

# Skin Tattoos Alter Sweat Rate and Na<sup>+</sup> Concentration

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## ABSTRACT

LUETKEMEIER, M. J., J. M. HANISKO, and K. M. AHO. Skin Tattoos Alter Sweat Rate and Na<sup>+</sup> Concentration. *Med. Sci. Sports Exerc.*, Vol. 49, No. 7, pp. 1432–1436, 2017. The popularity of tattoos has increased tremendously in the last 10 yr particularly among athletes and military personnel. The tattooing process involves permanently depositing ink under the skin at a similar depth as eccrine sweat glands (3–5 mm). **Purpose:** The purpose of this study was to compare the sweat rate and sweat Na<sup>+</sup> concentration of tattooed versus nontattooed skin. **Methods:** The participants were 10 healthy men (age = 21 ± 1 yr), all with a unilateral tattoo covering a circular area at least 5.2 cm<sup>2</sup>. Sweat was stimulated by iontophoresis using agar gel disks impregnated with 0.5% pilocarpine nitrate. The nontattooed skin was located contralateral to the position of the tattooed skin. The disks used to collect sweat were composed of Tygon® tubing wound into a spiral so that the sweat was pulled into the tubing by capillary action. The sweat rate was determined by weighing the disk before and after sweat collection. The sweat Na<sup>+</sup> concentration was determined by flame photometry. **Results:** The mean sweat rate from tattooed skin was significantly less than nontattooed skin (0.18 ± 0.15 vs 0.35 ± 0.25 mg·cm<sup>-2</sup>·min<sup>-1</sup>; *P* = 0.001). All 10 participants generated less sweat from tattooed skin than nontattooed skin and the effect size was -0.79. The mean sweat Na<sup>+</sup> concentration from tattooed skin was significantly higher than nontattooed skin (69.1 ± 28.9 vs 42.6 ± 15.2 mmol·L<sup>-1</sup>; *P* = 0.02). Nine of 10 participants had higher sweat Na<sup>+</sup> concentration from tattooed skin than nontattooed skin, and the effect size was 1.01. **Conclusions:** Tattooed skin generated less sweat and a higher Na<sup>+</sup> concentration than nontattooed skin when stimulated by pilocarpine iontophoresis. **Key Words:** PILOCARPINE, IONTOPHORESIS, HYPOHIDROSIS, DERMIS, ECCRINE GLAND

The practice of tattooing dates back to ancient civilizations but its popularity has escalated in recent times. A report by the Pew Research Center for the People and the Press, estimated that 45 million Americans had at least one tattoo (14). Furthermore, the report indicated 40% of young adults between the ages of 26 and 40 yr have one or more tattoos compared with 10% of older adults. Tattoos are particularly popular with college and professional athletes. One anecdotal report indicated that 53% of all NBA basketball players during the 2015 to 2016 season were tattooed and that two NBA teams, the Cleveland Cavaliers and the Houston Rockets, had prevalence rates of 73% (10). Military personnel are another population with a high incidence of tattooed individuals. A survey of 1835 U.S. basic recruits and advanced training personnel indicated that 36% were already tattooed and 48% were serious or very serious about getting a tattoo (1).

The tattooing process involves puncturing the skin with a cluster of needles at a frequency of 50 to 3000 punctures per minute. The needles are loaded with dye that is deposited 3 to 5 mm below the surface of the skin into the dermal layer. The punctures initiate an inflammatory response that includes the attraction of neutrophils and monocytes to the injured skin. Monocytes transition into macrophages and engulf some of the dye transporting it via the lymphatic system to lymph nodes. Other macrophages laden with dye remain in the dermis at the tattoo site, and together with dye filled dermal fibroblasts and unincorporated dye particles, form the basis of the permanent color (12).

The dermal layer of the skin is composed of collagen fibers, nerves, blood vessels, and glands including the eccrine sweat glands used to produce sweat for evaporative heat loss when internal heat generation exceeds thermoregulation by other means (15,16). Eccrine sweat glands actively draw fluid from the extracellular fluid into secretory coils located at the base of the glands. Eccrine sweat glands are stimulated primarily by sympathetic cholinergic nerve fibers using the neurotransmitter acetylcholine. Because the isosmotic primary sweat is extruded through the duct of the gland toward the skin surface, sodium chloride is reabsorbed causing the sweat Na<sup>+</sup> concentration to diminish (5).

