

October 2021

## Diversity and Evolution of Human Eccrine Sweat Gland Density

Andrew W. Best  
*University of Massachusetts Amherst*

Follow this and additional works at: [https://scholarworks.umass.edu/dissertations\\_2](https://scholarworks.umass.edu/dissertations_2)



Part of the [Biological and Physical Anthropology Commons](#), and the [Biology Commons](#)

---

### Recommended Citation

Best, Andrew W., "Diversity and Evolution of Human Eccrine Sweat Gland Density" (2021). *Doctoral Dissertations*. 2274.  
<https://doi.org/10.7275/23522558> [https://scholarworks.umass.edu/dissertations\\_2/2274](https://scholarworks.umass.edu/dissertations_2/2274)

This Open Access Dissertation is brought to you for free and open access by the Dissertations and Theses at ScholarWorks@UMass Amherst. It has been accepted for inclusion in Doctoral Dissertations by an authorized administrator of ScholarWorks@UMass Amherst. For more information, please contact [scholarworks@library.umass.edu](mailto:scholarworks@library.umass.edu).

University of Massachusetts Amherst

**ScholarWorks@UMass Amherst**

---

Doctoral Dissertations

Dissertations and Theses

---

## Diversity and Evolution of Human Eccrine Sweat Gland Density

Andrew W. Best

Follow this and additional works at: [https://scholarworks.umass.edu/dissertations\\_2](https://scholarworks.umass.edu/dissertations_2)



Part of the [Biological and Physical Anthropology Commons](#), and the [Biology Commons](#)

---

Diversity and Evolution of Human Eccrine Sweat Gland Density

A Dissertation Presented

By

ANDREW W. BEST

Submitted to the Graduate School of the  
University of Massachusetts Amherst in partial fulfillment  
of the requirements for the degree of

DOCTOR OF PHILOSOPHY

September 2021

Anthropology



Diversity and Evolution of Human Eccrine Sweat Gland Density

A Dissertation Presented

By

ANDREW W. BEST

Approved as to style and content by:

---

Jason M. Kamilar, Chair

---

Daniel. E. Lieberman, Member

---

Brigitte Holt, Member

---

Alexander R. Gerson, Member

---

Julie Hemment, Department Chair  
Department of Anthropology

DEDICATION:

To my wife Michaela, who agreed that pursuing a Ph.D. was an adventure I needed to take.

## ACKNOWLEDGEMENTS

In chronological order, I would like to thank: my wife Michaela for encouraging and supporting this process; Dr. Daniel Lieberman, whose work inspired me to pursue this degree and who has since served as a committee member and mentor; Dr. Brigitte Holt, who agreed to sponsor my candidacy as a graduate student after only a short meeting in her office discussing human evolution; Dr. Jason Kamilar, whose assistance to me and his other graduate students borders on Herculean; and Dr. Alexander Gerson, who provided expertise in methods essential to this project.

I'd also like to express my appreciation to the Anthropology Department and Graduate School for supporting my research with grant funding, the Leakey Foundation and Wenner-Gren Foundation for taking a chance on me and supplying the bulk of funding, my fellow Kamilar Lab graduate students for their friendship, and countless researchers who have offered advice and input along the way.

## ABSTRACT

### DIVERSITY AND EVOLUTION OF HUMAN ECCRINE SWEAT GLAND DENSITY

SEPTEMBER 2021

ANDREW W. BEST, B.A., SAINT MICHAEL'S COLLEGE

M.A., QUINNIPIAC UNIVERSITY

M.A., UNIVERSITY OF MASSACHUSETTS

Ph.D., UNIVERSITY OF MASSACHUSETTS AMHERST

Directed by: Associate Professor Jason M. Kamilar

Human eccrine density is highly derived. However, little is known about contemporary variation in this trait, what shapes it, and how it influences heat dissipation. This project explores 3 questions: 1) Is variation in functional eccrine density (FED) explained by childhood climate? 2) Is this variation patterned by geographic ancestry? 3) Is variation in FED associated with differences in heat dissipation capacity? We measured FED and sweat production in 6 body areas via pharmacological stimulation and impressions of sweating skin in 72 participants. Childhood climate variables were taken from the WorldClim database and geographic ancestry was estimated with 23andMe tests. The relationship between FED and heat dissipation capacity was measured in 7 heat-acclimatized endurance runners who cycled in a metabolic chamber at 30°C. Indirect calorimetry was used to calculate heat dissipation quotient (HDQ). Interindividual variation in 6-site FED was more than twofold, ranging from 60.9 to 132.7 glands/cm<sup>2</sup>. Variation in 6-site FED was best explained by body surface area (negative association,  $p < 0.001$ ), sex (higher FED in females,  $p = 0.19$ ), and age (higher FED in younger participants,  $p < 0.05$ ). FED of the forearm was associated with a high proportion of European ancestry ( $p = 0.07$ ); geographic ancestry and childhood climate conditions were otherwise not associated with variation in FED. Males on average produced twice as much sweat as females ( $p < 0.05$ ) and sweat production was unrelated to FED ( $r^2 = 0.004$ ). HDQ was best explained by  $\dot{V}O_2$ -peak and whole-body sweat loss and was not related to FED. Our results suggest that variation in per-gland sweat production renders differences in contemporary FED physiologically unimportant, and there is no tradeoff between heat dissipation and water conservation within the range of contemporary FED. In this view, eccrine density did not change via evolutionary adaptation or phenotypic plasticity as humans moved into novel climates; sweating capacity was instead altered via gland-level adaptations. Future research should measure effects of FED in dehydrated states, carefully control for effects of microclimate to rule out phenotypic plasticity in FED, and determine whether gland-level adaptations are sufficient to buffer against increased salt losses potentially incurred by lower FED.



## TABLE OF CONTENTS

	Page
ACKNOWLEDGMENTS.....	v
ABSTRACT.....	vi
LIST OF TABLES .....	ix
LIST OF FIGURES .....	x
CHAPTER	
I. BACKGROUND: HUMAN ECCRINE SWEAT GLAND DENSITY	
A. Introduction.....	1
B. Eccrine glands and human heat acclimation.....	3
C. Diversity in human eccrine gland density.....	5
D. Are there “inactive” glands?.....	7
E. Evolutionary origins of human eccrine gland density.....	11
F. Current evidence for factors influencing variation in modern human eccrine gland diversity.....	15
G. Proximate mechanisms influencing eccrine gland density.....	19
H. Does gland density matter?.....	22
II. A METHOD OF MEASURING FUNCTIONAL ECCRINE DENSITY	
A. Introduction.....	24
B. Methods.....	25
C. Results.....	28
D. Discussion.....	30
III. THE RELATIONSHIP BETWEEN FUNCTIONAL ECCRINE DENSITY AND HEAT DISSIPATION CAPACITY	
A. Introduction.....	32
B. Methods.....	34
C. Results.....	39
D. Discussion.....	41

#### IV. UNDERSTANDING VARIATION IN HUMAN FUNCTIONAL ECCRINE DENSITY

A. Introduction.....	45
B. Methods.....	46
C. Results.....	50
D. Discussion.....	51
E. Conclusions.....	60

#### APPENDICES

A. Tables.....	61
B. Figures.....	66

BIBLIOGRAPHY.....	71
-------------------	----

## LIST OF TABLES

Table	Page
1. Observed active eccrine gland densities vs. results reported in the literature.....	61
2. Summary of volunteer demographics, anthropometrics and performance characteristics.....	61
3. Functional eccrine gland densities (FED) and sweat production in response to pilocarpine.....	61
4. Heat dissipation test results.....	62
5. Results of the generalized linear mixed models showing associations between heat dissipation quotient and independent variables.....	62
6. Relative importance of independent variables in explaining variation in heat dissipation quotient.....	62
7. Demographics and anthropometrics.....	63
8. Gland densities and sweat weights.....	63
9. Results of the generalized linear mixed models.....	64
10. Relative importance of independent variables in explaining variation in dependent variables.....	65

## LIST OF FIGURES

Figure	Page
1. ImageJ analysis.....	66
2. Results of repeated counts of the same impressions by one researcher.....	66
3. Results of repeated counts of the same impressions by three researchers.....	67
4. Schematic of flow-through respirometry using the University of Massachusetts Human Testing Center's flex room calorimeter.....	67
5. Results of select simple linear regressions.....	68
6. Example heat test data from volunteer T7.....	68
7. 6-site average functional eccrine density vs. age, simple linear regression.....	69
8. 6-site average functional eccrine density vs. body surface area, simple linear regression.....	69
9. 6-site total sweat production.....	70

## **CHAPTER 1**

### **BACKGROUND: HUMAN ECCRINE SWEAT GLAND DENSITY**

#### **Introduction**

Human heat dissipation capacity is highly derived, far surpassing that of many other mammals (Carrier et al., 1984; Jablonski & Chaplin, 2000; Jablonski, 2004; Lieberman, 2015). The evolutionary roots of this exceptional thermoregulation reach back to basal catarrhine primates, which saw the expansion of eccrine sweat glands from the hands and feet to the body surface. The anthropoid (ape) lineage is characterized by reduced hair follicle density, and in hominins (human ancestors) body hair evolved into microscopic vellus hairs, further increasing the effectiveness of sweating (Kamberov et al., 2018).

Considerable attention has been paid to understanding the physiology of human thermoregulation, particularly the compensatory responses that accompany heat exposure and the acclimation that follows. Research on the human sweat apparatus, including the eccrine sweat glands, has primarily focused on the histology and biochemistry of individual glands. Comparatively little attention has been directed towards understanding interindividual variation in human eccrine sweat gland density. This measure should have significant physiological relevance due to its obvious potential relationship with heat dissipation. It also has evolutionary importance because increased eccrine gland density, and perhaps the ability to adjust active gland density to climate, were likely essential adaptations for human ancestors. At some point in human evolution, hominins started to walk and run long distances in open, semi-arid habitats (Blumenthal et al., 2017) with the genus *Homo* being especially characterized by increased locomotor

activity (Bramble & Lieberman, 2004). These thermoregulatory challenges were further compounded by bigger brains which demanded further adaptations to keep cool during physical activity (Lieberman, 2011).

Kuno (1956) was among the first to recognize the importance of variations in eccrine gland density among humans. Based on studies of living humans he hypothesized that eccrine sweat glands become fully functional by age 2.5 via cholinergic innervation, while some proportion of glands remain inactive for life (Kuno, 1956; Thomson, 1954). Further, he proposed that active gland density was phenotypically plastic, with hotter early childhood climates eliciting greater gland activation, resulting in higher gland density in adulthood. In addition to phenotypic plasticity, evolutionary forces may have shaped diversity in eccrine density as humans migrated out of Africa into novel environments. Sixty-three years later Kuno's hypotheses remain largely untested. Similarly, existing data on inter-population diversity in eccrine density are insufficient owing to small sample sizes, inconsistency in methodology across studies, and methodological limitations (Taylor, 2006).

Characterizing diversity in human eccrine gland density and assessing the contributions of phenotypic plasticity and evolutionary history to this diversity are essential to understanding the evolution of human thermoregulation. This chapter aims to: 1) provide context for understanding the role of eccrine glands in modern human thermoregulation, and in the span of human evolution; 2) summarize current methods for measuring variation in human eccrine gland density and what these variations imply; and 3) present potential mechanisms through which eccrine density may be shaped by both evolutionary forces and climate-driven plasticity.

### **Eccrine glands and human heat acclimation**

The base of the eccrine gland is a coiled structure, lying in the dermis and surrounded by a capillary cage that provides water and electrolytes for sweat production (Yamamoto, 1990). The secretory coil leads to a duct that extends through the epidermis and opens onto the skin surface as a pore. Each gland is innervated by multiple sudomotor nerve fibers of the sympathetic nervous system (Kennedy, Wendelschafer-Crabb & Brelje, 1994) using the neurotransmitter acetylcholine. When stimulated by these nerve fibers, sweat produced in the secretory coil is forced out onto the skin surface; evaporation of sweat cools the skin hence underlying venous blood, thus lowering core temperature. Sweat production in the secretory coil relies upon active transport which is fueled by intracellular and plasma glucose via both aerobic and anaerobic metabolism (Sato & Dobson, 1973; Sato, 1977; Smith & Dobson, 1966). During both passive heating and physical activity, greater numbers of sweat glands are gradually recruited (Kondo et al., 1998) and sweat output per gland increases (Amano et al., 2011). Some glands appear to be recruited earlier and more often than others, suggesting variation between glands in stimulation threshold for sweat production (Nishiyama et al., 2001). Recent evidence shows that eccrine glands are sometimes associated with a hair follicle (Poblet et al., 2018) and, like other skin appendages, aid in epidermis wound healing Kuno

The body of research on human heat acclimation is vast and beyond the scope of this review, but a brief summary may provide context for understanding the role of eccrine gland density in the processes of thermoregulation. (See Taylor, 2014 for a thorough treatment of the literature on this topic.) Acclimation (phenotypic response) to

heat involves multiple physiological and anatomical systems. Short-term acclimation includes increased sweat rate, lowered threshold for onset of sweating, and eccrine gland hypertrophy, as gland size can vary fivefold between individuals and correlates positively with maximum sweat rate (Sato et al., 1989). In contrast, long term acclimation is characterized by decreased sweat output, increased sweating efficiency (calories of heat lost per ml of sweat) via higher skin temperatures, and reduced metabolic heat production (Bae et al., 2006; Gale, 1973; Katsuura et al., 1993), responses seen in individuals with tropical ancestry and temperate peoples with long-term tropical residence. Several studies have claimed reduced sweat output in natives of tropical habitats, with lower reported maximum sweat rates compared to Europeans among Ugandans (McCance and Purohit, 1969), Nigerians (Thomson, 1954), Indians (Edholm et al., 1965; McCance and Purohit, 1969; Samueloff, 1987), New Guineans (Fox et al., 1974), Bedouin (McCance et al., 1974), and Malaysians (Duncan & Horvath, 1988). However, these studies did not control for phylogenetic and geographic ancestry, so they do not inform questions related to phenotypic plasticity vs. evolutionary/genetic adaptation. Finally, attenuated sweat responses may characterize hot-humid as opposed to hot-dry long-term acclimation (Taylor, 2014). Humid air reduces evaporation rate and leads to “wasteful” sweat production in which increased sweating rates fail to produce proportional cooling effects (Garden, Wilson, & Rasch, 1966). Nonetheless, increased sweat rate occurs with short-term humid heat exposure in tropical people just as in temperate people (Mitchell et al., 1976).



### **Diversity in human eccrine gland density**

Studies quantifying gland density take one of two approaches: counting anatomical glands through direct histological observation of excised or cadaver skin; or counting only “active” glands by inferring the presence of pores via sweat droplets, either through direct observation or indirectly via plastic or vinyl polysiloxane impressions, starch-iodine droplet visualization, or droplets recorded on iodine-impregnated paper. Vinyl polysiloxane impressions are the most sensitive and tend to produce the highest gland counts (Gibbons et al., 2008; Harris, Polk, & Willis, 1972); other methods also have high repeatability but direct comparisons cannot be made between methods. Because sweat glands are recruited incrementally during physical activity or heat exposure, an insufficient heat stimulus may produce misleadingly low active gland counts. Therefore pharmacological stimulation, usually with pilocarpine nitrate delivered via iontophoresis or injection, is preferred because it stimulates sweat production in all physiologically active glands (Webster & Rundell, 1982). However, several authors report comparable gland counts from pilocarpine compared to a sufficiently robust thermal exposure (Kuno, 1956; Sato et al., 1970). Additionally, glands that respond to pilocarpine but not thermal stimulation are effectively “inactive” because they don’t contribute to thermoregulation in real-world conditions, so this distinction may be unimportant.

Human eccrine gland density varies across body regions, with the highest densities in the fingers and toes. In these areas, glands function in psychologically induced sweating as well as thermoregulation. Eccrine gland densities on the body surface vary greatly between individuals. A comprehensive review suggests averages of

~200/cm<sup>2</sup> on the forehead and 70-140/cm<sup>2</sup> on the back, torso and limbs, with a total average gland number of 2.03 million glands for a “typical” individual with a 1.8m<sup>2</sup> body surface area (Taylor & Machado-Moreira, 2013). Differences among body regions may owe partly to, or partly result in, differing heat dissipation properties; however, gland density across individuals is correlated with body surface area (Kuno, 1956; Szabo, 1967; Thomson, 1954) suggesting that intersegmental differences in gland density are mostly resultant from differential body segment growth during development.

Sex and age may influence eccrine density modestly. Older studies on eccrine density tended to include only males, made no mention of the sex of the participants (e.g., the most thorough anatomical, cadaver study on eccrine density-Szabo, 1967) or aggregated multi-study data from males and females (e.g., Taylor and Machado-Moreira, 2013). Two exceptions are Knipp who found no sex differences, though her male and female samples represented different geographic ancestries and childhood climates, and Szabo who found no significant differences in thigh eccrine density in a sample of 7 males and 14 females (Knip, 1975; Szabo, 1962). While existing data are sparse several studies report higher gland densities in females. Hwang and Baik report higher anatomical (cadaver) densities in females in 7 of 30 tested body regions (Hwang and Baik, 1997). Gagnon and Kenny, using an exercise protocol, found higher functional gland densities in females on the back and chest but not the forearm, though the influence of body surface area on this difference was not measured (Gagnon and Kenny, 2012). Testing only the forearm, Gagnon, Grandall and Kenny similarly report no significant sex differences in functional eccrine density of the forearm when pharmacologically stimulated, but greater density in females was statistically significant when measured

after passive heating (Gagnon, Grandall and Kenny, 2013). Finally, Bar-Or et al. report twofold higher density and 150% higher estimated whole-body eccrine number in females, though repeated counts showed a coefficient of variation of 50% (Bar-Or et al., 1968). The effects of age on gland density are better understood. Anatomical gland density does not change with age, but some evidence suggests that fewer glands are recruited during thermal stress in older individuals, though most age-related decrements in whole-body sweat rate result from reduced sweat production per gland (Inoue, 1996; Larose et al., 2013).

### **Are there “inactive” eccrine glands?**

Since Kuno (1956), several researchers (Thomson, 1953; Folk and Semken, 1991; Taylor and Machado-Moreira, 2013; Machado-Moreira and Taylor, 2017) have proposed that some proportion of human eccrine glands are wholly inactive—that is, they appear normal in structure but fail to produce sweat in response to thermal exposure, physical activity, or pharmacological stimulation. If true, the existence of morphologically developed, non-functional glands presents the possibility of phenotypic plasticity in active eccrine gland density. It is therefore important to assess the evidence supporting this claim.

