Pacific Journal of Health

Volume 7 | Issue 1 Article 12

2024

A case study of endocrine and immune responses to traditional hand-tap tattooing

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Recommended Citation

Landgraf, Lauren A.; Nowak, Tomasz; Gassen, Jeffrey; Muehlenbein, Michael; and Lynn, Christopher D. (2024) "A case study of endocrine and immune responses to traditional hand-tap tattooing," *Pacific Journal of Health*: Vol. 7: Iss. 1, Article 12.
DOI: https://doi.org/10.56031/2576-215X.1060
Available at: https://scholarlycommons.pacific.edu/pjh/vol7/iss1/12

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Abstract

Tattooing is a stressor that could have adaptive benefits. Previous research indicates that endocrine and immune systems adjust to the stress of modern electric tattooing over lifetime experience, but it is unclear how these systems react to traditional hand-tap tattooing. The objective of this study was to explore how the body responds to this intense cultural stressor through examining traditional tattooing in Samoa, where saliva samples were collected throughout the first day from a Samoan man receiving the tattoo. Morning elevations and diurnal profiles of cortisol, C-reactive protein (CRP), secretory immunoglobulin A (sIgA), and bacteria killing activity (BKA) are described, and comparison is made between these data and a previous study including hand-tap and electric tattooing. Peaks in the diurnal cortisol slope correspond with anticipation of beginning an important tattoo, tattooing activity, and evening pain as stress-related analgesia diminishes and inflammation rises. Peaks in CRP levels may reflect normal moment-to-moment changes in salivary excretion. sIgA and BKA fluctuate similarly to one another throughout the day of tattooing. There were no significant differences in average pain ratings or biomarker levels between the two tattooing styles. Exploring tattooing and endocrine function is important to understanding how culture interacts with endocrine and immune function.

Keywords

tattooing, endocrine function, immune function

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Introduction

The process of getting a tattoo puts stress on the body that may have adaptive benefits, much like exercise [1-3]. Physical activity reduces allostatic load, or the collective negative consequences of lifelong wear and tear that influence health over time [4]. Even though physical activity itself is a stressor, the body adjusts to the stress of repeated bouts spaced in quick succession (daily or weekly exercise). Such habituation enables one to run longer or lift heavier weights with diminishing soreness and smaller refractory periods of recovery, all other things (effort, resistance, time) being equal [5]. Exercise also has benefits for immune function and inflammation; repeated, moderately intense exercise is associated with increased immunosurveillance and lower systemic inflammation [6]. Lynn and colleagues [3] suggest that lifetime experience with modern electric tattooing creates a similar allostatic adjustment as exercise. However, research into how the endocrine and immune systems respond to cultural stressors like the tattooing process is still in its infancy.

During the typical physiological stress response, whether activated because of distress (e.g., fear) or eustress (i.e., excitement), existing energy stores are broken into usable forms and released. Noncritical systems such as digestion, reproductive physiology, inflammation, and pain perception are curtailed. The release of glucocorticoids, after a short latency period, inhibits glucose storage and certain effector mechanisms of the immune response (e.g., cytokine and antibody production, lymphocyte activity). This temporary suppression of adaptive immunity likely functions to prevent over reactivity and autoimmunity. Aspects of innate immunity, however, do become activated during acute responses [7-9].

Research on physiological responses to tattooing has focused primarily on tissue trauma associated with modern techniques using electric devices. These studies have exclusively compared biomarker responses to receiving a new tattoo to previous tattoo experience. In four successive studies [1-3, 10], researchers explored whether people's previous tattoo experience impacted their cortisol and immune responses before and while they were getting tattooed. Previous tattoo experience was measured as the percent of body tattooed, hours spent being tattooed, number of tattoos, number of tattoo sessions, years since first tattoo, or variables made by combining these factors. The biomarkers of endocrine and immune response that were measured include immune levels (secretory immunoglobulin A), inflammation (C-reactive protein), and functional immunity (bacteria killing activity). In those studies, the hypothesis that the immune system adapts to cultural stressors over time was supported. The authors predicted that people with more previous tattoo experience would have a more consistent immune response without immunosuppression. The logic of this prediction is based in exercise science. Exercise is also a cultural stressor that people engage in to benefit their lives but comes with undesirable temporary muscle soreness, tiredness, and immunosuppression. Yet ongoing exercise is healthy, and the body adapts to the repeated stress [11-14].

