American Journal of Human Biology

The evolutionary adaptation of body art: Tattooing as honest signaling of enhanced immune response in American Samoa

Journal:	*American Journal of Human Biology*
Manuscript ID	AJHB-19-0121.R1
Wiley - Manuscript type:	Original Research Article
Date Submitted by the Author:	n/a
Complete List of Authors:	Lynn, Christopher; University of Alabama, Anthropology Howells, Michaela; University of North Carolina at Wilmington, Anthropology Herdrich, David; American Samoa Department of Historic Preservation; Federal Emergency Management Agency, Environmental Historic Protection Division Ioane, Joseph; Off Da Rock Tattoos Hudson, Duffy; Tatau Manaia Fitiao, Su'a Tupuola
Keywords:	tattooing, immune response, costly signaling, American Samoa, allostasis

Title: The evolutionary adaptation of body art: Tattooing as honest signaling of enhanced immune response in American Samoa

American Journal of Human Biology, Special Issue: Evolutionary Theory in Biocultural Anthropology

Abbreviated title: Tattoos as a costly honest signal

Authors: Christopher D. Lynn[1]*, Michaela Howells[2], David Herdrich[3#], Joseph Ioane[4], Duffy Hudson[5], Su'a Tupuola Uilisone Fitiao[6]
[1]Department of Anthropology, University of Alabama, Tuscaloosa, AL 35487
[2]Department of Anthropology, University of North Carolina Wilmington, NC 28403
[3]American Samoa Department of Historic Preservation, American Samoa
[4]Off Da Rock Tattoos, American Samoa
[5]Tatau Manaia, American Samoa
[6]*Tufuga tā tatau*, American Samoa

*Corresponding author: Christopher Lynn
Email: cdlynn@ua.edu
Box 870210
Tuscaloosa, AL 35487
Phone: (205) 348-4162

[#]Currently at Federal Emergency Management Agency (FEMA), Environmental Historic Protection Division.

Abstract

Tattooing has been practiced globally for thousands of years. From an evolutionary perspective, this tradition seems counterintuitive because it is a dermal injury that risks infection. Previous research indicates tattooing may habituate the immune system for subsequent stress, as with exercise or vaccination, an important benefit in high-risk areas. Visible injuries through tattooing may be a form of costly honest signaling—consciously or unconsciously drawing attention to immunological quality.

Objectives: We tested this habituation effect of tattooing in American Samoa, where its practice is common and extensive and infectious disease rates high. We hypothesized that people with more tattoo experience would have enhanced immune response related to the stress of being tattooed. We compared total and rate of tattoo experience to determine if tattooing is more analogous to exercise or vaccination.

Methods: We measured secretory immunoglobulin A (SIgA), cortisol, C-reactive protein (CRP), and tattoo experience in 25 adults receiving tattoos. We compared post-tattoo SIgA to total and rate of tattoo experience using ANCOVA, controlling for pre-tattoo SIgA, tattoo duration, age, marital status, and stress and baseline health (cortisol, CRP, BMI, and cigarette use).

Results: Post-tattoo SIgA positively correlated with total tattoo experience ($p < 0.05$). Furthermore, when dichotomized by experience, participants with low tattoo experience showed little to no stress-related immune change, whereas high-experience participants exhibited elevated SIgA, suggesting habituation to repeated tattooing.

Conclusions: The historical and cultural popularity of tattooing may be partly due to honest information tattoos convey about adaptive biology, similar to physical benefits of exercise.

Keywords:

Immune response, tattooing, costly signaling, allostasis, American Samoa

Introduction

Tattooing has been practiced throughout the world for thousands of years as self-decoration, status marker, rite of passage, therapy, and physical protection (DeMello, 2000). Although there are clear cultural benefits to tattooing, studies of biological aspects have largely focused on risk of disease transmission, drug use, and infection (Atkinson, 2004). However, scarification (tattooing, scratching, branding, etc.) may prime the immune system by habituating it to short-term stress (Sosis, Kress, and Boster, 2007). Reducing the immunosuppression normally associated with stress may be particularly important in environments that have higher pathogenicity and disease loads, such as the tropics. Here we explore tattooing as a cultural adaptation that may provide enhanced immune response through short-term stress and consider its potential as a costly signal of health in American Samoa.

Ethnographic and historical research reflects the use of tattooing to confer strength, prevent illness, construct and reinforce social identity, and reduce spiritual and physical malignancy (e.g., Caplan, 2000; DeMello, 1991). In traditional Samoan culture, selected men and women received extensive tattoos to indicate commitment and affiliation with their chiefs, families, and villages (Ausage, 2015; Gell, 1993; Mallon and Galliot, 2018). Tattooing is integral to Samoan cultural identity and is reported to toughen the mind and body and create thoughtful leaders and warriors (Ausage, 2015; Mallon and Galliot, 2018; Ruck, 2018). In this Pacific region, tattooing is common, celebrated, and extensive and includes traditional hand ("tapping") and modern electric techniques (Mallon and Galliot, 2018).

During the historic period, the Samoan Islands have been frequently exposed to infectious diseases (Crews, 1988), and tattooing may play a protective role. Prehistoric infectious disease exposure is unclear, though at least one source indicate that the malaria endemic in

Melanesia was rare in prehistoric Polynesia until Europeans arrived, suggesting a prolonged period of cultural expansion in the absence of this virulent threat associated with high mortality rates elsewhere (Buckley, 2006; Buckley, Kinsaston, Anson, Bedford, and Spriggs, 2013). On the other hand, there is pre-contact evidence of a dual-burden of yaws and metabolic disease in Polynesia that is consistent with later rates of yaws in the region (Buckley, 2000). Although biomedicine is now commonly utilized, exposure to these diseases paired with stress-related immunosuppression has been linked to elevated mortality and morbidity throughout the history of the island (Howells, Lynn, Weaver, Langford-Sesepesara, and Tufa, 2018). Tattooing may play an underappreciated role in biosocial buffering of environmental stressors by habituating the body to some types of adversity in ways analogous to other cultural stressors, such as exercise or vaccination.

Immune Response to Cultural Stressors

Immune changes occur in response to novel short-term stress as part of the normal fight-or-flight response (Coe, 2002), adaptations that can be culturally manipulated. Onset of physiological stress, including that triggered through exercise, vaccination, tattooing, or other sources, causes the deployment of leukocytes in the bloodstream to lymph nodes and sites of damage, vaccination, or wounding (Dhabhar, 2014). Contrary to suggestion that novel stress is immunosuppressive (Coe, 2002), leukocyte proliferation in localized areas can result in reduced numbers elsewhere in the bloodstream and give the appearance of immunosuppression in serum assays (Dhabhar, 2014). The confusion about the valence of immune response (enhanced or suppressed) to stress is due to lack of clarity about the type of stress. Short-term or acute stress (minutes to hours) is generally immune-enhancing, whereas chronic stress (hours to days) is immunosuppressant (Bosch, Ring, de Geus, Veerman, and Amerongen, 2002; Dhabhar, 2014).