Kuno (1956) was among the first to report the existence of inactive sweat glands. He observed sweating with a microscope on the fingertip, where sweat pores are readily visible on the dermal ridges. Some pores never discharged sweat after repeated applications of varying heat stimulus- for example, six out of 29 pores in a 5 mm<sup>2</sup> area on the index finger. However, these glands are not primarily used in thermoregulation, and pharmacological stimulation may have recruited more of them. Ogata (1936, as reported

by Kuno 1956) found comparable counts of active sweat glands by applying “extreme” heat to acclimated subjects and from stimulation with acetylcholine delivered via iontophoresis, concluding that repeated high thermal stress produced gland activation equal to that of pharmacological stimulation. Ogata then used passive heating at 45°C, coated the skin of his own forearm, thigh, and leg, and the forearm of another volunteer in oil and directly observed sweat droplet formation via microscope. Following this, he excised 0.3 cm<sup>2</sup> of skin from these areas to count glands histologically. He found that these anatomical counts always exceeded gland counts obtained from counting sweat droplets, and concluded that some glands fail to respond to any stimulation. These data, though limited, revealed active to inactive gland ratios ranging from nearly 1:1 to 16:1. Glands which failed to produce sweat were identical in structure to functional glands. Finally, to rule out the possibility that glands with very low sweat production were mistaken as inactive, Ogata observed sweat droplet formation during passive heating in 10 consecutive sessions during winter. Sweat output per gland increased, as expected with heat acclimation, but the location of active sweat pores was consistent across the 10 trials.

Since Ogata’s work we are aware of only one other study showing evidence for completely inactive glands. Thomson (1953) chemically burned skin, removed the blistered layers, counted the eccrine glands present, and compared these figures with sweat droplet counts made from plastic impressions during heat-induced sweating on the same areas of skin. In three individuals all droplet impressions corresponded to an eccrine gland. In two individuals, two out of 48 glands and three out of 60 glands respectively did not have a corresponding hole in the impression. This was taken as

evidence that these glands were inactive, though conceivably these observations may have resulted from an insufficient heat stimulus or lack of fidelity in the impressions.

One potential method for identifying inactive glands would entail locating eccrine pores in vivo and noting those which do not produce sweat. Thomson (1953) attempted this by pressing plastic impression material into non-sweating skin, but despite the high resolution of the material (which recorded hair follicles), pore openings were not visible except on the fingers and toes. We recently confirmed these results by pressing a vinyl polysiloxane material into non-sweating skin and cadaver skin and searching the resulting impressions with laser confocal microscopy (Best and Kamilar, unpublished data). Even at skin locations where an active gland was confirmed through sweat stimulation pores were not recorded in the impressions. We concluded that existing noninvasive measures are insufficient to identify pores when not producing sweat, perhaps because pores are forced open during sweat expulsion and are effectively closed otherwise (Sato, Nishiyama & Kobayashi, 1979). Thus, we argue that sweat droplet quantification followed by skin excision and histological gland counting is the best way to confirm the presence of physiologically inactive sweat glands. To our knowledge Ogata (1936, as reported by Kuno, 1956) and Thomson (1953) are the only researchers to have used this approach, perhaps due to its invasiveness, but references to inactive glands are nonetheless found in published literature. Folk and Semken (1991) claimed that some human eccrine glands appear normal but “are actually functionless”, but this statement is made without reference to data or published literature. Several other papers (Randall, 1946; Willis, Harris and Moretz, 1973) have been erroneously cited as providing evidence for inactive glands. Randall (1946) reported that “extreme” thermal

stimulation was required to attain gland recruitment numbers equal to that of pharmacological stimulation, and suggested that Ogata incorrectly inferred the presence of inactive glands because his thermal regime was not sufficiently strenuous; this objection appears unlikely, though Ogata's quantification methods (observing sweat droplets under a layer of oil and wax) may be insensitive. Willis, Harris and Moretz (1973) provided validation of a silicone impression technique, finding that at least 95% of the same sweat ducts were identified in impressions spanning a 4-8 week period.

Several researchers doubt the existence of inactive eccrine glands. Harris, Polk and Willis (1972) suggest that Kuno's technique was insensitive and he simply missed some active glands. Gordon and Maibach (1968) claimed that the outer layer of keratinized skin cells may block sweat ducts, but Johnson and Shuster (1970) refuted this and provided evidence that sweat duct occlusion is not a common occurrence in healthy skin. In short: the existence of inactive, fully-formed sweat glands is unresolved.

Finally, it is worth considering whether a gland must be wholly incapable of producing sweat to merit interest when considering diversity in gland density. Glands which consistently produce only small amounts of sweat will contribute little to thermoregulation, making them just as relevant to this discussion as "inactive" glands, and several studies demonstrate their existence. Saito (1934, as reported by Kuno, 1956) characterized some glands in the palm as poorly active. Using pilocarpine iontophoresis and a silicone impression technique, Willis, Harris and Morte (1973) made successive gland counts over 4-8 weeks on the same body sites, and described <10% of active glands as "poorly functional": glands which were found to produce only tiny holes in the impression material in week 1 continued to produce low or variable output over the

succeeding weeks. This implies that low-functioning glands may not be observed in single-session gland counts and may therefore be mistakenly labeled as “inactive”. From a physiological perspective this distinction may be overstated because 1) phenotypic plasticity may result in poorly-active glands via the same mechanisms as wholly inactive glands; and 2) poorly active glands will contribute little to thermoregulation. Thus, for the purposes of assessing differences in active gland density and the physiological relevance of these differences, we will maintain the use of “inactive” to mean both wholly-inactive and poorly active.

### **Evolutionary origins of human eccrine gland density**

Eccrine glands do not fossilize, but evolutionary questions are informed by the comparative biology of eccrine glands in extant species. Mammals have eccrine glands on the volar surfaces of the paws which are thought to aid in frictional gripping (Adelman, Taylor, and Heglund, 1975). In humans and some other primate species, eccrine sweat glands are also found over most of the body surface and are principally used in thermoregulation. Eccrine glands should not be confused with the apocrine sweat glands, also found in the skin of some body areas (axillae and pubic regions in humans). Apocrine glands are associated with a hair follicle; a few ungulates such as horses and camels employ these glands in thermoregulation (Bullard, Dill, and Yousef, 1970; Whittow, 1971), and some evidence suggests that apocrine glands also perform this role in nonhuman primates (Whitford, 1976).

Eccrine gland profusion on the body surface first evolved near the base of the catarrhine primate clade (Montagna and Yun, 1963; Montagna, Yun and Machida, 1964; Montagna and Machida, 1966; Folk and Semken, 1991; Best and Kamilar, 2018), a group

comprised of monkeys and apes from Africa and Asia. Eccrine density continued to increase during the evolution of apes and even more so in fossil hominins (human ancestors). Several primates are known to perform thermoregulatory sweating (Johnson and Elizondo, 1979; Lemaire, 1967; Mahoney, 1980; Sato et al., 1990) though none match the heat dissipation capacity of humans.

Kamberov et. al (2018) provides the most rigorous comparative study to date on eccrine density in extant primates. Chimpanzees and macaques were found to have similar eccrine density while that of humans is 10 times higher. Macaques were found to have higher hair follicle density than either chimpanzees or humans, who intriguingly had similar hair follicle density. The near-naked skin of humans, then, is due not to fewer hair follicles but a shift from terminal pigmented hair to microscopic, unpigmented vellus hair. This observation has important implications for the evolution of eccrine sweating in the human lineage. First, hair follicle density decreased in the ape lineage, which was then followed by a terminal-to-vellus hair transition and increased eccrine density in the hominin lineage (Kamberov et al., 2018). Hair follicles and eccrine glands develop from the same epithelial buds, and a shift in the timing and location of the changeover from hair follicles to eccrine glands endowed humans with greater eccrine density. Together, these adaptations are likely the product of selection for increased heat dissipation capacity.

Until Kamberov et al.'s work, most information on primate eccrine gland counts came from a series of papers in the 1960's and 1970's, e.g., (Ellis and Montagna, 1962; Ford and Perkins, 1970), and others, which recorded qualitative and semi-quantitative histological characteristics of primate skin, including the distribution, structure, and



histochemical composition of eccrine glands. We compiled these data (Best and Kamilar, 2018) for a phylogenetic analysis finding that glycogen concentration (a fuel substrate powering eccrine gland metabolism) and degree of capillarization (enabling greater water, glucose and electrolyte delivery) were significantly correlated with climate across species. Specifically, primate taxa living in warm and dry climates tended to have greater eccrine glycogen stores and greater capillarization surrounding the eccrine glands. These results provide evidence for the evolution of increased sweating capacity in hot-dry climates but not hot-humid. Importantly, thermoregulatory sweating in extant primates occurs despite pronounced terminal (vs. vellus) body hair, suggesting that some increases in sweating capacity are not necessarily dependent upon increased physical activity or reduced terminal hair.

Since early hominins such as *Sahelanthropus* and *Ardipithecus* (~7-3 mya) diverged from a last common ancestor with chimpanzees, it is likely they had apelike eccrine gland densities. Given their many other apelike characteristics (Pilbeam and Lieberman, 2017), it is not unreasonable to infer that thermoregulatory sweating in early hominins was comparable to chimpanzees. Humanlike thermoregulation evolved later, but it is unclear whether this shift began with *Australopithecus* or *Homo*.

Although *Ardipithecus* and other early hominins were probably facultative bipeds that climbed frequently and did not walk like humans, there are multiple lines of evidence that species in the genus *Australopithecus* (~4-1 mya) were effective, efficient striding bipeds while retaining some adaptations for climbing trees, perhaps for a measure of safety while sleeping (Barak et al., 2013; Raichlen, Pontzer, and Sockol, 2008; Sellers et al., 2005; Ward, 2013). We can infer only a little about thermoregulation in these

hominins. Bipedalism might have conferred at least some thermoregulatory benefit if they were walking in hot, open habitats because upright posture reduces the body surface area exposed to solar radiation (Wheeler, 1991). However, it is unknown how long *Australopithecus* day ranges were and other skeletal traits associated with long-distance locomotion, including running, had not yet evolved (Bramble and Lieberman, 2004). These observations, together with evidence for a diet based largely on plants (Peterson et al., 2018; Quinn, 2019) have led to a consensus opinion that *Australopithecus* ranging was limited. However, Lieberman (Lieberman, 2015) has suggested there might have been selection for enhanced thermoregulatory capacities in *Australopithecus*. Like all bipeds, australopiths were necessarily slow because they can generate speed with only two legs, and thus must have been vulnerable to predation. It would therefore have been advantageous for them to travel to foraging locations in the middle of the day when it was hot and predators are less active, thus favoring individuals with increased eccrine gland density and body hair reduction. (Lieberman, 2015).

For the time being we can only hypothesize about australopith thermoregulatory capacities, but there are many indications that by the time of the genus *Homo* more modern human sweating capacities had evolved. First, skeletal adaptations associated with long distance walking and running ability evolved in *Homo erectus* in Africa by 1.8 mya. This behavior was likely necessary to enable hominins to hunt and scavenge in an increasingly open savannah environment where food is widely dispersed, a hypothesis supported by butchery evidence and studies of contemporary hunter-gatherers who successfully run prey to heat exhaustion (Bramble and Lieberman, 2004; Liebenberg, 2006). Running in hot ambient temperatures poses an enormous thermoregulatory

challenge and selection would have strongly favored enhanced heat dissipation, particularly via hair reduction and increased eccrine density. Second, increased body size in *Homo* produced a less favorable body surface area to volume ratio, compounding the thermoregulatory challenge imposed by increased locomotor behavior. Third, there is no doubt that the environment in Africa where early *Homo* has been found was hot and semi-arid (Blumenthal et al., 2017) and even walking, let alone running, would have produced great thermal strain. Still, questions remain about eccrine evolution in our genus. Sweating depletes body water reserves, leading us to wonder how selection produced a compromise between the need to cool and the need to avoid dehydration, a question addressed later (see “Does gland density matter?”).

Finally, along with other age-related physical decrements such as reduced muscular strength and aerobic capacity, reduced ability to dissipate heat would have further impaired ranging and locomotor activity in older hominins relative to that of younger individuals. Though, the impact that this would have on hominin life history evolution would probably be minimal since most individuals would reproduce well before 40 years old.

### **Current evidence for factors influencing variation in modern human eccrine gland diversity**

Kuno (1956) hypothesized that a hot environment during infancy prompts more glands to become physiologically active (i.e. capable of sweating) while a cooler environment produces lower active gland density, presumably because some glands were seldom recruited for sweating and became “inactive”. Further, he proposed a critical developmental window of approximately 2.5 years, based on his cross-sectional study of

16 individuals aged 35 days to 35 years. Kuno found that total gland number over the body surface increased up through age 2.5 and did not change thereafter. These results should be interpreted with caution because this study was not longitudinal and therefore the observed gland count differences may be the result of inter-individual variation. But his hypothesis is plausible. The specific phenotype for many biological traits is determined within a set developmental period (West-Eberhard, 1989) and there is evidence that active gland number does not increase with heat acclimation (Inoue et al., 1999).

Kuno's hypothesis for phenotypic plasticity is based on two studies. First, Kawahata and Sakamoto (1951) found higher active gland density in tropical southeast Asians than in Russians and Ainu, indigenous peoples of Japan and Russia. Unfortunately, this study design does not allow us to distinguish the effects of ancestry or phenotypic plasticity. Second, Kuno tested 26 people who were ethnically Japanese but raised in tropical Southeast Asian locations. Using a thermal stimulus and direct microscopic observation of the skin to count active sweat glands, Kuno found that these people had greater numbers of active sweat glands than Japanese-born individuals that were raised in Japan but emigrated to tropical climates in adulthood. It appears that he did account for body surface area as gland counts are reported as whole-body estimates but effect sizes and statistical significance are not reported. Only a handful of researchers have tested Kuno's hypothesis since these initial findings. Ojikutu (1965) observed higher active gland counts in 108 Nigerian-born males living in Nigeria compared to 13 Ghanaian and Liberian-born males who moved to Germany as adults and resided there for 1-5 years. Interestingly, while Kuno's data suggest phenotypic plasticity in gland

density during childhood, Knip (1975) provides evidence for increases in active gland density during adulthood. Studying people born and living in the Netherlands compared to those born in the Netherlands but living as adults in Suriname for four months to nine years, he found the latter to have more active glands after adjusting for body surface area. Further, he used a sensitive plastic imprint technique and sampled up to 144cm<sup>2</sup> of skin, an enormous area compared with other studies. Still, it is hard to reconcile these results with existing dogma stating that active gland density cannot be increased after childhood. Knip suggests that such increases are possible only with long-term acclimation, though to our knowledge these results have not been replicated.

Existing evidence for Kuno's phenotypic plasticity hypothesis appears to be limited to three studies. Yet, Kuno's hypothesis is influential and often repeated. In fact, the 2016 edition of *Gray's Anatomy* states, "People indigenous to warmer climates tend to have more sweat glands than those indigenous to cooler regions". Unfortunately, there is scant evidence to support this statement. While existing direct evidence on eccrine gland data in populations from varying climatic conditions is insufficient, a growing body of data on mechanisms of phenotypic plasticity (how traits with the same genetic basis can be altered due to different environments of development) lends credence to this idea, as we will discuss later (see "proximate mechanisms influencing eccrine gland density".)

The combined results of Best et al. (2018) and Kamberov et al. (2018) reinforce earlier hypotheses that natural selection drove eccrine evolution in hominins and early humans. More research is needed, but selection may have continued to shape eccrine density in *Homo sapiens* as they expanded out of Africa and into novel environments. In cooler environments where heat dissipation was less important, selection may have been

relaxed, and neutral evolutionary processes such as genetic drift and gene flow may also have influenced eccrine density in the human lineage. Regardless of the evolutionary force, such gland density diversity across populations should be measurable today. Existing data is sparse and problematic but worth a brief consideration and is summarized below.

Limited research assessing differences in total and active eccrine gland density across human populations generally shows low variation, with a few exceptions. Kawahata and Sakamoto (1951) reported higher active gland counts in people indigenous to tropical regions in Asia compared to indigenous peoples of Japan and Russia, though again he does not provide statistical analyses. Kawahata and Adams (1961) found more active glands in European-Americans than African-Americans, though Taylor (2006) re-analyzed these data and found this difference to be statistically insignificant. Using iontophoresis of carbachol (a chemical which apparently induced unpleasant physical side effects) and a starch-iodine paper method, Toda (1967) reported greater active gland density in Indonesian compared to Japanese populations. However, it is not clear if he accounted for body surface area, and puzzlingly the iontophoresis current applied to Indonesians was greater than that used with Japanese volunteers. At the molecular level, Kamberov et al. (2013) found evidence of positive selection for a gene variant (EDARV370A) of the EDAR gene, discussed further in the next section. While this work is important for connecting genotype to gland density, selection for this allele appears to have occurred within one population and the specific trait (e.g. hair follicle density, mammary duct branching) selected for remains unknown. This limits our ability to make inferences about gland evolution across populations.

Other studies have sought population level (evolutionary) differences in anatomical and active gland density based on geographic ancestry and have found little or no differences. In a comprehensive review Taylor (2006) concluded that small sample sizes and methodological limitations render the existing body of work on gland differences across populations almost immaterial, and concludes that any observed differences result from phenotypic plasticity. We agree that existing data is problematic and insufficient, but as such, we also cannot confidently attribute variation to plasticity.

### **Proximate mechanisms influencing eccrine gland density**

Recent work expands our understanding of the proximate causes underlying variation in eccrine density. Eccrine glands develop from the same epidermal placodes as hair follicles, and an earlier differentiation from the latter to the former, driven by increased expression of EN1, could result in more eccrine glands at the expense of hair follicles (Lu et al., 2016). Aldea et al. identified an EN1 enhancer in humans, hECE18, which may largely explain human's derived eccrine density (Aldea et al., 2021). Multiple independent mutations in this enhancer each increase EN1 expression, suggesting sustained selection for increased eccrine density in the human lineage, and when part of this sequence was replaced with the orthologous chimpanzee sequence in mice, EN1 activity was reduced. Intriguingly this research team identified hECE18 polymorphisms found in low frequencies in some populations, hinting at possible inter-population diversity in eccrine density. Another candidate for genotypic diversity in human eccrine density across populations is a variant of the Ectodysplasin receptor with pleiotropic effects, *EDARV370A*, which is associated with increased functional eccrine density on the thumb and index finger in a sample of Han Chinese. Modeling suggests

that this allele arose through natural selection ~30,000 years ago, though the phenotypic result targeted by selection is unclear: increased gland density may have permitted enhanced heat dissipation, helpful for East Asian hunter gatherers in the summer; alternatively, increased ductal branching of the mammary gland may have been the target (Kamberov et al., 2013). The later explanation is favored by Hlusko et al. who pin the selection event at 20,000 years ago and suggest that *EDARV370A* is an adaptation to increase nutrient delivery in vitamin-D deficient latitudes (Hlusko et al., 2018). As this allele seems to have been the target of selection only once and well after humans dispersed from Africa, and it is unlikely to explain most variation in contemporary human eccrine density or how eccrine density increased in the hominin lineage much earlier.