Tattooing seems to follow a similar pattern as exercise. People will often note that they feared the pain when getting their first tattoo, but it was not as bad as they anticipated. In two of the previous studies of tattooing and immune and endocrine function, cortisol increased significantly from before the tattoo to after, suggesting that the fear or the pain of the tattoo may have triggered stress responses [1, 10]. In a third study, conducted at a tattoo festival, there was a slight mean change in cortisol from pre- to post-test, but the difference was not statistically significant. The authors suggest this lack of change may be because people who attend tattoo festivals generally know what they are going to experience. There may have been less anticipation or fear, or those feelings may have been blunted by other substances (that study was conducted in a state where marijuana use is legal, and several participants reported being under the influence during their tattooing sessions) [3]. Secondary analysis with combined samples from two of the previous studies also found no significant changes in cortisol [2].

These studies found that sIgA levels were higher after the tattoo (or at post-test, when repeat samples were taken one hour into the tattoo) for participants with more tattoo experience. People with less tattoo experience exhibited immunosuppression. This has been interpreted as an allostatic change in the mechanisms mediating immune function—adjustments by the immune and endocrine systems in response to the tattoo experience [1-3, 10]. Studies of immunological responses to moderate repeated bouts of exercise show similar improvements in immunosurveillance [6]. Another possibility is that the appraisal of the experience may be more influential in how the body responds than the physiological experience of being tattooed.

C-reactive protein (CRP) was used as a control for pre-existing inflammation or a health proxy in three of the previous studies, though salivary CRP is not consistently correlated with serum CRP and therefore is questionable as a marker of systemic inflammation. The biggest problem with the collection of salivary CRP is the oral environment [15], which, as described in these studies, was not controlled for. Another use of salivary CRP is in comparison to other biomarkers of immunity, as a function of serum CRP is to trigger further immune responses [16]. Serial sampling of salivary CRP and other immune biomarkers could test for this role of salivary CRP.

While the aforementioned studies provide some sense of how the cultural stress of tattooing (using modern electrical devices) impacts endocrine and immune function, it is less clear how the body reacts to intensive traditional tattooing methods, like hand-tapping, hand-poking, incision, and stitching. Of all these methods, hand-tapped and incised tattoos are reportedly the most painful, according to ethnographic interviews with tattooists and tattoo collectors around the world. The larger project investigating tattooing in the Samoan Islands (including the independent country Samoa and the U.S. territory American Samoa) of which the current case study is a part was initiated in 2016, but previous analyses included mostly modern electric with some limited hand-tap tattooing [1-3].

Depending on the length of the tattoo session, hand-tapped and incised tattoos potentially exert the most physical and mental stress. Hand-tapped tattoos seem to be particularly intense stressors because the process entails a constant tapping, especially compared to the modern wireless tattoo pens designed to minimize vibration. Furthermore, the Samoan *pe'a* specifically takes longer to administer than many modern tattoos. The *pe'a* is a tattoo traditionally given to titled Samoan men as their rite of passage into adulthood [17, 18], according to interviews with contemporary *tufuga tā tatau* (Samoan master hand-tap tattooists, who are also chiefs in charge of the craft) and other Samoans. Today, the *pe'a* is not always given to titled males (title connotes high status in local village) but can be given to others at the discretion of the *tufuga*. However, both the *pe'a* and *malu*, the female equivalent to the *pe'a*, have traditional symbolic meanings in Samoa associated with status that persist to varying degrees [17-20].

The *pe'a* covers a sizeable portion of the lower body, including much of the torso and thighs, and takes *tufuga* approximately 30–32 combined hours to complete.

This estimate is complicated because usually at least two *pe'a* and other *tatau* (Polynesian word adopted as "tattoo" in English) are being given at a time in overlapping sessions and because *tufuga* work at different rates, depending on client constraints. Clients from the Samoan diaspora often bring their families and are financially pressed by the extended hotel visits and other social expectations around the *tatau* process. Some want the entire *pe'a* done in as little as five days to save money, which contributes to greater daily physical stress from longer tattooing sessions. By contrast, native Samoans can afford to draw the process out and will take around two weeks of shorter sessions with days off to heal and recover.

The person receiving *tatau* lays on a leaf mat they have brought specifically for the purpose (they are instructed on certain rules beforehand). Fig. 1 shows a person on the first day of receiving a *tatau*, which begins with a representation of the Samoan flying fox (*Pteropus samoensis*), a type of bat called *pe'a* or *pe'a vao* in Samoan. In Samoa, *tatau* are generally administered outside in a *fale* (open-air bungalow). Most people are accompanied by family members and friends, who sit with the person being tattooed and fan them, keeping them calm as much as fending the flies and mosquitos away. Pillows wrapped in fresh plastic are used to prop the person's head up, as well as position them for the *tufuga*. Another plastic-coated pillow is used by the *tufuga* as a handrest and pivot as he works. The *tosos* (assistants) stretch the skin at the instruction of the *tufuga* and wipe the excess ink away with towels that are changed out between every client. A steady playlist of Samoan and pop music and the *tufuga*, *tosos*, and Samoan families chit-chatting throughout provide a continual soundtrack.