Whether stress enhances or suppresses immune response depends on duration of the stress, leukocyte distribution in the body, glucocorticoid (cortisol in humans) distributions in the body, and the timing of the stressor (Dhabhar, 2014). The latency and degree of increased immune function caused by short-term stress appears to depend on previous exposure to the stressor in conjunction with other health factors (Campbell and Turner, 2018; Sapolsky, 2002).

For instance, exercise induces stress response and increases circulating epinephrine, norepinephrine, cortisol, and other stress-related factors. Studies mimicking exercise-like, repeated short-term stress in mice demonstrate immune benefits (Dhabhar, 2014). Energy flux changes with respect to physical condition, which involves corresponding changes that impact the immune system and allow it to adjust to the exercise stress (Campbell and Turner, 2018). Similarly, though initial response to a vaccine can result in mild pathogenic symptoms, short-term stress accompanying the vaccine ultimately improves vaccine efficacy (Dhabhar, 2014). This acute stress has been found to enhance immune response for a large portion of the organism's lifetime through increase in effector and memory helper T cells (Dhabhar and Viswanathan, 2005). During this time, the immune system produces antibodies that increase the likelihood of rapid detection of future pathogen exposures related to the vaccine.

This principle has recently been harnessed by administering vaccines through electric tattoo machines (Kim, 2017). Vaccinations administered using tattoo machines work better than intramuscular injection through the acute stress of repeated shallow puncturing (Pokorna, Rubio, and Müller, 2008). This immune effect via tattoo machine administration is likely linked to tertiary immune surveillance, which triggers system-wide response to localized encounters (Kupper and Fuhlbrigge, 2004). These surveillance mechanisms in the skin interact via dendritic cells with primary immune monitoring in lymph nodes. Tertiary immune surveillance hedges

bets on the encounter being system-wide instead merely a localized dermal encounter by providing enhanced adaptive immune responses to antibodies in other body tissues (Kupper and Fuhlbrigge, 2004). Thus, adaptive immune response caused by localized stress can be detected throughout the body, including saliva.

However, with cosmetic and cultural tattooing, it is not clear if there is a prolonged immune system effect from one tattooing session (as with the acute stress accompanying immunization), if repetition is necessary (as with the exercise-like benefit achieved through repeated short-term stress episodes), or what mechanisms are involved in the process. Testing these effects requires measurement of total tattoo experience and rate of tattoo experience with regard to endocrine and immune markers.

Measuring the Body's Response to Tattooing

Assessment of various endocrine and immunological biomarkers, such as immunoglobulin A, cortisol, and C-reactive protein (CRP), can address these tertiary mechanisms of immune response to tattooing. They are rapidly produced and easily collected through non-invasive means, such as saliva. IgA is an acute-phase biomarker and mucosal antibody that is a frontline defense against common bacteria and viruses. IgA undermines bacterial adherence to mucosal surfaces through nonspecific interactions (Marcotte and Lavoie, 1998).

Cortisol is released as part of the hypothalamic-pituitary-adrenal (HPA) axis and plays a role in suppression of immune response and restoration to baseline (Sapolsky, 2002). Cortisol is positively associated with secretory immunoglobulin A (SIgA) under conditions of short-term stress and inversely related under long-term or excessive stress (Bosch et al., 2002). Cortisol seems to play a regulatory function for lymphocyte proliferation, such that abnormally high

doses of corticosteroid added to lymphocyte cultures decreases cellular activity, whereas subphysiological levels of corticosteroid augment immune response (Coe, 2002). Gleeson (2007) proposes that long-term or chronic stress causes increases in cortisol and epinephrine production, among other actions, resulting in decreased macrophage and T helper 1 cell cytokine production. This diminished production (i.e., immunosuppression) can increase risk for viral infection but reduce risk of chronic disease (Gleeson, 2007).

CRP is an acute-phase biomarker that reflects general inflammation and can be indicative of infection, as well as potential cardiovascular and metabolic disorders (Dehghan et al., 2007; Pearson et al., 2003; Volanakis, 2001). A previous study found that total tattoo experience (an index of number of hours tattooed, tattoos, tattoo sessions, body covered, and years tattooed) positively predicts post-tattoo SIgA levels (Lynn et al., 2016). In that study, changes in cortisol and SIgA were inversely related in participants with lower tattoo experience but positively associated in those with higher tattoo experience. However, the authors were unable to control for pre-existing conditions that might produce physiological stress or add to an already taxed immune response. A minimally invasive way to assess baseline health is to assay CRP from the same saliva samples.

Tattooing as a Costly Signal of Health

Multiple scholars have suggested that tattooing may be a permanent exterior "costly signal" of health that demonstrates the ability to quickly heal (Koziel, Kretschmer, and Pawlowski, 2010; Sosis et al., 2007; Wohlrab, Fink, Kappeler, and Brewer, 2009). Although this can be particularly important where infectious disease risk is relatively high, tattooing occurs all over the world, not just in dangerous environments. Carmen and colleagues (2012) suggest that, even where disease rates are not particularly high, tattooing may be a means of "upping the ante"

by injuring the body in a relatively safe way, thereby highlighting underlying biological quality or group commitment. Bearing the cost of injuries to the body through tattooing may suggest stamina through pain tolerance and a robust ability to heal.

Such an ability to rebound from injury would be especially important for populations that experience high disease burden, like those living in the Samoas. We tested this costly signaling model of tattooing in American Samoa. We predicted that those with more tattoo experience would have enhanced immune response to their tattoo when controlling for overall health. Similarly, consistent with the physical manifestation of health embodied through exercise, we predicted that tattooing has a comparable impact on the immune system, requiring repeated exposure for benefits. We tested this second hypothesis by comparing immunological impacts of total tattoo experience (analogous to exercise) and rate of tattoo experience (analogous to vaccination).

Methods

Location

American Samoa is the southernmost territory of the USA. Located in the South Pacific, this chain of seven islands and atolls is home to over 51,000 inhabitants, the majority (89%) of which is of Samoan descent (CIA, 2018). In 2017, we recruited participants in collaboration with a *tufuga tā tatau* (traditional Samoan tattoo master using the tapping method) and two electric-machine tattooists/owners at their professional studios (Off Da Rock Tattoos and Tatau Manaia) on the largest island of Tutuila.

Hand-tap tattoos were administered in the *fale'* (open-air hut) of the *tufuga tā tatau* in two cases and on a community member's open-air back porch in another case. In both situations, tattoo recipients lay on woven mats made of leaves and were positioned by assistants with

pillows (wrapped in new plastic wrap at the beginning of each session). The *tufuga tā tatau* sat cross-legged on the floor to administer the tattoo with tools (*'au*) of various sizes he had crafted from boar tusk, turtle shell, wood, and coconut fiber. Two assistants stretched the recipients' skin with the palms of their hands and wiped away ink and bodily fluids, and another assistant fanned the tattoo recipient. The *tufuga tā tatau* and stretchers wore latex gloves throughout tattooing. Friends and family were welcome to watch but expected to sit, wear a *lavalava* (cloth wrap), and remain quiet. Tattoos were administered with traditional Samoan music playing.