A potential though less likely mechanism for altering eccrine density is that thermoregulatory stress experienced by the mother from the external climate is signaled to the embryo, a phenomenon shown to impact many developmental processes in humans (Barker, 2003). As predicted by the hypothesis of symmorphosis, phenotypes of costly physiological traits such as muscle hypertrophy and the pathway for oxygen are under selection to match capacity with demand (Weibel, Taylor, & Hoppeler, 1991). However, for sweat gland development to be suppressed or augmented to accommodate future demands, these signals would need to occur very early in development before placode differentiation, and the cost of making glands is probably trivial compared to the costs of body water loss and the risk of hyperthermia.

A more likely mechanism of phenotypic plasticity that affects eccrine gland function density is modulating the innervation of glands during infancy. Eccrine glands are fully formed at birth, but early in the postnatal period the neurons innervating the



glands change phenotype from noradrenergic to cholinergic (Taylor and Machado-Moriera, 2013) and thereafter respond to acetylcholine. In paw eccrine glands of the rat, it appears that this change occurs in the population of nerve fibers that originally innervated the gland, rather than replacement with a new cholinergic population of fibers; this nerve change is induced by the gland itself. Further, responsiveness of the gland's secretory coil is dependent upon continued cholinergic innervation (Landis, 1990). Thus, for a gland to be functional, it must induce a phenotypic change in the nerve fibers innervating it early in postnatal life, and this innervation in turn induces changes in the gland enabling it to produce sweat (Landis, 1990). Factors influencing this process could result in modified active gland number. Here is perhaps the most plausible mechanism for Kuno's climate-driven developmental window hypothesis: glands that aren't recruited for sweating early in life may not acquire the mature phenotype, and therefore remain unresponsive to stimuli thereafter. This model of gland maturation may also explain development of poorly-active glands. Glands are innervated by multiple nerve fibers (Kennedy et al., 1994) and sweat production may be dependent upon the summed stimulation from these multiple inputs (Machado-Moreira & Taylor, 2017). Perhaps poorly-active glands are those innervated by a low ratio of mature to immature nerve fibers.

A final possibility is that previously active glands may lose function later in life. This fits with the "use it or lose it" nature of exercise capacity, but there is little evidence to suggest this occurs. The reverse process is unlikely as well, as active gland density has not been seen to increase following acclimation (Inoue et al., 1999), with the exception of Knip's data described previously (Knip, 1975).

### **Does gland density matter?**

Sweating is not the only component determining human thermoregulatory capacity, and improvements in heat dissipation following acclimation which can be attributed to sweat glands appear to be due largely to increased sweat rate per gland, enabled in part by gland hypertrophy (Sato et al., 1989). Still, all else being equal, increased gland density should permit a higher maximal sweating capacity. Several observations support this idea. First, progression of sweating during thermoregulatory challenge involves the gradual recruitment of more glands (Kondo et al., 1998), indicating that active gland number (or density) directly impacts heat dissipation. Second, gland density correlates with thermoregulatory capacity across primate taxa, suggesting both that selection favors higher gland density as a solution to thermoregulatory challenges.

As mentioned previously, a potential consequence of higher gland density—or at least higher whole-body sweat production—is greater body water loss. Attenuated sweat responses in living peoples of long-term tropical residence suggest that humans evolved phenotypic plasticity to make sweating more efficient. Still, the adaptive advantage of increased gland density (be it evolutionary or phenotypic plasticity) is surely to permit higher maximal sweating rates. Thus, increased sweat gland density evolved in hominins despite increasing the risk of dehydration. While hyperthermia and dehydration were both physiological challenges faced by human ancestors, the former was clearly more deleterious to evolutionary fitness, perhaps because there are fewer avenues through which it can be ameliorated. Active hominins on the African savannah necessarily faced unavoidable thermal strain. Meanwhile, the human body has multiple responses to

prevent and cope with dehydration. Heat acclimation improves ability to buffer against dehydration: body water increases 5-7%, due to increases in fluid-conserving hormones and changes in renal function; and thirst improves, leading to greater fluid intake (Périard, Racinais, and Sawka, 2015). Additionally, behavioral adaptations can help prevent dehydration, while similar options for preventing hyperthermia are few if midday physical activity is required. For example, the digestive system can be used as a sort of canteen, banking water for several hours' worth of physical activity. Before a persistence hunt covering 15-40 km in extreme heat, Kalahari hunter-gatherers consume large quantities of water and complete the hunt with no further drinking and without apparent dehydration (Liebenberg, 2006).

How this informs predictions about variation in modern human eccrine density depends upon which proximate mechanism(s) are responsible for this variation. Because sweating is less efficient in humid environments (increased sweat production is not related in a linear fashion to heat dissipation), we may expect selection and/or plasticity to produce lower gland densities in these climates. However, the most likely proximate mechanism for phenotypic plasticity—gland recruitment in infancy leading to nerve fiber maturation and subsequent gland activation—would be blind to humidity. In this model, glands that are recruited for sweating become active for life; as discussed previously, thermal stress is met with copious sweating even in humid air. While this is conjecture, we predict that phenotypic plasticity leads to higher active gland density in hot climates, both dry and humid.

## CHAPTER 2

### A METHOD OF MEASURING FUNCTIONAL ECCRINE DENSITY

#### Introduction

Variations in human skin are a highly visible, obvious and important first line of adaptation to different environments. While skin characteristics such as pigmentation and hair attributes are conspicuous (Jablonski, 2004) the features of another skin appendage essential to the evolution of our species - the eccrine sweat gland- are more difficult to measure. Existing data on sweat gland density may be sufficient to characterize variation across body regions. However, differences in gland density across sex, age, and populations remain poorly sampled, despite a handful of studies from the mid to late 20<sup>th</sup>-century whose utility are hampered by small sample sizes, lack of consistent methodology across studies, and other limitations (Best, Lieberman, & Kamilar, 2019). The most direct and ostensibly accurate method for quantifying eccrine glands is skin excision and microscopic dissection, but the highly invasive nature of this procedure limits its application to cadavers (e.g., (Hwang & Baik, 1997; Szabo, 1967). It is also difficult to quantify the total number of eccrine pores *in vivo* without excision. As a result researchers have focused on counting only functional eccrine glands (those which produce sweat) indirectly by inducing sweating via thermal or pharmacological stimulation, followed by making imprints of the sweating skin with iodine-impregnated paper or impression materials to record sweat droplets.

Here we describe a non-invasive, partly-automated methodology for quantifying functional eccrine gland density using pilocarpine iontophoresis and vinyl polysiloxane sweat impressions. This procedure permits sampling and analysis of multiple body

regions and large study populations, both of which are necessary for characterizing intra- and inter-individual variation in eccrine density.

## **Methods**

### *Study population*

As part of an ongoing research project cataloging contemporary diversity in eccrine gland density, we recruited 76 volunteers (35 M, 41 F; average age 21.2±4.6 years) from the Amherst, MA area, primarily undergraduate and graduate students. We recorded height, body mass, and circumference of measurement locations on the forearm, upper arm, thigh and lower leg, and estimated body surface area (BSA) using Haycock's (1978) derived equation based on height and body mass:  $BSA (m^2) = \text{body mass (kg)}^{0.5378} \times \text{height (cm)}^{0.3964} \times 0.024265$ . All study procedures were approved by the University of Massachusetts Human Subjects Review Board and written informed consent was obtained from each volunteer.

### *Sweat stimulation*

While passive or exercise-induced thermal stimuli may be sufficient to activate all physiologically responsive eccrine glands (Kuno, 1956; Sato & Dobson, 1970), we chose pharmacological stimulation via pilocarpine nitrate iontophoresis, a method known to stimulate sweating in all functional glands (Webster & Rundell, 1982). We prepared the skin in 6 body areas on the left side of the body- lateral upper arm, medial forearm, abdomen (immediately inferior to rib 10), back (scapular), lateral thigh, and posterior lower leg (gastrocnemius head)- by cleaning with alcohol and shaving. We followed an established protocol for sweat stimulation, the Cystic Fibrosis Sweat Test (LeGrys,

Yankaskas, Quittell, Marshall, & Mogayzel, 2007), to standardize equipment and pilocarpine administration. This protocol is used by hospitals to induce sweating in patients, usually young children, so that sweat may be collected for diagnosis of cystic fibrosis. Pilogel discs (Elitech Group, Puteaux, France) comprised of 0.5% pilocarpine nitrate in an agar base are placed between the skin and electrodes of an iontophoresis machine, the Webster Sweat Inducer (Elitech Group, Puteaux, France). This uses a mild electrical current equivalent to 1.5mA to drive pilocarpine nitrate into the sweat pores for 5 minutes, at which time the machine automatically shuts off. As pilocarpine is only delivered from the gel disc under the positive electrode we determined that the disc under the negative electrode may be reused in the next test, a cost-saving practical consideration. Pilocarpine nitrate is an acetylcholine agonist and induces the secretory coil of the eccrine gland to produce sweat.

#### *Sweat impressions*

Vinyl polysiloxane materials manufactured for dental impressions have previously been employed in functional gland measurement (e.g., (Gibbons, Illigens, Centi, & Freeman, 2008; Knip, 1975). Because available materials change over time, Vilches and Navarro (Vilches & Navarro, 2002) evaluated the efficacy of 3 vinyl polysiloxane materials for use in making sweat impressions, and recommended Silasoft (Detax, Germany) as a standard material for future researchers. This product was unavailable and we settled on another produced with the name Silasoft (Microsonic Inc., Ambridge, PA, USA). Intended for making casts of the auditory canal in the production of custom hearing aids, Silasoft is a 2-component system which begins to polymerize when mixed. After waiting 15 minutes post-iontophoresis we mixed the 2 Silasoft

components together on 0.76 mm thick cardstock which was then held against the skin for 5 minutes with a medical bandage. Sweat droplets produce holes in the Silasoft as they emerge from sweat ducts permitting indirect quantification of functional eccrine density. We made 2 impressions from each body site to ensure production of at least one viable impression.

### *Imaging and analysis*

Impressions were taped to an 8.5"x11" piece of cardstock with 3 cm delineations marked to provide scale, then scanned at a resolution of 600 dpi in grayscale. We found that a higher-resolution digital camera produced images with "noise" which erroneously inflated hole counts. Resulting PDF files were converted to JPEG format and digitally cut into 6 files, one file per impression. Impression images were processed using ImageJ (Schindelin et al., 2012). Only one researcher (A. Best) performed image processing and analysis to avoid introducing inter-observer error (measurements of which we provide later). First, light gradient was homogenized using the bandpass filter with the setting "filter small structures up to 3 pixels"; this setting must be kept constant across samples as filtering to fewer than 3 pixels can inflate hole counts through inclusion of noise. Scale was set using the 3 cm demarcations on the cardboard as a template (analyze → set scale). A circular region of interest was defined using the oval selection tool, between roughly 4 cm<sup>2</sup> and 10cm<sup>2</sup> depending on the usable area of the impression image, and the area outside of this region was cleared (edit → clear outside) (figure 1a). Threshold was adjusted manually (image → adjust → threshold) to make sweat droplet holes appear as black dots against a white background. Here it is possible to introduce subjectivity, as over-thresholding results in some holes merging together, and texture in the impression

material may falsely appear as holes. We found that thresholding such that sweat droplet holes appear but only minimally merge together produced highly repeatable counts, aided by using an unthresholded copy of the image to visually gauge how well the threshold setting highlighted holes and minimized noise (figure 1a and 1b). We then excluded from analysis any thresholded image that failed to produce a hole pattern visibly matching that of the unthresholded image, due either to inferior image resolution or low sweat droplet fidelity in the impression. We obtained 424 usable impressions from the 6 tested body sites in our sample of 76 volunteers. Due to a defective batch of impression material, all samples taken from 4 volunteers were unusable, bringing our gland-count sample size to 72. Of the remaining 44 unusable samples, 19 were obtained from 4 temperate-climate volunteers. The most frequently unusable impressions were taken from the lower leg suggesting that this body site is difficult to sample with our methodology. After thresholding, the image was converted to binary (process → binary → make binary) and the watershed feature (process → binary → watershed) was used to re-delineate most of the holes (if any) that had merged together with thresholding (figure 1b). Finally, automated hole counting was performed using the analyze particles feature (analyze → analyze particles), defining countable particles as between 0.0001 and 0.25 mm in diameter and between 0.20 and 1.0 in circularity, and excluding holes on the edges (figure 1c). Resulting counts were divided by the region of interest area to yield sweat droplet holes per cm<sup>2</sup>.

## **Results**

Functional eccrine density was highest on the ventral forearm ( $114.4 \pm 18.5$  glands/cm<sup>2</sup>) and lowest on the posterior lower leg ( $82.8 \pm 14.5$  glands/cm<sup>2</sup>). Density



averaged over the 6 body areas ranged from 60.1 glands/cm<sup>2</sup> in one volunteer to 132.7 glands/cm<sup>2</sup> in another (table 1). Without excising skin from tested body regions and comparing histological gland counts with functional gland densities obtained from impressions, we cannot perform a test of accuracy. However, the range of variation observed with our methodology agrees with published literature on functional gland densities. Table 1 shows functional eccrine gland densities reported by a meta-analysis of 31 studies prior to 2013 (Taylor & Machado-Moreira, 2013) and the single more recent study. We chose not to include results from one recent study (Lee & Shin, 2017) due to highly suspect gland density results which are far lower than any others reported in the literature. Additionally, their failure to account for body surface area led them to erroneously conclude that Koreans have higher functional eccrine density than Africans.

To assess repeatability of image analysis we chose 10 impressions (3 forearm, 2 upper arm, 2 back, 2 thigh, and 1 torso) from 9 volunteers representing relatively low, moderate and high sweat production. One researcher (A. Best) analyzed these 10 impressions twice, and we calculated the intraclass correlation coefficient (ICC) and coefficient of variation (CV) to estimate intra-observer repeatability (figure 2). The resulting ICC value was 0.99 and the CV was 2.21%. Next, two undergraduate research assistants, given a brief visual demonstration and a text description of the image analysis methodology similar to that presented here, analyzed these 10 images. These gland density results were compared with those of A. Best to estimate inter-observer repeatability, with a resulting ICC value of 0.82 and CV of 7.73% (figure 3).

## **Discussion**

We have demonstrated a method of quantifying functional eccrine gland density via pilocarpine iontophoresis, vinyl polysiloxane sweat impressions, and partly-automated computer-assisted analysis. The pharmacological stimulation used here provides a standardized stimulus for sweat production and Silasoft impression material produces a permanent record of sweat droplet density. Analysis with ImageJ enables more rapid counting and removes much of the observer error inherent in manual counting methods. Our data collection protocol sampling 6 body sites takes about 75 minutes per volunteer; imaging and analysis of resulting sweat impressions can be completed in 25 minutes. Intra-observer repeatability is extremely robust with an ICC score of 0.99 and CV of 2.21%. Inter-observer repeatability is lower but still strong with an ICC value of 0.82 and CV of 7.73%, perhaps reflecting the single step in our methodology- image thresholding- where observer subjectivity can be introduced. This may also be skewed by observer 2's analysis of a single impression- impression #3 -which differs dramatically from counts obtained by observers 1 and 3. For reference, ICC scores above 0.80 are considered to demonstrate "good" or "excellent" repeatability (Liljequist, Elfving, & Skavberg Roaldsen, 2019). Thus we suggest that this methodology can be applied to produce results which can be directly compared across studies. The particular brand of vinyl polysiloxane impression material used may influence gland counts (Vilches & Navarro, 2002), so cross-study comparisons are most valid if the same impression material is used.

Rapid data collection and analysis with high repeatability will enable construction of a body of data that, if aggregated, will provide adequate statistical power for

investigating unresolved questions about human eccrine density. These include: How is diversity in functional eccrine gland density patterned across sex, age, and geographic ancestry? Can any of this variation be explained in part by phenotypic plasticity resultant from climate or other factors? Did functional eccrine density change as humans moved to novel climates outside of Africa? What are the physiological implications of functional eccrine density, including total sweat production and ability to dissipate endogenous heat? In our view these unresolved questions deserve more attention- and more data on this variation, collected using a standardized and repeatable methodology, are a necessary next step.

## CHAPTER 3

### THE RELATIONSHIP BETWEEN FUNCTIONAL ECCRINE DENSITY AND HEAT DISSIPATION CAPACITY

#### Introduction

Most mammals dump excess metabolic heat through panting, a process of evapotranspiration in the upper respiratory tract. However, quadrupeds cannot pant while galloping due to coupling of the gait cycle with respiration and running duration in most mammals is therefore limited in part by the buildup of metabolic heat (Bramble and Jenkins, 1993; Entin, Robertshaw and Rawson, 1999). Horses and camels have solved this problem by repurposing apocrine sweat glands, normally associated with hair follicles and sebaceous glands, to secrete sweat onto the skin surface (Whittow, 1971). Catarrhine primates evolved an analogous strategy by elaborating eccrine sweat glands, found on the paws of all mammals, to other regions of the body. The effectiveness of eccrine sweating was further improved in the ape lineage with reduced hair follicle density and again in the hominin lineage with a tenfold increase in eccrine gland density (Kamberov et al., 2018). Eccrine glands do not fossilize but the locomotor anatomy and inferred ecology of *Homo erectus* suggest that modern human sweating capacity had evolved by at least 1.79 million years ago, the result of selective pressures for dissipation of endogenous heat produced during sustained physical activity in hot arid climates (Lieberman, 2015). Human heat dissipation capacity makes us among the only animals capable of long distance running in 30°C.

Onset of sweating during thermal strain includes elevated skin temperatures via increased skin blood flow, and increased sweat production via progressive recruitment of

more eccrine glands and increased sweat output per gland (Amano et al., 2011; Kondo et al., 1998). Heat acclimation status can dramatically change thermoregulatory capacity through changes in sweat response sensitivity (earlier onset of sweating), increased blood plasma volume, changes in electrolyte resorption from sweat, and dramatically increased whole-body and per-gland sweat volume (Taylor, 2014). Increased sweat production following heat acclimation is accomplished largely due to increased sweat output per gland, which is associated with increased gland size (hypertrophy) following 9-week and 9-month heat acclimation in patas monkeys and mitochondrial biosynthesis in the eccrine secretory coil following 1-month heat acclimation in macaques (Okuda et al., 1981; Sato et al., 1990). Other factors influencing heat dissipation include levels of subcutaneous adipose tissue, which increases heat retention and blunts conductive cooling; age, with impaired heat dissipation in older individuals; sex, as women tend to have lower evaporative heat dissipation capacity than men, mostly from reduced sweat output per gland but not fewer activated glands or reduced skin blood flow; and aerobic capacity, though associated factors such as heat production may conflate its effects (Dervis et al., 2016; Gagnon and Kenny, 2012; Lamarche et al., 2018; Meade et al., 2019; Savastano et al., 2009; Stapleton et al., 2015; Jay et al., 2011).

