

## Original Research Article

## Tattooing to “Toughen Up”: Tattoo Experience and Secretory Immunoglobulin A

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**Objectives:** A costly signaling model suggests tattooing inoculates the immune system to heightened vigilance against stressors associated with soft tissue damage. We sought to investigate this “inoculation hypothesis” of tattooing as a costly honest signal of fitness. We hypothesized that the immune system habituates to the tattooing stressor in repeatedly tattooed individuals and that immune response to the stress of the tattooing process would correlate with lifetime tattoo experience.

**Methods:** Participants were 24 women and 5 men (aged 18–47). We measured immune function using secretory immunoglobulin A (SIgA) and cortisol (sCORT) in saliva collected before and after tattoo sessions. We measured tattoo experience as a sum of number of tattoos, lifetime hours tattooed, years since first tattoo, percent of body covered, and number of tattoo sessions. We predicted an inverse relationship between SIgA and sCORT and less SIgA immunosuppression among those with more tattoo experience. We used hierarchical multiple regression to test for a main effect of tattoo experience on post-tattoo SIgA, controlling for pretest SIgA, tattoo session duration, body mass, and the interaction between tattoo experience and test session duration.

**Results:** The regression model was significant ( $P = 0.006$ ) with a large effect size ( $r^2 = 0.711$ ) and significant and positive main ( $P = 0.03$ ) and interaction effects ( $P = 0.014$ ).

**Conclusions:** Our data suggest that the body habituates over time to the tattooing stressor. It is possible that individuals with healthy immune systems heal faster, making them more likely to get multiple tattoos. *Am. J. Hum. Biol.* 00:000–000, 2016. © 2016 Wiley Periodicals, Inc.

Despite growing popularity of tattooing among all social classes since the 1970s (DeMello, 2000; Hill, 1972; Rubin, 1988), biological studies of tattooing have been restricted largely to health risks. Yet, historic and ethnographic accounts have long associated some forms of tattooing with “hardening” or protection against sickness (DeMello, 2000; Ludvico and Kurland, 1995). In fact, because of the health risks involved, such as skin cellulitis, bacterial infection, blood-borne disease transmission, hepatitis, allergic reactions to carcinogenic colors, and hazardous pigment concentrations (Kluger and Koljonen, 2012; Kluger et al., 2012; Laux et al., 2015; Wohlrab et al., 2009), successful tattooing might actually indicate resistance to such dangers (Koziel et al., 2010).

Tattooing may stimulate the immune system in a manner similar to a vaccination to be less susceptible to future pathogenic infiltration. Tattoo aficionados widely report becoming “addicted” to tattooing (e.g., <http://www.newloohouston.com/blog/2010/06/28/15-reasons-someone-could-become-addicted-to-tattoos/>), which we believe occurs primarily among those whose tattoos remain attractive because they heal quickly and cleanly. Tattoos may not literally harden or protect a body, but they may signal underlying immunological and genetic quality (Koziel et al., 2010). We tested this inoculation hypothesis in a sample in the U.S. South using a pre-post tattoo session design. Secretory immunoglobulin A (SIgA) and cortisol (sCORT) in saliva were collected and compared to lifetime tattoo experience. We sought to determine if tattooing could play a role as a costly honest signal of quality by influencing immunosuppression in response to the stress of tattooing.

#### Costly honest signaling

Tattoos are a form of body ornamentation that has been used throughout human history (Gilbert, 2000). Wester-

marck ([1891] 2003) suggested that such human self-adornment serves as means of attraction, as with the bright colors of birds and other sexually selected characteristics in nonhuman animals. But tattooing is not merely decorative; it is communicative, painful, and dangerous. Costly signaling is one of the most salient themes found in studies of body ornamentation (Carmen et al., 2012). Signaling refers to communication between individuals whose interests may be in conflict, such as between potential mates or predator and prey. Costly signaling occurs when the production of the signal entails some handicap to the signaler (Zahavi and Zahavi, 1997). The health risks associated with tattoos are examples of such costs (Laux et al., 2015). Death has even been reported as a result of receiving tattoos as part of traditional, extensive, and temporally intensive rites. For example, a Samoan *pe'a* is a tattoo for males covering the entire lower torso and thighs that is administered by hand tools. The continual stress of this administration, which was once the only way to tattoo, has been reported to weaken the abdominal walls and cause umbilical hernia (Moe, 1989). The ability to withstand such painful forms of tattooing was often linked to ritual change in status (Stirn, 2003).