Because of the close proximity of the eccrine sweat glands to the location where dye permanently resides after obtaining a tattoo, it is reasonable to question whether possessing a tattoo interferes with the basic functions of the sweat gland; that is,

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producing sweat and/or reabsorbing Na<sup>+</sup> from the sweat. This is an especially relevant concern for heavily tattooed athletes who exercise in the heat or military personnel who conduct training and combat operations in hot environments. Therefore, the purpose of this study was to determine whether tattooed skin altered sweat rate and sweat Na<sup>+</sup> concentration compared with nontattooed skin. An additional purpose of the study was to examine the relationship between the age of the tattoo and changes in sweat rate and Na<sup>+</sup> concentration between tattooed and nontattooed skin.

## METHODS

The institutional review board of Alma College approved the experimental protocol used in this study and written informed consent was obtained from all volunteers before their participation in the study. The participants of the study consisted of 10 apparently healthy male volunteers, and all candidates met both parts of the following entry criteria. 1) The participant had a tattoo on one side of their body located on the upper back, shoulder, upper torso, upper arm, or lower arm that covered 100% of a circular area extending 5.2 cm<sup>2</sup>. 2) The participants had nontattooed skin on opposite side of their body (contralateral) at the same anatomical position as the tattoo. Table 1 provides a list of the height, weight, age, tattoo location, and tattoo age for each participant along with means ± SD where appropriate.

All measurements were conducted in the Human Performance Research Laboratory at Alma College where the temperature and relative humidity were controlled at 19°C to 21°C and 50% to 60%, respectively. Upon arrival at the laboratory, a coin toss randomly determined whether the tattooed skin or nontattooed skin was tested first. This procedure resulted in a 7:3 split meaning seven subjects tested their tattooed skin first and three subjects tested their nontattooed skin first. A subsequent *t*-test was performed on sweat rate and sweat [Na<sup>+</sup>] and determined that this ordering was not significant to the final results. The area of skin with the highest density of ink was chosen to represent tattooed skin whereas the area of skin contralateral to the tattooed skin was chosen to represent nontattooed skin. The distance away from prominent anatomical landmarks, such as the acromion process, was used to help locate the contralateral position of the nontattooed skin. It is generally accepted that contralateral measures of sweat rate are

interchangeable and appropriate for studies requiring a comparable control (7). The two skin sites were wiped clean with 70% isopropyl alcohol, rinsed with distilled water, and allowed to dry. Participants assumed a sitting position throughout both collection periods.

Sweat was induced using the Macroduct® Sweat Collection System, model 3700-sys by Wescor (Logan, UT) (2). During both tests, the subjects were instrumented with two 5.2-cm<sup>2</sup> agar gel disks (Pilogel®) impregnated with 0.5% pilocarpine nitrate (17). Pilocarpine is an acetylcholine agonist used to promote sweating clinically as a diagnostic test for cystic fibrosis. The disks were attached to electrodes; one positive, one negative, that were used to deliver the pilocarpine into the skin using two 5-min cycles of iontophoresis. The electrodes containing the disks were attached the participant using Velcro straps and adhesive tape.

After the second cycle, the electrodes were removed and a sweat collection disk (5.2 cm<sup>2</sup>) was fitted over the skin surface where the positive electrode was before and sweat was collected for 20 min. The sweat collection disks were composed of Tygon® tubing wound into spiraling circles so that the sweat was drawn into the tubing by capillary action. The sweat rate was determined from the change in weight of the collection disk measured before and after sweat collection. After the sweat rate was measured, the sweat was blown out of the collector disk using a sterile syringe filled with 1 mL of distilled water. The diluted sweat samples were brought up to exactly 2.0 mL with distilled water and the sodium concentration was measured using a clinical flame photometer, model PFP7, by Jenway, Staffordshire, UK. The actual concentration of sodium in the sweat was determined by multiplying the concentration of the dilute sweat by the appropriate dilution factor.

The sample size of *n* = 10 was chosen based on a series of power analyses using a pilot study of three samples. Normal parameter estimates needed for a one sample *t*-test power analysis were estimated by splitting the 95% mean confidence interval from the pilot observations into five equidistant points as well as expanding the pilot standard deviation by magnitudes of 20%, 50%, and 100%. Sample size estimates ranged for 3 to 16 to achieve a power (1 - β) of 0.80. Sweat rates and sweat Na<sup>+</sup> concentrations were compared using a two-tailed paired *t*-test (Microsoft Excel). Rejection of the null hypothesis was set at *P* < 0.05. Effect sizes were calculated using the mean differences between tattooed and nontattooed skin divided by the pooled standard deviation. Pearson product-moment correlation coefficients were calculated using standard statistical practices for assessing the relationship between the ages of the tattoo with sweat rate and sweat Na<sup>+</sup> concentrations.

