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Tattoos do not affect exercise-induced localised sweat rate or sodium concentration

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1 **Abstract**

2 OBJECTIVES: Skin tattoos have been shown to reduce localised sweat rate and increase sweat
3 sodium concentration ($[Na^+]$) when sweating is artificially stimulated. This study investigated
4 whether similar responses are observed with exercise-induced sweating. DESIGN: Unblinded,
5 within-participant control, single trial. METHODS: Twenty-two healthy individuals (25.1 ± 4.8
6 y (Mean \pm SD), 14 males) with a unilateral tattoo ≥ 11.4 cm² in size, ≥ 2 months in age, and
7 shaded $\geq 50\%$ participated in this investigation. Participants undertook 20 min of intermittent
8 cycling (4 x 5 min intervals) on a stationary ergometer in a controlled environment
9 ($24.6 \pm 1.1^\circ C$; $64 \pm 6\%$ RH). Resultant sweat was collected into absorbent patches applied at two
10 pairs of contralateral skin sites (pair 1: Tattoo vs. Non-Tattoo; pair 2: Control 1 vs. Control 2
11 (both non-tattooed)), for determination of sweat rate and sweat $[Na^+]$. Paired samples *t*-tests
12 were used to determine differences between contralateral sites. RESULTS: Tattoo vs. Non-
13 Tattoo: Neither sweat rate (Mean \pm SD: 0.92 ± 0.37 vs 0.94 ± 0.43 mg \cdot cm⁻² \cdot min⁻¹, respectively;
14 $p=.693$) nor sweat $[Na^+]$ (Median(IQR): 37(32–52) vs 37(31–45) mM \cdot L⁻¹, respectively;
15 $p=.827$) differed. Control 1 vs. Control 2: Neither sweat rate (Mean \pm SD: 1.19 ± 0.53 vs
16 1.19 ± 0.53 mg \cdot cm⁻² \cdot min⁻¹, respectively; $p=.917$) nor sweat $[Na^+]$ (Median(IQR): 29(26–41) vs
17 31(25–43) mM \cdot L⁻¹, respectively; $p=.147$) differed. The non-significant differences for sweat
18 rate and $[Na^+]$ between Tattoo vs. Non-Tattoo were inside the range of the within participant
19 variability (sweat rate CV_i=5.4%; sweat $[Na^+]$ CV_i=4.4%). CONCLUSION: Skin tattoos do
20 not appear to alter the rate or $[Na^+]$ of exercise-induced sweating. The influence of skin tattoos
21 on localised sweat responses may have previously been over-estimated.

22 **Key words:** eccrine gland, thermoregulation, physical activity, fluid loss

23 1.0 Introduction

24 Sweating is a crucial thermoregulatory mechanism in humans due to its facilitation of
25 evaporative heat loss ¹. In response to exercise-induced heat production, eccrine sweat glands
26 can produce whole body sweat rates up to 3 L·h⁻¹ ², with sodium concentrations ([Na⁺]) between
27 15 to 120 mM·L⁻¹ ^{3,4}. Factors that compromise the function of the sweat gland, therefore, have
28 the potential to impair thermoregulation during exercise.

