimental sessions. In addition, participants were instructed to abstain from caffeine the day of the experiment, to eat breakfast before 9:30 a.m., and to eat or drink nothing after that point (except for water).

Upon arrival at the School of Dentistry Research Clinic at OSU, participants filled out questionnaires to assess psychological traits and states, demographics, and health behaviors, and saliva was subsequently collected.

2.3 Questionnaires

Psychological measures—The Perceived Stress Scale (PSS) consists of 10-items on a 5-point Likert scale, with values ranging from 0 to 4; it assesses the degree in which situations in one's life over the past week are appraised as taxing and stressful. The questionnaire assesses thoughts and feelings about stressful events, control, overload, experienced stress, as well as how often the individual felt or thought in a stressful manner. The scale allows for the examination of stress pathology relationships and has been found to predict mental and physical health outcomes (Cohen et al., 1993). In this study, internal reliability for PSS was high (Cronbach's α=0.85).

The Beck Depression Inventory (BDI) Short Form is a widely used and well-validated self-report measure of depressive symptoms that boasts good reliability statistics, correlates well with other measures of depression, and predicts clinical ratings of depression. In the present study, internal reliability was high (Cronbach's α=0.84). The scale comprises 13 items assessing the severity of depressive symptoms experienced during the past week, on a four-point Likert scale, with values ranging from 0 to 3.

Loneliness, the negative emotional experience of being socially isolated, was measured using the Revised UCLA Loneliness Scale (UCLA-R). For this 20-item scale participants again responded using a four-point Likert scale format, with answers ranging from "never" to "often" and values ranging from 1 to 4. Scores correlate with measures of shyness and self-esteem, and high scores on this measure have been associated with alterations of immune system functioning (Hawkley and Cacioppo, 2003; Uchino et al., 1996). This scale

is acknowledged to have good psychometric properties (Russell et al., 1980). In this study, internal reliability was again high (Cronbach's α=0.94).

An occasional missing questionnaire item was replaced by interpolation (replacing item by item average); if more than two items were missing the entire questionnaire was excluded from the analyses (<2%).

Demographic and health behavior measures—Questionnaires were used to assess variables that might confound the relation between psychosocial factors and saliva secretory immunity. These included demographic factors such as gender, age, and ethnicity, as well as health practices and lifestyle variables such as alcohol, caffeine and tobacco use/consumption (described in (Kiecolt-Glaser et al., 2007)). Weight and height were measured with a digital scale using a height rod.

2.4 Saliva collection

Saliva was collected and assayed for all 113 participants. Participants were allowed to drink water until 10 minutes before the start of the collection trial, at which point participants were requested to rinse the mouth with water. Unstimulated saliva was collected by the "spitting method" (Navazesh, 1993). Unstimulated saliva was collected for 10 minutes in polypropylene cups kept on ice. After collection, saliva was homogenized using a vortex mixer and centrifuged (10,000 × g, 6 min, 4°C) to eliminate buccal cells and oral microorganisms. The clear supernatant was divided into small aliquots and stored at −80°C for later use.

2.5 Assessment of S-IgA, IgA subclasses, and Secretory Component

Total S-IgA, IgA1, IgA2 and SC determined by sandwich ELISA (Bosch et al., 2001). All assays used the same capture antibody that was directed against the Secretory Component (Clone GA-1, Sigma-Aldrich, Saint Louis, MO, USA). The high specificity and quality of this monoclonal antibody has been established in a multicenter WHO/NIUIS study (Mestecky et al., 1996) and corroborated in our laboratory by Western blot and ELISA. For detection, we used HRP-conjugated mouse anti-human monoclonal antibodies directed against S-IgA, IgA1, IgA2, or SC (all obtained from Nordic Immunology, Tilburg, Netherlands). These monoclonal antibodies have been determined to be of high specificity in several comparative studies (de Fijter et al., 1995; Mestecky et al., 1996). All standards (for S-IgA, IgA1, IgA2, and SC) were also obtained from Nordic. The intra-assay variability of each assay was <4% (inter-assay CV% < 6%). The ELISAs were all found to be of very high sensitivity, with upper detection limits (>200pg/ml) that were several thousand-fold above the lowest observed concentrations.

2.6 Determination of total salivary protein

Total protein was determined using the bicinchoninic acid assay (BCS - Bicinchoninic acid solution, Sigma, Saint Louis, MO, USA; Copper (II) sulfate solution, Sigma-Aldrich, Saint Louis, MO, USA). The intra-assay variability of each assay was < 5% (inter-assay CV% < 10%).