Both electric tattoo studios were in enclosed, air-conditioned rooms and equipped similarly to those of the US mainland. Customers were positioned on stools or padded benches that the artists covered with new plastic wrap between each client. Artists would sit on padded stools with wheels to move around customers. Electric tattoo machines with sterile needles were utilized in these shops, and tattooists wore disposable latex gloves while giving tattoos. Each artist worked independently, though friends and family were invited to support the tattoo recipient. Artists would frequently have rock or hip-hop music playing during the tattooing process.

Procedures

We recruited participants by working in collaboration with the *tufuga tā tatau* and electric tattoo artists and used radio, TV, and newspaper stories to introduce the community to the study. The *tufuga tā tatau* and tattoo artists would contact us when they had clients scheduled. They would tell their clients about the study and ask if they would like to hear more about the project. If the customer agreed, our research team would provide additional explanations. All clients approached participated in the study.

After obtaining informed consent from participants, a team member administered a short survey querying demographic information, tattoo experience, perceived stress, and other potential endocrine and immune covariates (e.g., recent sickness, cigarette and alcohol use, medications, etc.) (Appendix A). We obtained previous tattoo experience by asking when participants had received each of their tattoos, how long each had taken, and the extent of the body tattoos covered (using female and male body outlines with grid overlays to calculate percentages). We measured perceived stress using the 4-item Perceived Stress Scale (Cohen, Kamarck, and Mermelstein, 1983) to control for non-tattoo-related elevations in cortisol caused by personal stress (lowest score = 0, highest score = 16; high scores associated with greater stress). All written materials were available in English and Samoan.

We then collected participant weight and body fat percentage using a Tanita body composition analyzer (Model TBF 310) and calculated BMI using self-reported height. We collected handgrip strength (mean of duplicate measures for each hand) using a Detecto hand dynamometer (Model DHS 174). Finally, we collected 1.8mL saliva immediately before and after tattooing sessions via the passive drool method using 2mL cryovials and saliva collection aids (SalivaBio LLC) and recorded the time of each sample to account for tattoo duration.

We stored saliva samples in a consumer-grade freezer before transporting them to the University of North Carolina Wilmington, where they were stored in a medical freezer (-20° F) until assayed. We shipped samples overnight with dry ice to Salimetrics LLC (State College, PA), where they were assayed according to published enzyme-linked immunosorbent assay (ELISA) protocols. SIgA and cortisol units are reported as micrograms per milliliter (µg/mL), whereas CRP units are reported as picograms per milliliter (pg/mL).

The study design was approved by the Institutional Review Boards of the University of Alabama (#17-OR-156-ME) and American Samoa Department of Health (IRB #1249, FWA #24252).

Participants

We recruited 9 women and 16 men (ages 19-69, mean ± SD = 37.7 ± 16.31). Seven participants were residents of American Samoa, while 16 were visiting for work, family, or vacation. Three participants received hand-tapped tattoos and 22 via electric tattoo machines. Self-reported ethnicity was evenly split between Pacific Islander (n = 14) and non-Pacific Islander (n = 11). All participants were at least 18 years old.

Analysis

To gain better understanding of the effects of previous tattoos on immune and stress response, we created two tattoo experience variables—an additive and a rate variable. We considered the additive variable (total tattoo experience) analogous to the impact of physical exercise on the immune system, as something that is cumulative or repeated throughout life. We created the total tattoo experience variable by summing:

Number of tattoos + Tattoo sessions + Lifetime hours spent receiving tattoos + Years tattooed + Percent of body tattooed.

The rate variable (rate of tattoo experience) was comparable to an inoculation, such that one or a few administrations has long-term effects on immune response. We calculated the rate of tattoo experience variable as follows:

(Number of tattoos + Tattoo sessions + Lifetime hours spent receiving tattoos + Percent of body tattooed)/ Years tattooed.

To assess immune and endocrine response, SIgA, cortisol, and CRP were assayed from saliva samples. SIgA may be sensitive to amount of saliva a person can produce in a given time,

so we controlled for flow rate by dividing SIgA values by amount of total protein per sample (which is not flow sensitive) following Lynn et al. (2016). We tested for differences between pre- and post-tattoo measures of all three biomarkers using paired samples *t*-tests and then transformed them using $\log_{10}$ because of skewed distributions typical of biomarkers.

To test the impact to total and rate of tattoo experience on post-tattoo $SIgA_{log10}$, we conducted separate analyses of covariance (ANCOVA) using the linear regression function of SPSS Version 25 for Windows (IBM Corp., Armonk, NY). We included pre-tattoo SIgA, pre-tattoo CRP, post-tattoo cortisol, age, BMI, and duration of the tattoo received in the study as *a priori* covariates in the models. Pre-tattoo SIgA controlled for variability in antibody levels before tattooing began and pre-tattoo CRP for baseline health, while post-tattoo cortisol was included to control for effects of circulating cortisol on SIgA. We included BMI in the models because of established relationships between body size and immune response and the importance of body weight in previous tattoo research (Lynn et al., 2016; 2018). Age was included since "years tattooed" was a measure in the tattoo experience variables and tattoo duration as a control for time between pre- and post-tattoo measures. We used bivariate correlations with SIgA to determine other covariates for the models. We considered all statistics significant if $p < 0.05$.

Results

While tattooing is an important cultural practice in American Samoa, the *tufuga tā tatau* and artists in our study relied on tourists and visitors to supplement their incomes, and participants in the study were primarily non-residents of American Samoa ($n = 16$). Most were also well educated—18 had at least some college, while only one participant had less than a high school diploma. Half were in a committed relationship of some type ($n = 12$), while 10 were single or in a casual relationship. All participants self-reported SES as either high ($n = 12$) or

middle class (n = 11). Participants worked on average 33.9 hours per week (SD = 22.60); but, consistent with being mostly visitors to the island, they had generally worked fewer the week they received the tattoo for this study (4.8 ± 11.75).

Table 1 outlines the psycho- and anthropometric data collected for dependent variables and potential fixed factors and covariates for regression modeling. Of note, only 16% (n = 4) of our participants classified as normal/healthy BMI according to Centers for Disease Control and Prevention criteria (CDC, 2017), while another 16% were classified as overweight and 68% (n = 17) as obese. A large positive correlation between fat percentage and BMI (r = 0.67, p < 0.001) suggests that these categories are not due to high muscle mass. Additionally, mean SIgA and cortisol increased from pre-to post-tattoo measures, though only the change in cortisol was significant (p = 0.04). CRP decreased from pre- to post-tattoo, but the change was not significant.