Even given the apparent efficacy of plasticity in gland-level characteristics to improve thermoregulatory capacity, existing variation in eccrine density within *Homo sapiens* may influence heat dissipation. To our knowledge the data needed to explore this question have never been reported. Here we investigate the effect of interindividual variation in functional eccrine density (FED; the number of eccrine sweat glands per square cm which produce sweat in response to thermal or pharmacological stimulation)

on heat dissipation capacity in a sample of heat-trained endurance athletes using indirect calorimetry with flow-through respirometry. We hypothesized that, after controlling for factors known to influence heat dissipation, higher eccrine density would be associated with increased ability to dissipate metabolic heat through evaporative cooling.

## **Methods**

### *Study population*

Participant demographics, anthropometrics and performance characteristics are shown in table 2. We recruited 9 male endurance runners from the Amherst, MA, USA area. Data from two participants were discarded and are not reported here: one participant failed to produce a reliable  $\dot{V}O_2$ -peak measure, and inaccurate water production measurement rendered another participant's data unusable. The remaining 7 participants were aged 23-37 years (average  $29.4 \pm 4.6$ ). In an effort to control for endurance sport specificity all participants were “sub-elite” trail runners which we defined as finishing in the top 5 in competitive trail running competitions within the prior year. Volume of cycling training varied, but running comprised at least 75% of each participant's training volume in the preceding 2 years. As the chamber could not accommodate a treadmill, testing was conducted on a cycle ergometer. To help ensure that all participants were heat acclimated data collections were conducted in summer (June through September 2019), and in the 2 weeks leading up to heat dissipation testing participants completed at least 5 endurance training sessions of 60+ minutes in temperatures exceeding 30°C.

### *Functional eccrine density measurement*

Measurements were taken within 30 days prior to heat dissipation testing. FED was assessed using pilocarpine iontophoresis and vinyl polysiloxane sweat impressions. Six sites on the left side of the body were chosen for counting functional eccrine density: dorsal forearm, lateral upper arm, scapular region of the back, abdomen near rib 10, lateral thigh, and lower leg at the gastrocnemius head. Each area was shaved and cleaned with alcohol. Sweating was induced at each body site via iontophoresis of pilocarpine nitrate, an acetylcholine agonist that recruits all functional eccrine glands in the target skin area (Webster and Rundell, 1982), following a protocol developed for the Cystic Fibrosis sweat test (LeGrys et al., 2007). Pilogel discs (Elitech Group, Inc.) containing 0.5% pilocarpine nitrate in an aqueous agar base were placed between the skin and electrodes of the Webster Sweat Inducer iontophoresis machine (Wescor, Inc.) which delivers an electric current equivalent to 1.5 mA for 5 minutes driving the pilocarpine nitrate cation into eccrine sweat ducts in the skin. After allowing 15 minutes for sweating to begin we applied a thin layer of Silasoft vinyl polysiloxane impression material (Microsonic, Inc.) spread on a piece of cardstock, held against the skin with a medical bandage for 5 minutes. Sweat droplets form holes in the impression material as they emerge from pores, and after 5 minutes the material begins to set, forming a stable record of sweat droplet density, with each hole corresponding to one functional eccrine gland. Sweat was then collected for 10 minutes with pre-weighed gauze to estimate sweat production. Impressions were scanned with 3 cm demarcations for scale and the resulting images were processed with ImageJ (Schindelin et al., 2012). We applied a bandpass filter to even the light gradient using the setting “filter small structures up to 3 pixels”, defined a circular region of interest between 4 and 10 cm<sup>2</sup>, and used the adjust threshold

feature to highlight holes as black dots against a white background. A second copy of the image file was used as a reference to help ensure that threshold was set to reveal sweat droplet holes and minimize noise. We used the watershed feature to separate any holes that had merged due to thresholding. Finally, we used the analyze particles feature to count holes in an automated fashion, with the following settings: particle size- between 0.0001 and 0.25 mm in diameter; particle circularity: between 0.20 and 1.0; and excluding holes on the edges. Resulting counts were divided by the region of interest area to yield FED/cm<sup>2</sup>. All 42 impressions taken from the 7 participants yielded high-quality images suitable for analysis, results of which are shown in table 3.

#### *Body composition and VO<sub>2</sub>-peak assessment*

Participants arrived at the University of Massachusetts Human Testing Center for body composition and vO<sub>2</sub>-peak assessments between 5 and 14 days prior to heat dissipation testing. Results are shown in table 2. After recording body mass and height, participants were scanned with iDXA (GE Lunar), a radiometric technique permitting accurate and highly repeatable measurement of body composition and adiposity (Hind et al., 2011). Next, vO<sub>2</sub>-peak was measured using a Velotron cycle ergometer (Racermate/SRAM) with electronic power control in the Human Testing Center's "flex" calorimeter, a metabolic chamber utilizing push-system respirometry explained further in the next section. Temperature was set to 21°C. The vO<sub>2</sub>-peak test began with a 7-minute warmup cycling at self-selected easy effort followed by 3-minute ramped stages starting at 100W external power and increasing by 30W until volitional exhaustion, or until further increases in external power did not increase vO<sub>2</sub>. VO<sub>2</sub>-peak was determined from the average vO<sub>2</sub>-value observed in the final 60 seconds of the test. Though we used



a ramp protocol typical of  $\dot{V}O_2$ -max tests we use the term “ $\dot{V}O_2$ -peak” because runners rarely achieve their true aerobic capacity in cycling tests (Corry and Powers, 1982; Niemelä et al., 1980).

#### *Indirect calorimetry with flow-through respirometry*

The University of Massachusetts Human Testing Center’s “flex” room calorimeter permits measurement of exercising metabolic rate (and therefore heat production) indirectly from measured differences in  $\dot{V}O_2$  and  $\dot{V}CO_2$  in inflow vs excurrent air (Lighton, 2008, Melanson et al., 2010; figure 4). A LICOR model LI-840A gas analyzer (LICOR, Lincoln, NE USA) was connected to the excurrent gas sampling piping of the metabolic chamber to record water vapor concentration in parts per thousand. The main dependent variable of interest was heat dissipation quotient (HDQ), a simple ratio defined as metabolic heat production/evaporative heat loss.

#### *Heat dissipation protocol*

Participants were instructed to arrive well-hydrated and to have avoided alcohol for 12 hours pre-test. Before entering the metabolic chamber participants were weighed on a force platform wearing only shorts. Next, they were given a pre-weighed water bottle to drink *ad libitum* during the test. Participants sat on the cycle ergometer for 5 minutes to establish baseline  $O_2$  and  $CO_2$  levels in the chamber, followed by a 3-minute warmup cycling period of 3 minutes at 100 watts external power, followed by 50 minutes of cycling at an external power production equivalent to 50% of the maximal power achieved in the  $\dot{V}O_2$ -max test. In this way we standardized for relative aerobic effort rather than heat production as other heat dissipation studies often do, which our setup would not permit. Chamber temperature was set at 30°C with an air inflow rate of

200L/min. Humidity is not controlled; the medical-grade air pumped into the chamber is near 0% water vapor and humidity in the chamber rises as a function of evaporative cooling from the participant inside. Participants took a baseline auditory canal temperature with a Braun Thermoscan thermometer and repeated this measurement every 10 minutes during the test as a safety precaution. These values were assumed to be a rough approximation of core temperature and were not used for analysis. After exiting the chamber participants towel-dried and were again weighed on the force platform. After subtracting the weight of water consumed during the test these data were used to estimate whole-body sweat loss (WBSL).

#### *Data processing and analysis*

VO<sub>2</sub> data were smoothed to eliminate peaks and a Haldane transformation was applied, standard practice for calculating v<sub>O2</sub> in flow-through respirometry systems (Haugen et al., 2007). Licor water production data were time-aligned and merged with metabolic chamber data, after which a 90-second centered derivative was applied to calculate water production rate. VO<sub>2</sub> values were converted to watts of metabolic heat production as follows, where RQ is the respiratory quotient, CO<sub>2</sub> production/O<sub>2</sub> consumption (Lighton, 2008):

$$\text{Metabolic heat production (Watts)} = \text{VO}_2 (\text{ml per min}) * [16 + 5.164 * (\text{RQ})]$$

VH<sub>2</sub>O was converted to grams/minute using the following equations (Lighton, 2008):

$$\begin{aligned} \text{VH}_2\text{O} (\text{mg/min}) &= \text{inflow rate (mL/min)} * (\text{H}_2\text{O ppt/1000}) * 0.803 \\ \text{VH}_2\text{O} (\text{g/min}) &= \text{VH}_2\text{O} (\text{mg/min}) / 1000 \end{aligned}$$

VH<sub>2</sub>O was then used to calculate evaporative heat loss (EHL) using the latent heat of vaporization of water as follows (Lighton, 2008):

$$\text{Evaporative heat loss (Watts)} = \text{VH}_2\text{O (g/min)} * 2430 \text{ (J/g)} * 60$$

Heat dissipation quotient (HDQ) was then defined as metabolic heat production/evaporative heat loss. We used average HDQ for the 5 minutes leading up to maximum observed evaporative heat loss (EHL). Simple linear regressions consisting only of 2 variables at a time were performed to assess relationships between HDQ and independent variables before including multiple variables together in a generalized linear mixed model (GLM). Next, we calculated estimated whole-body FED by multiplying 6-site average density by BSA, essentially standardizing for BSA, which is known to negatively associate with eccrine density. We emphasize that these figures are underestimates because we did not sample body regions with the highest eccrine density such as the forehead, hands and feet, but they serve as valid within-group comparisons. Finally, because our small sample size and multiple predictor variables risk overfitting the model, we used an information theoretic approach to rank predictors by how well they explain observed variation in HDQ. Using the MuMin package in R, we generated a set of models produced from all possible combinations of predictor variables, and then used the “importance” function to produce the sum of corrected Akaike Information Criterion (AICc) weights for each predictor variable (Burnham and Anderson, 2003). Sums of AICc weights closer to 1 indicate an increased ability to predict the dependent variable.

## **Results**

Heat dissipation test results are shown in table 3. Figure 6 shows curves of  $\dot{V}O_2$  (used to calculate metabolic heat production), external power production, chamber humidity, and water production from evaporation from characteristic participant T7.

Maximum evaporative heat loss (EHL) was observed for all participants early in the heat test, between 289-899 seconds after power production was increased from 100 watts (warmup) to power corresponding to 50% of VO<sub>2</sub>-peak (140-185W). Chamber humidity for the 5-minute period leading up to maximum EHL- the range in which we sampled heat dissipation quotient- ranged from 21.5-28.3%. Average HDQ during this period ranged from 1.1464 (the best observed HDQ) to 1.6198. After this point, EHL fell dramatically as chamber humidity continued to increase. All participants continued to sweat visibly as EHL declined. Whole-body sweat loss (WBSL) ranged from 0.75-2.40% body mass.

Simple linear regressions (figure 5) revealed moderate negative correlations between HDQ and VO<sub>2</sub>-peak ( $r^2=0.44$ ,  $p=0.11$ ) and WBSL ( $r^2=0.35$ ,  $p=0.16$ ; fig. 2) indicating that higher aerobic capacity and whole-body sweat loss for the duration of the 50-minute heat test were associated with improved heat dissipation during the 5 minutes leading up to maximum observed EHL. Sweat production in response to pilocarpine was closely associated with WBSL during the thermal test ( $r^2=0.82$ ,  $p<0.01$ ). HDQ was insignificantly and poorly correlated with 6-site FED ( $r^2=0.01$ ,  $p=0.82$ ), estimated whole-body FED ( $r^2=0.001$ ,  $p=0.94$ ), age ( $r^2=0.09$ ,  $p=0.51$ ), BSA/body mass ( $r^2=0.13$ ,  $p=0.44$ ), heat production/kg ( $r^2=0.12$ ,  $p=0.44$ ), body fat ( $r^2=0.0004$ ,  $p=0.97$ ), limb FED ( $r^2=0.05$ ,  $p=0.62$ ), and trunk FED ( $r^2=0.05$ ,  $p=0.62$ ). Limb and trunk FED were highly colinear ( $r^2=0.62$ ) and independently with 6-site density ( $r^2=0.79$  and  $0.97$  respectively) and were thus omitted from GLM's. Running all 8 independent variables overfitted the model so we split them among 2 GLM's (note that all variables were log<sub>10</sub> transformed). Heat

production/kg was colinear with V02-peak ( $r^2=0.65$ ) so these variables were included in different GLM's, with the following structure:

1. *HDQ (metabolic heat production/evaporative heat loss) = 6-site FED + V02-peak + BSA/body mass + age*
2. *HDQ (metabolic heat production/evaporative heat loss) = estimated whole-body FED + WBSL + heat production/kg + body fat*

Output results from the GLM are shown in table 5. No independent variables explained significant variation in HDQ. Model selection results are shown in table 6. For both GLM's, the model best explaining variation in HDQ (indicated by the highest AICc values, each -14.7) contained only the intercept and none of the predictor variables. VO2-peak and WBSL were the most important predictors in GLM's 1 and 2 respectively, with AICc sum of weights of 0.150 and 0.140.

## **Discussion**

This study assessed in a sample of fit runners during cycling in warm (30°C) conditions the relationship between functional eccrine sweat gland density (FED) and the heat dissipation quotient (HDQ, the ratio of metabolic heat production to evaporative heat loss, EHL). Our results do not support the hypothesis that higher FED is associated with improved HDQ. After accounting for factors known to influence heat dissipation- including vO2-peak, body surface area to body mass ratio (BSA/body mass), age, whole body sweat loss (WBSL), and body fat percentage- absolute FED averaged across 6 body sites, and split into limb and trunk densities, did not explain variation in HDQ. Similarly, a measure of FED standardized for BSA- estimated whole-body FED- was not associated with HDQ. Instead, variation in HDQ was best explained by vO2-peak, whole body

sweat production over the duration of the 50-minute cycling test, and BSA/body mass. VO<sub>2</sub>-peak was not significantly associated with HDQ but it was the most important of the tested variables in GLM 1 in model selection. Similarly, WBSL was an insignificant but most-important predictor in GLM 2. The association between BSA/body mass and HDQ was positive, the opposite of predictions based on the known association between surface area relative to body mass and heat dissipation (Epstein et al., 1983; Notley et al., 2019). Model selection, however, revealed this factor to be far less important than the p-value suggests.

Previous studies have found associations between aerobic capacity and heat dissipation capacity (Lamarche et al., 2018, Notley et al., 2019). Jay et al. argue that this relationship reflects effects of heat production, body mass, and BSA rather than an independent effect of aerobic capacity, and other acclimation responses including increased plasma volume may also partly explain this relationship (Jay et al., 2011). While our participants with higher measured vO<sub>2</sub>-peak cycled at a higher absolute and mass-adjusted metabolic heat production (a consequence of our study design), heat production per kg had little bearing on HDQ. VO<sub>2</sub>-peak is a measure of the summed capacity of many physiological components that deliver and use oxygen, and enhancement of many of these components will result in superior heat dissipation. For example, larger stroke volume of the heart means that more cardiac reserve is available for pumping blood to the skin to facilitate heat dissipation. Additionally, differences between individuals in aerobic capacity may result partly from endurance training. Because endurance training produces heat adaptation -both directly via challenges to core-temperature maintenance and also indirectly, e.g., adaptation in the vascular beds of

the skin which aid in blood perfusion- higher aerobic capacity often co-occurs with heat dissipation capacity (Green et al., 2004).

Unsurprisingly, in conditions permitting full evaporation, greater sweat production was associated with superior heat dissipation. However, we measured HDQ before humidity increased and blunted sweat evaporation, while our measure of WBSL encompasses the entire 50-minute cycling test, much of which was conducted in humid conditions in which sweat visibly dripped off of the participants. Dripping sweat removes much less heat from the skin than evaporated sweat and thus water lost early in the test contributed more to heat dissipation than water lost as humidity rose and EHL declined (Garden et al., 1966). Still, the correlation between WBSL and EHL indicates that, despite “wasteful” sweating later in the test, the heavy sweat-producers in our sample were also the most effective at dissipating heat in the initial dry conditions.

Volume of sweat production and capacity for whole-body evaporative cooling are unrelated to observed variation in functional eccrine density in our experiment. Higher FED does not appear to increase dehydration risk through additional water loss during exercise in hot dry conditions (the first ~15 minutes of our test), humid conditions where evaporation is blunted (the final ~35 minutes of our test), or when sweating is induced via pilocarpine. Our results suggest that variation in the sweat response at the level of individual eccrine glands appears to explain variation in heat dissipation and water loss better than eccrine gland density. Most of the variation in per-gland sweat response is likely the result of phenotypic plasticity due to both short-term and long-term acclimatization to local climate and physical activity. Gland-level phenotypic changes likely include hypertrophy and increased mitochondrial density in the secretory coil

(Okuda et al., 1981; Sato et al., 1990). While data are sparse, one study found that gland size varies fivefold between individuals, and size correlates positively with sweat rate, though to our knowledge the influence of plasticity vs. genotypic factors on gland size is unexplored (Sato and Sato, 1983). Further research is needed to confirm our observed uncoupling between FED, sweat production and heat dissipation, and to determine if low FED incurs a heat dissipation penalty in dehydrated states where sweat production per gland decreases. In such conditions individuals with low FED may be unable to maintain skin coverage with sweat, reducing evaporative heat dissipation.



## **CHAPTER 4**

### **UNDERSTANDING VARIATION IN HUMAN FUNCTIONAL ECCRINE DENSITY**

#### **Introduction**

In the early to mid-20<sup>th</sup> century Yas Kuno developed a hypothesis for climate-driven phenotypic plasticity in functional eccrine gland density, based largely on higher active gland densities in tropical southeast Asians vs. Russians and Ainu, indigenous peoples of Russia and Japan, and results of his own study of 26 Japanese individuals raised in tropical southeast Asia, who had higher gland densities than Japanese-born Japanese (Kuno, 1956). To our knowledge only two studies have tested this hypothesis and they provide some evidence for changes in functional gland density in adulthood rather than early childhood. Ojikutu reported higher functional gland densities in 108 Nigerian males living in Nigeria vs. 13 Ghanaian and Liberian males born in Africa but living in Germany as adults (Ojikutu, 1965). Knip observed higher functional gland densities in Dutch individuals who moved to Suriname as adults vs. Dutch individuals residing in the Netherlands (Knip, 1975). Interestingly, not only are these the only studies directly testing Kuno's phenotypic plasticity hypothesis, they are also the only (albeit indirect) evidence of functional gland density increasing during adulthood.