The *tatau* process involves dipping an *'au*, a serrated comb hafted perpendicularly to a wooden handle, into the tattooing ink. Then, the skin is stretched by the *tosos*, and the *'au* is repeatedly tapped into the skin using a *sausau* or wooden mallet

[18]. Such conditions are much less hygienic seeming than most contemporary Euromerican tattoo studios, though modern *tufuga* sterilize their equipment, use gloves, and cover everything touching the clients in disposal plastic (changed between each client) in accordance with health standards (e.g., [22]). Before they established modern hygiene measures, according to one older, high-status *tufuga*, infection was a common side effect, and a healed *pe'a* was a sign of vigour.

Each day after tattooing, a person abstains from drinking alcohol or having sex, sleeps on their mat, and receives massages to prevent the tattoo from scabbing and clotting while still being administered. The massaging may facilitate healing and prevent scabbing but is reportedly extremely painful on the fresh tattoo.

The purpose of this study was to explore how the body adapts to the stress of this experience by examining the endocrine and immune responses to hand-tapping of *pe'a* over multiple days. Saliva samples were collected from Samoans receiving the *pe'a* over multiple days, but from one individual, it was possible to collect the diurnal profile of biomarker activity for the first day of tattooing. These data make it possible to describe how salivary cortisol, sIgA, CRP, and bacteria killing activity (BKA) change throughout the *pe'a* process. Furthermore, these data are compared to findings from a previous study that included participants receiving hand-tap and electric tattooing (separately) [3] available via The University of Alabama Institutional Repository [23].

Materials and Methods

Case Study

In 2019, biomarker data were collected from a then 41-year-old Samoan male on the first day of the *tatau* process. The participant was a local schoolteacher living in Apia, the capital city of Samoa and largest city on ‘Upolu, the main island of Samoa. He had been interested in getting his *pe'a* so he could sit among his elders during rites in his home village on Savai'i, the other main island of Samoa. He was recruited via a mutual colleague to be part of this study.

Demographic information was collected to compare his data to those from previous studies, including hours worked, self-rated social status [24], and current perception of life stress [25]. He reported working an average of 40 hours per week (though had taken the week off to get tattooed), considered himself upper middleclass, and reported low perceived stress.

Ten saliva samples were collected throughout the day of sampling. The time taken to collect each saliva sample was recorded to account for the effects of flow rate on large analytes like sIgA [26].

Biomarkers

Saliva was assayed for cortisol, sIgA, CRP, and BKA as indicators of physiological stress, inflammation, and immune function. Cortisol is a steroid hormone produced in the adrenal glands that influences stress, digestion, mood, sexual desire, energy expenditure, and importantly for this study, the immune system [27]. Cortisol levels typically rise and fall in association with circadian rhythms, peaking in the morning right after rising from bed then falling over the course of the day (diurnal slope) [28]. Cortisol awakening response (CAR) has also emerged as an important aspect of the hypothalamic-pituitary-adrenal (HPA) axis and is regulated different than the rest of the diurnal cycle [29]. CAR is the sharp increase in cortisol caused by the cortisol awaking response, which has different sensitivities than diurnal cortisol. However, valid CAR assessment relies on participants closely following a timed schedule of self-sampling of saliva, starting the moment they awaken, followed by samples at strict time increments (e.g., 10 or 15 minutes) over the following 30-60 minutes [30]. This strict protocol for

CAR was not possible in the current study, so this measure is referred to as morning cortisol rather than CAR.

sIgA is an antibody found in mucosal tissue, and it is an important part of the immune system's reaction to invasive pathogens [31]. Generally, under acute stress, levels of cortisol and sIgA are negatively correlated with one another [32]. As cortisol increases during chronic stress, sIgA decreases. sIgA, like cortisol, follows a diurnal pattern in which levels peak in the morning upon awakening and fall over the course of the day [33].

CRP is an important acute-phase protein produced in the liver that correlates positively with systemic inflammation [34]. CRP takes 4-6 hours to rise in response to stress [35], so it is unclear if CRP is associated with cortisol levels or responding to other mechanisms. However, under chronic stress, CRP levels rise significantly as cortisol levels rise and remain high [36]. There is conflicting information about whether CRP follows a diurnal pattern like cortisol and sIgA [37]. Wetterö and colleagues [37] found that average levels of CRP are much higher in the morning, around 56,000 pg/mL, than they are in the evening, around 6,000 pg/mL.