Costly *honest* signals are those produced with high degrees of fidelity because they are particularly hard or impossible to fake (Zahavi and Zahavi, 1997). Tattoos that heal with no signs of deleterious reaction are considered

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beautiful and tattoo care websites commonly discuss how to care for new tattoos so they heal properly without losing color and clarity (e.g., <http://www.skin-artists.com/tattoo.htm>). But, tattoos are wounds and must be cared for like any wound to heal properly. Small lesions heal relatively quickly, but deeper or more extensive surface injuries take longer to heal. This time puts individuals at greater risk of infection and scarring (Galili, 2015). Thus, quick recovery from a tattoo may be a costly honest signal of immunological health and phenotypic vigor, especially when the tattoo is large or administration is intensive.

Several studies of this costly signaling model of tattooing have been conducted with mixed results. Ludvico and Kurland (1995) found some ethnologic relationships between sexual selection and scarification, which included tattooing as one form. In a study rating phenotypically male and female computer-simulations with and without tattoos (Wohlrab et al., 2009), tattooed men were considered healthier than nontattooed men by female raters and more dominant than nontattooed men by both sexes. In a study correlating tattooing and piercing with biological quality, Koziel et al. (2010) found tattooing but not piercing significantly and positively associated with greater body symmetry, an indication of developmental stability and health that is highly correlated with attractiveness.

#### *Immunosuppression and stress*

A more direct test of the costly signaling model would involve assessing the effect of tattooing on the immune system. Onset of stress, such as is involved in tattooing, is followed within the first few minutes by enhancement of immune function above baseline. Cortisol is released in humans as part of the hypothalamic-pituitary-adrenal stress response 30–60 min after stressor onset and functions, in part, to suppress this immune response and restore it to baseline (Sapolsky, 2002). Studies of SIgA and sCORT responses to stress indicate that they display inverse patterns before and after stress response (Ng et al., 2003; Watanuki and Kim, 2005). However, this inverse association is only statistically significant in awakening diurnal levels (Hucklebridge et al., 1998). This lack of correlation is likely due to their asynchronous onset/offset systems—unlike the latency of cortisol production, SIgA is immediately responsive.

Immunoglobulin A is a polymeric antibody produced in bodily mucosa and serum and the frontline defense of the upper respiratory and gastrointestinal tracts (Trochimiak and Hübner-Wozniak, 2012; Woof and Kerr, 2006). IgA antibodies provide protection against a range of pathogens and toxins through binding to immunoglobulin receptors on the basolateral surfaces of epithelial cells of the mucosa (Woof, 2013). SIgA elevation is generally associated with acute, temporary stress (Bristow et al., 1997), with immunosuppression occurring 30–60 min after stress onset. This is followed by SIgA elevation again above baseline (Trochimiak and Hübner-Wozniak, 2012) when cortisol production ceases (again, with the same 30–60 min latency after stressor offset). The degree of immunosuppression during stress response is, importantly, dependent on a number of factors, such as valence of and habituation to the stressor (Beck et al., 2000; Gleeson, 2007).

Prolonged stress or physical activity exposure and stress associated with negative mood have been repeatedly associated with prolonged immunosuppression and

low SIgA levels (Bristow et al., 1997; Trochimiak and Hübner-Wozniak, 2012). Studies of overtraining in the military (Carins and Booth, 2002) and athletics (Spence et al., 2007) support this, as they find extended physical strain associated with upper respiratory tract infections (URTIs—e.g., common colds) and immunosuppression in SIgA. Tattooing presents a similar paradigm. Tattooists consulted for this study indicate the tattoo process can produce a feeling of being “wiped out.” Symptoms akin to URTIs are anecdotally reported in conjunction with getting new tattoos, especially extensive tattoos taking long periods of time or covering much of the body. Given that tattooing sessions last long enough for the immunosuppressive phase of the stress response to be engaged by the end of the session, we would generally expect people undergoing the acute stress of tattooing to show signs of immunosuppression immediately upon completion of a tattooing session. But, as research demonstrates that exposure to repeated stressors can result in immune response habituation (Gleeson, 2007; McEwen, 2004), we predict less immunosuppression among individuals with more tattoo experience.