2.7 Statistical analyses

Linear regression analyses were performed to assess associations among psychosocial factors and salivary parameters with the following outcomes: S-IgA, IgA1, IgA2, SC, S-IgA/SC ratio, IgA1/SC ratio and IgA2/SC ratio. Initially, simple linear regression was used to establish whether there were associations between psychosocial factors and salivary levels of S-IgA, IgA1, IgA2 and SC. In subsequent hierarchical regression analysis, gender and age (demographics variables) were entered in model 1. After that, adjustments for smoking, alcohol and caffeine consumption (health behaviors) were performed in model 2. Values were corrected for salivary protein concentrations in model 3 to account for dilution effects. Finally, the psychological parameters (PSS, BDI or UCLA) were entered in model 4. The same analyses were performed on S-IgA/SC, IgA1/SC, and IgA2/SC ratio outcomes. Values of S-IgA, IgA1, IgA2 or SC that were >3 SD from the mean (<2% of samples) were considered as outliers, and removed from the analyses.

Because salivary flow rate (ml/min) was not recorded, total protein was used as a covariate for the analyses on S-IgA/SC, IgA1/SC, IgA2/SC ratios (Nyklicek et al., 2005). The rationale behind this approach is that dilution effects due to flow rate similarly affect total protein concentrations and S-IgA concentrations. Hence, any variance in S-IgA concentrations that occurs due to variations in flow rate is removed by controlling for total protein concentration.

3. Results

Means for scores on the psychosocial questionnaires, as well as for physiological measurements in saliva, are listed in Table 1. Correlations between the three psychosocial scales were significant ($p<.001$ for each) and ranged from .613 to .622 (Pearson's r).

Unadjusted regression analyses

Results of Univariate linear regression analyses are presented in Table 1— IgA1 was negatively associated with perceived stress ($\beta=-1.74$, $t=-1.454$, $R^2=0.063$, $p<0.01$), whereas SC was positively associated with both depressive symptoms ($\beta=69.71$, $t=2.95$, $R^2=0.084$, $p<0.01$) and loneliness ($\beta=17.08$, $t=2.48$, $R^2=0.059$, $p<0.05$). With regard to IgA ratios, there were negative associations between the IgA1/SC ratio and perceived stress ($\beta=-0.004$, $t=-2.51$, $R^2=0.061$, $p<0.05$), depressive symptoms ($\beta=-0.011$, $t=-2.467$, $R^2=0.060$, $p<0.05$), and loneliness ($\beta=-0.011$, $t=-2.467$, $R^2=0.060$, $p<0.05$). Depressive symptoms were additionally negatively associated with IgA2/SC ratio ($\beta=-0.010$, $t=-2.076$, $R^2=0.043$, $p<0.05$). No other significant relationships were found between IgA parameters and psychosocial measures.

Stepwise adjusted analyses

Results of Multiple linear regression analyses are presented in Tables 2–4— After adjustments for age and gender (model 1), smoking, alcohol and caffeine intake (model 2), and additional adjustment of total saliva protein concentration (model 3) perceived stress remained negatively associated with IgA1 level (ß=−2.09, t=−2.29,

ΔR^2=0.04, p<0.05), and the IgA1/SC ratio (ß=−0.003, t=−2.10, ΔR^2=0.04, p<0.05) after adjustment.

Loneliness was positively associated with SC level (ß=17.67, t=2.65, ΔR^2=0.06, p<0.01), and negatively associated with the IgA1/SC ratio (ß=−0.003, t=−2.69, ΔR^2=0.06, p<0.05) after adjustment for confounders. Depressive symptoms were positively associated with SC level (ß=61.88, t=2.61, ΔR^2=0.06, p<0.05), and negatively associated with both the IgA1/SC ratio (ß=−0.01, t=−2.42, ΔR^2=0.05, p<0.05) and the IgA2/SC ratio (ß=−0.01, t=−2.05, ΔR^2=0.04, p<0.05), also after adjustment for confounders (Tables 2 and 3). Table 4 displays a summary of these findings.