## RESULTS

The mean sweat rate from tattooed skin was significantly less than from nontattooed skin (0.18 ± 0.15 vs 0.35 ± 0.25 mg·cm<sup>-2</sup>·min<sup>-1</sup>; *P* = 0.001). Figure 1 illustrates the

TABLE 1. Subject characteristics.

Subject	Height, cm	Weight, kg	Age, yr	Location of Tattoo	Tattoo Age
1	165	75.0	21	Left scapula	3.5
2	180	81.8	24	Left deltoid	4.0
3	180	118.2	21	Right scapula	2.0
4	173	70.5	20	Right lower lateral rib	1.5
5	175	86.4	21	Left scapula	3.0
6	193	102.3	21	Left pectoral	3.0
7	185	100.0	21	Right upper lateral rib	4.0
8	168	72.7	21	Left deltoid	2.5
9	191	100.0	21	Left scapula	0.7
10	188	140.0	21	Left forearm	0.2
Mean	180	94.7	21		2.4
SD	10	22.1	1		1.3

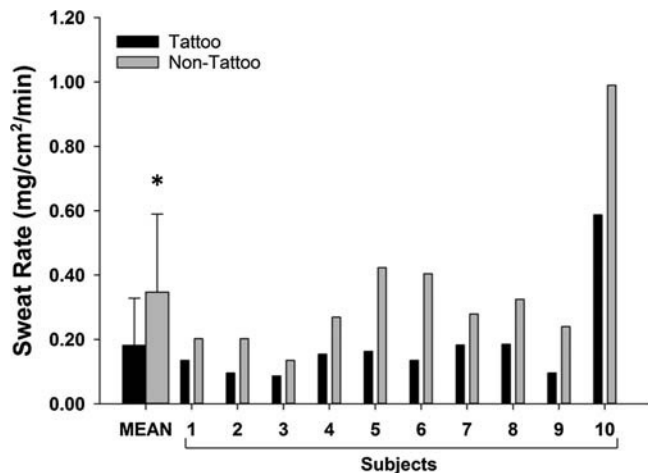


FIGURE 1—The bars on the left represent mean sweat rates ( $\pm$  SD) from tattooed and nontattooed skin following pilocarpine iontophoresis. The \* indicates that the sweat rate was significantly lower for tattooed vs contralateral nontattooed skin,  $P = 0.001$ . The thinner bars to the right represent the individual responses from each of the 10 subjects.

mean sweat rates ( $\pm$ SD) from tattooed and nontattooed skin as well as the individual values for each participant. All ten participants in the study generated a lower sweat rate from their tattooed skin compared to their contralateral nontattooed skin. The mean difference in sweat rate between tattooed skin and nontattooed skin was  $-0.17 \pm 0.11 \text{ mg}\cdot\text{cm}^{-2}\cdot\text{min}^{-1}$  and the effect size (Cohen's  $d$ ) was  $-0.79$ . This means that the mean sweat rate from tattooed skin was 0.79 standard deviations lower than the mean sweat rate of nontattooed skin. The mean ratio of sweat rates from tattooed and nontattooed skin (tattooed:nontattooed) was  $0.53 \pm 0.12$ . Therefore, on average, the sweat rate from tattooed skin was about half of the sweat rate from nontattooed skin. The correlation coefficient for the relationship between the age of the tattoo and the mean ratio of sweat rates was  $r = 0.05$  ( $r^2 = 0.002$ ) meaning that less than 1% of the variance in the ratio of sweat rates was explained by the age of the tattoo. Thus, any decrement in sweat rate due to tattooing did not appear to diminish over time.

The mean sweat  $\text{Na}^+$  concentration from tattooed skin was significantly higher than for tattooed versus nontattooed skin ( $69.1 \pm 28.9$  vs  $42.6 \pm 15.2 \text{ mmol}\cdot\text{L}^{-1}$ ,  $P = 0.02$ ; see Fig. 2). Nine of 10 participants had higher sweat  $\text{Na}^+$  concentration values for tattooed skin than nontattooed skin. The mean difference in sweat  $\text{Na}^+$  concentration between tattooed skin and nontattooed skin was  $26.5 \pm 29.7 \text{ mmol}\cdot\text{L}^{-1}$  and the effect size (Cohen's  $d$ ) was 1.01. This means that the mean sweat  $\text{Na}^+$  concentration from tattooed skin was 1.01 standard deviations higher than the mean sweat  $\text{Na}^+$  concentration from nontattooed skin. The mean ratio of  $\text{Na}^+$  concentrations between the tattooed and nontattooed skin (tattooed:nontattooed) was  $1.73 \pm 0.83$ . Therefore, the mean sweat  $\text{Na}^+$  concentration from tattooed skin was 1.73 times higher than that of nontattooed skin. The correlation coefficient for the relationship between tattoo age and ratio of  $\text{Na}^+$  concentrations was  $r = 0.25$  ( $r^2 = 0.06$ ) meaning that 6% of the variance in sweat