This can be tested by comparing SIgA and sCORT levels before and after tattooing in individuals with varying tattoo experience. Both biomarkers can be sampled easily from saliva (Kugler et al., 1992). Saliva can provide a general reflection of the entire mucosal immune system because salivary glands in the mouth are dense with immunoglobulin A-producing plasma cells and ductal and acinar cells with high levels of IgA receptors (Mestecky, 1993). SIgA is flow-dependent, meaning that the rate of salivation is important in measuring its quantity, but flow rate can be controlled for by timing saliva flow or measuring IgA against the quantity of other elements of the saliva sample, such as total protein (Brandtzaeg, 1971). Immune response has also been associated with body mass (Nazmi and Victora, 2007), age-related factors (Blackwell et al., 2010), and baseline immunological health (Koziel et al., 2010; Manning, 2002; Palmer and Strobeck, 1986; Schaap et al., 2006), so these must be accounted for.

As part of a larger study of cultural models held about tattooing, we sampled saliva among participants immediately before and after tattooing sessions to test for the degree of immunosuppression related to the tattoo. We predicted that people with more extensive previous tattooing experience would display the habituation or inoculation effect and suffer less immunosuppression as a result of the tattoo stress.

## METHODS

From May through December 2012, we collected data from three tattoo studios in Leeds and Tuscaloosa, Alabama that had granted permission to collect data on their premises. Since the larger study was focused on cultural models about tattooing among females in the U.S. Southeast, the majority of the sample comprises women. The University of Alabama Institutional Review Board approved research protocols.

#### *Participants and recruitment*

We used snowball sampling to recruit 24 women and 5 men (aged 18–47) receiving tattoos at the three studios. We used social media (Facebook and Twitter) to find

participants who would be receiving tattoos at the Tuscaloosa studios and made arrangements to be on site to collect data when they received the tattoo. The owner of the Leeds studio alerted us when clients were scheduled and gave permission to recruit on site. Three participants received multiple tattoos (2–3) during the study and contributed data each time. Each of those participants' data were averaged to provide one statistic per variable. Participation was voluntary, and all participants gave informed consent.

#### *Tattoo experience and saliva collection*

We collected demographic, tattoo experience, and anthropometric data in the tattoo studios before each participant's tattoo session. Using paper and pencil surveys, we queried number of tattoos, number of tattoo sessions, lifetime hours spent receiving tattoos, years since first tattoo, and percent of body tattooed. These values were summed to create a *tattoo experience* variable. We collected saliva samples immediately before and after each session using commercially available SalivaBio inert polymer oral swabs and storage tubes (Salimetrics LLC, State College, PA). Following manufacturer recommendations (Salimetrics and SalivaBio, 2015), participants were asked to place the swab under their tongues for 1–2 min without chewing to ensure saturation. They then placed the swab in the basket insert in the upper portion of the SalivaBio storage tube. We recorded the times of the pre- and post-tattoo measures to control for the length of time of the tattoo session, duration between biomarker measures, and diurnal patterns of sIgA and cortisol. We stored tubes in an insulated bag with a frozen ice pack while at the field sites, and then transferred them to  $-30^{\circ}\text{C}$  freezers at the University of Alabama, where they were stored until analysis.

Large tattoos often involve longer and multiple sessions. Therefore, people with more tattoo experience may also sit for longer sessions, creating an interaction between tattoo experience and session duration. We tested this by creating interaction terms for inclusion in analysis.

#### *Immunosuppression analysis*

We measured immunosuppression using posttest sIgA while controlling for pretest sIgA. Based on the principles of immunosuppression during stress outlined above, we predicted sIgA levels would be lower after the tattoo session (posttest) than before it (pretest) in people with limited tattoo experience and neutral or elevated in people with more tattoo experience, who would be habituated to such stress. We controlled for flow rate by measuring the total amount of protein in the saliva following Brandtzaeg (1971), calculating the sIgA pretest measure as  $\text{sIgA}_{\text{pretest}}/\text{Protein}_{\text{pretest}}$ , the posttest measure as  $\text{sIgA}_{\text{posttest}}/\text{Protein}_{\text{posttest}}$ , and mean sIgA change as:

$$\frac{\left(\frac{\text{sIgA}_{\text{posttest}}}{\text{Protein}_{\text{posttest}}}\right) - \left(\frac{\text{sIgA}_{\text{pretest}}}{\text{Protein}_{\text{pretest}}}\right)}{\left(\frac{\text{sIgA}_{\text{pretest}}}{\text{Protein}_{\text{pretest}}}\right)}$$