Group comparisons: BDI

We performed a follow-up analysis comparing individuals with BDI scores <8 and ≥8, as 8 is often used as a clinical cut-off (red flag) for suspected clinical depression on the BDI-sf (Beck et al., 1972; Beck et al., 1988). Comparing these two groups, individuals with higher depressive symptoms had significantly higher SC levels (1852 vs. 1106 μg/mL; p<.001), a significantly lower IgA1/SC ratio (p=.030), and close to significantly lower IgA/SC and IgA2/SC ratios (P<.10). These results reveal group differences that mirror the regression analyses and support the finding that depressive symptoms are associated with higher SC levels.

4. Discussion

The present study provides the most extensive analysis of secretory immunity to date, assessing total salivary S-IgA, S-IgA subclasses, antibody/SC ratios, and associations with measures of psychological distress. This also is the first study to investigate stress, depression, or loneliness in the context of S-IgA subclasses and their transporter molecule. Higher levels of perceived stress were related to lower IgA1 concentrations and a lower IgA1/SC ratio, but were unrelated to SC concentrations. This pattern of findings indicate that the lower IgA1 did not occur secondary to reduced transport activity by the saliva glands, but instead signify lower availability of IgA1 (Brandtzaeg, 2013). A possible explanation for this reduced availability is lower immunoglobulin production by local B-lymphocytes.

A lower S-IgA/SC ratio was also observed among those who reported higher levels of loneliness and depressive symptoms. However, analyses of the separate components of the secretory system suggest that this outcome involves a different mechanism. Individuals reporting higher depression or loneliness had higher SC levels, suggesting that transport activity was enhanced; however this was not matched by availability of S-IgA or its subclasses, resulting in lower antibody/SC ratios. In contrast, the lower IgA/SC ratio associated with higher perceived stress appeared secondary to lower immunoglobulin availability. Although both results indicated that immunoglobulin availability was not keeping pace with transport activity of the saliva glands, under conditions of stress this could be attributed to lower availability of IgA1 (i.e., a lower *absolute* IgA availability) versus a higher transport demand (i.e., a lower *relative* IgA availability) under conditions of loneliness or depression. In this latter instance, one may speculate that a mismatch between

availability and demand, when protracted, may eventually result in actual S-IgA depletion, as was observed for perceived stress and IgA1. Further research is needed to test this speculation. By statistically adjusting for salivary protein concentrations, we were also able to establish that the observed relationships between psychosocial measures and secretory immunity were not driven by diluting effects of salivary flow rate (Nyklicek et al., 2005).

The results from this study showed somewhat stronger associations for S-IgA1 than for S-IgA2. For example, IgA1/SC ratios were negatively associated with all three psychological measures, but IgA2/SC ratios were negatively associated only with depressive symptoms. Further, while perceived stress was linked to lower S-IgA, this was only significant for the S-IgA1 subclass. These observations are consistent with studies on acute stress and physical exercise (Bosch et al., 2001; Gleeson et al., 1999). For example, Bosch *et al.* found that stressors, such as mental arithmetic or viewing a distressing surgical video, increased total salivary S-IgA and IgA1, but not IgA2 (Bosch et al., 2001). At this point it is not clear how psychological stress exerts differential effects on salivary IgA subclasses. One possible explanation is a differential neuroendocrine sensitivity of subclass-producing B lymphocytes. For example, Coqueret *et al.* showed that incubation of beta-adrenergic agonists affected IgE production in stimulated B lymphocytes, but not production of other immunoglobulin classes (Coqueret et al., 1995). The observed selectivity for the IgA1 subclass is of potential clinical relevance, as this subclass appears to provide better protection to mucosal infections (e.g., upper respiratory tract) than IgA2 (Gleeson et al., 1999; Moreira et al., 2008; Nakamura et al., 2006).

At present it is not entirely clear what mechanisms underlie the observed effects. Experimental animal studies have shown that the mucosal IgA system is highly sensitive to neuroendocrine mediators and this is a needed focus for future studies. For example, dexamethasone administration in mice decreases SC levels in mucosal secretions (Wira et al., 1990). Similarly, repeated restraint stress in mice has been shown to reduce levels of intestinal S-IgA, although without reducing the number of lamina propria IgA+ plasma cells, indicating that the immunosuppressive effect was due to reduced IgA production per cell (Jarillo-Luna et al., 2007). In the same study, removal of the adrenal glands restored the production of IgA, whereas dexamethasone or epinephrine treatment reduced levels of intestinal S-IgA, indicating a moderating role of adrenal stress hormones on IgA levels. Studies using repeated restraint stress have also found reduced IgA levels in the mucosal (nasal) secretions of mice that related to elevations in epinephrine and corticosterone levels (Oros-Pantoja et al., 2011). Human neuroendocrine studies, although mostly observational, confirm these animal findings by showing depressing effects of long-term stress and cortisol on salivary IgA levels (Nieman et al., 2002; Papacosta and Nassis, 2011; Tsujita and Morimoto, 1999). The present findings add to this literature, suggesting that non-acute psychological stress reduces the IgA level available for transport, whereas psychosocial factors such as depressive symptoms and loneliness reduce IgA transport activity, into saliva. In the face of an immune challenge, such as infection, these alterations have potential negative consequences for mucosal immunity.