[TABLE 1]

We collected data related to recent illness and alcohol, cigarette, and medication use because of potential for these factors to influence on endocrine or immune activity. Two participants reported being sick within two days of the study. Most participants (64%) had no alcoholic drinks in the 24 hours before being tattooed, but five (21%) participants had 1-2 drinks and three (12%) as many as 5-9. Similarly, 16 participants did not smoke any cigarettes in the previous week, but eight were smokers who reported having anywhere from one to 80 cigarettes the previous week. One participant reported being on a stimulant medication (Adderall); one on hormonal medication (birth control); one on a dietary supplement; and five on medication for blood pressure, cholesterol, or diabetes.

We conducted bivariate correlations with pre- and post-tattoo SIgA, post-tattoo cortisol, and pre-tattoo CRP to determine which of these additional covariates to include in ANCOVA modeling (Table 2). Marital status, SES, and cigarette use were significantly related to SIgA, but marital status and SES were also positively associated with each other ($r = 0.60$, $p = 0.002$). We included cigarette use in regression analysis, but, to avoid confounding effects, we retained marital status over SES because marital status explains more variance in pretest SIgA ($r = -0.53$). Education, previous tattoo-related infections, and recent alcohol consumption were significantly associated with pre-tattoo CRP. Since inflammation is associated with increased CRP levels, we considered pre-tattoo CRP a proxy of problems with tattoo healing or alcohol use and, to avoid multicollinearity, did not use tattoo problems or alcohol use in regression analysis. The association with education is less clear, but the correlation was negative, so it may indicate other factors associated with low education (e.g., poor nutrition, sleep, or drug use). Bivariate correlations with biomarkers for other variables were not significant and therefore not included in regression modeling.

[TABLE 2]

We conducted ANCOVA on post-tattoo $SIgA_{log10}$ with pre-tattoo $SIgA_{log10,}$ pre-tattoo CRP_{log10}, post-tattoo $cortisol_{log10}$, tattoo experience, tattoo duration, age, cigarette use, BMI (grouped by classifications), and marital status as independent variables. We tested separate models with tattoo experience as an additive and as a rate variable, but neither the rate of tattoo experience model ($F_{9,7} = 3.02$, $p = 0.08$, $r^2 = 0.80$) nor any of the variables in that model were predictive of SIgA, so we do not report on rate of tattoo experience further. By contrast, the additive model was significant ($F_{9,15} = 5.07$, $p = 0.003$, $r^2 = 0.75$); and total tattoo experience, cortisol, BMI, marital status, and cigarette use were all significant predictors with large effect

sizes (Table 3). Total tattoo experience had the largest effect on SIgA after cigarette use, BMI, and marital status. We chose to control for cigarette use instead of removing those participants since the sample was so small, but we double-checked their influence by removing them from analysis. Removing those seven participants did not change the models in any significant ways. While being sick in the past 48 hours was not correlated with biomarker measures, we similarly checked the influence of recent illness by rerunning the regression model without the two recently sick participants and found no difference.

[TABLE 3]

We explored the elements that comprised the total tattoo experience variable (hours tattooed, number of tattoos, number of tattoo sessions, percent of body tattooed, and years tattooed) by substituting each independently into the ANCOVA model in place of total tattoo experience (because of limited statistical power, we could not include them in the model simultaneously). Number of sessions ($\beta = 0.53$, $p = 0.001$), total hours tattooed ($\beta = 0.52$, $p = 0.001$), percent of body tattooed ($\beta = 0.36$, $p = 0.01$), and number of tattoos ($\beta = 0.51$, $p = 0.01$) were all significant with moderate to large effect sizes. Years tattooed was not significant ($\beta = 0.29$, $p = 0.30$). Combined, this suggests that the repeated stimulation and extent of tattooing had the greater effect on SIgA than the amount of time the body had to adjust.

To explore the direction of the impact of the overall model on SIgA, we dichotomized the additive tattoo experience variable into two groups based on the distribution (low tattoo experience = 0-39, high tattoo experience = 40+). We then graphed this dichotomized variable against estimated marginal means (which includes all independent variables in the model) of pre- and post-tattoo SIgA (Fig 1). The estimated marginal means for pre- and post-tattoo measures in the low tattoo experience group (2.274 and 2.264, respectively) showed a moderate decrease,

whereas there was an increase in the high tattoo experience group (2.508 to 2.578). We conducted paired samples *t*-tests on pre-tattoo, post-tattoo, and change in SIgA, cortisol, and CRP; no differences between high and low tattoo groups were significant. When controlling for other variables by replacing total tattoo experience in ANCOVA with the dichotomized variable, the difference between high and low tattoo experience was significant with a moderate effect size ($p = 0.01, \beta = 0.36$).

[FIGURE 1]

Discussion

We explored tattooing as a cultural adaptation that provides benefits to immune response through short-term stress while considering its potential as a costly signal of health. Quick immunological response to dermal injury may be a one mechanism of costly honest fitness signaling and an adaptation for populations experiencing high disease burdens. As such, the biological cost of the tattoo as a wound is offset by enhancement of immune response and eye-catching qualities of tattoos. This combination is consistent with Zahavi and Zahavi's (1997) model for costly honest signaling (aka, "handicap principle"). As Getty (2002) points out, a costly honest signal of biological quality is one wherein an individual can "waste" energy or viability but have more to spare, resulting in a positive correlation between that wasted and remaining surplus energy/viability.

We hypothesized that tattooing is an honest signal of fitness because the immune systems of relatively fit individuals would adjust over time to being tattooed. We predicted that those with more total tattoo experience would have enhanced immune response associated with their newest tattoo when controlling for other variables; heavily tattooed individuals would have immune responses that are more robust after receiving a dermal injury relative to those with less tattooing. Consistent with that prediction, we found people with low total tattoo experience

showed no immunological enhancement, while those with high total tattoo experience showed immune enhancement. It is unclear if these effects are both clinically and statistically significant, but they are consistent with previous research on tattooing and immune benefits (Lynn et al., 2016).

Despite this support for the research hypothesis, the positive association among total tattoo experience, BMI, and SIgA is puzzling. While BMI does not distinguish fat from muscle mass, measurement of fat density confirms that high BMI is consistent with overweight and obese phenotypes in our study. In previous research, overweight and obese BMIs were associated with higher rates of tattoo-related infections (Lynn et al., 2018). Studies of immune response in overweight and obese phenotypes indicates higher risks of infection and increased levels of some peripheral immune factors, consistent with those observed in autoimmune diseases or infections (Dobner and Kaser, 2018; Ilavská et al., 2012). It is not clear these concentrations extend to secretory levels in saliva, but increased rates of circulating stress and immune factors in overweight and obese people is associated with asthma and autoimmune disorders (Hersoug and Linneberg, 2007). As with chronic stress, these persistently high levels contribute to allostatic load, an accumulation of negative health factors. Previous reports indicated low rates of asthma and autoimmune disease in developing countries, but lifestyles and health have changed quickly in many of these countries. There are few data on adult asthma in the Pacific (Beran, Zar, Perrin, Menezes, and Burney, 2015), and rates of autoimmune disorders vary significantly by disease.