Cortisol analysis was not part of the original study design, but, because of its direct effect as an immunosuppressant, we assayed it to obtain a picture of the interaction among the tattoo and stressor, physiological stress,

and sIgA response. However, there were not sufficient salivary volumes in all remaining samples. Thus, we obtained sCORT sufficient to calculate pre-posttest difference in 22 participants. In assessing the influence of cortisol on immune response, we predicted sCORT would increase pre-posttest and have an inverse pre-posttest relationship with sIgA in participants with lower lifetime tattoo experience.

#### *Anthropometrics and potential covariates*

We collected information about demographics and other factors that might influence immune and stress response, such as alcohol, tobacco, marijuana, or medication use; recent illness; and perceived stress (Cohen and Williamson, 1988). Demographic information included age, ethnicity, relationship status and appraisal, education, and social status (Singh-Manoux et al., 2003). We collected body mass, handgrip strength, digit ratios, and bilateral symmetry data to control for baseline immune response (Innes, 1999; Koziel et al., 2010; Manning, 2002; Nazmi and Victora, 2007; Palmer and Strobeck, 1986; Schaap et al., 2006). We measured participant weight in light clothing (without shoes) using a Tanita Model TBF 310 portable bioimpedance analyzer that calculated body mass index (BMI,  $\text{kg}/\text{m}^2$ ) using self-reported height. We measured handgrip strength with a Detecto Model DHS 174 portable hand dynamometer (kg). The results of two trials on each hand were averaged together to determine a rating. We measured 2nd-to-4th digit ratios (2D4D) and bilateral symmetry by scanning participant hands using a Canon CanoScan LiDE 700F flatbed scanner. We used the measuring tool in Adobe Acrobat Pro Version 11.0.11 to calculate the length of the 2nd and 4th finger of each hand from tip to basal crease. We calculated 2D4D as  $\frac{\sum (2D/4D)_i}{2}$ , where  $i$  = respective hand. We calculated fluctuating asymmetry (FA or deviation from symmetry) following Palmer and Strobeck (1986), as  $\frac{\sum (R_i - L_i)}{\text{Number of pairs}}$ , where  $i$  = respective finger. Unfortunately, handscans of six participants were not properly saved and 2D4D and FA could not be calculated for them.

#### *Biomarker assays*

We assayed the samples with commercially available sIgA, cortisol, and total protein kits (Salimetrics LLC, State College, PA and Pierce Biotechnology, Rockford, IL). Prior to analysis, we centrifuged the samples at 3,000 rpm for 15 min to remove mucins, and all samples were assayed in duplicate. We pipetted 25  $\mu\text{L}$  of saliva into 96-well microtiter plates precoated with highly purified human sIgA, followed by a goat anti-human sIgA antibody conjugated to horseradish peroxidase. We washed the wells and detected free antihuman sIgA by adding tetramethylbenzidine to each well, stopping the reaction following incubation using a sulfuric acid solution, and determining optical density (450 nm) via a PowerWave HT Microplate Spectrophotometer (BioTek Instruments, Winooski, VT). All standards, controls, and unknowns were run in duplicate, and outcomes represent the averages. We used wells containing known high and low sIgA concentrations to correct for multiple plate comparisons. Intra- and inter-assay coefficients of variation (CVs) were below 12%.