Some limitations of this study must be considered. The sample consisted of young and healthy adults and the generalizability to other groups needs to be established. Phillips and

colleagues consistently found a negative correlation between major stressful life events and salivary S-IgA in a large epidemiological study that consisted of two substantial age cohorts (middle aged and elderly) with a broad social-economic range (Phillips et al., 2006). Hence, there is reason for some confidence that the present findings in young adults are relatively stable across conditions of age and SES. It also remains to be determined to what degree these findings extend to other models of psychological distress, such as work stress or psychiatric disorders (e.g., clinical depression). Lastly, we controlled for salivary flow rate by correcting for changes in total protein concentrations. This approach follows the arguments that any diluting (or concentrating) effects of changes in saliva flow would affect all salivary constituents equally (Nyklicek et al., 2005). One drawback of this approach is a potential over-adjustment; this would weaken the observed associations, due to the fact that protein and secretory immunoglobulin concentrations may share some covariance other than changes in flow rate (Matos-Gomes et al., 2010; Nyklicek et al., 2005). However, the present analyses provided no indication of over-adjustment, since the associations reported were only minimally affected when protein concentrations were entered as a covariate.

In summary, the findings of the current study observed a consistent negative association between various measures of psychological distress and IgA/SC levels, indicative of a stress-related mismatch between IgA availability and transport. We speculate these associations may reflect the early stages of IgA depletion, as appeared the case for perceived stress which showed a negative association with IgA concentrations. Also consistent was the observation that associations were found to be stronger for the IgA1 subclass, and few significant associations were detected for IgA2 or the IgA2/SC ratio. Given the importance of S-IgA1 in immune defense at mucosal surfaces, and the fact that most infections are initiated at these surfaces, selective dysregulation of IgA1 availability may form a pathway by which psychological distress could increase susceptibility to local infections Further studies are needed to determine the exact mechanisms by which IgA subclasses are modulated by different psychosocial stressors, and to provide direct evidence for links with infectious disease.

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Highlights

- Psychological distress exerts differential effects across salivary IgA subclasses.
- Higher perceived stress relates to lower salivary IgA1, but not IgA2, levels.
- Loneliness and depression relate to higher levels of secretory component (SC).
- Results suggest that stress affects secretory immunity directly by modulating B-cell secretion of IgA.
- Loneliness and depression appear to modify secretory immunity indirectly by altering the IgA transport mechanism.

Table 1

Descriptive analysis of salivary measures and psychosocial scores.

			Range	
	Mean (μg/ml)	**± SEM**	**Minimum**	**Maximum**
Salivary S-IgA	197.17	9.45	54.13	697.61
Salivary IgA1	157.16	7.40	32.90	445.76
Salivary IgA2	145.11	8.73	25.08	611.02
Salivary SC	1186.20	67.46	236.53	3981.54
	Mean (score)	**± SEM**	**Minimum**	**Maximum**
Beck Depression Inventory	3.16	0.33	0	13
UCLA Loneliness Scale	35.04	1.09	20	66
Perceived Stress Scale	15.24	0.84	1	35

IgA1: Immunoglobulin A subclass 1; IgA2: Immunoglobulin A subclass 2; SC: Secretory Component;

Table 2

Summary of significant associations: Univariate linear regression

Salivary Measure	Positive Association	Negative Association	Gender Effect
S-IgA	Protein Concentration		
IgA1	Protein Concentration	PSS (Stress)	
IgA2	Protein Concentration Age		Higher in Men
SC	Protein Concentration BDI (Depression) UCLA (Loneliness)		
S-IgA/SC			
IgA1/SC		PSS (Stress) BDI (Depression) UCLA (Loneliness)	Higher in Women
IgA2/SC	Age	BDI (Depression)	

IgA1: Immunoglobulin A subclass 1; IgA2: Immunoglobulin A subclass 2; S-IgA: Secretory Immunoglobulin A; SC: Secretory Component; BDI: Beck Depression Inventory; PSS: Perceived Stress Scale; UCLA: University of California at Los Angeles Loneliness Scale.