29 Skin tattooing involves the deposition of ink into the skin via repeated microneedle
30 penetration and has the potential to compromise eccrine sweat gland function, and
31 consequently thermoregulation ⁵. Given that tattoos are common, (~10% of populations in
32 some countries (e.g. France, Finland and Australia) ⁶), particularly, among physically active
33 individuals (e.g. athletes and military personnel ⁷), it is surprising that only two published
34 studies (~25 year apart) have explored the effect of tattoos on sweat responses ^{5,8}. The earliest
35 of these, a case report (*n*=1), described a ~50% reduction in sweat rate responses to passive
36 heat exposure. The recent investigation compared sweat samples taken from tattooed and non-
37 tattooed skin of 10 participants, following electrochemical sweat gland stimulation (pilocarpine
38 iontophoresis) using a commercial sweat collection system (Macroduct[®], Wescor, Logan, UT).
39 Again, results indicated tattooed skin had significantly compromised sweat gland function,
40 with ~50% reduced sweat rate (Non-tattooed skin = 0.35±0.25 vs Tattooed skin 0.18±0.15
41 mg·cm⁻²·min⁻¹) and increased [Na⁺] by ~35% (Non-tattooed = 42.6±15.2 vs Tattooed =
42 69.1±28.9 mM·L⁻¹), implicating the gland's distal tubule function ⁹. These effects appeared
43 independent of the tattoo's age (range 0.2-4 years), suggesting that skin tattooing may
44 immediately impair regional thermoregulatory responses, and that these impairments are
45 unlikely to resolve over time.

46 To date, no study has explored if skin tattoos influence exercise-induced sweating beyond
47 the typical contralateral variation observed between two non-tattooed skin regions¹⁰. This is
48 important, as sweat rates are considerably larger (up to 1.2 mg·cm⁻²·min⁻¹ ³) than those

49 observed during artificial sweat stimulation, and the thermoregulatory load associated with
50 exercise triggers a cascade of neural and physiological responses that determine the sweating
51 response ¹.

52 Therefore, the aim of this study was to explore the effect of skin tattoos on exercise-
53 induced sweat responses. Specifically, we endeavoured to understand if the presence of a tattoo
54 compromised exercise-induced sweat response beyond the normal variation observed between
55 contralateral non-tattooed sites. We hypothesised that sweat samples taken from tattooed skin
56 would demonstrate compromised function (i.e. reduced sweat rates and increased [Na⁺])
57 compared to contralateral non-tattooed skin samples, irrespective of the age of the tattoo, in
58 keeping with previous reports using artificial sweat stimulation. Furthermore, we anticipated
59 that this variation would be greater than the typical variation observed between samples
60 collected from two contralateral non-tattooed sites within individuals.

61 **2.0 Methods**

62 Twenty-two healthy volunteers (14M/8F, ht = 176±9 cm, body weight = 75±13 kg)
63 participated in this investigation involving a single laboratory visit. Individuals were eligible
64 to participate if they met the following criteria: 1) aged between 18 and 45 years, 2) had a
65 unilateral tattoo that was ≥2 months old, ≥11.4 cm² in size, and ≥50% shaded, and 3) deemed
66 medically safe to undertake aerobic exercise. Table 1 describes participant and tattoo
67 characteristics. Tattoos were categorised as ‘Dense’ if >90% of the sample area was considered
68 shaded (*n*=14), or ‘Partial’ if shading covered 50 to 90% of the sample area (*n*=8) (based on
69 visual inspection). All participants provided written informed consent prior to commencing the
70 study. All data were collected in the summer months (January-March). This investigation was
71 approved by the XXXX (removed for blinding) University Human Research Ethics Committee
72 (Ref No. 2017/955).

73 *Insert table 1 about here*

74 On arrival to the laboratory ($24.6 \pm 1.1^\circ\text{C}$, $64 \pm 6\%$ RH), participants provided a urine
75 sample for the determination of urine specific gravity (U_{SG}) and a baseline nude body weight
76 measure to allow for subsequent determination of whole body fluid loss. If U_{SG} was ≥ 1.024
77 ($n=1$), indicating likely dehydration ¹¹, participants were asked to consume a bolus of water
78 (~ 400 mL) prior to providing a second U_{SG} measure ~ 30 minutes later.