We assayed salivas for cortisol using the Salimetrics Expanded Range High Sensitivity Salivary Cortisol

TABLE 1. Parameters of untransformed study variables

		Mean $\pm$ SD	Min	Max
Tattoo experience	Years	4.26 $\pm$ 6.27	0	26
	Number	2.76 $\pm$ 3.07	0	10
	Sessions	4.92 $\pm$ 11.91	0	60
	Hours	20.08 $\pm$ 57.70	0	240
	Percent body covered	0.17 $\pm$ 0.57	0	2
Session duration (hours)		1.49 $\pm$ 1.15	0.09	4.04
SIgA	Pretest	0.016 $\pm$ 0.007	0.00	0.03
	Posttest	0.017 $\pm$ 0.008	0.00	0.04
sCORT	Pretest	0.106 $\pm$ 0.102	0.004	0.404
	Posttest	0.140 $\pm$ 0.133	0.003	0.428
BMI (kg/m <sup>2</sup> )		25.47 $\pm$ 6.47	16.20	39.67
Handgrip strength (kg)		61.88 $\pm$ 17.36	39.83	108.45
Digit ratio (2D4D)		0.977 $\pm$ 0.03	0.92	1.03
Fluctuating asymmetry		0.010 $\pm$ 0.006	-0.10	0.10
Perceived stress		4.43 $\pm$ 3.56	0	14

SIgA values expressed in relation to total protein.

enzyme immunoassay (cat #1-3002). This is a competitive immunoassay in which cortisol in the sample competes for anti-cortisol antibody binding sites with a known quantity of horseradish peroxidase-linked cortisol. The horseradish peroxidase substrate tetramethylbenzidine develops color, measured at 450 nm, in inverse proportion to the quantity of cortisol in the original sample. We used a BioTek Powerwave HT microplate reader for absorbance detection. Intra-assay CVs were below 10%, and inter-assay CVs were below 13%.

We assayed for total protein using the Pierce BCA protein assay kit. This assay relies on the reduction of copper by protein that occurs in an alkaline solution. The quantity of protein, directly proportional to the reduction of Cu<sup>+2</sup> to Cu<sup>+1</sup>, is calculated against an albumin standard curve by quantifying Cu<sup>+1</sup> through a timed reaction with bicinchoninic acid that generates an absorbance peak at 562 nm.

### Analysis

We calculated descriptive statistics for all variables to characterize the sample. Data for subjects who contributed multiple times were tested independently and as averages, and there were no significant differences in analyses. Therefore, averages for these three participants' multiple tattoo sessions were retained for analysis. We conducted tests for normality, linearity, and homoscedasticity to ensure the underlying assumptions of multivariable analysis were met and transformed non-normal variables using log<sub>10</sub> after adding 1 to ensure constancy in valence. Transformed variables included tattoo experience measures, session duration, and sCORT. We used bivariate correlations to compare sCORT and SIgA to tattoo experience and test for predicted inverse relationships. To test the hypothesis that tattoo experience is associated with immunosuppression, we conducted hierarchical multiple regression on posttest SIgA. The first block included pretest SIgA and any demographic or anthropometric covariates that fit the model. Other covariates were chosen using stepwise methods. Block 2 included tattoo experience and session duration. Block 3 included the tattoo experience-by-session duration interaction term. All variables were standardized using Z-scores. Interaction term was calculated as the cross-product of the standardized

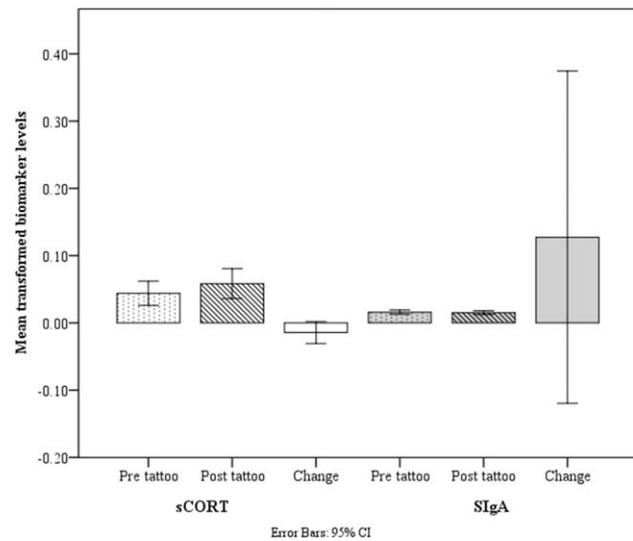


Fig. 1. Pretest, posttest, and difference levels of sCORT (log<sub>10</sub>) and SIgA (proportion of protein). Note that SIgA change is not calculated directly as pre-posttest but as proportion of whole protein. Differences are not significant.

Block 2 variables. All analyses were conducted using SPSS Version 21 (IBM Corp., 2012) and  $\alpha = 0.05$ .