The BKA assay measures how much bacteria are killed by the various immunological components of saliva. The major antibodies present in saliva include sIgA and sIgG, but saliva also contains peptides with direct antimicrobial effects like defensins, cathelicidins, and histatins, among others [38, 39]. Complementary activity and white blood cells likely also play roles in inhibiting pathogen growth in saliva. BKA is measured by incubating diluted saliva with an enumerated number of bacteria, with colony inhibition determined relative to positive and negative controls. Lynn and colleagues [2] found that participants getting tattooed had an average BKA of 11% beforehand and 19% after one hour of tattooing.

Salivary collection and analysis

The biomarker data measured in this study were collected at awakening (5:45 AM), 15-minutes later (6:00 AM), one hour later (7:00 AM), 15 minutes before beginning the tattoo (1:30 PM), two during the tattoo process (2:50 PM, 3:42 PM,), then four more times over the course of the day (4:40 PM, 6:00 PM, 8:00 PM, 11:30 PM). The tattooing lasted from 1:44-3:37 PM.

Saliva was donated via the passive drool method using 1mL cryovials (Salimetrics LLC, State College, PA). Time to fill the cryovial was recorded to control for flow rate. Samples were kept in a standard small refrigerator during data collection and kept in a cold storage bag with ice packs to return to the U.S. The samples were packed in dry ice and shipped to the Laboratory for Evolutionary Medicine Lab at Baylor University, where they were stored at -80°C until assayed.

Samples were thawed, centrifuged for 15 min at 1500rcf at room temperature, aliquoted to prevent repeatable freeze/thaw cycles, and assayed. Salivary cortisol, sIgA, and CRP were analysed with commercially available ELISA kits (#3002, #1602, #2102) from Salimetrics, LLC (State College, PA). Sensitivities for these assays were < 0.007 μg /dL, 2.5 μg /mL, and 9.72 pg/mL, respectively. Correlation coefficients for each standard curve were better than 0.999. Intra-assay CVs (based on sample duplicates within plates) were 5.46%, 4.54%, and 1.67%, respectively. Inter-assay CVs (based on high and low control duplicates between plates) were 8.23%, 10.04%, and 3.96%, respectively.

In vitro bacteria killing assays were used with saliva to measure innate immunity. Saliva was diluted 1:2 in CO2 Independent Media (Gibco #18045). A single lyophilized *E. coli* pellet (MicroBiologics Epower Microorganisms #0483E7) was reconstituted in sterile phosphate buffered saline and then diluted into a working solution, which produced approximately 200–300 colonies per 20 µL of aliquot. Aliquots of bacteria working solution were added to diluted saliva in a microcentrifuge tube, vortexed, and incubated for 30 minutes. After incubation, the samples were spread on trypticase soy agar plates (BD BBL #211043) in triplicate and incubated overnight at 37°C. The number of colonies on each plate the next day were counted, and the percent bacteria killed for each sample relative to a positive control (media and bacteria only) was calculated.

Biomarker levels were standardized using Z-scores for ease in interpretation during statistical analyses.

Comparative studies

Since control data were not collected in Samoa, the case study was compared to data collected in 2018 at the Northwest Tatau Festival in Puyallup, WA. There were four hand-tap artists working at that festival, including two Samoan *tufuga*, one Hawaiian *kakau* artist (*tatau* and *kakau* are allophones), and one Filipino *batok* (name of traditional Filipino style) artist. However, all hand-tap artists currently working in these Pacific traditions use the Samoan tools and hand-tap methods [18]. Data from that festival include 6 hand-tapped and 42 electric tattoos and were analysed for the same biomarkers as the current case study.

Statistical analysis

This study largely uses descriptive statistics to explore the influence of traditional hand-tap tattooing on endocrine and immune function. However, mean biomarker levels for electric and hand-tap tattooing were compared using independent samples *t*-test. SPSS Version 28 (IBM Corp. Armonk, New York) was used for all statistical analysis, and differences were considered significant if $p < .05$.

Results

As outlined in Table 1 and depicted in Fig. 2 cortisol levels first peaked at 6:00AM, reflecting the typical elevation that takes place upon awakening. Cortisol was also elevated at the 1:30 PM sample, just before starting the tattooing process, and again in the evening at 6:00 PM and 8:00 PM. It appears to have decreased throughout the afternoon despite the ongoing tattoo process. On average, cortisol levels decreased slightly over the course of the day.

Table 1. Biomarker levels across the first day of tattooing, adjusted for flow rate.

Sample Time	Cortisol (μg/dL)	CRP (pg/mL)	sIgA (μg/dL)	BKA (%)
05:45 AM	0.0089	7.009	9.894	89.53
06:00 AM	0.0178	9.041	11.178	61.12
07:00 AM	0.0054	58.988	4.089	-76.07
1:30 PM	0.0099	4.412	2.947	32.28
2:50 PM	0.0057	10.714	0.848	-15.93
3:42 PM	0.0058	9.819	2.912	28.57
4:40 PM	0.0026	2.467	0.595	-6.66
6:00 PM	0.0110	8.294	4.324	60.40
8:00 PM	0.0119	11.726	1.868	-17.97
11:30 PM	0.0085	26.548	3.888	46.31

sIgA levels peaked upon awakening and at 6:00 AM. It was elevated as tattooing began and ended, and it continued to rise and fall until the day was over. On average, it followed its expected diurnal pattern by declining over the course of the day.