Though we are cautious about over-interpreting from a small sample, tattooing may mitigate some of the negative impacts of increased adipose tissue on immunocompetence. This effect could be produced in a manner similar to how exercise ameliorates deleterious impacts of

chronic stress (Dhabhar, 2014). Finally, there is an emerging literature on metabolically healthy obesity, which concerns a subgroup of obese phenotypes not at higher risk for cardiometabolic disorders (Stefan, Häring, Hu, and Schulze, 2013). Though Samoans suffer from high rates of obesity-related metabolic disorders (Lin et al., 2017), they may also be genetically predisposed to metabolically healthy large phenotypes (Minster et al., 2016). The role of tattooing as a costly honest signal of health is thus relative to the normal distribution of phenotypes in the Samoa population and to population health.

Again, we are cautious in that only part of our sample included Pacific Islanders, but in the Samoas and the United States, tattooing may be a means of "upping the ante," as Carmen and colleagues (2012) point out, when other factors are relatively equal. Thus, in American Samoa and USA, where is obesity epidemic, tattooing may highlight *relative* immunological fitness despite other negative indications of health—i.e., signaling "healthy enough." This is consistent with how natural selection actually works, favoring all phenotypes able to survive and differentially produce by simply gaining an edge on competitors, even a small one.

Cortisol levels in our study showed significant increase from pre- to post-tattoo, which is consistent with a short-term stress response. CRP exhibited a non-significant decrease. While we primarily assayed CRP to establish baseline health at the beginning of the tattoo, we thought tattoo-related inflammation would cause an overall increase in CRP. However, the inverse relationship between change in CRP and change in SIgA is consistent with one that measured changes with regard to short-term stress (Tauler, Martinez, Moreno, Martínez, and Aguilo, 2013) and another that showed a non-significant trend of an increase in SIgA and lack of change in CRP (Campisi, Bravo, Cole, and Gobeil, 2012). Thus, the utility of cortisol and CRP in

understanding the biological dynamics of tattooing remain unclear save for baseline controls of stress and inflammation.

In addition to examining a signaling function, we tested whether tattooing impacts the immune system over time, as with vaccinations. Research on the mechanisms by which the immune systems holds tattoo ink in place suggests that immune response may continue after even one tattoo (Baranska et al., 2018). However, there was no relationship between immune response and either the rate of tattoo experience or years tattooed (years since first tattoo). By contrast, people with more total tattoo experience had elevated immune responses that may be related to multiple exposures to tattooing, similar to studies of repeated exercise or other forms of repeated short-term stress (Dhabhar, 2014; Dhabhar et al., 2010). In our regression modeling, immune response and total tattoo experience (both continuous and dichotomized versions of the variable) had a positive, significant relationship.

These mechanisms are likely part of the allostatic interaction among endocrine and immune systems and the environment. Allostasis is the maintenance of stability in changing internal or external environments throughout the life course (McEwen and Wingfield, 2010). Results from previous studies focusing on the impact of allostatic mechanisms of tattooing on immune response have been mixed. One recent case study reported complications from tattooing in an immunosuppressed woman (Wilson, O'Boyle, and Leach, 2018), which is consistent with other research demonstrating that short- and long-term stress can exacerbate proinflammatory and autoimmune diseases (Dhabhar, 2014). Conversely, Baranska and colleagues' (2018) study of the interaction between inks and the immune system was conducted on mice but suggests macrophages and fibroblasts of immune response may play roles.

Our findings are somewhat consistent with other studies of SIgA and stress, indicating that tattooing follows the normal pattern of short-term stress. For instance, laboratory studies that assess immune responses to induced psychological stress (e.g., variations of the Trier Social Stress Test, which involves anticipatory waiting and public speaking, mental arithmetic) indicate that SIgA increases from pre-posttest then returns to baseline (Benham, Nash, and Baldwin, 2009; Birkett, Johnson, and Gelety, 2017; Campisi et al., 2012; De Andrés-García, Moya-Albiol, and González-Bono, 2012; Fan et al., 2009; Takatsuji et al., 2008; Trueba, Mizrachi, Auchus, Vogel, and Ritz, 2012). Our findings differ in that SIgA returned to baseline shortly after the stress in those studies. While we did not test SIgA levels after a post-tattoo refractory period, comparison of low and high tattoo experience participants indicates an accrued affect. It is possible that SIgA returns to baseline a short time after the stress of tattooing is over, but the baselines of low and high tattoo experiences is somewhat different (though the differences are not significant).

However, significant positive correlation between SIgA and total tattoo experience in our study and previous research (Lynn et al., 2016) suggests habituation occurs due to repeated dermal stress of being tattooed. This lends support to our hypothesis that tattooing is more like exercise than inoculation in how it affects the immune system. Like tattooing, exercise is culturally moderated eustress with positive emotional valence. Experimental studies of a single exercise bout on immune response are inconclusive, but regular physical activity or frequent exercise is consistently associated with enhanced immune response in general (Campbell and Turner, 2018) and increased SIgA in particular (Akimoto et al., 2003; Leicht, Bishop, and Goosey-Tolfrey, 2011). Mechanisms are still unclear, but one recently favored hypothesis suggests that exercise benefits thymopoiesis (differentiation of thymocytes into mature T cells in

the thymus) and the balance of naïve and memory T cells in the bloodstream (Simpson, 2011; Simpson and Guy, 2010). Similar mechanisms may entail from tattooing but be affected by cultural differences. If such benefits accrue, they are most likely to occur by extended dermal resistance to viruses, bacteria, and tumors, as has been observed in rodents and non-human primates (Dhabhar, 2014).

Limitations

Our interpretations are limited because a small sample size lacked the variation in tattoo experience needed to address all research questions. Nonetheless, our analysis supports previous findings among a different demographic with greater variation in gender and age parity. Additionally, despite consistent associations between total tattoo experience and SIgA measures in the current and past studies (Lynn et al., 2016), there are some challenges to the utility of oral SIgA as a reliable marker of stress-related immune response. Salivary IgA is involved in maintaining homeostasis in the host-bacteria ecology of the mouth, but optimal oral hygiene is not common in adults, resulting in high rates of between-person variation in SIgA. SIgA also varies due to circadian rhythms, psychological stress, sex differences, diet, ethnicity, disease, medications, tobacco use, and menstrual cycle phase, among other factors (Campbell and Turner, 2018).

Future research would benefit from using markers with potential for greater precision that can yield more insight into the mechanisms involved in allostatic change. The costly signaling model could also benefit from ratings of signaling (in terms of healthful appearance, attractiveness, etc.) by objective observers. Finally, refinements in methodology should include sampling at controlled time intervals instead of simply before and after tattooing; many tattoos

involve breaks or are completed over multiple days, leading to confusion in operationalizing the beginning and end of tattoo "sessions."