Kuno's hypothesis for climate-driven phenotypic plasticity in eccrine gland density occurring within a narrow developmental window is plausible, and given the immediate and profound impact of gland density on fitness via heat dissipation and water loss, inter-population differences due to divergent selective pressures or neutral evolutionary processes similarly cannot be ruled out without further testing. Here we test Kuno's climate hypothesis and investigate possible population-level differences in

functional eccrine density (hereafter referred to as FED) with a sample of 72 adults aged 18-39 spanning various early childhood climate regimes and geographic ancestries. In addition to helping fill a gap in our understanding of human biological diversity, we hoped that these data would permit inference into the evolution of human sweating.

## **Methods**

### *Study population*

We recruited 76 participants in the Amherst, MA, USA area, 35 males age  $21.8 \pm 5.1$  years and 41 females age  $20.9 \pm 4.1$  years (table 7). Participants were selected on the basis of having spent birth through at least age 5 in one of the following climate regimes: a) hot year-round without air conditioning, including both hot-dry and hot-humid climates, with average annual temperatures ranging from 18.0°C to 28.9°C ; b) temperate climates, mostly the Northeastern United States but with several volunteers from the American Pacific Northwest, Great Britain, and Bogota Columbia, with average annual temperatures of 7.0°C-13.6°C. Data were collected between April 2019 and September 2020. Informed consent was obtained in-person and all study procedures were approved by the University of Massachusetts Amherst Human Research Protection Office.

### *Assessment of childhood climate and geographic ancestry*

Average annual temperature and average annual water vapor pressure for the geographic coordinates of each volunteer's childhood residence were extracted from WorldClim files ([www.worldclim.org](http://www.worldclim.org)) (Fick and Hijmans, 2017). To account for possible effects of heat acclimatization on sweat production we took average daily temperatures from the month prior to each data collection using published National Atmospheric and Oceanic Administration data.

Participants were provided with a complementary 23andMe genetic ancestry test kit and asked to report results back to the researchers. Self-reported ancestry was used for 18 participants who failed to report their 23andMe results. The 23andMe test involves genotyping sections of the genome known to contain loci differing between populations, and assigning “percentage ancestry” based on identified variants. Ancestry as defined by 23andMe samples recent ancestry, perhaps several hundred years. For example, “Polish” as defined by 23andMe means having genetic variants commonly found in their “Polish” reference database, consisting of the genomes of individuals who have 4 grandparents with confirmed residence in Poland. Thus these test results do not sample the deep ancestry (thousands of years) where any divergent evolution in eccrine density would most likely have occurred. Given this limitation we collapsed fine-grain ancestry into 22 larger categories- e.g., “Northwestern European”, “Sub-Saharan African”, etc. We reduced the dimensionality of the 23andMe reports (e.g. geographic ancestry categories) using non-metric multidimensional scaling (NMDS). NMDS is a rank-based ordination method that is more flexible than other methods such as principal coordinates analysis. We used the resulting NMDS axis values as additional predictors in our general linear model (GLM) to represent the ancestry of our participants.

#### *Anthropometric and demographic measurements*

Participants filled out a survey regarding early childhood residence history, presence or absence of air conditioning in their childhood home (which participants were asked to verify with their childhood caretaker), self-reported geographic ancestry of all grandparents (to be included in analysis if genetic ancestry results were not reported), and current physical activity habits, which was assigned a relative value of “low” “moderate”

or “high” corresponding to self-reported minutes per week of moderate to vigorous physical activity (<60 min, 60-180 min, and >180 min respectively). This estimate of current physical activity level and average temperature of the preceding 30 days were included in an effort to account for heat acclimatization status which is known to influence sweat production. Stature, body mass, limb length (arm: acromion process to ulna head; leg: femoral head to lateral malleolus of fibula), and distal and proximal limb circumference were recorded. Body surface area (BSA) was estimated using the following regression equation developed by Haycock et al. (1978):  $BSA (m^2) = \text{body mass (kg)}^{0.5378} \times \text{height (cm)}^{0.3964} \times 0.024265$ .

#### *Quantification of functional eccrine density (FED)*

We have previously described our methods in greater detail, including a discussion of the various methods for quantifying eccrine density. Briefly, sweating was induced at 6 sites on the left side of the body, including lateral upper arm, medial forearm, abdomen (immediately inferior to rib 10), back (scapular), lateral thigh, and posterior lower leg (gastrocnemius head) with iontophoresis of 0.5% pilocarpine nitrate. Impressions of sweating skin were made using a vinyl polysiloxane impression material (Silasoft, Microsonic Inc.) with a 0.76 mm thick cardstock, held against the skin for 5 minutes with a medical bandage. Resulting impressions were scanned and analyzed with ImageJ to automatically count holes produced by sweat droplets, with each hole corresponding to a functional sweat gland (Schindelin et al., 2012). Repeated analysis by the same observer showed a coefficient of variation of 2.21%; analysis repeated by a second observer showed a coefficient of variation of 7.73%.

### *Statistical analysis*

All analyses were carried out in R (Team, 2013). A GLM with the following formula was run for each of the 6 body regions, and examining relationships between body regions (limbs vs. trunk, distal vs. proximal limbs, and distal limb vs. trunk):

$$\text{FED} = \text{BSA} + \text{limb circumference (for limb GLM's only)} + \text{age} + \text{sex} + \text{avg temp childhood climate} + \text{avg vapor pressure childhood climate} + \text{ancestry V1} + \text{ancestry V2} + \text{ancestry V3}$$

Our linear models contained a large number of predictors, which could lead to spurious results due to overfitting. To account for this potential problem, we used an information theoretic approach to understand the best predictors explaining variation in our dependent variables. We used the MuMIn package to generate a set of models produced from all combinations of our predictors. Then we used the “importance” function to assess the relative importance of each predictor variable. This function yields the sum of corrected Akaike Information Criterion (AICc) weights for each predictor variable (Burnham and Anderson, 2003). The sum of AICc weights ranges from zero to one, with increasing ability to predict the dependent variable.

Relationships between 6-site sweat production in response to pilocarpine and predictor variables were assessed using the following GLM, followed by model selection:

$$\text{6-site sweat production} = \text{6-site FED} + \text{age} + \text{sex} + \text{avg temp childhood climate} + \text{avg vapor pressure childhood climate} + \text{ancestry V1} + \text{ancestry V2} + \text{ancestry V3} + \text{current PA} + \text{avg temp of previous 30 days}$$

Finally, we examined differences in mean FED between the 6 tested body regions using an ANOVA followed by post-hoc Tukey HSD.

## **Results**

We obtained 424 usable impressions from 72 participants. Sweat weight data were collected from 75 of the 76 participants. Ancestry composition was estimated from demographic surveys for 18 participants who failed to share 23andMe reports. Nonmetric multidimensional scaling produced 4 variables from 23andMe ancestry percentages with a stress value of 12.7. Collapsing results into broad geographic regions defined by 23andMe, 51 participants had majority ancestry from Europe, 7 from India or Pakistan, 9 from Asia or the Americas, and 9 from Africa or Northern West Asia. Higher values for ancestry variable 1 (V1) corresponded to majority ancestry from Europe. Participants with high values for V2 had low percentages of European ancestry, but did not have other geographic ancestries in common.

### *Functional eccrine gland density*

FED averaged over the 6 sampled body sites showed a more than twofold range of variation, from 60.9 glands/cm<sup>2</sup> to 132.7 glands/cm<sup>2</sup> (table 8). FED was highest in the ventral forearm at  $114.4 \pm 18.5$  glands/cm<sup>2</sup>, significantly greater than all other body regions ( $p < 0.01$ ). FED was higher in the abdomen than in the lower leg ( $93.0 \pm 16.4$  vs.  $82.8 \pm 14.5$ ,  $p < 0.01$ ). No other between-region differences were statistically significant. Tables 9 and 10 show results of generalized linear models and model selection, respectively. Body surface area was the best predictor of FED in each model it was included in (6-site FED,  $p = 0.0008$ ; abdomen FED,  $p = 0.0015$ ; back FED,  $p = 0.0012$ ; figure 8). Where limb circumference was included instead, this measure was among the best predictors with an AICc sum of weights over 0.50 (forearm,  $p = 0.015$ ; upper arm,  $p = 0.083$ ; thigh,  $p = 0.004$ ; lower leg,  $p = 0.014$ ). Age was negatively associated with FED

and was an important predictor of six-site FED, though this relationship was largely driven by 6 participants over age 30 ( $p=0.037$ ; figure 7). Age was an important predictor of FED in the forearm ( $p=0.015$ ), upper arm ( $p=0.016$ ), abdomen ( $p=0.145$ ), thigh ( $p=0.152$ ), and lower leg ( $p=0.126$ ). Sex was an important predictor of 6 site FED ( $p=0.187$ ) and FED in the upper arm ( $p=0.144$ ), back ( $p=0.160$ ), and thigh ( $p=0.003$ ); women had higher densities in these regions. Ancestry variable 1 was important in predicting forearm density ( $p=0.072$ ) and distal/proximal density ratio ( $p=0.223$ ), and ancestry variable 2 was an important predictor of lower leg density ( $p=0.089$ ). Childhood climate variables (average annual temperature and water vapor pressure) did not explain variation in FED.

#### *Sweat production*

Males produced on average twice as much sweat per unit BSA as females in response to pilocarpine ( $0.052\text{g} \pm 0.062$  vs.  $0.026\text{g} \pm 0.020$ ,  $p<0.05$ ; figure 9). After removing 3 outliers this difference remained significant. Important predictors in model selection included sex, age (positive association), and average temperature of the preceding 30 days (positive association). Six-site FED did not explain variation in sweat production, with a correlation coefficient ( $r^2$ ) of 0.016 ( $p=0.30$ ), and no significant effects of childhood climate variables were observed.

### **Discussion**

Functional eccrine density (FED) in our sample of 72 participants was not associated with average annual temperature or humidity of childhood residence, nor was geographic ancestry predictive of FED in most tested body regions. Instead, variation in

FED was best explained by body surface area (BSA), a negative relationship; age, with higher densities in younger participants; and sex, with higher densities in females. Sweat production in response to pilocarpine was significantly higher in males as has been previously reported. The uncoupling of sweat production from FED confirms results from a small study using passive heating (Sato and Dobson, 1970) and indicates that sweat production in our participants was a function more of per-gland sweat output than gland density. Pilocarpine-induced sweat production is highly correlated with “real-world” sweat responses observed during thermal strain so our results can be cautiously extended as a proxy for thermal sweating. Taken together our results suggest that contemporary variation in FED observed in our sample ( $\sim 61\text{--}133$  glands/cm<sup>2</sup>) may have minimal fitness consequence, and selective pressures acting on FED were relaxed once some minimum density was reached in the hominin lineage. We hypothesize that humans adapt to new thermal regimes by altering sweat production at the gland level as rather than via changes in gland density.

*Sweating phenotype is explained by BSA, sex, and age*

Sex differences in eccrine density, or lack thereof, have seldom been reported, but our results roughly agree with those of Hwang and Baik in showing higher densities in the thigh and back of females (Hwang and Baik, 1997). These differences are not explained by BSA, nor is a candidate genetic or developmental mechanism immediately apparent as the handful of genes known to associate with eccrine density do not show sex linkage. Sweat production showed an even more striking sex difference. Men produced on average twice the sweat of females during the 10-minute post-iontophoresis collection period, a result that remains significant after removing 3 outliers. Two of these outliers



are competitive endurance athletes and were tested in the summer and during training, so their heightened sweat response is not surprising; endurance training in the heat is known to produce substantial increases in sweat output, and we have observed that 7 male endurance athletes produced on average twice as much sweat in response to pilocarpine as our male non-endurance athletes. Yet several female participants also perform endurance training and their sweat production was far below that of the male athletes, further suggesting a sex effect. This is not the first study to report sex differences in sweat response but it is among the largest to use the standardized stimulus of pilocarpine iontophoresis, and these results agree with literature showing higher maximal sweating rate in males in both passive and exercise-induced heating (Gagnon and Kenny, 2012; Gagnon et al., 2013). These differences were not explained by FED, and in fact, males in our study produced more sweat from fewer glands, echoing a small study showing this result in the forearm, indicating higher sweat output per gland in males (Gagnon et al., 2013).

Body surface area (BSA) was negatively associated with FED in our sample, agreeing with previous findings and underscoring the need to account for BSA when comparing eccrine density between individuals (Kuno, 1956; Szabo, 1967; Thomson, 1954). Extrapolating from regional densities to estimate total eccrine number over the body surface does not solve this problem as it relies on BSA, which is malleable during life and therefore uninfluenced by any of the factors hypothesized to associate with eccrine density, such as age, sex, childhood climate, geographic ancestry and genotype for genes known to impact eccrine development.

Older participants in our study had lower functional gland densities and age was a predictor of sweat production in model selection, with older individuals producing more sweat. Heat dissipation capacity declines with age, perhaps beginning as early as age 40, with lower whole-body sweat rate due to reduced sweat output per gland; some evidence also implicates recruitment of fewer glands, though these data come from men aged 60+ years (Inoue, 1996; Larose et al., 2013). Our study design was intended to minimize effects of age and therefore our sample included only 5 individuals over age 30, all of whom were younger than 40, and the positive association between age and sweat production was driven by a single outlier. An experimental design including a larger age range of participants is needed to clarify these findings.

#### *Sweating phenotype and childhood climate*

Our data do not support Kuno's hypothesis of phenotypic plasticity due to childhood climate. While our study design did not permit direct testing of the developmental effects Kuno envisioned, adult FED should reflect the plasticity he hypothesized. We suggest several explanations for this negative result. First, perhaps outdoor climate during childhood is unlikely to influence phenotype as strongly as it may have prior to the advent of cultural adaptations that buffer environmental extremes, and effects of climate may therefore be undetectable in adult FED and sweat production phenotypes. Humans employ a range of strategies to increase thermal comfort such as seeking shade or indoor spaces and adjusting physical activity schedules to avoid the hottest parts of the day. Even within a geographic region, indoor climatic conditions vary widely (Wang et al., 2018). This variation in behavior and indoor environment alters an individual's "microclimate" (the layer of air immediately surrounding the body) and core

temperature by influencing rate of convective heat loss and exogenous heat gain and endogenous heat production, blunting the need for homeostatic thermal responses such as sweating and increased skin perfusion, effectively reducing the variation in climate factors across populations and geographic regions. Further investigation into possible phenotypic plasticity will therefore require careful measurement and consideration of individual microclimate. Second, phenotypic responses in FED due to climate may still occur but were not detected in our sample due to inability of our study design to adequately control or account for childhood thermal regime. Testing this idea could be accomplished with a longitudinal study of FED in a hot climate where part of the population lives with air conditioning while others do not due to wealth disparities; depending on the study population, this could also effectively control for geographic ancestry. Finally, an alternative explanation, and one that we favor, is that FED is not altered in response to early childhood climate. While our sample of hot-climate participants was small (n=20), the uncoupling between sweat production and gland density leaves little reason to expect that FED should change to accommodate a new thermal regime. Rather, variation in per-gland sweat production- whether due to genotypic adaptation, phenotypic plasticity, or both- is sufficient to match sweat capacity to demand, and provides a mechanism of adaptation that is highly malleable beyond the early developmental window Kuno hypothesized.

#### *Sweating phenotype and geographic ancestry*

The results presented here support Taylor's (2006) conclusion that eccrine density is not patterned by geographic ancestry, with several possible exceptions. Ancestry variable 1 -a measure of European ancestry composition- was a moderately-important

predictor of forearm eccrine density and ratio of glands in distal vs. proximal limbs, suggesting higher density in the forearm, and lower leg vs. upper arm and thigh, in participants with majority European ancestry. The ventral forearm had the highest FED of any tested body region, and together with a high surface area to volume ratio, this suggests an important role in cooling for this body site. Further research should quantify the contribution of the distal limbs to cooling, and more data are needed on non-Europeans, who are underrepresented in our sample. This would provide context for our currently inexplicable result of higher forearm FED in Europeans. Ancestry variable 2 was a moderately-important predictor of lower leg density, with low European ancestry associated with high FED, but this body region yielded the fewest usable impressions and this result may not be robust. Additionally, participants with high values for variable 2 shared only one ancestry element in common: low percentage of European ancestry. Clearly “not European” is not a cogent population on which evolutionary forces can act, and we suggest this result is spurious.

Our findings do not preclude the possibility of derived eccrine density in specific populations- for example, the EDAR variant which increases eccrine density in the thumb and index finger which is present in Han Chinese in high frequencies, likely due to selection for another of its pleiotropic effects, and hECE18 polymorphisms influencing EN1 expression and altering eccrine density (Aldea et al., 2021). Future work should explore relationships between genotypes for these loci and eccrine density via targeting candidate populations and genotyping individuals from broader studies of eccrine density such as this one. Still, the lack of clear differences in eccrine density across populations stands in contrast to other phenotypic and genotypic differences such as skin

pigmentation, lactase persistence, and malaria resistance resultant from divergent “niche specific selective pressures” encountered as humans moved into new environments (Kamberov et al., 2013).

Previous research has demonstrated an attenuated sweating response in temperate-climate natives who have lived for years in the tropics (Bae et al., 2006; Katsuura et al., 1993). This is also seen in individuals with long-term tropical residence though this is best interpreted as phenotypic plasticity rather than genetic adaptation (Duncan and Horvath, 1988; Edholm et al., 1965; Fox et al., 1974; McCance and Purohit, 1969; McCance et al., 1974; Samueloff, 1987; Thomson, 1954). Our data show little effect of temperature or humidity of childhood residence or geographic ancestry on sweat production. This may be explained by the varying length of temperate-climate residence of our hot-climate participants: some only recently came to Massachusetts while others have resided here since late childhood.

*Does functional eccrine gland density matter?*

The roughly tenfold increase in eccrine density in the hominin lineage was almost certainly the product of selection for increased heat dissipation capacity, but variation within the range of contemporary humans (~61 to ~133 glands/cm<sup>2</sup> observed in this study) appears to have little bearing on sweat production and water loss. Instead, we suggest that per-gland sweat production can be altered to match demand regardless of total eccrine gland density or number. For example, if 60 eccrine glands/cm<sup>2</sup> is sufficient to promptly coat the skin surface as the sweat response initiates because each gland has high sweat output, then further increases in density will not result in more sweat being evaporated. Per-gland sweat production can also be reduced to avoid excess water loss,

as evidenced in the attenuated sweat responses seen in individuals with long-term hot-climate residence. Given that population differences in gland density have yet to be consistently reported, these sweat reductions must therefore be achieved through reductions in per-gland sweat production.