CRP levels rose at 7:00 AM and again after the tattooing process was finished for the day, though, on average, CRP levels decrease slightly over the course of the day.

The results from the BKA assays fluctuated between positive and negative percentages of BKA throughout the course of the day. They peaked at the beginning and end of the tattooing process, but they were negative as tattooing occurred. The highest positive percentage was at 89.53% activity, which occurred at 5:45 AM, upon awakening. The lowest negative percentage was -76.07% at 7:00 AM, an hour after the cortisol levels were at their peak. On average, BKA declined over the course of the day.

Negative BKA values are the result of bacteria growing better when media contained participant saliva than when it was the media alone (positive control).

Comparison of hand-tap and electric tattooing

During the tattooing process, the participant rated his pain level as 8/10. Mean pain ratings were compared in the previous study as well, and there was a slightly higher rating for those receiving hand-tapped tattoos (mean ± SD = 5.08 ± 2.65) relative to electric (4.65 ± .32), but the difference was not statistically significant (p = .32). Biomarker levels of electric and hand-tap tattooing in a previous study were also compared, and no significant differences (equal variances not assumed) were found (Table 2).

Table 2. Independent sample t-test comparison of hand-tap and electric tattooing on cortisol, C-reactive protein (CRP), and secretory immunoglobulin A (sIgA) (Data from https://ir.ua.edu/handle/123456789/8256).

	Style	*N*	Mean	SD	*P*
Cortisol$_{\text{pretattoo}}$	Handtap	6	.4417	1.3872	.14
	Electric	40	-.0373	.9537	
Cortisol$_{\text{1-hour}}$	Handtap	6	-.0559	.6522	.43
	Electric	40	.0281	1.0683	
CRP$_{\text{pretattoo}}$	Handtap	5	.6048	1.9323	.24
	Electric	39	-.0632	.8572	
CRP$_{\text{1-hour}}$	Handtap	6	-.0002	.7592	.48
	Electric	40	.0211	1.0589	
sIgA$_{\text{pretest}}$	Handtap	6	-.6004	.2266	.07
	Electric	40	.0546	1.0477	
sIgA$_{\text{1-hour}}$	Handtap	6	1.0868	1.9327	.08
	Electric	40	-.2080	.6594	

Discussion

The purpose of this study was to provide a basic description of changes in salivary biomarkers of endocrine and immune responses throughout a day of *pe'a* tattooing. Despite common refrains from people who have received both hand-tap and electric tattooing, there are no statistically significant differences in average pain ratings or biomarker levels distinguishing the two styles based on these limited data.

Morning cortisol levels shortly after waking are significantly elevated relative to the rest of the day, as is expected of a waking response [40]. In the current study, the next peak may indicate high levels of stress and excitement in anticipation of tattoo pain. In previous studies [2, 3], there was no significant change in cortisol between pre- and post-tattoo measures, suggesting that either tattooing did not hurt or stress the participants or that their cortisol levels were already high due to anticipation of the event.

The fluctuations in CRP are more difficult to account for since it typically takes four to six hours for levels to rise in response to tissue damage. While the peak at 11:30 PM could be associated with dermatological trauma caused by the *pe'a*, since it would have occurred over four hours prior, it would not explain the peaks in the morning or afternoon. It is possible that alcohol consumption within the past 24 hours of testing impacted CRP levels, as there is some evidence that acute alcohol exposure can increase systemic inflammation [41]. An alternative and more likely explanation is that there are normal moment-to-moment changes in salivary excretion of CRP, and similar though less pronounced patterns may be observed for sIgA and BKA.

The results from the BKA assay follow a pattern similar to the fluctuations in sIgA levels [38]. Since sIgA has previously been used as a representative of immune activity, and BKA directly measures functional immune response, this result was expected. However, Muehlenbein and colleagues [42] suggest that BKA may constitute a better model of innate immune response than sIgA in isolation, as other components of the immune system may play a more important role in reacting to the bacteria used. The negative percentages of BKA indicate that the bacteria continued to grow even in the presence of the subject's saliva.