Conclusion

Although the number of heavily tattooed Pacific Islanders in our study was small, conducting this work in American Samoa was important from cultural and health perspectives. Samoans have a vibrant cultural tradition of tattooing. In the Samoas, as with many other cultures, tattoos are viewed as protective against illness and an indicator physical stamina (Krutak, 2012). Tattooing is a matter of pride and a cultural behavior for which Samoans are globally celebrated (Mallon, 2005; 2010; Mallon and Galliot, 2018). Psychosocial benefits of tattooing may extend to both social prestige and enhanced immune response, despite negative trends in obesity and other cardiometabolic health factors (Lin et al., 2017). Our data suggest there are biological underpinnings of these cultural understandings. This region continues to experience significant challenges with infectious and non-communicable diseases (Howells et al., 2018). We propose that the short-term risks of tattooing may be minor compared to a long-term benefits of bolstering of the immune system.

Our findings support a model for tattoos as a signal of immunocompetence and physical stamina. The persistent notion that tattooing is a visible embodiment of resilience is supported by the positive relationship between total tattooing experience and SIgA. This research is important because of the contemporary, historical, and cultural ubiquity of tattooing. When compared to other cultural stressors, such as exercise and vaccination, tattooing appears to depend on multiple applications to facilitate allostatic habituation. This response is more similar to the influence exercise has on immune response, as there is no vaccine left in the body that has a lasting impact on immune response. We predict that, as with repeated exercise, the benefits of multiple tattoo

experiences on immune response extend beyond tattoo sessions, rendering highly tattooed individuals relatively more resistant to other dermal stress. Our findings can be applied in sports, exercise, and health sciences and clinical research areas wherein cultural practices have salient, visible impacts on immune function and health. Combined, these avenues of research will improve knowledge about the mechanisms of short-term stress and allostatic habituation.

Author Contributions

Lynn designed study, analyzed data, and drafted manuscript. Lynn and Howells collected all data and revised manuscript. Herdrich assisted with field logistics. Ioane, Hudson, and Fitiao assisted with participant recruitment. All authors reviewed and approved final manuscript.

Acknowledgements

We thank the participants who took part in this study and members of the community who supported this research. In addition, we would like to thank the American Samoa Government, American Samoa Department of Public Health, American Samoa Historic Preservation Office, *Samoa News*, KVZK TV, and 98KHJ Radio for their support. We would especially like to thank Telei'ai Fanaeā Christian Ausage, Letitia "Tish" Peau-Folau, Motusa Tuileama Nua, Reggie Meredith, EmBlu Fruean-Chen, Leuila Ioane, Niko AhFoon, Tolua Christle Tavai, Whitey Chen, Brian Peck, and Michael Muehlenbein for their support and insights during this research. Lynn is grateful to the University of Alabama Research Grants Committee and College of Arts and Sciences for funding to support this project. Howells would like to acknowledge support from the University of North Carolina Wilmington and Holly Wood for research support.

References Cited

Akimoto T, Kumai Y, Akama T, Hayashi E, Murakami H, Soma R, Kuno S, Kono I. 2003. Effects of 12 months of exercise training on salivary secretory IgA levels in elderly subjects. British Journal of Sports Medicine 37(1):76-79.

Atkinson M. 2004. Tattooing and Civilizing Processes: Body Modification as Self-Control. Canadian Review of Sociology 41(2):125.

Ausage TaFC. 2015. Laei a Samoa. Pago Pago, American Samoa: Lavea'i Samoan Language Institute.

Baranska A, Shawket A, Jouve M, Baratin M, Malosse C, Voluzan O, Vu Manh TP, Fiore F, Bajenoff M, Benaroch P and others. 2018. Unveiling skin macrophage dynamics explains both tattoo persistence and strenuous removal. JExpMed 215(4):1115.

Benham G, Nash MR, Baldwin DR. 2009. A comparison of changes in secretory immunoglobulin A following a stress-inducing and stress-reducing task. Stress and Health: Journal of the International Society for the Investigation of Stress 25(1):81-90.

Beran D, Zar HJ, Perrin C, Menezes AM, Burney P. 2015. Burden of asthma and chronic obstructive pulmonary disease and access to essential medicines in low-income and middle-income countries. The Lancet Respiratory Medicine 3(2):159-170.

Birkett M, Johnson L, Gelety C. 2017. Investigation of sex differences in sIgA response to the trier social stress test. Stress Health 33(2):158-163.

Bosch JA, Ring C, de Geus EJ, Veerman EC, Amerongen AVN. 2002. Stress and secretory immunity. International review of neurobiology 52:213-253.

Buckley HR. 2000. Subadult health and disease in prehistoric Tonga, Polynesia. AmJPhysAnthropol 113(4):481-505.

Buckley HR. 2006. The predators within': investigating the relationship between malaria and health in the prehistoric Pacific Islands. Bioarchaeology of Southeast Asia 43:309.

Buckley HR, Kinsaston R, Anson D, Bedford S, Spriggs M. 2013. Differential pathogen loads affecting quality of life during Lapita colonisation of the Pacific: Skeletal pathology in the SAC Watom and Teouma samples. Homo-Journal of Comparative Human Biology 64(2):144.

Campbell JP, Turner JE. 2018. Debunking the Myth of exercise-induced immune Suppression: Redefining the impact of exercise on immunological Health Across the Lifespan. Frontiers in immunology 9(Article 648):648.

Campisi J, Bravo Y, Cole J, Gobeil K. 2012. Acute psychosocial stress differentially influences salivary endocrine and immune measures in undergraduate students. Physiology & behavior 107(3):317-321.

Caplan J, editor. 2000. Written on the body: The tattoo in European and American history. Princeton: Princeton University Press.

Carmen RA, Guitar AE, Dillon HM. 2012. Ultimate answers to proximate questions: The evolutionary motivations behind tattoos and body piercings in popular culture. Review of General Psychology 16(2):134.

Centers for Disease Control and P. 2017. About Adult BMI. U.S. Department of Health & Human Services.

Coe CL. 2002. Neuroendocrine and behavioral influences on the immune system. In: Becker JB, Breedlove SM, Crews D, McCarthy MM, editors. Behavioral endocrinology. Cambridge: MIT Press. p 373.