We propose a hypothesized scenario for the evolution of human functional eccrine density and thermal adaptation to new environments, as follows: 1) Variation in per-gland sweat production, whether resultant from genotypic adaptation or phenotypic plasticity or both, is sufficient in scope to render variation in contemporary human FED physiologically unimportant, and there is no tradeoff between heat dissipation and water loss based on FED; and therefore, 2) once FED in hominins reached the values seen in contemporary humans, selection on FED was relaxed and other physiological characteristics- including those influencing per-gland sweat production- became the primary targets of selection and/or phenotypic plasticity as humans experienced novel thermal regimes.

Our hypothesis generates testable predictions. First, FED should be uncoupled not just from pilocarpine-induced sweating but also from sweat production during exercise or passive heating. Our metabolic chamber data confirm previous results showing this relationship (Sato and Dobson, 1970). Second, because salt loss has significant physiological implications, differences in FED must not associate with differences in net salt loss. Increased sweat rate per gland (which is necessary for low-FED individuals to produce a high sweat rate) leads to decreased sodium reabsorption per unit sweat volume, presenting the possibility that low FED may incur a salt loss penalty (Buono et al., 2008). Heat acclimatization reduces salt content of sweat and increases

sodium reabsorption, and some evidence indicates that these gland-level acclimatization responses may be sufficient to fully compensate for the theoretical penalty of lower FED (Amano et al., 2016; Buono et al., 2007; Sato and Dobson, 1970). If our hypothesis that contemporary variation in FED has little physiological consequence is to be supported, experimental data must demonstrate that gland-level adaptations are sufficient to minimize salt loss- just as they appear sufficient to minimize water loss and maximize whole-body sweat production- at the lower end of contemporary FED.

An alternative interpretation of our data is that low eccrine density incurs a heat-dissipation penalty during physical activity in dehydrated states. If per-gland sweat rate drops enough, perhaps individuals with low gland density cannot maintain skin coverage with sweat in these conditions; and as selection often acts on rare or extreme circumstances we may then expect higher gland density to confer a fitness advantage. Modern elite marathoners lose up to 10% body mass in sweat during competition, conditions which *Homo erectus* may have encountered in persistence hunts which may have been central to the ecology of our genus (Beis et al., 2012; Hora et al., 2020). This is near the hypothesized physiological limit of 12% where the ability to swallow is lost (Schmidt-Nielsen, 1964). Data indicating reductions in sweat rate during dehydration are mixed (Pearcy et al., 1956). Further research should seek to identify the point at which dehydration impedes sweat rate and whether low gland density impairs evaporative heat dissipation in this state. Finally, further research should explore whether other factors known to influence heat dissipation, such as skin blood flow and skin temperature during thermal stress, compensate for differences in FED and partly explain the results of our study.

## **Conclusions**

Functional eccrine gland density (FED) in our sample of 72 adults showed a wide range of variation (60.9 glands/cm<sup>2</sup> to 132.7 glands/cm<sup>2</sup>) which is best explained by body surface area (BSA), sex, and age. Younger participants and females had higher FED. Sweat production in response to pilocarpine was twice as high in males, agreeing with previous work showing sex differences in sweat output and heat dissipation capacity. Most importantly, our results indicate that sweat production and FED are uncoupled, and differences in sweat production must therefore be attributable to sweat output per eccrine gland. We hypothesize that, while dramatically increased eccrine density was central to human evolution, selection on variation in FED was relaxed once some minimum FED was attained. After this point, phenotypic plasticity in aspects of thermoregulation other than eccrine gland density, including per-gland sweat production, are the primary means through which humans have and continue to adapt to new thermoregulatory environments. Remaining questions include whether low eccrine density is associated with increased salt loss, or impaired evaporative heat dissipation in conditions of dehydration and physical activity.



## TABLES

Author/Method	Study sample	Summed over multiple body sites	Forearm (ventral)	Upper arm (dorsal)	Abdomen	Back (scapula)	Thigh (lateral)	Lower Leg (posterior)
This study/pilocarpine iontophoresis, vinyl polysiloxane impression	77 males and females age 21.3 ± 4.6 years	92.4 ± 13.4 (range: 60.1-132.7)	114.4 ± 18.5	90.9 ± 14.6	93.0 ± 16.4	86.6 ± 17.9	86.2 ± 16.2	82.8 ± 14.5
Taylor and Machado-Moreira 2013/various	Various; meta-review of 31 studies	---	103.3	98.3	102.5	100.3	76.3	--
Gagnon and Kenny 2012/active thermal, iodine-paper*	16 males and females age 27.5 ± 5.5	---	117.5	---	---	75.5	---	---

**Table 1.** Observed active eccrine gland densities vs. results reported in the literature. Gland densities are given in glands/cm<sup>2</sup>, unadjusted for body surface area. Standard deviation not available when we pooled data originally presented in subgroups. \*Dorsal or ventral not specified; values are pooled averages for data originally presented in sex subgroups.

Volunteer	Age (years)	Height (cm)	Body mass (kg)	BSA (m <sup>2</sup> )	BSA/body mass	Body fat (%)	vO <sub>2</sub> peak (ml/kg/min)	Power production at vO <sub>2</sub> peak (watts)	Relative power production at vO <sub>2</sub> peak (watts/kg body mass)
3	23.7	170.0	63.7	1.735	0.0272	13.2	58.6	310	4.87
4	27.5	183.0	63.0	1.776	0.0282	8.0	56.2	310	4.92
5	25.9	168.0	59.8	1.670	0.0279	14.0	51.2	280	4.68
6	31.0	195.3	74.2	1.990	0.0268	11.5	41.7	280	3.77
7	37.3	167.5	66.5	1.766	0.0266	8.1	56.6	370	5.56
8	26.4	167.6	60.5	1.679	0.0277	15.1	54.6	310	5.12
9	34.3	174.0	62.7	1.737	0.0277	13.6	50.0	280	4.47
Avg	29.4	175.1	64.3	1.800	0.0275	11.9	52.7	305.7	4.80
± SD	± 4.6	± 9.7	± 4.5	± 0.100	± 0.0006	± 2.6	± 5.3	± 29.7	± 0.50

**Table 2:** Summary of volunteer demographics, anthropometrics and performance characteristics.

Volunteer	Pilocarpine sweat production (g)	6-site average FED/cm <sup>2</sup>	Estimated whole-body FED/cm <sup>2</sup>	Forearm FED/cm <sup>2</sup>	Upper arm FED/cm <sup>2</sup>	Abdomen FED/cm <sup>2</sup>	Back FED/cm <sup>2</sup>	Thigh FED/cm <sup>2</sup>	Lower leg FED/cm <sup>2</sup>
3	0.042	88.7	1,539,289	93.7	92.9	98.8	87.7	76.3	82.8
4	0.040	78.4	1,393,163	101.6	79.1	96.1	73.2	58.7	61.9
5	0.166	111.0	1,852,937	153.7	109.2	119.4	104.3	95.0	84.3
6	0.006	85.6	1,702,699	92.9	86.3	100.8	97.8	60.5	75.0
7	0.270	76.0	1,340,976	98.6	66.7	79.5	75.0	77.5	58.4
8	0.146	98.3	1,650,204	129.0	96.5	102.1	89.6	77.0	95.7
9	0.072	81.6	1,416,213	102.6	64.6	100.6	83.1	74.0	64.4
Avg	0.106	88.5	1,556,497	110.3	85.0	99.6	87.2	74.1	74.6
± SD	± 0.086	± 11.5	± 173,673	± 21.0	± 15.0	± 10.8	± 10.5	± 11.2	± 12.7

**Table 3:** Functional eccrine gland densities (FED) and sweat production in response to pilocarpine. Note that estimated whole-body FED are likely underestimates because we did not sample the forehead, hands and feet, body regions with the highest eccrine density.

Volunteer	Heat dissipation quotient	Heat production (W)	Heat production (W)/body mass (kg)	Total water loss (L)	Total water loss as % of body mass
3	1.1464	631.8	9.919	0.889	1.40
4	1.4679	608.7	9.661	0.642	1.02
5	1.4251	607.0	10.150	1.396	2.33
6	1.4965	564.4	7.606	0.558	0.75
7	1.2204	628.3	9.448	1.594	2.40
8	1.2313	556.9	9.205	1.225	2.02
9	1.6198	576.5	9.195	0.567	0.90
Avg	1.3725	596.2	9.312	0.982	1.55
± SD	± 0.16151	± 28.1	± 0.769	± 0.393	± 0.65

**Table 4:** Heat dissipation test results. Average heat dissipation quotient (metabolic heat production/evaporative heat loss) was taken from the 5-minute period leading up to each volunteer's maximum observed evaporative heat loss. Water loss and temperature increase were measured by comparing measurements taken pre- and post- 50-minute cycling protocol.

Fixed effect	Estimate	Std. error	t value	P
<i>Model 1</i>				
(Intercept)	9.0253	2.1253	4.247	0.0512
log10 (6-site FED)	-0.2184	0.2366	-0.923	0.4534
log10 (VO2-peak)	-0.8161	0.2207	-3.698	0.0660
log10 (BSA/body mass)	4.8328	1.2125	3.986	0.0576
log10 (Age)	0.3302	0.2076	1.591	0.2527
<i>Model 2</i>				
(Intercept)	-1.81295	5.32980	-0.340	0.766
log10 (estimated whole-body FED)	0.29890	0.83510	0.358	0.755
log10 (WBSL)	-0.20375	0.19284	-1.057	0.401
log10 (heat production/kg)	0.21020	0.97934	0.215	0.850
log 10 (body fat)	-0.07123	0.35808	-0.199	0.861

**Table 5:** Results of the generalized linear mixed models showing associations between heat dissipation quotient and independent variables.

Model 1	log10 (VO2-peak)	log10 (BSA/body mass)	log10 (Age)	log10 (6-site FED)
	0.150	0.038	0.035	0.024
Model 1	log10 (WBSL)	log10 (heat production/kg)	log10 (estimated whole-body FED)	log10 (body fat)
	0.140	0.038	0.024	0.024

**Table 6:** Relative importance of independent variables in explaining variation in heat dissipation quotient. Reported values are sum of AICc weights. Values closer to 1 indicate increased ability to predict heat dissipation quotient.

	All (n≤76)	Hot childhood climate (n≤20)	Temperate childhood climate (n≤56)	Males	Females
male	35	8	27		
female	41	12	29		
age	21.3 ± 4.6	22.6 ± 6.0	20.8 ± 3.8	21.8 ± 5.1	20.9 ± 4.1
BSA	1.77 ± 0.29	1.71 ± 0.25	1.79 ± 0.30	1.89 ± 0.28	1.68 ± 0.26
SA:body mass	0.0269 ± 0.0027	0.0272 ± 0.0027	0.268 ± 0.0028	0.0262 ± 0.0026	0.0275 ± 0.0027
leg length:BMI	3.596 ± 0.687	3.555 ± 0.623	3.612 ± 0.710	3.625 ± 0.720	3.574 ± 0.660
arm length:BMI	2.376 ± 0.485	2.276 ± 0.442	2.412 ± 0.494	2.344 ± 0.425	2.404 ± 0.529

**Table 7:** Demographics and anthropometrics. Means are followed by standard deviation.

	All (n≤76)	Hot childhood climate (n≤20)	Temperate childhood climate (n≤56)	Males	Females
sweat weight (g)	0.038 ± 0.046	0.034 ± 0.020	0.039 ± 0.052	0.052 <sup>a</sup> ± 0.062	0.026 ± 0.020
6-site avg. gland density (cm <sup>2</sup> )	92.9 ± 13.0	95.3 ± 16.2	91.8 ± 11.4	88.2 ± 10.5	97.5 ± 13.7
forearm gland density (cm <sup>2</sup> )	114.4 <sup>c</sup> ± 18.5	112.6 ± 21.4	115.1 ± 17.1	119.8 ± 17.1	108.6 ± 18.1
upper arm gland density (cm <sup>2</sup> )	90.9 ± 14.6	89.2 ± 14.9	91.5 ± 14.4	86.7 ± 14.0	94.8 ± 14.1
abdomen gland density (cm <sup>2</sup> )	93.0 ± 16.4	97.2 ± 20.3	91.3 ± 14.0	89.7 ± 15.1	96.3 ± 17.0
back gland density (cm <sup>2</sup> )	86.6 ± 17.9	89.9 ± 18.6	85.3 ± 17.5	79.5 ± 13.1	92.8 ± 19.3
thigh gland density (cm <sup>2</sup> )	86.2 ± 16.2	88.9 ± 18.5	85.2 ± 15.1	80.5 ± 12.8	91.5 <sup>b</sup> ± 17.1
lower leg gland density (cm <sup>2</sup> )	82.8 ± 14.5	87.4 ± 17.0	81.3 ± 13.1	80.5 ± 12.5	84.9 ± 15.8
gland density limbs:trunk	1.059 ± 0.121	1.035 ± 0.116	1.069 ± 0.122	1.065 ± 0.125	1.053 ± 0.118
gland density distal:proximal	1.147 ± 0.140	1.147 ± 0.151	1.148 ± 0.135	1.133 ± 0.128	1.159 ± 0.148
gland density distal:trunk	1.129 ± 0.150	1.151 ± 0.157	1.120 ± 0.146	1.144 ± 0.129	1.116 ± 0.165

**Table 8:** Gland densities and sweat weights. Means are followed by standard deviation. <sup>a</sup> Significantly higher than females (p<0.05). <sup>b</sup> Significantly higher than males (p<0.01). <sup>c</sup> Significantly higher than all other body regions (p<0.01). Additional significant effects are highlighted in tables 3 and 4.

Sweat Production				
	Estimate	Std. Error	t value	P
(Intercept)	-4.279e-02	6.729e-02	-0.636	0.5273
6-site FED	1.604e-04	4.937e-04	0.325	0.7464
age	1.960e-03	1.558e-03	1.258	0.2132
sex (male)	2.970e-02	1.233e-02	2.408	<b>0.0192 *</b>
temp	-7.362e-05	4.533e-04	-0.162	0.8715
vapor	3.375e-03	4.909e-02	0.069	0.9454
V1	-5.331e-04	1.348e-03	-0.396	0.6939
V2	-7.522e-04	1.246e-03	-0.604	0.5484
V3	-1.755e-03	1.241e-03	-1.414	0.1625
PA (low)	-3.420e-03	2.178e-02	-0.157	0.8757
PA (mod)	-1.061e-02	1.752e-02	-0.605	0.5473
30 day temp	5.341e-04	4.785e-04	1.116	0.2689

6-site FED				
	Estimate	Std. Error	t value	P
(Intercept)	136.86745	11.03329	12.405	<b>&lt; 2e-16 ***</b>
BSA	-18.62065	5.23995	-3.554	<b>0.000755 **</b>
age	-0.64545	0.30246	-2.134	<b>0.037008 *</b>
sex (male)	-3.86909	2.89810	-1.335	0.186991
temp	0.11156	0.10055	1.109	0.271729
vapor	-7.79669	10.78830	-0.723	0.472720
V1	0.47047	0.33605	1.400	0.166749
V2	0.38402	0.28518	1.347	0.183270
V3	0.08005	0.28527	0.281	0.779995

Forearm FED				
	Estimate	Std. Error	t value	P
(Intercept)	184.89567	22.82914	8.099	<b>3.72e-11 ***</b>
forearm circ.	-2.21009	0.88258	-2.504	<b>0.0151 *</b>
age	-0.94229	0.45764	-2.059	<b>0.0439 *</b>
sex (male)	-3.35110	4.95990	-0.676	0.5019
temp	-0.02478	0.15469	-0.160	0.8733
vapor	6.58195	16.59053	0.397	0.6930
V1	0.91483	0.50016	1.829	0.0724 .
V2	0.22480	0.41978	0.536	0.5943
V3	-0.44795	0.43482	-1.030	0.3071

Upper Arm FED				
	Estimate	Std. Error	t value	P
(Intercept)	136.65302	14.58007	9.373	<b>3.83e-13 ***</b>
upperarm circ.	-0.81269	0.46063	-1.764	0.0830 .
age	-0.92837	0.37350	-2.486	<b>0.0159 *</b>
sex (male)	-5.30439	3.58091	-1.481	0.1440
temp	0.03489	0.13309	0.262	0.7942
vapor	-4.28721	14.21806	-0.302	0.7641
V1	0.10191	0.39331	0.259	0.7965
V2	0.49733	0.35351	1.407	0.1649
V3	-0.04747	0.36632	-0.130	0.8973

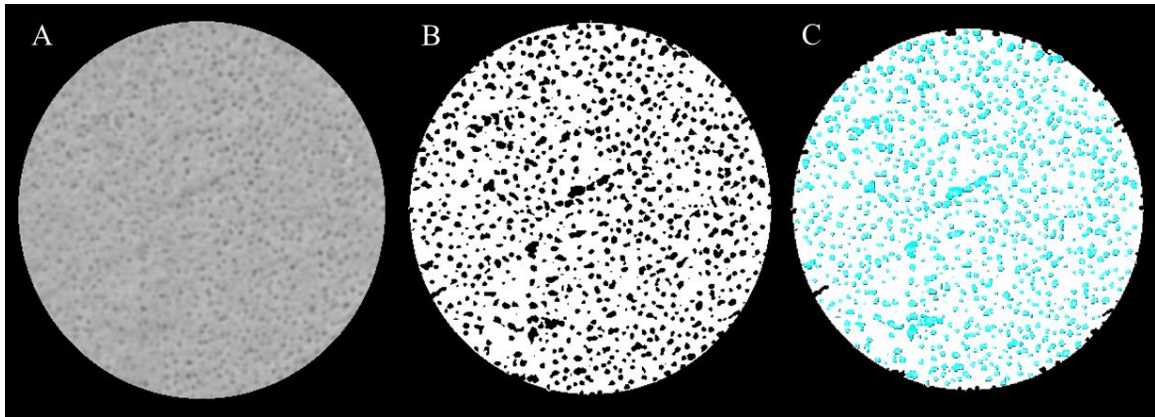
Abdomen FED				
	Estimate	Std. Error	t value	P
(Intercept)	141.363153	15.662489	9.026	<b>1.92e-12 ***</b>
BSA	-24.770751	7.389611	-3.352	<b>0.00146 **</b>
age	-0.628220	0.424957	-1.478	0.14503