79 Following the hydration measures, the skin of participants was inspected to identify the
80 most suitable sample sites. The most densely shaded tattoo site (Tattoo) with a non-tattooed
81 contralateral (Non-tattoo) area was identified. A direct distance (Lufkin[®] 2m metal tape) to the
82 nearest prominent anatomical landmark was used as a reference point to identify the
83 appropriate contralateral site. For the control sites (Control 1 & 2), priority was given to a
84 forearm location, ~ 10 cm from the wrist flexion crease, except when this area was tattooed.
85 Once identified all sites were cleaned with ethanol, followed by distilled water, and thoroughly
86 dried. Pilot testing of the Macroduct[®] sweat collection system proved unreliable in our
87 exercise-induced sweating context. Hence, a more common exercise-sweat collection protocol
88 was employed ¹⁰; the application of pre-weighed (HT-120, A&D Company, Japan, Precision =
89 0.01 g), sterile absorbent patches (Tegaderm[™] +Pad (5 cm x 7 cm), 3M Deutschland GmbH,
90 Germany, which contain an absorbance area of 2.75 cm x 4.16 cm (11.445 cm²), with a
91 maximum capacity 1.34 g. The remaining area is comprised of a non-absorbent adhesive film).
92 The use of absorbent patches has been shown to reflect ventilated capsule methods for
93 measuring local sweat rates with limits of detection of (~ 0.12 mg \cdot cm⁻² \cdot min⁻¹) suitable for the
94 expected changes in exercise-induced sweating ¹². Patches at each contralateral skin site were
95 applied simultaneously, shortly (i.e. < 5 min) before commencing exercise.

96 To induce sweating, participants completed 4×5 min intervals (with 1 min rest between
97 each) on an electronically braked cycle ergometer (Lode Excalibur Sport; Lode BV,
98 Groningen, the Netherlands). The timed cycling began at a fixed power output intended to elicit
99 a 'hard' rating of perceived exertion (RPE ~ 15 ¹³). Heart rate (Suunto Ambit, Finland) and RPE

100 were recorded at the end of each 5 min interval, at which point power output was adjusted to
101 suit individual participant responses.

102 Following exercise, a short period of rest (~3-5 min) was undertaken to ensure the
103 patches absorbed any sweat resulting from the residual heat load. Subsequently, the time from
104 exercise commencement was recorded, and the patches were removed and weighed for
105 determination of sweat rate using the following formula:

106

107
$$\text{Sweat rate (mg}\cdot\text{cm}^{-2}\cdot\text{min}^{-1}) = \frac{(\text{Post-exercise patch (mg)} - \text{Pre-exercise patch (mg)})}{11.445 \text{ cm}^{-2}} \div \text{Collection period (min}^{-1})$$

108

109 Used patches were then placed into sterile tubes and centrifuged at 3400 rpm for 5 min
110 to extract a sweat sample for subsequent $[\text{Na}^+]$ analysis using a calibrated sodium ion meter
111 (LAQUA-Twin B-722, Horiba, Japan), previously validated for sweat $[\text{Na}^+]$ analysis¹⁴. All
112 measures were performed in duplicate. Finally, participants towel dried before providing a
113 nude body weight for the determination of whole body fluid loss.

114 Statistical procedures were performed using IBM SPSS, Version 25.0. All measures
115 were examined for normality (Shapiro-Wilk test). When normally distributed, differences in
116 mean data was analysed using parametric tests (e.g. paired-samples *t*-tests). When assumptions
117 of normality were violated, differences were assessed using nonparametric measures (e.g.
118 Wilcoxon signed rank test). The co-efficient of variation (CV) across the control sweat sites
119 was considered as analytical (CV_a), within (CV_i), and between (CV_g) participant variation
120 using traditional methods¹⁵. The CV_a of the sodium analyser has been previously determined
121 (3.7%)¹⁶. The relationship between tattoo age and change in sweat responses between tattooed
122 and non-tattooed skin was assessed using the correlation coefficient. All normally distributed
123 data are presented as means and standard deviations (Mean±SD), while skewed data are
124 presented as medians and interquartile ranges (Median(IQR)). Statistical significance was
125 accepted as $p < 0.05$.