Limitations

Case studies are important in biological and social sciences because they flesh out the phenomenological and embodied aspects of cultural behaviour that can be hard to discern through analyses of sample statistics [43]. Furthermore, case studies of biological systems in response to specific stimuli can provide insights into the dynamics of immune function not readily visible when simply measuring biomarkers across time points [44]. Yet a first case study like this one is limited in that it lacks comparative data. Future studies of daily biomarker patterns for endocrine and immune function regarding electric tattooing will enable researchers to compare these results to other forms of tattooing. Repeated sampling in the morning would enable one to determine CAR, a robust marker of HPA axis activity [29]. Furthermore, it will be important to determine if individual styles of tattooing (faster or slower, heavy colour tattooing or black line work) make significant biological impacts.

Controlling the conditions around biomarker sampling in field settings is difficult, and participants cannot reasonably be asked to curtail normal activities. The participant did not smoke or take any medication during the week prior to beginning his *pe'a*, but food or beverage intake may have impacted his biomarker levels. Neither food nor caffeine intake was recorded but were consumed in the eight hours from awakening to beginning the tattoo and could have resulted in some contamination [45, 46]. Furthermore, it is common to have meals between tattooing sessions. Future research should at minimum conduct oral health examinations, which can be accomplished with a brief questionnaire [15]. Another option could be to use dried blood spot samples rather than saliva to minimize contamination concerns [47].

There are many potential avenues for expanding upon this case study. It would likely provide useful context if one were to collect samples throughout the entire tattooing process, rather than just one day, to observe how biomarker levels change when additional stress is added in the following days. Collecting saliva samples at regular intervals throughout the day, rather than only at crucial points in the process, may provide insight into any additional missed fluctuations [48]. It would also be worthwhile to gather in-depth data from more participants. Doing so would provide insight into whether biomarker levels are impacted by lifetime tattoo experience. Additionally, there are many gender issues around cultural tattooing practices that have yet to be explored; for instance, the Samoan *malu* is applied only to women and is generally completed in 1–2 sittings. While traditionally important, some Samoans see the meaning of the *malu* becoming dissolute compared to the *pe'a*, perhaps because the *malu* is smaller [20]. Future studies can investigate the *pe'a* and the *malu* as aspects of Samoan identity, including biological and cultural analyses.

Conclusion

The activities of the endocrine and immune systems were characterized based on a limited set of biomarkers with respect to beginning a traditional Samoan *pe'a* tattoo. It was anticipated that the biological responses to the *pe'a* would be more intense than those of modern electric tattoos based on anecdotal descriptions by people who have experienced both types of tattooing. The case study supports those descriptions in part; rates of pain were slightly higher than average for hand-tap tattooing, and hand-tap tattooing exerts a clear influence on biomarkers during tattooing. However, these influences do not appear exaggerated relative to responses observed in previous studies. The more striking influences of hand-tap tattooing appear to be due to the anticipation before beginning the tattoo and the pain of massaging the fresh tattoo in the evening. Since this is the first study to examine biomarkers of endocrine and immune function over a full day including diurnal profiles of multiple biomarkers, how these anticipatory and latent responses to the tattooing compare to other tattooing paradigms remains to be seen.

Acknowledgments

Thanks to Sulu’ape Tatau for their collaboration and to Su’a Sulu’ape Alaiva’a for supporting this research. Thanks to Whitey Chen for recruiting a participant for this study and an accompanying film project. Kat Beidler assisted with research support in the field, as did filmmaker Adam Booher. The Olaga Project were gracious in loaning equipment and providing emergency logistic support. Muiz Awan, Liana Donsbach, Rebecca Modisette, Vy Nguyen, and Alexandria Henderson assisted with laboratory assays. Finally, the Samoa Ministry of Health and Centre for Samoan Studies provided research approval and general support of this project.

Author Contributions

Alex Landgraf composed the first draft, analysed data, prepared figures and tables, edited and revised all drafts, and approved the final version of the manuscript.

Tomasz Nowak and Jeffrey Gassen performed biomarker assays, edited and revised manuscript, and approved the final version of the manuscript.

Michael Muehlenbein co-designed the research, performed biomarker assays, edited and revised manuscript, and approved the final version of the manuscript.

Christopher Lynn conceived and designed the research; collected and analysed data and interpreted results; drafted manuscript, edited and revised manuscript, and approved the final version of the manuscript.

Financial Support

This project was supported by funding from the Wenner-Gren Foundation (Grant No. 7985665216).

Competing Interests

The authors have no competing interests.

Ethical Approval

The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institution committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008. Free and informed consent of participants was obtained, and all research protocols were approved by The University of Alabama Institutional Review Board (#19-OR-167) and the Samoa Ministry of Health.

Data availability

Source data for this study are available upon request from the corresponding author.