Table 3

Beta values and ΔR^2 of multiple linear regression using perceived stress (PSS), depressive (BDI) or loneliness symptoms (UCLA) to predict S-IgA, IgA1, IgA2 and SC

PSS Model		**S-IgA**			**IgA1**			**IgA2**			**SC**		
		β	**t**	**ΔR^2**	**β**	**t**	**ΔR^2**	**β**	**t**	**ΔR^2**	**β**	**t**	**ΔR^2**
Model 1	Gender	−23.78	−1.70	0.04	−17.83	−1.23	0.04	−14.44	−0.82**	0.09	38.62	0.27	0.006
	Age	−6.18	−2.09*		1.47	0.63		7.79	2.76**		−15.70	−0.69	
Model 2	Smoking	−22.16	−1.00	0.007	−12.52	−0.72	0.005	18.60	0.88	0.03	62.36	0.37	0.005
	Alcohol	3.81	0.19		−0.40	−0.03		21.58	1.13		−11.88	−0.08	
	Caffeine	−0.82	−0.04		7.05	0.46		−21.18	−1.14		2.48	0.02	
Model 3	Protein	0.13	4.14**	0.13	0.09	3.80**	0.11	0.05	1.73	0.02	0.52	2.19*	0.05
Model 4	PSS	−2.15	−1.85	0.03	**−2.09**	**−2.29***	**0.04**	−1.20	−1.08	0.01	12.64	1.41	0.02
BDI Model		*S−IgA*			*IgA1*			*IgA2*			*SC*		
		β	t	ΔR^2	β	T	ΔR^2	β	t	ΔR^2	β	t	ΔR^2
Model 1	Gender	−30.12	−1.64	0.04	−22.87	−1.58	0.04	−15.10	−0.88**	0.09	35.89	0.26	0.006
	Age	−5.78	−1.93		1.875	0.74		7.73	2.75**		−14.01	−0.63	
Model 2	Smoking	−22.28	−0.99	0.007	−11.39	−0.64	0.005	21.72	1.03	0.03	17.54	0.11	0.005
	Alcohol	1.40	0.07		1.18	−0.07		24.24	1.27		−56.31	−0.35	
	Caffeine	−1.48	−0.08		7.23	0.46		−19.46	−1.05		−21.62	−0.15	
Model 3	Protein	0.13	4.16**	0.14	0.10	3.97**	0.12	0.06	2.05*	0.03	0.38	1.82	0.04
Model 4	BDI	−1.34	−0.42	0.01	−2.82	−1.13	0.01	−4.62	−1.55	0.02	**61.88**	**2.61***	**0.06**
UCLA Model		*S−IgA*			*IgA1*			*IgA2*			*SC*		
		β	t	ΔR^2	β	t	ΔR^2	β	t	ΔR^2	β	t	ΔR^2
Model 1	Gender	−32.74	−1.76	0.04	−29.05	−2.02*	0.04	−18.84	−1.07**	0.09	148.44	1.07	0.006
	Age	−5.89	−1.95		1.39	0.59		8.03	2.81**		−9.91	−0.44	
Model 2	Smoking	−24.00	−1.07	0.007	−15.37	−0.88	0.005	17.79	0.84	0.03	94.66	0.57	0.005
	Alcohol	−0.35	−0.02		−5.05	−0.32		19.38	1.02		24.95	0.17	

PSS Model		S-IgA			IgA1			IgA2			SC		
		β	**t**	**ΔR^2**	**β**	**t**	**ΔR^2**	**β**	**t**	**ΔR^2**	**β**	**t**	**ΔR^2**
	Caffeine	−0.77	0.04		9.57	0.61		−21.66	−1.15		−56.96	−0.32	
Model 3	Protein	0.13	4.18 **	0.14	0.10	3.91 **	0.12	0.05	1.76	0.03	0.48	2.07 *	0.04
Model 4	UCLA	−0.43	−0.48	0.002	−1.16	−1.66	0.02	−0.09	−0.10	0.00	**17.67**	**2.65** **	**0.06**

*
p<.05;

**
p<.01

IgA1: Immunoglobulin A subclass 1; IgA2: Immunoglobulin A subclass 2; S-IgA: Secretory Immunoglobulin A; SC: Secretory Component; BDI: Beck Depression Inventory; PSS: Perceived Stress Scale; UCLA: University of California at Los Angeles Loneliness Scale.