## RESULTS

The sample was mostly white, young, educated, in a committed relationship, and middle-class. Mean age ( $\pm$ SD) was 26.38  $\pm$  7.15. Twenty-seven participants were white (84%), and 17 (64%) had at least some college. Participants averaged 5.33  $\pm$  1.66 on a 10-rung scale in self-reported social status. Forty-one percent were married or in a committed relationship. These demographic data did not significantly correlate with any SIgA variables and were not used in subsequent analysis. Participants ranged widely in self-reported tattoo experience (Table 1). Several were getting their first tattoo (38%), while one had  $\sim$ 240 h under the needle.

We used bivariate correlations (two-tailed) to test for significant influences of medication, alcohol, tobacco, or other drug use on SIgA, sCORT, and perceived stress; to test relationships between sCORT and SIgA; and to choose regression model variables. There were significant relationships between pre-tattoo sCORT and alcohol in the last 24 h ( $r = 0.67$ ,  $P < 0.001$ ) and post-tattoo sCORT and alcohol per week ( $r = 0.42$ ,  $P = 0.04$ ) and in the last 24 h ( $r = 0.51$ ,  $P = 0.01$ ). Since alcohol and cigarette use were not correlated with any SIgA measures in bivariate correlations or model testing, they were not used in SIgA analyses. Although we imagine the anticipation of getting a tattoo would influence pretest cortisol, there were no other significant relationships with pretest sCORT. There were no significant relationships between pre-posttest differences in sCORT and SIgA ( $r = -0.23$ ,  $P = 0.30$ ) (Fig. 1).

Comparison of SIgA and sCORT and tattoo experience variables (Table 2) indicates significant positive correlations between posttest SIgA and percent of body covered ( $P = 0.02$ ) and significant inverse correlations between SIgA change and number of sessions tattooed ( $P = 0.003$ ), hours tattooed ( $P = 0.002$ ), percent of body tattooed

TABLE 2. Bivariate correlations between  $\log_{10}$ -transformed sIgA, sCORT, and tattoo experience variables

		Tattoo experience						Session duration
		Years	Number	Sessions	Hours	Percent body covered	Total	
sIgA	Pretest	-0.283	-0.239	-0.280	-0.263	-0.148	-0.279	-0.017
	Posttest	0.154	0.229	0.402	0.430	0.534 <sup>b</sup>	0.356	0.359
	Change	-0.379	-0.419	-0.624 <sup>a</sup>	-0.638 <sup>a</sup>	-0.650 <sup>a</sup>	-0.577 <sup>a</sup>	-0.260
sCORT	Pretest	-0.286	-0.316	-0.297	-0.274	-0.095	-0.301	-0.257
	Posttest	-0.189	-0.171	-0.228	-0.188	-0.230	-0.206	-0.288
	Change	-0.194	-0.224	-0.044	-0.026	0.360	-0.084	0.180

<sup>a</sup> $P < 0.01$ .<sup>b</sup> $P < 0.05$  (two-tailed).

TABLE 3. Hierarchical multiple regression of tattoo experience on posttest sIgA, controlling for pretest sIgA, tattoo session duration (h), BMI, and tattoo experience-by-session duration interaction

	Standardized $\beta$	$P$	$r^2$	
<b>Block 1</b>				
sIgA <sub>pretest</sub>	0.452	0.080	0.254	
BMI	0.451	0.081		
<b>Block 2</b>				
sIgA <sub>pretest</sub>	0.493	0.036	0.510	
BMI	0.330	0.156		
Tattoo experience	0.445	0.050		
Tattoo session duration	0.285	0.166	0.711	
<b>Block 3</b>				
sIgA <sub>pretest</sub>	0.459	0.018		
BMI	0.385	0.049		
Tattoo experience	0.406	0.030		
Tattoo session duration	0.206	0.215		
Tattoo experience-x-session duration	0.461	0.014		

Note: All variables transformed using  $\log_{10}$ .

( $P = 0.002$ ), and total tattoo experience ( $P = 0.04$ ). Body density, handgrip strength, and FA were not associated with sIgA, but 2D4D was significantly and negatively associated with pretest sIgA ( $r = -0.475$ ,  $P = 0.04$ ) and sIgA change ( $r = -0.626$ ,  $P = 0.004$ ).