126 3.0 Results

127 Throughout exercise, participants reported a mean RPE of 14 ± 1.6 (average HR =
128 165 ± 22 bpm, av. workload = 135 ± 38 (range = 65-250) W). The exercise task resulted in a
129 mean body weight loss of $0.55 \pm 0.33\%$ from the participant's initial nude body weight, which
130 equated to an average sweat rate of 1.2 ± 0.6 L·h⁻¹. The mean sweat rate from contralateral
131 control sites was not significantly different (Control 1 = 1.19 ± 0.53 vs. Control 2 = 1.19 ± 0.53
132 mg·cm⁻²·min⁻¹, respectively, $t(21) = -0.106$, $p = 0.917$). Similarly, the median [Na⁺] from the
133 control sites was not significantly different (Control 1 = 29(26–41) vs. Control 2 = 31(25–43)
134 mM·L⁻¹, $Z = -1.450$, $p = 0.147$). The CV_i of participants' sweat rates and [Na⁺] across these sites
135 was 5.4% and 3.8%, respectively. The CV_g of participants' sweat rates and [Na⁺] across
136 forearm sites only ($n = 16$) was 47% and 43%, respectively.

137 Participants' individual sweat rates are displayed in Figure 1. The mean sweat rate from
138 tattooed skin was not significantly different from contralateral non-tattooed skin (Tattoo =
139 0.92 ± 0.37 vs. Non-tattoo = 0.94 ± 0.43 mg·cm⁻²·min⁻¹, $t(21) = -0.400$, $p = 0.693$). Furthermore,
140 when considering only densely tattooed skin ($n = 14$), sweat rates were not different from the
141 corresponding non-tattooed skin (Dense tattoo = 0.97 ± 0.44 vs. Non-Tattoo = 0.97 ± 0.50
142 mg·cm⁻²·min⁻¹, $t(13) = 0.164$, $p = 0.872$).

143 Participant's individual sweat [Na⁺] are displayed in Figure 2. The median sweat [Na⁺]
144 from tattooed skin was not significantly different from contralateral non-tattooed skin (Tattoo
145 = 37(32–52) vs Non-tattoo = 37(31–45) mM·L⁻¹, $Z = -0.218$, $p = 0.827$). When considering only
146 densely tattooed skin, sweat [Na⁺] was not significantly different from non-tattooed skin
147 (Dense tattoo = 37(30-39) vs Non-tattoo = 36(31-39) mM·L⁻¹, $Z = -0.051$, $p = 0.959$).

148 No significant correlation was observed between tattoo age and percentage change in
149 sweat rate ($r = 0.007$, $p = 0.975$) or sweat [Na⁺] ($r = -0.141$, $p = 0.532$) (Supplementary Figure).

150 4.0 Discussion

151 This study investigated the effect of skin tattoos on the localised sweat response during
152 exercise employing a sweat patch collection method. Contrary to our hypothesis, results from
153 the present study suggest that sweat rate and $[Na^+]$ do not differ between tattooed skin and
154 contralateral non-tattooed skin. Indeed, any variance observed between sites was within the
155 typical contralateral (non-tattooed skin) variability of individuals. Thus, tattoos are unlikely to
156 influence sweat-mediated thermoregulation in exercising individuals.

157 Two previous studies have raised concerns that skin tattoos may negatively influence
158 sweat-mediated thermoregulation ^{5,8}. The most recent (and more rigorous) of these
159 investigations employed artificial sweat gland stimulation and indicated that tattoos
160 substantially reduced sweat rate (Cohen's $d=0.79$) and increased sweat $[Na^+]$ (Cohen's
161 $d=1.01$). Given that these responses were independent of tattoo age, and were consistent across
162 all participants, the authors attributed the compromised sweat response to damage to, or
163 blockage of, the sweat glands caused by the repeated needling process involved in tattooing.
164 Indeed, fractional micro-needling radiofrequency treatment, (a procedure similar to tattooing
165 with concurrent thermal energy delivery), has recently been described as an effective treatment
166 modality for axillary osmidrosis ¹⁷. Collectively, this supports the logic of a probable
167 attenuation of sweating response in tattooed skin during exercise.