Cohen S, Kamarck T, Mermelstein R. 1983. A global measure of perceived stress. JHealth SocBehav 24(4):385.
Crews DE. 1988. Multiple causes of death and the epidemiological transition in American Samoa. Social Biology 35(3-4):198-213.
De Andrés-García S, Moya-Albiol L, González-Bono E. 2012. Salivary cortisol and immunoglobulin A: responses to stress as predictors of health complaints reported by caregivers of offspring with autistic spectrum disorder. Hormones and behavior 62(4):464-474.
Dehghan A, Kardys I, de Maat MPM, Uitterlinden AG, Sijbrands EJG, Bootsma AH, Stijnen T, Hofman A, Schram MT, Witteman JCM. 2007. Genetic Variation, C-Reactive Protein Levels, and Incidence of Diabetes. 56(3):872-878.
DeMello M. 1991. Anchors, Hearts and Eagles: From the Literal to the Symbolic in American Tattooing. In: Schmidt D, Smith J, editors. Literacies: Writing Systems and Literate Practices. Davis: University of California. p 93.
DeMello M. 2000. Bodies of inscription: A cultural history of the modern tattoo community. Durham, NC: Duke University Press.
Dhabhar FS. 2014. Effects of stress on immune function: the good, the bad, and the beautiful. Immunologic research 58(2-3):193-210.
Dhabhar FS, Saul AN, Daugherty C, Holmes TH, Bouley DM, Oberyszyn TM. 2010. Short-term stress enhances cellular immunity and increases early resistance to squamous cell carcinoma. Brain, behavior, and immunity 24(1):127-137.
Dhabhar FS, Viswanathan K. 2005. Short-term stress experienced at time of immunization induces a long-lasting increase in immunologic memory. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology 289(3):R738-R744.
Dobner J, Kaser S. 2018. Body mass index and the risk of infection - from underweight to obesity. Clinical Microbiology and Infection 24(1):24-28.
Fan Y, Tang Y, Lu Q, Feng S, Yu Q, Sui D, Zhao Q, Ma Y, Li S. 2009. Dynamic changes in salivary cortisol and secretory immunoglobulin A response to acute stress. Stress Health 25(2):189-194.
Gell A. 1993. Wrapping in images: Tattooing in Polynesia. New York: Oxford University Press. - xi, 374 p.
Getty T. 2002. Signaling health versus parasites. AmNat 159(4):363.
Gleeson M. 2007. Immune function in sport and exercise. JApplPhysiol 103(2):693.
Hersoug L-G, Linneberg A. 2007. The link between the epidemics of obesity and allergic diseases: does obesity induce decreased immune tolerance? Allergy 62(10):1205-1213.
Howells ME, Lynn CD, Weaver LJ, Langford-Sesepesara M, Tufa J. 2018. Zika virus in American Samoa: challenges to prevention in the context of health disparities and non-communicable disease. AnnHumBiol 45(3):229.
Ilavská S, Horváthová M, Szabová M, Nemessányi T, Jahnová E, Tulinská J, Líšková A, Wsolová L, Staruchová M, Volkovová K. 2012. Association between the human immune response and body mass index. Human Immunology 73(5):480-485.
Kim Y-C. 2017. Skin Vaccination Methods: Gene Gun, Jet Injector, Tattoo Vaccine, and Microneedle. In: Dragicevic N, Maibach HI, editors. Percutaneous Penetration Enhancers Physical Methods in Penetration Enhancement. Berlin: Springer. p 485.
Koziel S, Kretschmer W, Pawlowski B. 2010. Tattoo and piercing as signals of biological quality. Evolution and Human Behavior 31(3):187.

Krutak LF. 2012. Spiritual skin: Magical tattoos and scarification: wisdom. Healing. Shamanic power. Protection. Aschaffenburg, Germany: Edition Reuss.

Kupper TS, Fuhlbrigge RC. 2004. Immune surveillance in the skin: mechanisms and clinical consequences. 4:211.

Leicht CA, Bishop NC, Goosey-Tolfrey VL. 2011. Mucosal immune responses to treadmill exercise in elite wheelchair athletes. Medicine and Science in Sports and Exercise 43(8):1414-1421.

Lin S, Naseri T, Linhart C, Morrell S, Taylor R, McGarvey ST, Magliano DJ, Zimmet P. 2017. Trends in diabetes and obesity in Samoa over 35 years, 1978–2013. Diabetic Med 34(5):654.

Lynn CD, Dominguez JT, Decaro JA. 2016. Tattooing to "Toughen up": Tattoo experience and secretory immunoglobulin A. AmJHumBiol 28:603.

Lynn CD, Howells ME, Herdrich D, Ioane J, Hudson D, Fitiao SaTW. 2018. Tatau and the Hygiene Hypothesis: Retesting the Inoculation Effect of Tattooing in American Samoa. AmJHumBiol 30(2):215.

Mallon S. 2005. Samoan tatau as global practice. Tattoo: Bodies, Art, and Exchange in the Pacific and the West:145.

Mallon S. 2010. Samoan Tattooing, Cosmopolitans, Global Culture. Tatau: Samoan Tattoo, New Zealand Art, Global Culture:15.

Mallon S, Galliot S. 2018. Tatau: A history of Samoan tattooing. Honolulu: University of Hawaii Press.

Marcotte H, Lavoie MC. 1998. Oral Microbial Ecology and the Role of Salivary Immunoglobulin A. 62(1):71-109.

McEwen BS, Wingfield JC. 2010. What is in a name? Integrating homeostasis, allostasis and stress. Hormones and behavior 57(2):105.

Minster RL, Hawley NL, Su C-T, Sun G, Kershaw EE, Cheng H, Buhule OD, Lin J, Tuitele J, Naseri T. 2016. A thrifty variant in CREBRF strongly influences body mass index in Samoans. NatGenet 48(9):1049.

Pearson TA, Mensah GA, Alexander RW, Anderson JL, Cannon RO, Criqui M, Fadl YY, Fortmann SP, Hong Y, Myers GL and others. 2003. Markers of Inflammation and Cardiovascular Disease. 107(3):499-511.

Pokorna D, Rubio I, Müller M. 2008. DNA-vaccination via tattooing induces stronger humoral and cellular immune responses than intramuscular delivery supported by molecular adjuvants. Genetic Vaccines & Therapy 6:1.

Ruck R. 2018. The tropic of football: The long and perilous journey of Samoans in the NFL. New York: New Press.

Sapolsky RM. 2002. Endocrinology of the stress-response. In: Becker JB, Breedlove SM, Crews D, McCarthy MM, editors. Behavioral endocrinology. Cambridge: MIT Press. p 409.

Simpson RJ. 2011. Aging, persistent viral infections, and immunosenescence: can exercise" make space"? Exercise
sport sciences reviews 39(1):23-33.

Simpson RJ, Guy K. 2010. Coupling aging immunity with a sedentary lifestyle: has the damage already been done?–a mini-review. Gerontology 56(5):449-458.

Sosis R, Kress HC, Boster JS. 2007. Scars for war: evaluating alternative signaling explanations for cross-cultural variance in ritual costs. Evolution and Human Behavior 28(4):234.

Stefan N, Häring H-U, Hu FB, Schulze MB. 2013. Metabolically healthy obesity: epidemiology, mechanisms, and clinical implications. The lancet Diabetes & endocrinology 1(2):152.
Takatsuji K, Sugimoto Y, Ishizaki S, Ozaki Y, Matsuyama E, Yamaguchi Y. 2008. The effects of examination stress on salivary cortisol, immunoglobulin A, and chromogranin A in nursing students. Biomedical Research 29(4):221-224.
Tauler P, Martinez S, Moreno C, Martínez P, Aguilo A. 2013. Changes in salivary hormones, immunoglobulin A, and C-reactive protein in response to ultra-endurance exercises. Applied Physiology, Nutrition, and Metabolism 39(5):560-565.
Trueba AF, Mizrachi D, Auchus RJ, Vogel PD, Ritz T. 2012. Effects of psychosocial stress on the pattern of salivary protein release. Physiology & behavior 105(3):841-849.
Volanakis JE. 2001. Human C-reactive protein: expression, structure, and function. Molecular Immunology 38(2):189-197.
Wilson WT, O'Boyle M, Leach WJ. 2018. Unusual complication of a tattoo in an immunosuppressed patient. 2018:bcr-2018-224968.
Wohlrab S, Fink B, Kappeler PM, Brewer G. 2009. Perception of human body modification. Personality and Individual Differences 46(2):202.
Zahavi A, Zahavi A. 1997. The handicap principle: A missing piece of Darwin's puzzle. New York: Oxford University Press.