References

[1] Lynn CD, Howells M, Herdrich D, Ioane J, Hudson D, Fitiao STU. The evolutionary adaptation of body art: Tattooing as costly honest signaling of enhanced immune response in American Samoa. Am.J.Hum.Biol. 2020;32:e23347.
[2] Lynn CD, Howells ME, Muehlenbein MP, Nowak T, Gassen J, Henderson A. Tattooing as a phenotypic gambit. American Journal of Biological Anthropology 2023;182:7-11.
[3] Lynn CD, Howells ME, Muehlenbein MP, Wood H, Caballero GW, Nowak TJ, et al. Psychoneuroimmunology and tattooing. Adaptive Human Behavior and Physiology 2022;8:355-69.
[4] Guidi J, Lucente M, Sonino N, Fava GA. Allostatic load and its impact on health: a systematic review. Psychother.Psychosom. 2020;90:11-27.
[5] Bonilla DA, Pérez-Idárraga A, Odriozola-Martínez A, Kreider RB. The 4R's framework of nutritional strategies for post-exercise recovery: a review with emphasis on new generation of carbohydrates. International Journal of Environmental Research and Public Health 2021;18:103.
[6] Nieman DC, Wentz LM. The compelling link between physical activity and the body's defense system. J Sport Health Sci 2019;8:203.
[7] Munck A, Guyre PM, Holbrook NJ. Physiological functions of glucocorticoids in stress and their relation to pharmacological actions. Endocrine reviews 1984;5:25-44.
[8] Sapolsky RM. Endocrinology of the stress-response. in: JB Becker, SM Breedlove, D Crews and MM McCarthy (Eds.), Behavioral endocrinology, MIT Press, Cambridge, 2002, p. 409.
[9] Sapolsky RM, Romero LM, Munck AU. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. Endocrine reviews 2000;21:55-89.
[10] Lynn CD, Dominguez JT, Decaro JA. Tattooing to "Toughen up": Tattoo experience and secretory immunoglobulin A. Am.J.Hum.Biol. 2016;28:603.
[11] Cotman CW, Berchtold NC. Exercise: a behavioral intervention to enhance brain health and plasticity. Trends in neurosciences 2002;25:295.
[12] Donges C, Duffield R, Drinkwater E. Effect of resistance or aerobic exercise training on Interleukin-6, C-Reactive protein, and body. Medicine and Science in Sports and Exercise 2010;42:304-13.
[13] Eöry A, Békési D, Eöry A, Rózsa S. Physical exercise as a resilience factor to mitigate COVID-related allostatic overload. Psychother.Psychosom. 2021;90:200-6.
[14] Gleeson M. Immune function in sport and exercise. J.Appl.Physiol. 2007;103:693.
[15] Pay JB, Shaw AM. Towards salivary C-reactive protein as a viable biomarker of systemic inflammation. Clinical Biochemistry 2019;68:1-8.
[16] Riis JL, Byrne ML, Hernández LM, Robles TF. Salivary bioscience, immunity, and inflammation. Salivary bioscience: Foundations of interdisciplinary saliva research and applications 2020;177-213.
[17] Mallon S. Samoan tatau as global practice. Tattoo: Bodies, Art, and Exchange in the Pacific and the West 2005;145.
[18] Mallon S, Galliot S. Tatau: A history of Samoan tattooing. University of Hawaii Press, Honolulu, 2018.
[19] Mallon S. Against Tradition. The Contemporary Pacific 2010;362.
[20] Samau B. Perceptions on the Commercialization of the Malu: A Case of Samoa. Global Journal of Arts, Humanities and Social Sciences 2016;4:69-80.