Table 4

Beta values and ΔR^2 of multiple linear regression using perceived stress (PSS), depressive (BDI) or loneliness symptoms (UCLA) to predict S-IgA/SC, IgA1/SC and IgA2/SC

PSS Model		**S-IgA/SC**			**IgA1/SC**			**IgA2/SC**		
		β	**t**	**ΔR^2**	**β**	**t**	**ΔR^2**	**β**	**t**	**ΔR^2**
Model 1	Gender	−0.05	−1.54	0.03	−0.04	−1.45 *	0.06	0.03	−1.18 **	0.09
	Age	0.00	−0.17		0.005	1.28		0.01	2.88 **	
Model 2	Smoking	−0.04	−1.05	0.01	−0.03	−0.97	0.01	0.01	0.23	0.01
	Alcohol	0.008	0.22		0.01	0.44		0.03	0.85	
	Caffeine	−0.01	−0.40		−0.01	0.40		−0.03	−0.85	
Model 3	Protein	−0.00	0.84	0.007	−0.00	0.25	0.01	−0.00	−0.59	0.003
Model 4	PSS	−0.003	−1.61	0.02	**−0.003**	**−2.10** *	**0.04**	−0.001	−0.67	0.004
BDI Model		*S-IgA/SC*			*IgA1/SC*			*IgA2/SC*		
		β	t	ΔR^2	β	t	ΔR^2	β	t	ΔR^2
Model 1	Gender	−0.06	−1.69	0.03	−0.04	−1.64 *	0.06	−0.03	−1.11 **	0.09
	Age	0.00	−0.17		−0.05	1.30		0.01	2.83 **	
Model 2	Smoking	−0.04	−0.89	0.01	−0.02	−0.75	0.01	0.02	0.45	0.01
	Alcohol	0.01	0.35		0.02	0.61		0.03	1.11	
	Caffeine	−0.01	−0.31		−0.01	−0.27		−0.02	−0.71	
Model 3	Protein	0.00	1.25	0.007	0.00	0.80	0.001	−0.00	−0.16	0.003
Model 4	BDI	−0.01	−1.83	0.03	**−0.01**	**−2.42** *	**0.05**	**−0.01**	**−2.05** *	**0.04**
UCLA Model		*S-IgA/SC*			*IgA1/SC*			*IgA2/SC*		
		β	t	ΔR^2	β	t	ΔR^2	β	t	ΔR^2
Model 1	Gender	−0.07	−2.22	0.03	−0.06	−2.43	0.06	−0.05	−1.60 **	0.09
	Age	−0.002	−0.28		0.005	1.09		0.01	2.74 **	
Model 2	Smoking	−0.05	−1.20	0.01	−0.04	−1.20	0.01	0.004	0.13	0.01
	Alcohol	0.00	−0.01		0.003	0.13		0.02	0.74	

PSS Model		S-IgA/SC			IgA1/SC			IgA2/SC		
		β	**t**	**ΔR^2**	**β**	**t**	**ΔR^2**	**β**	**t**	**ΔR^2**
	Caffeine	−0.001	−0.20		−0.002	−0.08		−0.02	−0.66	
Model 3	Protein	−0.00	0.98	0.007	0.00	0.44	0.001	−0.00	−0.51	0.003
Model 4	UCLA	−0.003	−1.77	0.03	**−0.003**	**−2.69** **	**0.06**	−0.002	−1.41	0.02

* p<.05;

** p<.01

IgA1: Immunoglobulin A subclass 1; IgA2: Immunoglobulin A subclass 2; S-IgA: Secretory Immunoglobulin A; SC: Secretory Component; BDI: Beck Depression Inventory; PSS: Perceived Stress Scale; UCLA: University of California at Los Angeles Loneliness Scale.

Table 5

Summary of significant associations: Multiple linear regression

Psychosocial Test	Positive Association	Negative Association
PSS (Stress)		IgA1 IgA1/SC
BDI (Depression)	SC	IgA1/SC IgA2/SC
UCLA (Loneliness)	SC	IgA1/SC

IgA1: Immunoglobulin A subclass 1; IgA2: Immunoglobulin A subclass 2; SC: Secretory Component; BDI: Beck Depression Inventory; PSS: Perceived Stress Scale; UCLA: University of California at Los Angeles Loneliness Scale.