$\text{Na}^+$  concentration was explained by the age of the tattoo. Therefore, it is unlikely that sweat glands resume normal sodium reabsorption for years after obtaining a tattoo.

## DISCUSSION

These data clearly demonstrate a diminished sweat rate and a higher sweat  $\text{Na}^+$  concentration from tattooed versus nontattooed skin that has undergone cholinergic stimulation. They also provide preliminary evidence that these changes to sweating are unrelated to the age of the tattoo. To the authors' knowledge, this is the first study of its kind to document alterations in sweating function associated with tattooing so comparisons with similar research are not possible. Likewise, determination of the mechanism to explain these findings is premature. Yet, this study may provide a "proof of concept" for the development and advancement of future research.

In the present study, the stimulus for sweating was pilocarpine, which is an acetylcholine agonist. The administration of pilocarpine by iontophoresis provided a dose-controlled stimulus delivered locally to the sweat glands and was not temperature dependent since the ambient temperature was controlled at  $\sim 20^\circ\text{C}$ . Therefore, it was highly unlikely that the differences in sweat rate or sweat  $\text{Na}^+$  seen in the present study were due to any temperature related mechanism. If the observed changes in sweating function were unrelated to temperature, then it was most likely that they were due to alterations within the sweat glands themselves. One could speculate that sweat glands from tattooed skin were less responsive to the cholinergic stimulation. This, too, is unlikely since both sweat rate and sweat  $[\text{Na}^+]$  were affected. The binding of the cholinergic stimulus, pilocarpine, signals the production of primary sweat but it is not involved with the reabsorption of sodium and chloride within the straight duct

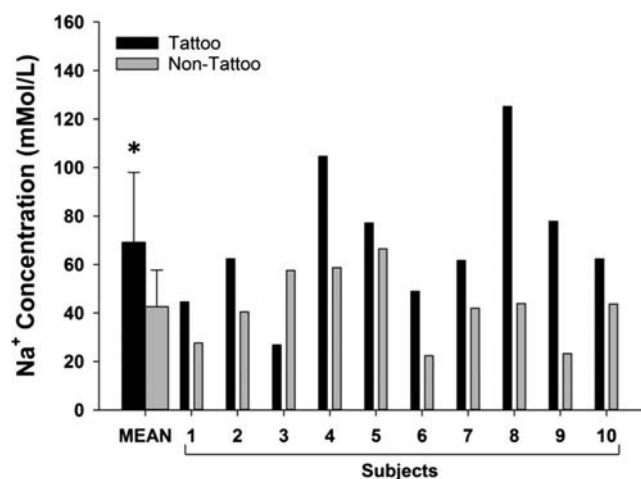


FIGURE 2—The bars on the left represent mean sweat  $[\text{Na}^+]$  ( $\pm$  SD) from tattooed and nontattooed skin following pilocarpine iontophoresis. The \* indicates that the sweat  $[\text{Na}^+]$  was significantly higher for tattooed vs contralateral nontattooed skin,  $P = 0.02$ . The thinner bars to the right represent the individual responses from each of the 10 subjects.

leading up to the skin. The primary sweat that is formed in the secretory coil is isotonic to plasma with a sodium concentration of  $\sim 140 \text{ mmol}\cdot\text{L}^{-1}$ . As the primary sweat leaves the secretory coil and traverses up the straight duct toward the skin, sodium and chloride ions are reabsorbed resulting in a final  $[\text{Na}^+]$  of 70 to  $80 \text{ mmol}\cdot\text{L}^{-1}$ . The sodium concentration of the final sweat varies according to sweat rate with higher sweat rates resulting in a higher sweat  $[\text{Na}^+]$  (4). This is thought to be due to the reduced time that  $\text{Na}^+$  ions remain in the duct and accessible for reabsorption. If sweat glands from tattooed skin are less responsive to the pilocarpine, it would be reasonable to expect a diminished sweat rate but a not an elevated sweat  $[\text{Na}^+]$ . In fact, it would be more reasonable to expect a lower sweat  $[\text{Na}^+]$  due to the increased time that the sodium ions remain in the duct available for reabsorption but, in the present study, this was not the case. Using the same logic, it is also unlikely that differences in sweat function were due to any specific ion channel except, perhaps the  $\text{Na}^+/\text{K}^+$  ATPase pumps that are operational in both the secretion of primary sweat and the alteration of final sweat.