We used hierarchical multiple regression to test the influence of tattoo experience on posttest sIgA. As indicated in Table 3, we included pretest sIgA as a control in Block 1 and used stepwise methods to select from among potential demographic and anthropometric covariates. BMI provided the best fit and is also included in Block 1. Neither predictor nor the Block 1 model were significant ( $F_{2,15} = 2.548$ ,  $P = 0.112$ ). Block 2 includes tattoo experience and session duration. The Block 2 model ( $F_{4,13} = 3.381$ ,  $P = 0.042$ ) was significant, as were the variables pretest sIgA and tattoo experience. Block 3 included the tattoo experience-by-session duration interaction term; the model ( $F_{5,12} = 5.909$ ,  $P = 0.006$ ), pretest sIgA, BMI, tattoo experience, and the interaction were significant predictors in block 3.

To examine the nature of the interaction effect, we plotted tattoo experience and tattoo experience-by-session duration at  $\pm 1$  SD (<http://www.jeremydawson.co.uk/slopes.htm>). As Figure 2 illustrates, there is a greater elevation in posttest sIgA among those with more tattoo experience during longer tattoo session.

Because our sample size for 2D4D and sCORT was smaller, we used stepwise methods to test their influences on posttest sIgA, using Bonferroni correction for multiple

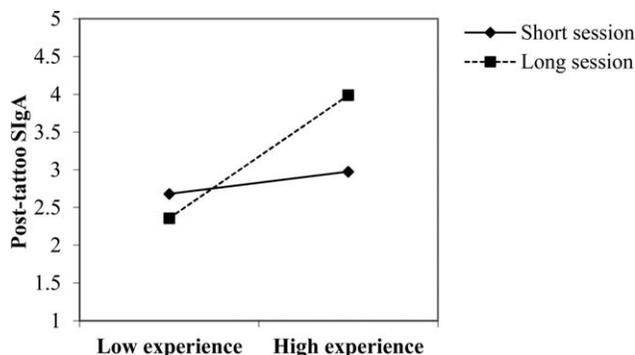


Fig. 2. Tattoo experience (unstandardized  $\beta = 0.380$ ) and session duration (0.192) plotted at  $\pm 1$  SD to visualize interaction effect (0.359). Variables are transformed ( $\log_{10}$ ) and standardized. (<http://www.jeremydawson.co.uk/slopes.htm>).

analyses ( $\alpha = 0.025$ ). We tested 2D4D, pretest sCORT, posttest sCORT, and sCORT change in models including pretest sIgA, tattoo experience, session duration, and tattoo experience-by-session duration. Only the model including digit ratio was significant ( $F_{5,9} = 4.576$ ,  $P = 0.024$ ,  $r^2 = 0.718$ ). The only variable that significantly predicted posttest sIgA in these models was tattoo experience ( $\beta = 0.531$ ,  $P = 0.025$ ) in the model that included sCORT change ( $F_{5,9} = 4.386$ ,  $P = 0.027$ ,  $r^2 = 0.709$ ).

## DISCUSSION

We tested the hypothesis that there would be less immunosuppression among those with more tattoo experience. Tattoo experience correlated positively with items comprising post-tattoo experience but no pre-tattoo measures. Although there was a nonsignificant decrease from pre-posttest in sIgA, there was a significant positive correlation between tattoo experience and posttest sIgA when controlling for the pretest measure. The sample size was small, but these data are important in providing into the body's physiological response to tattooing. There are a few ways to interpret these findings. One possibility is that sIgA is generally downregulated but more specifically responsive in older participants, who were generally those with the most tattoo experience and whose tattoo sessions were longer in our study. Studies show that life history factors influence trade-offs in immune response and that energy allocation for sIgA production may be diminished in older participants because of reduction in age-related innate immune function. For instance,

Blackwell et al. (2010) have found greater negative effects for older ages in immunoglobulin E response. Immunoglobulin E is associated mainly with allergic responses and, like IgA, is found extensively in mucous membranes. However, the relationships between age and SIgA measures and age and tattoo experience in our study were not statistically significant, supporting the interpretation that it is the tattoo experience that is important.