Tables

Table 1. Variable descriptives and *t*-test comparisons of pre- and post-tattoo biomarker measures.

		Mean	SD	Min-max
Perceived stress		5.1	1.83	0-8
BMI		32.0	6.23	20.2-43.4
Fat %		30.9	9.58	12.1-49.0
Handgrip strength	**Right**	90.7	24.38	47.5-138.0
	Left	84.5	22.38	45.4-133.2
	Mean	87.6	22.81	46.5-125.9
Tattoo experience	**Years tattooed**	12.5	11.96	0-39
	Number of tattoos	2.4	2.48	0-11
	Percent of body	8.8	14.10	0-50
	Total time tattooed (hours)	7.2	11.20	0-49.3
	Number of sessions	2.8	3.70	0-15
	Total tattoo experience	25.2	28.31	0-105.9
	Rate of tattoo experience	0.43	0.501	0-2.0
Tattoo duration (hours)		4.0	2.40	1.2-10.4
Pain rating		7.0	1.65	5-10
SIgA (µg/mL)	**Pre-tattoo**	263.6	134.59	34.9-546.4
	Post-tattoo	325.3	199.93	92.01-896.4
Cortisol (µg/mL)*	**Pre-tattoo**	0.143	0.09	0.03-0.39
	Post-tattoo	0.218	0.16	0.02-0.60
CRP (pg/mL)	**Pre-tattoo**	7042.7	19038.50	152.9-88139.7
	Post-tattoo	5021.1	99.04	92.3-45959.7

*$p < 0.05$

Table 2. Bivariate correlations of regression biomarkers and potential covariates.

	SIgA		$CRP_{pre\text{-}tattoo}$	$Cortisol_{post\text{-}tattoo}$
	Pre-tattoo	**Post-tattoo**		
Gender	0.09	0.14	0.25	-0.30
Age	-0.15	0.27	0.11	-0.23
Resident	-0.04	0.06	0.18	0.36
Ethnicity	-0.32	-0.15	0.12	0.03
Education	0.26	0.16	-0.50*	0.13
Marital status	-0.53**	0.25	0.10	0.01
SES	-0.48*	-0.06	0.07	-0.14
Perceived stress	0.36	0.29	-0.18	0.18
Tattoo problems	-0.08	0.001	0.89**	-0.11
Recently sick	0.20	-0.22	-0.05	-0.12
Alcohol use	-0.15	-0.01	0.51*	-0.32
Cigarette use	0.01	0.63**	-0.09	0.36
Medications	0.25	0.20	-0.14	-0.16
Recent hours worked	0.02	-0.26	0.20	-0.23
Fat percentage	0.25	0.05	-0.09	-0.13
Handgrip strength	0.12	0.09	0.15	-0.20
Social support	0.14	0.19	0.31	0.11
Tattoo artist	-0.14	0.13	-0.18	0.19
Pain rating	-0.24	0.05	0.01	0.03

*$p <$ 0.05 (2-tailed), **$p <$ 0.01 (2-tailed)

Table 3. Analysis of covariance of post-tattoo $SIgA_{log10}$.

	Standardized *ß*	***P***
Constant		<0.001
Pre-tattoo $SIgA_{log10}$	-0.10	0.485
Pre-tattoo CRP_{log10}	-0.42	0.003
Post-tattoo $cortisol_{log10}$	0.52	0.001
Total tattoo experience	0.48	0.004
Tattoo duration	-0.18	0.195
Age	0.54	0.003
Cigarette use	0.71	<0.001
BMI	0.81	<0.001
Marital status	-0.42	0.062

Figure Legends

Fig 1. Low tattoo experience participants (0-39) display slight decrease in estimated marginal means of pre- (2.274) to post-tattoo $SIgA_{log10}$ (2.264), whereas high tattoo experience participants (40+) show a general increase in immune response (2.508 to 2.578) over the course of the tattoo. Estimated marginal means represent adjustment for other model variables (pre-tattoo CRP_{log10}, post-tattoo $cortisol_{log10}$, tattoo experience, tattoo duration, age, cigarette use, BMI, and marital status).

Appendix A

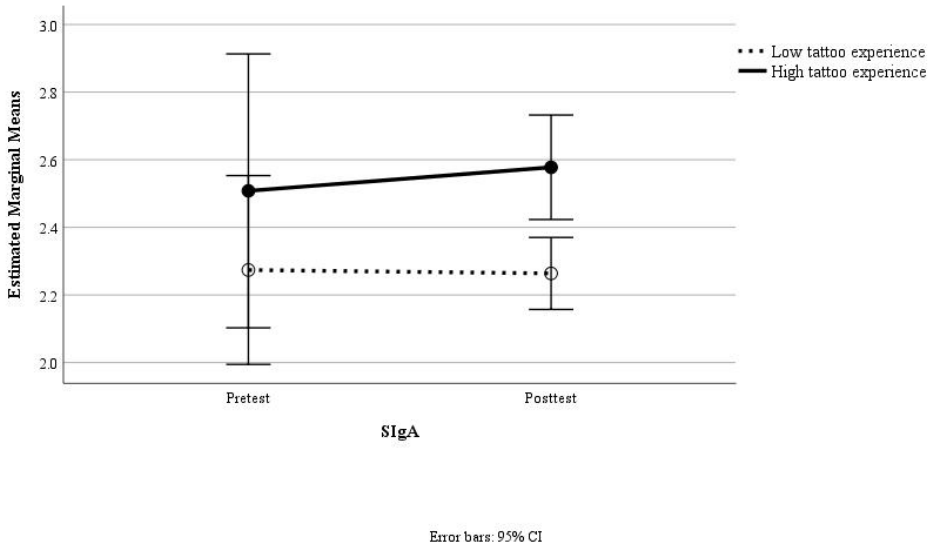

Fig 1. Low tattoo experience participants (0-39) display slight decrease in estimated marginal means of pre- (2.274) to post-tattoo $SIgA_{log10}$ (2.264), whereas high tattoo experience participants (40+) show a general increase in immune response (2.508 to 2.578) over the course of the tattoo. Estimated marginal means represent adjustment for other model variables (pre-tattoo CRP_{log10}, post-tattoo $cortisol_{log10}$, tattoo experience, tattoo duration, age, cigarette use, BMI, and marital status).

300x176mm (72 x 72 DPI)