It is also possible that sweat function was affected by trauma due to repeated punctures to the dermal layer of the skin. It is well documented that trauma from burns (9,19) and artificially induced sunburn (11) diminishes sweat rates. However, this was not the case in a study by Wing et al. (18) who examined the effects of repeatedly puncturing the skin with a stamp pad containing rows of microneedles (pretreatment) before pilocarpine iontophoresis. They reported that after pretreating the skin with microneedles, sweat production increased significantly without altering sweat osmolarity. They concluded that pretreatment with microneedles decreases skin resistance thereby improving drug deliverer by iontophoresis. It should be noted that this study dealt with the immediate effects of puncturing the skin and does not consider the long-term effects that may likely include scarring.

Another conceivable mechanism involves an obstruction of the opening of the sweat glands that reduces the amount of sweat emanating from the pore. This is thought to be the mechanism associated with antiperspirant deodorants containing aluminum chlorohydrate or aluminum zirconium chlorohydrate glycine complex. Quatral et al. (13) reported histological evidence that application of a antiperspirant with these active ingredients caused an amorphous electron dense material to the lumen of the sweat gland duct thereby blocking the release of sweat. While the composition of tattoo ink differs from artist to artist, small quantities of aluminum and other metals are common (3). One cannot rule out the possibility of a sweat gland obstruction but it is unlikely that these effects are permanent.

A more plausible mechanism for the present findings involves an innate immune response that is triggered by the insertion of ink under the skin. **The tattooing process initiates an inflammatory response** known as chemotaxis, which is a multistage process that attracts neutrophils and monocytes from the blood to the injured tissue. The monocytes are transformed into functional macrophages that engulf a portion

of the dye by phagocytosis. Neutrophils and macrophages are known to secrete inflammatory mediators and other toxic chemicals into the injured area that are not specific with regard to their target. Consequently, **normal tissue including sweat glands may be damaged during an inflammatory response** that is directed at invading pathogens.

Lea and Palowski evaluated ultrathin serial sections of human biopsy specimens, taken at 24 h, 1 month, and 1, 3, and 40 yr post-tattooing under electron microscope. They discovered that an acute inflammatory reaction characterized the tattooed area and many cells became involved in “cleaning up” the cellular debris resulting from the tattoo needles, while simultaneously taking up ink particles (8). Karanth (6) also demonstrated an increased number of Langerhans cells in frozen sections of skin biopsies taken from tattooed skin compared to control skin. Langerhans cells are dendritic cells that reside in the basal layer of the epidermis and serve as the lookouts of the adaptive immune system. They continuously scan the surface layers of skin and transport foreign antigen for presentation to responsive T lymphocytes in regional lymph nodes. As such, Langerhans cells play essential roles in the initiation of cutaneous immune responses, including immune responses to chemical allergens encountered at skin surfaces. In the same study, Karanth, found a persistently high number of Langerhans cells in tattooed skin for years after obtaining a tattoo.

Although attenuation of sweat rate observed in the present study may ultimately have relevance to thermoregulation, it is wise to interpret these initial finding as preliminary. First, the ultimate purpose of sweating is to contribute to a person’s ability to manage a heat load. **In the present study, the participants were not challenged with a heat load** and, thereby, it remains unknown whether similar alterations in sweating would accompany treatments involving external heating or internal metabolic heat production. **Second, most individuals who obtain a tattoo will do so to only a small proportion of their overall skin surface area.** In that case, one would expect that their **nontattooed skin would compensate** for any diminished sweating by their tattooed skin and that they would be able to manage a moderate heat load without any problem. However, it remains to be determined whether a high heat load combined with a large proportion of tattooed skin would sufficiently limit whole-body heat loss to an extent that heat balance could not be achieved resulting in a continuous rise in core temperature. This is particularly relevant with occupations (military and mining) and sporting endeavors (running and triathlon) that require high metabolic rates for prolonged periods in very hot/humid conditions, where individuals with increasing amounts of tattooed skin may be at further risk of noncompensable heat stress.