168 The current results are in contrast to our hypothesis; failing to demonstrate a tattoo-
169 mediated impaired sweating response local to the site of the tattoo. Moreover, no effect was
170 observed when the analysis was performed exclusively on the most densely shaded tattoos (i.e.
171 involving the greatest number of skin penetrations and ink deposition). The disparity in
172 observations between studies may, in part, be due to a number of methodological differences.
173 For instance, pilocarpine iontophoresis ⁵ induces sweating via local cholinergic stimulation,
174 whereas exercise-induced sweating triggers a combination of local and central mediators ¹⁸,
175 which results in considerably (~3-5 fold) higher sweat rates ³. Furthermore, the current
176 investigation employed absorbent patches to collect sweat, while a sodium ion-selective

177 electrode analyser was used to assess $[\text{Na}^+]$. These approaches were employed based on pilot
178 testing and recommendations as preferred methods of sweat collection during exercise due to
179 their accuracy, validity and practicality ^{10,12,14}. For example, the current method resulted in
180 sweat volumes comfortably within the absorbance capacity of the patch (e.g. maximum sweat
181 rate from any individual site = 2.65 mg/cm²/min, or 57% of the 1.34 g capacity (based on ~25
182 min exposure). The impact of these methodological differences was not directly investigated
183 as the sweat collection system used in Luetkemeier et al ⁵ proved unreliable in our exercising
184 conditions.

185 Results from the current investigation do not indicate a need for altered cooling, and/or
186 nutritional (i.e. fluid administration) advice to tattooed individuals undertaking exercise. That
187 said, it is important to acknowledge that the studies to date (including this investigation) have
188 monitored typically small tattoos in locations not necessarily associated with anatomical
189 regions known to have the largest sweat rates (e.g. head or back ¹⁹). Future investigations
190 should confirm the presence of localised anatomical and/or neurological changes associated
191 with tattooing, in particular the impact of different tattooing techniques, equipment, materials
192 (inks), and reactions ²⁰ which may, in turn, influence sweat gland function. At present, it is
193 unknown if large surface area tattoos, covering regions of high sweat rates, compromise skin
194 temperature and/or whole body thermoregulatory responses to standardised thermal loads (e.g.
195 sweating onset (via ventilated sweat capsule) or sudomotor responsiveness (via axon reflex
196 tests).

197 **5.0 Conclusion**

198 Overall, the present data indicate that tattooed skin was capable of rapidly producing
199 sweat, without influencing Na^+ resorption, in response to variable metabolic heat loads.

200 **6.0 Practical Implications**

- 201 • Previous studies have raised concerns that tattoos impair sweating responses and
202 therefore could expose individuals to greater risks of heat-related illnesses.
- 203 • Under the exercising and environmental conditions employed in this study, our data
204 suggest that skin tattoos do not appear to alter the amount (rate) or sodium concentration
205 (type) of sweat produced.
- 206 • The influence of skin tattoos on sweat responses to exercise may have previously been
207 over-estimated.

208 **Conflict of Interest:** The authors have no conflicts of interest to disclose and confirm the
209 data within is presented honestly and without fabrication, falsification, or inappropriate data
210 manipulation.

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261

262 **Figure Captions**

263 **Figure 1.** Individual sweat rates (n=22) at different skin locations. Dashed lines with open circles
264 represent partially shaded tattoos. Includes Mean±SD (thick solid line).

265 **Figure 2.** Individual sweat sodium concentrations [Na⁺] (n=22) at different skin locations. Dashed
266 lines with open circles represent partially shaded tattoos. Includes Median (IQR) (thick solid line).

267 **Supplementary Figure.** Correlations between tattoo age (y) and change in sweat responses
268 (%) between tattooed and non-tattooed skin (n=22).