Thus, the second interpretation of these data is in light of the inoculation model of tattooing. We predicted a greater influence on immune response among those with more tattoo experience, which was supported. The effect was greater when the duration of the tattoo session was longer. Tattoo collectors tend to get larger tattoos that take longer to administer, often over multiple sessions. The immunological boost associated with this interaction between tattoo experience and session duration has been born out in animal studies. Administration of vaccinations via the same technique used in tattooing to inject ink under the skin is a more effective method of vaccination than intramuscular injection. Rather than restrict the inoculation effect to the specific agent being injected, tattooing transfects more cells due to its larger application area and produces a more generalized immune response (Pokorna et al., 2008; van den Berg et al., 2014). Our data may confirm the numerous historical and cultural beliefs associating tattoos with protecting the body, rather than injuring it (DeMello, 2000; Ludvico and Kurland, 1995). Accounts dating to the 19th century report of young people skirting legality to get tattooed for purposes of “toughening up” (e.g., Parry, [1933] 2006; Smeaton, 1898; Steward, 1990; Vale and Juno, 1989). Among military personnel and others who value toughness for their safety or livelihoods, tattoos have represented the ability to withstand sickness or disease and to protect against and recover from illness (Parry, [1933] 2006).

Tattooing is not unique in this protective effect, as similar relationships have been observed between SIgA and exercise (Bishop and Gleeson, 2009; Gleeson and Pyne, 2000; Leite et al., 2013) and choral singing (Beck et al., 2000). Among elite athletes, postexercise IgA suppression is associated only with prolonged bouts (> 1.5 h) and low food intake (Gleeson, 2007), whereas, among highly trained choral singers, positive stress was more likely to lead to elevated SIgA than negative stress and extended relaxation practices were more associated with higher SIgA than shorter term practices (Beck et al., 2000). Based on those studies, we can consider our data in two ways. First, participants with greater tattoo experience may be more excited than anxious about a tattooing session, resulting in reduced immunosuppression. Another explanation, which is not mutually exclusive, is that people with higher tattoo experience might also display reduced IgA suppression after tattooing, similar to elite athletes who habituate to moderate and high intensity exercise stress over time (Gleeson, 2000).

The relationship among tattooing, immune response, and athletics is no coincidence. Competition and tattooing are ways to demonstrate fitness, and tattoos may amplify the fitness signal. For instance, Mayers et al. (2002) found male athletes significantly more likely than male nonathletes to be tattooed. In contemporary North America and Europe, healthcare innovations have changed the grain and visibility of disparities (Sridhar, 2005) in a way that potentially lessens the salience of subtle signals of biological quality. Thus, tattooing may “up the ante,” as Carmen

et al. (2012) suggest, by drawing attention to one’s capacity to undergo hardship and heal. Although we did not test the tendency of individuals who heal well to be more likely to collect tattoos, tattoo artists consulted for this study indicate that tattoo collectors do tend to heal quickly from tattoos. Well-done tattoos that heal cleanly draw compliments from others, which contributes to positive feedback, leading them to get even more tattoos. In contrast, tattooing is an injury to the skin that can become infected or stimulate immune responses that damage the site and thus the ultimate appearance of tattoo (e.g., keloids or granulomas) (Goldstein, 1979; LeBlanc et al., 2012).

There are several limitations of this study, leading us to be cautious lest we over-interpret these findings. The sample was exclusively young, white, educated, middle-class, and predominantly female tattoo novices, so follow-up studies should include more varied demographic comparisons and people with more extensive tattoo experience. The region from which participants were recruited involved only cities in Alabama, where tattooing hygiene standards are high. Future research should examine tattooing culture in regions where tattoo infections are more common due to poor tattoo sanitation and with longstanding traditions of extensive tattooing. Finally, SIgA may be sensitive to dietary and diurnal influences (Gleeson, 2000) that field conditions made it difficult to control for but which should be accommodated where possible. Yet, through these data, we can better understand the relationship between tattooing and stress and why tattooing could be a reliable signal of quality. Tattooing takes a toll on the body, so more fit individuals may have immune systems better able to adapt to the additional strain. We anticipate future research will validate this immunological importance of tattooing as embodied culture.

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#### AUTHOR CONTRIBUTIONS

CDL designed the study and directed the implementation and data collection. JTD collected the data. JAD conducted the biochemical analysis, edited the manuscript, and provided critical feedback. CDL and JTD analyzed the data and drafted the manuscript.

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