In summary, this study examined the differences in sweat rate and  $\text{Na}^+$  concentration between tattooed and nontattooed skin. Upon stimulation with pilocarpine, tattooed skin was associated with a lower sweat rate and a higher sweat  $\text{Na}^+$  concentration than nontattooed skin. These changes in sweating function appeared to be unrelated to the age of the tattoo.



Additional studies need to be conducted to determine the mechanism associated with these changes in sweat function and the extent that they may affect thermal balance.

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no conflicts of interest including financial, consultant, institutional, and other relationships that led to bias or a conflict of interest. The authors made every attempt to present the results of the study clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation. The authors also acknowledge that the present study does not constitute endorsement by the American College of Sports Medicine.

## REFERENCES

1. Armstrong ML, Murphy KP, Sallee A, Watson MG. Tattooed Army soldiers: examining the incidence, behavior, and risk. *Mil Med*. 2000;165(2):135–41.
2. Barlow WK, Webster HL. A simplified method of sweat collection for diagnosis of cystic fibrosis. In: *Proceedings of the Cystic Fibrosis: Horizons, Proceedings of the 9th International Cystic Fibrosis Congress*. Brighton, England; 1984. p 204.
3. Beute TC, Miller CH, Timko AL, Ross EV. In vitro spectral analysis of tattoo pigments. *Dermatol Surg*. 2008;34(4):508–15, discussion 15–6.
4. Buono MJ, Ball KD, Kolkhorst FW. Sodium ion concentration vs. sweat rate relationship in humans. *J Appl Physiol*. 2007;103(3):990–4.
5. Cui C-Y, Schlessinger D. Eccrine sweat gland development and sweat secretion. *Exp Dermatol*. 2015;24(9):1600–25.
6. Karanth SS. Tattooing increases the number of Langerhans cells in skin: an immunocytochemical study. *Exp Dermatol*. 1996;5(3):168–72.
7. Kenefick RW, Chevront SN, Elliott LD, Ely BR, Sawka MN. Biological and analytical variation of the human sweating response: implications for study design and analysis. *Am J Physiol Regul Integr Comp Physiol*. 2012;302(2):R252–8.
8. Lea PJ, Pawlowski A. Human tattoo. Electron microscopic assessment of epidermis, epidermal-dermal junction, and dermis. *Int J Dermatol*. 1987;26(7):453–8.
9. Ma K, Tan Z, Zhang C, Fu X. Mesenchymal stem cells for sweat gland regeneration after burns: From possibility to reality. *Burns*. 2016;42(3):492–9.
10. NBA tattoos. 2016. Available from: <http://nbatattoos.tumblr.com/>.
11. Pandolf KB, Gange RW, Lutzka WA, Blank IH, Kraning KK 2nd, Gonzalez RR. Human thermoregulatory responses during heat exposure after artificially induced sunburn. *Am J Physiol*. 1992; 262(4 Pt 2):R610–6.
12. Poli D, Fleenor M, Rearick M. Drawing on Popular Culture: Using Tattooing to Introduce Biological Concepts. *Am Biol Teach*. 2012; 74(6):381–5.
13. Quatralo RP, Coble DW, Stoner KL, Felger CB. The mechanism of antiperspirant action of aluminum salts. II. Histological observations of human eccrine sweat glands inhibited by aluminum zirconium chlorohydrate glycine complex. *J Soc Cosmet Chem*. 1981;32(May/June):1905–221.
14. Research Center for the People & The Press. *How Young People View Their Lives, Futures and Politics: A Portrait of "Generation Next"*. Washington, D.C.; 2007. pp. 1–43.
15. Sato K. The physiology, pharmacology, and biochemistry of the eccrine sweat gland. *Rev Physiol Biochem Pharmacol*. 1977;79:52–123.
16. Sato K, Kang WH, Saga K, Sato KT. Biology of sweat glands and their disorders. I. Normal sweat gland function. *J Am Acad Dermatol*. 1989;20(4):537–63.
17. Webster HL. Improved pilocarpine reservoir for use in sweat testing. *Clin Chem*. 1982;28:2182.
18. Wing D, Prausnitz MR, Buono MJ. Skin pretreatment with microneedles prior to pilocarpine iontophoresis increases sweat production. *Clin Physiol Funct Imaging*. 2013;33(6):436–40.
19. Zhang C, Chen Y, Fu X. Sweat gland regeneration after burn injury: is stem cell therapy a new hope? *Cytotherapy*. 2015;17(5):526–35.