[21] Mallon S. Samoan Tattooing, Cosmopolitans, Global Culture. Tatau: Samoan Tattoo, New Zealand Art, Global Culture 2010;15.
[22] Samoa Ministry of Health. Health Guidelines for Tattooing. in: LTTN Naseri and RM Carney (Eds.), Samoa Ministry of Health, Apia, Samoa, 2017.
[23] Lynn C, Howells M. Inking of Immunity Seattle Dataset. Adaptive Human Behavior and Physiology, 2017.
[24] Cohen S. Measures of psychological stress. MacArthur Research Network on SES & Health, MacArthur Foundation, 2000.
[25] Cohen S, Kamarck T, Mermelstein R. A global measure of perceived stress. J.Health Soc.Behav. 1983;24:385.
[26] Kugler J, Hess M, Haake D. Secretion of salivary immunoglobulin A in relation to age, saliva flow, mood states, secretion of albumin, cortisol, and catecholamines in saliva. J.Clin.Immunol. 1992;12:45.
[27] Hellhammer DH, Wust S, Kudielka BM. Salivary cortisol as a biomarker in stress research. Psychoneuroendocrinology 2009;34:163.
[28] Adam EK, Quinn ME, Tavernier R, McQuillan MT, Dahlke KA, Gilbert KE. Diurnal cortisol slopes and mental and physical health outcomes: A systematic review and meta-analysis. Psychoneuroendocrinology 2017;83:25, 6.
[29] Steptoe A, Serwinski B. Cortisol Awakening Response. in: G Fink (Ed.), Stress: Concepts, Cognition, Emotion, and Behavior, Academic Press, San Diego, 2016, pp. 277-83.
[30] Stalder T, Kirschbaum C, Kudielka BM, Adam EK, Pruessner JC, Wüst S, et al. Assessment of the cortisol awakening response: Expert consensus guidelines. Psychoneuroendocrinology 2016;63:414-32.
[31] Bonner A, Almogren A, Furtado PB, Kerr MA, Perkins SJ. Location of secretory component on the Fc edge of dimeric IgA1 reveals insight into the role of secretory IgA1 in mucosal immunity. Mucosal Immunology 2009;2:74.
[32] Hucklebridge F, Clow A, Evans P. The relationship between salivary secretory immunoglobulin A and cortisol: neuroendocrine response to awakening and the diurnal cycle. International Journal of Psychophysiology 1998;31:69.
[33] Li T-L, Gleeson M. The effect of single and repeated bouts of prolonged cycling and circadian variation on saliva flow rate, immunoglobulin A and ??-amylase responses. Journal of sports sciences 2004;22:1016.
[34] Pepys MB, Hirschfield GM. C-reactive protein: a critical update. J Clin Invest 2003;111:1805.
[35] Bray C, Bell LN, Liang H, Haykal R, Kaiksow F, Mazza JJ, et al. Erythrocyte Sedimentation Rate and C-reactive Protein Measurements and Their Relevance in Clinical Medicine. Wmj 2016;115:319.
[36] Tolmay CM, Malan L, van Rooyen JM. The relationship between cortisol, C-reactive protein and hypertension in African and Causcasian women: the POWIRS study. Cardiovasc J Afr 2012;23:78.
[37] Wetterö J, von Löhneysen S, Cobar F, Kristenson M, Garvin P, Sjöwall C. Pronounced Diurnal Pattern of Salivary C-Reactive Protein (CRP) With Modest Associations to Circulating CRP Levels. Frontiers in Immunology 2021;11:2.
[38] Brandtzaeg P. Do salivary antibodies reliably reflect both mucosal and systemic immunity? Ann.N.Y.Acad.Sci. 2007;1098:288-311.
[39] Vila T, Rizk AM, Sultan AS, Jabra-Rizk MA. The power of saliva: Antimicrobial and beyond. PLoS Pathog 2019;15:e1008058.
[40] Fries E, Dettenborn L, Kirschbaum C. The cortisol awakening response (CAR): facts and future directions. International Journal of Psychophysiology 2009;72:67-73.

[41] Gacouin A, Roussel M, Le Priol J, Azzaoui I, Uhel F, Fest T, et al. Acute alcohol exposure has an independent impact on C-reactive protein levels, neutrophil CD64 expression, and subsets of circulating white blood cells differentiated by flow cytometry in nontrauma patients. Shock 2014;42:195.
[42] Muehlenbein MP, Prall SP, Chester E. Development of a noninvasive salivary measure of functional immunity in humans. Am.J.Hum.Biol. 2011, p. 267.
[43] George AL, Bennett A. Case studies and theory development in the social sciences. mit Press, 2005.
[44] Blackwell AD, Garcia AR. Ecoimmunology in the field: Measuring multiple dimensions of immune function with minimally invasive, field-adapted techniques. Am.J.Hum.Biol. 2022;34:e23784.
[45] Klein R. Limiting your child's fire time: A guide for concerned paleolithic parents. New Yorker, 2018.
[46] Schwartz EB, Granger DA, Susman EJ, Gunnar MR, Laird B. Assessing Salivary Cortisol in Studies of Child Development. Child Development 1998;69:1503-13.
[47] McDade TW, Williams S, Snodgrass JJ. What a drop can do: Dried blood spots as a minimally invasive method for integrating biomarkers into population-based research. Demography 2007;44:899-925.
[48] Adam EK, Kumari M. Assessing salivary cortisol in large-scale, epidemiological research. Psychoneuroendocrinology 2009;34:1423.

DOI: 10.56031/2576-215X.1060

Figure Captions

Figure 1. Providing passive drool saliva sample after day 1 of the *pe'a* tattoo (Tattoo by Su'a Sulu'ape Paulo III, photo by C. Lynn).

Figure 2. Morning and diurnal patterns of cortisol, secretory immunoglobulin A (sIgA), C-reactive protein (CRP), and bacteria killing activity (BKA) in response to receiving a traditional hand-tapped tattoo (tattooing took place during shaded period).