**Table 9:** Results of the generalized linear mixed models. \*\*\*p<0.0001. \*\*p<0.01. \*p<0.05

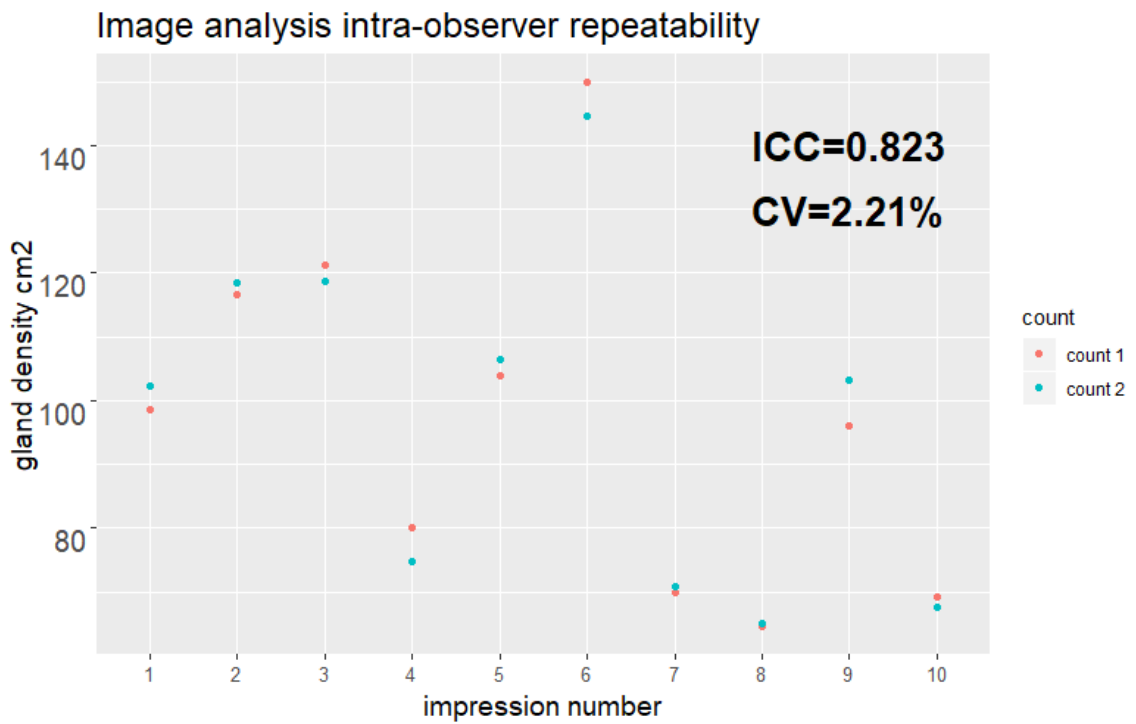
Dependent variable	Independent variable (AICc sum of weights)
sweat weight (g)	sex <sup>a</sup> (0.87), age (0.67), 30 day avg. temp. (0.53)
6-site avg. gland density (cm <sup>2</sup> )	BSA <sup>b</sup> (1.00), age <sup>c</sup> (0.91), sex (0.51)
forearm gland density (cm <sup>2</sup> )	forearm circumference <sup>c</sup> (0.97), ancestry V1 (0.75), age (0.74) <sup>c</sup>
upper arm gland density (cm <sup>2</sup> )	age <sup>c</sup> (0.96), upper arm circumference (0.71), sex (0.61)
abdomen gland density (cm <sup>2</sup> )	BSA <sup>d</sup> (0.99), age (0.56)
back gland density (cm <sup>2</sup> )	BSA <sup>d</sup> (1.00), sex (0.57)
thigh gland density (cm <sup>2</sup> )	thigh circumference <sup>d</sup> (0.99), sex <sup>d</sup> (0.99), age (0.61)
lower leg gland density (cm <sup>2</sup> )	ancestry V2 (0.72), age (0.61), lower leg circumference (0.50)
gland density limbs:trunk	no predictors with sum of weights <0.50
gland density distal:proximal	ancestry V1 (0.52)
gland density distal:trunk	no predictors with sum of weights <0.50

**Table 10:** Relative importance of independent variables in explaining variation in dependent variables. Only independent variables with a sum of AICc weights  $\geq 0.50$  are reported. <sup>a</sup> Significantly greater in males ( $p < 0.05$ ). <sup>b</sup>  $p < 0.001$ . <sup>c</sup>  $p < 0.05$ . <sup>d</sup>  $p < 0.01$ .

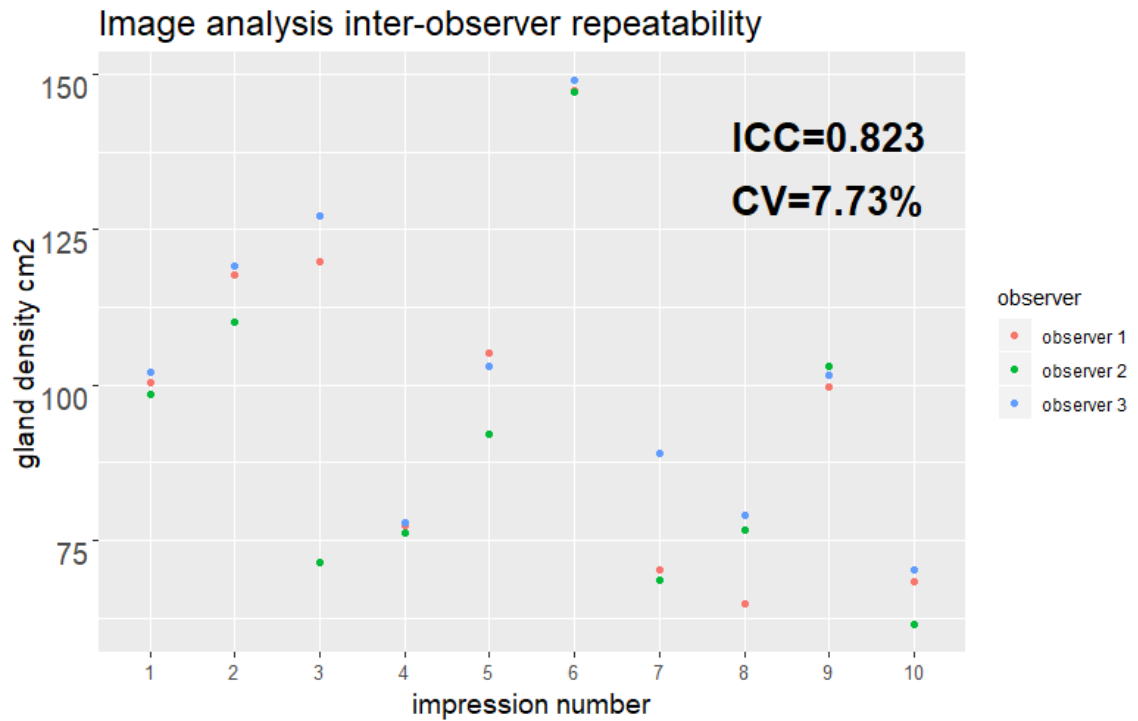
## FIGURES



**Figure 1.** ImageJ analysis of a sweat impression taken from the ventral forearm of a volunteer: After scanning, bandpass filtering and cropping (A); with threshold adjusted to highlight holes produced by sweat droplets (B); and after counting holes with the analyze particles plugin (C).



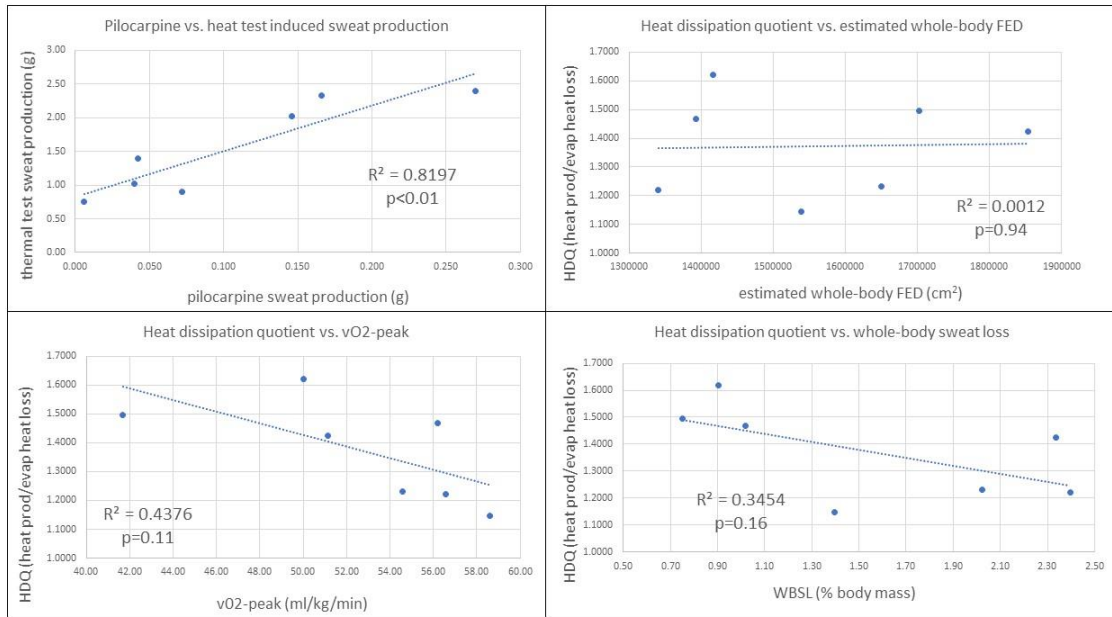
**Figure 2.** Results of repeated counts of the same impressions by one researcher.



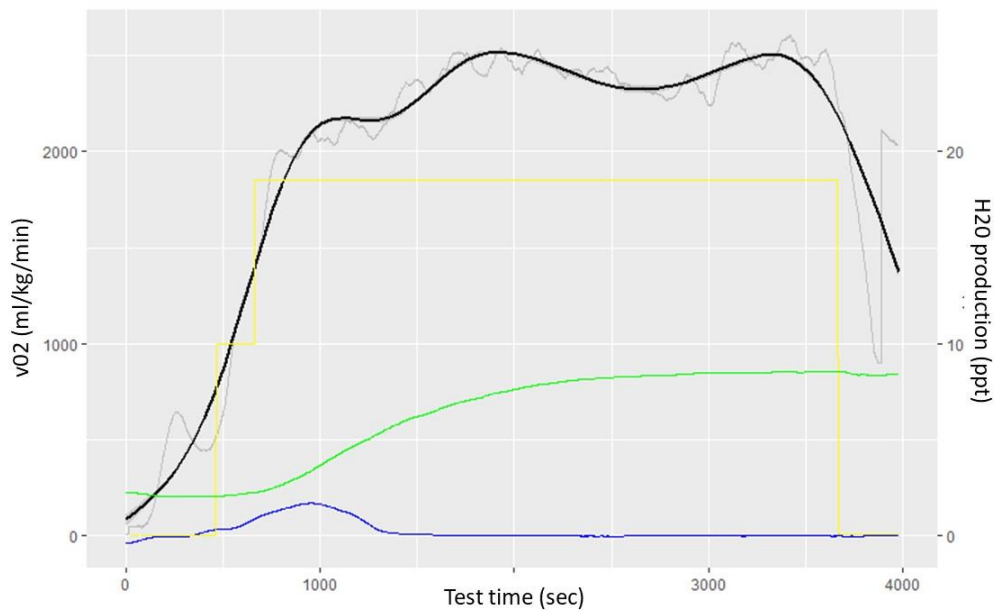
**Figure 3.** Results of repeated counts of the same impressions by three researchers.



**Figure 4:** Schematic of flow-through respirometry using the University of Massachusetts Human Testing Center's flex room calorimeter.

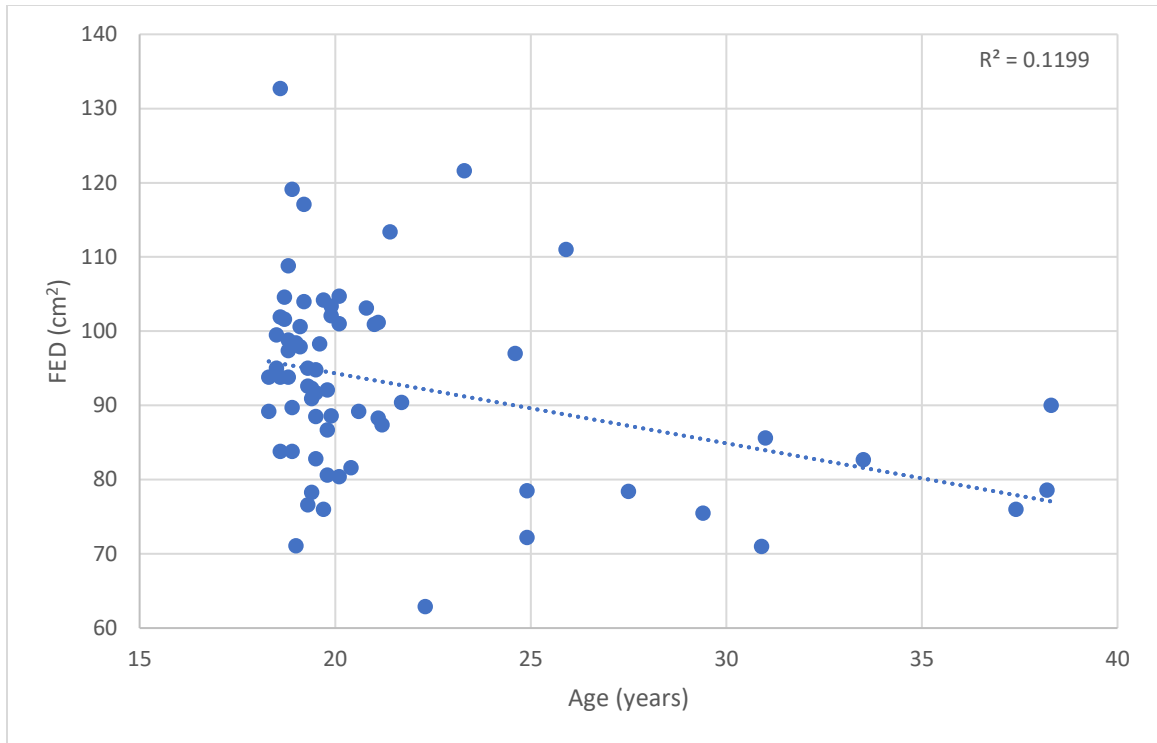


**Figure 5:** Results of select simple linear regressions.

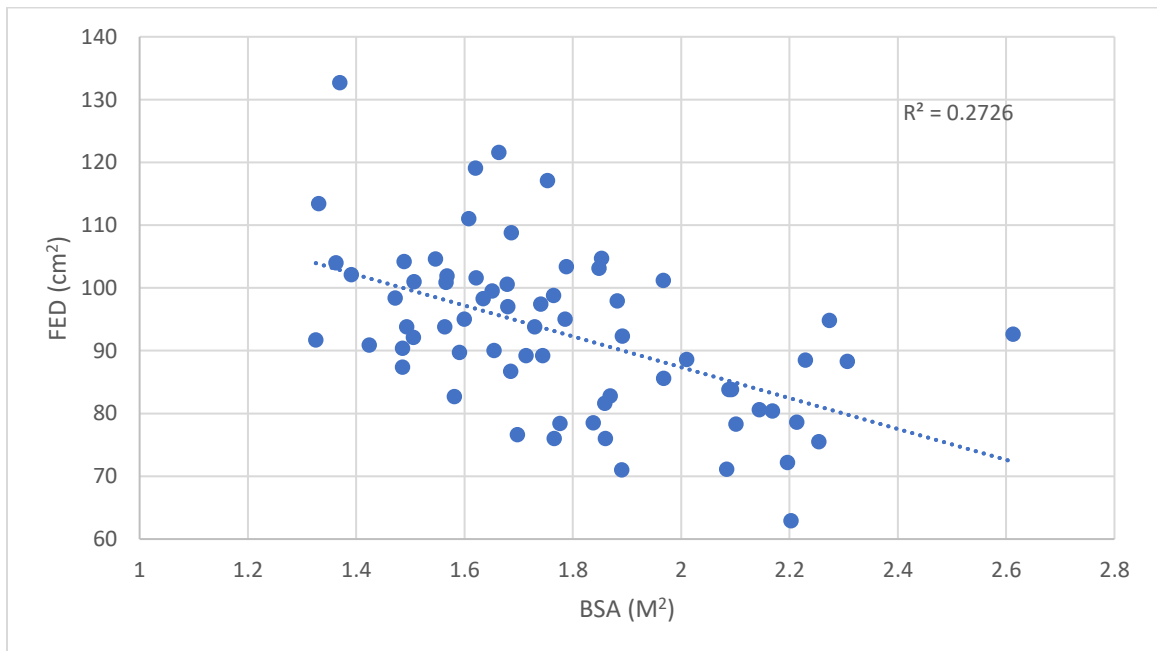


**Figure 6:** Example heat test data from volunteer T7. Grey: vO2 (ml/min); black: smoothed vO2, which was multiplied by  $[16 + 5.164 \cdot (RQ)]$  to yield metabolic heat production in watts; yellow: external power production (watts, units not shown); green: chamber humidity (%; units not shown); blue: H2O concentration in excurrent airflow (parts per thousand). Note that the X-axis shows test time starting with resting baseline measurements; time to maximum evaporative heat loss was measured from the moment each volunteer began cycling an external power production corresponding to 50% of their vO2-peak, where the yellow line jumps.

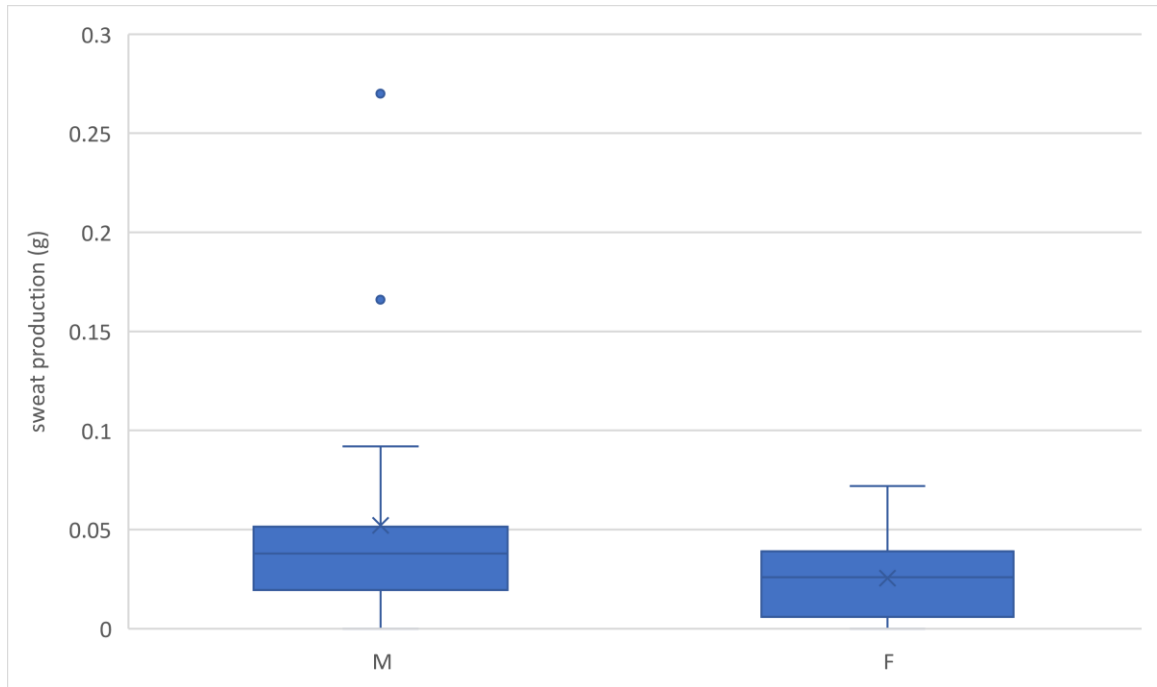




**Figure 7:** 6-site average functional eccrine density vs. age, simple linear regression.



**Figure 8:** 6-site average functional eccrine density vs. body surface area, simple linear regression.



**Figure 9:** 6-site total sweat production.

## BIBLIOGRAPHY

- Adelman, S., Taylor, C. R., & Heglund, N. C. (1975). Sweating on paws and palms: What is its function? *American Journal of Physiology-Legacy Content*, 229(5), 1400-1402.
- Aldea, D., Atsuta, Y., Kokalari, B., Schaffner, S. F., Prasasya, R. D., Aharoni, A., . . . Kamberov, Y. G. (2021). Repeated mutation of a developmental enhancer contributed to human thermoregulatory evolution. *Proceedings of the National Academy of Sciences*, 118(16), e2021722118. doi:10.1073/pnas.2021722118
- Amano, T., Kato, Y., Machado-Moreira, C. A., Taylor, N. A., Inoue, Y., Nishiyasu, T., & Kondo, N. (2011). Changes in eccrine sweating on the glabrous skin of the palm and finger during isometric exercise. *Acta Physiologica*, 202(4), 649-655.
- Bae, J., Lee, J., Matsumoto, T., Othman, T., Min, Y., & Yang, H. (2006). Prolonged residence of temperate natives in the tropics produces a suppression of sweating. *Pflügers Archiv*, 453(1), 67-72.
- Barak, M. M., Lieberman, D. E., Raichlen, D., Pontzer, H., Warrener, A. G., & Hublin, J. (2013). Trabecular evidence for a human-like gait in australopithecus africanus. *PloS One*, 8(11), e77687.
- Barker, D. (2003). The midwife, the coincidence, and the hypothesis. *British Medical Journal*, 327(7429), 1428-1430.
- Best, A., & Kamilar, J. M. (2018). The evolution of eccrine sweat glands in human and nonhuman primates. *Journal of Human Evolution*, 117, 33-43.
- Best, A., Lieberman, D. E., & Kamilar, J. M. (2019). Diversity and evolution of human eccrine sweat gland density. *Journal of Thermal Biology*, 84, 331-338.
- Biggs, L. C., & Mikkola, M. L. (2014). Early inductive events in ectodermal appendage morphogenesis. *Seminars in Cell & Developmental Biology*, 25, 11-21. Academic Press.
- Blumenthal, S. A., Levin, N. E., Brown, F. H., Brugal, J. P., Chritz, K. L., Harris, J. M., Jehle, G.E., & Cerling, T. E. (2017). Aridity and hominin environments. *Proceedings of the National Academy of Sciences of the United States of America*, 114(28), 7331-7336. doi:10.1073/pnas.1700597114 [doi]
- Bramble, D. M., & Jenkins, F. A. (1993). Mammalian locomotor-respiratory integration: implications for diaphragmatic and pulmonary design. *Science*, 262(5131), 235-240.
- Bramble, D. M., & Lieberman, D. E. (2004). Endurance running and the evolution of homo. *Nature*, 432(7015), 345-352.

- Buono, M. J., Ball, K. D., & Kolkhorst, F. W. (2007). Sodium ion concentration vs. sweat rate relationship in humans. *Journal of Applied Physiology*, 103(3), 990-994.
- Buono, M. J., Claros, R., DeBoer, T., & Wong, J. (2008). Na secretion rate increases proportionally more than the na reabsorption rate with increases in sweat rate. *Journal of Applied Physiology*, 105(4), 1044-1048.
- Bullard, R. W., Dill, D. B., & Yousef, M. K. (1970). Responses of the burro to desert heat stress. *Journal of Applied Physiology*, 29(2), 159-167.
- Burnham, K. P., & Anderson, D. (2003). Model selection and multi-model inference. *A Pratical Information-Theoretic Approach*. Sringer, 1229
- Carrier, D. R., Kapoor, A. K., Kimura, T., Nickels, M. K., Satwanti, Scott, E. C., So, J.K., & Trinkaus, E. (1984). The energetic paradox of human running and hominid evolution [and comments and reply]. *Current Anthropology*, 25(4), 483-495.
- Corry I., Powers N. (1982). Maximal aerobic power measurement in runners and swimmers. *British Journal of Sports Medicine*, 16, 154-60.
- Dervis S., Coombs G.B., Chaseling G.K., Filingeri D., Smoljanic J., Jay O. (2016). A comparison of thermoregulatory responses to exercise between mass-matched groups with large differences in body fat. *Journal of Applied Physiology*, 120, 615-23.
- Duncan, M. T., & Horvath, S. M. (1988). Physiological adaptations to thermal stress in tropical Asians. *European Journal of Applied Physiology and Occupational Physiology*, 57(5), 540-544.
- Edholm, O. G., Fox, R. H., Goldsmit R, Hampton, I., & Pillai, K. V. (1965). A comparison of heat acclimatization in Indians and Europeans. *Journal of Physiology-London*, 177(1), 15.
- Ellis, R. A., & Montagna, W. (1962). The skin of primates. VI. the skin of the gorilla (gorilla gorilla). *American Journal of Physical Anthropology*, 20(2), 79-93.
- Entin, P. L., Robertshaw, D., & Rawson, R. E. (1999). Effect of locomotor respiratory coupling on respiratory evaporative heat loss in the sheep. *Journal of Applied Physiology*, 87(5), 1887-1893.
- Epstein Y., Shapiro Y., Brill S. (1983). Role of surface area-to-mass ratio and work efficiency in heat intolerance. *Journal of Applied Physiology*, 54, 831-6.
- Fick, S. E., & Hijmans, R. J. (2017). WorldClim 2: New 1-km spatial resolution climate surfaces for global land areas. *International Journal of Climatology*, 37(12), 4302-4315.

- Folk, G. E., & Semken, A. (1991). The evolution of sweat glands. *International Journal of Biometeorology*, 35(3), 180-186.
- Ford, D. M., & Perkins, E. M. (1970). The skin of the chimpanzee. *The Chimpanzee*, 3, 82-119.
- Fox, R. H., Budd, G. M., Woodward, P. M., Hackett, A. J., & Hendrie, A. L. (1974). A study of temperature regulation in new guinea people. *Philosophical Transactions of the Royal Society of London. B, Biological Sciences*, 268(893), 375-391.
- Gagnon, D., Crandall, C. G., & Kenny, G. P. (2013). Sex differences in postsynaptic sweating and cutaneous vasodilation. *Journal of Applied Physiology*, 114(3), 394-401.
- Gagnon, D., & Kenny, G. P. (2012). Sex differences in thermoeffector responses during exercise at fixed requirements for heat loss. *Journal of Applied Physiology*, 113(5), 746-757.
- Gale, C. C. (1973). Neuroendocrine aspects of thermoregulation. *Annual Review of Physiology*, 35(1), 391-430.
- Garden, J. W., Wilson, I. D., & Rasch, P. J. (1966). Acclimatization of healthy young adult males to a hot-wet environment. *Journal of Applied Physiology*, 21(2), 665-669.
- Gibbons, C. H., Illigens, B. M., Centi, J., & Freeman, R. (2008). QDIRT: Quantitative direct and indirect test of sudomotor function. *Neurology*, 70(24), 2299-2304.
- Gluckman, P. D., & Hanson, M. A. (2004). The developmental origins of the metabolic syndrome. *Trends in Endocrinology & Metabolism*, 15(4), 183-187.
- Gordon, B. I., & Maibach, H. I. (1968). On the mechanism of the inactive eccrine human sweat gland. *Archives of Dermatology*, 97(1), 66-68.
- Green D.J., Maiorana A., O'Driscoll G., Taylor R. (2004). Effect of exercise training on endothelium-derived nitric oxide function in humans. *Journal of Physiology London*, 561, 1-25.
- Haugen H.A., Chan L., Li F. (2007). Indirect calorimetry: A practical guide for clinicians. *Nutrition in Clinical Practice*, 22, 377-88.
- Harris, D. R., Polk, B. F., & Willis, I. (1972). Evaluating sweat gland activity with imprint techniques. *Journal of Investigative Dermatology*, 58(2), 78-84. Inoue, Y., Havenith, G., Kenney, W. L., Loomis, J. L., & Buskirk, E. R. (1999). Exercise-and methylcholine-induced sweating responses in older and younger men: Effect of heat acclimation and aerobic fitness. *International Journal of Biometeorology*, 42(4), 210-216.

- Haycock, G. B., Schwartz, G. J., & Wisotsky, D. H. (1978). Geometric method for measuring body surface area: A height-weight formula validated in infants, children, and adults. *The Journal of Pediatrics*, 93(1), 62-66.
- Hiley, P. G. (1976). The thermoregulatory responses of the galago (*galago crassicaudatus*), the baboon (*papio cynocephalus*) and the chimpanzee (*pan stayrus*) to heat stress. *The Journal of Physiology*, 254(3), 657-671.
- Hind K., Oldroyd B., Truscott J.G. (2011). In vivo precision of the GE lunar iDXA densitometer for the measurement of total body composition and fat distribution in adults. *European Journal of Clinical Nutrition*, 65, 140-2.
- Hwang, K., & Baik, S. H. (1997). Distribution of hairs and sweat glands on the bodies of korean adults: A morphometric study. *Cells Tissues Organs*, 158(2), 112-120.
- Inoue, Y., & Shibasaki, M. (1996). Regional differences in age-related decrements of the cutaneous vascular and sweating responses to passive heating. *European Journal of Applied Physiology and Occupational Physiology*, 74(1-2), 78-84.
- Inoue, Y., Shibasaki, M., Hirata, K., & Araki, T. (1998). Relationship between skin blood flow and sweating rate, and age related regional differences. *European Journal of Applied Physiology and Occupational Physiology*, 79(1), 17-23.
- Jablonski, N. G. (2004). The evolution of human skin and skin color. *Annu. Rev. Anthropol.*, 33, 585-623.
- Jablonski, N. G., & Chaplin, G. (2000). The evolution of human skin coloration. *Journal of Human Evolution*, 39(1), 57-106.
- Jablonski, N. G., & Chaplin, G. (2010). Human skin pigmentation as an adaptation to UV radiation. *Proceedings of the National Academy of Sciences*, 107(Supplement 2), 8962-8968.
- Jay O., Bain A.R., Deren T.M., Sacheli M., Cramer M.N. (2011). Large differences in peak oxygen uptake do not independently alter changes in core temperature and sweating during exercise. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* 301, R832-41.
- Johnson, C., & Shuster, S. (1970). The patency of sweat ducts in normal-looking skin. *British Journal of Dermatology*, 83(3), 367-370.
- Johnson, G. S., & Elizondo, R. S. (1974). Eccrine sweat gland in macaca mulatta: Physiology, histochemistry, and distribution. *Journal of Applied Physiology*, 37(6), 814-82

- Johnson, G. S., & Elizondo, R. S. (1979). Thermoregulation in macaca mulatta: A thermal balance study. *Journal of Applied Physiology: Respiratory, Environmental and Exercise Physiology*, 46(2), 268-277.
- Kamberov, Y. G., Karlsson, E. K., Kamberova, G. L., Lieberman, D. E., Sabeti, P. C., Morgan, B. A., & Tabin, C. J. (2015). A genetic basis of variation in eccrine sweat gland and hair follicle density. *Proceedings of the National Academy of Sciences of the United States of America*, 112(32), 9932-9937. doi:10.1073/pnas.1511680112 [doi]
- Kamberov, Y. G., Guhan, S. M., DeMarchis, A., Jiang, J., Wright, S. S., Morgan, B. A., Sabeti, P.C., Tabin, C.J., & Lieberman, D. E. (2018). Comparative evidence for the independent evolution of hair and sweat gland traits in primates. *Journal of Human Evolution*, 125, 99-105.
- Kamberov, Y. G., Wang, S., Tan, J., Gerbault, P., Wark, A., Tan, L., Chen, H., & Powell, A. (2013). Modeling recent human evolution in mice by expression of a selected EDAR variant. *Cell*, 152(4), 691-702.
- Katsuura, T., Tachibana, M. E., Okada, A., & Kikuchi, Y. (1993). Comparison of thermoregulatory responses to heat between Japanese Brazilians and Japanese. *Journal of Thermal Biology*, 18(5-6), 299-302.
- Kawahata, A., & Adams, T. (1961). Racial variations in sweat gland distribution. *Proceedings of the Society for Experimental Biology and Medicine*, 106(4), 862-865.
- Kawahata, A., & Sakamoto, H. (1951). Some observations on sweating of the Aino. *The Japanese Journal of Physiology*, 2, 166-169.
- Kennedy, W. R., Wendelschafer-Crabb, G., & Brelje, T. C. (1994). Innervation and vasculature of human sweat glands: An immunohistochemistry-laser scanning confocal fluorescence microscopy study. *Journal of Neuroscience*, 14(11), 6825-6833.
- Knip, A. S. (1975). Acclimatization and maximum number of functioning sweat glands in Hindu and Dutch females and males. *Annals of Human Biology*, 2(3), 261-277.
- Kondo, N., Takano, S., Aoki, K., Shibasaki, M., Tominaga, H., & Inoue, Y. (1998). Regional differences in the effect of exercise intensity on thermoregulatory sweating and cutaneous vasodilation. *Acta Physiologica Scandinavica*, 164(1), 71-78.
- Kuno, Y. (1956). *Human perspiration*. Thomas.
- Landis, S. C. (1990). Target regulation of neurotransmitter phenotype. *Trends in Neurosciences*, 13(8), 344-350.

- Lamarche D.T., Notley S.R., Louie J.C., Poirier M.P., Kenny G.P. (2018). Fitness-related differences in the rate of whole-body evaporative heat loss in exercising men are heat-load dependent. *Experimental Physiology*, 103, 101-10.
- Larose, J., Boulay, P., Sigal, R. J., Wright, H. E., & Kenny, G. P. (2013). Age-related decrements in heat dissipation during physical activity occur as early as the age of 40. *PLoS One*, 8(12), e83148.
- Lee, J., & Shin, Y. (2017). Comparison of density and output of sweat gland in tropical africans and temperate koreans. *Autonomic Neuroscience*, 205, 67-71.
- LeGrys, V. A., Yankaskas, J. R., Quittell, L. M., Marshall, B. C., & Mogayzel, P. J. (2007). Diagnostic sweat testing: The cystic fibrosis foundation guidelines. *The Journal of Pediatrics*, 151(1), 85-89.
- Lemaire, R. (1967). Modes of thermolysis in the monkey macacus nemestrina. [Les modes de thermolyse chez le singe Macacus nemestrina] *Journal De Physiologie*, 59(1 Suppl), 257-258.
- Lighton J.R. (2008). Measuring metabolic rates: A manual for scientists. Oxford University Press.
- Liebenberg, L. (2006). Persistence hunting by modern hunter-gatherers. *Current Anthropology*, 47(6), 1017-1026.
- Lieberman, D. (2011). *The evolution of the human head*. Harvard University Press.
- Lieberman, D. E. (2015). Human locomotion and heat loss: An evolutionary perspective. *Comprehensive Physiology*, 5(1), 99-117.
- Liljequist, D., Elfving, B., & Skavberg Roaldsen, K. (2019). Intraclass correlation—A discussion and demonstration of basic features. *PloS One*, 14(7), e0219854.
- Lu, C. P., Polak, L., Keyes, B. E., & Fuchs, E. (2016). Spatiotemporal antagonism in mesenchymal-epithelial signaling in sweat versus hair fate decision. *Science*, 354(6319), aah6102.
- Machado-Moreira, C. A., & Taylor, N. A. (2017). Thermogenic and psychogenic recruitment of human eccrine sweat glands: Variations between glabrous and non-glabrous skin surfaces. *Journal of Thermal Biology*, 65, 145-152.
- Mahoney, S. A. (1980). Cost of locomotion and heat balance during rest and running from 0 to 55 degrees C in a patas monkey. *Journal of Applied Physiology: Respiratory, Environmental and Exercise Physiology*, 49(5), 789-800.



- McCance, R. A., & Purohit, G. (1969). Ethnic differences in the response of the sweat glands to pilocarpine. *Nature*, 221(5178), 378.
- McCance, R. A., Rabiya, Y. A., Beer, G., Edholm, O. G., Even-Paz, Z., Luff, R., & Samueloff, S. (1974). Have the bedouin a special 'desert' physiology? *Proceedings of the Royal Society of London. Series B. Biological Sciences*, 185(1079), 263-271.
- Meade R.D., Notley S.R., Kenny G.P. (2019). Aging and human heat dissipation during exercise-heat stress: An update and future directions. *Current Opinion in Physiology*, 10, 219-25.
- Mitchell, D., Senay, L. C., Wyndham, C. H., Van Rensburg, A. J., Rogers, G. G., & Strydom, N. B. (1976). Acclimatization in a hot, humid environment: Energy exchange, body temperature, and sweating. *Journal of Applied Physiology*, 40(5), 768-778.
- Montagna, W., & Machida, H. (1966). The skin of primates. XXXII. The Philippine tarsier (*Tarsius syrichta*). *American journal of physical anthropology*, 25(1), 71-83.
- Montagna, W., Machida, H., & Perkins, E. (1966). The skin of primates XXVIII. the stump-tail MACAQUE (*macaca speciosa*). *American Journal of Physical Anthropology*, 24(1), 71-85.
- Montagna, W., & Parakkal, P. F. (1974). Eccrine sweat glands. *The Structure and Function of Skin*, , 366-441.
- Montagna, W., & Yun, J. S. (1963). The skin of primates. XVI. The skin of Lemur mongoz. *American journal of physical anthropology*, 21(3), 371-381.
- Montagna, W., Yun, J. S., & Machida, H. (1964). The skin of primates XVIII. The skin of the rhesus monkey (*Macaca mulatta*). *American journal of physical anthropology*, 22(3), 307-319
- Newman, R. W. (1970). Why man is such a sweaty and thirsty naked animal: A speculative review. *Human Biology*, 12-27.
- Niemelä K., Palatsi I., Takkunen J. (1980). The oxygen uptake-work-output relationship of runners during graded cycling exercise: Sprinters vs. endurance runners. *British Journal of Sports Medicine*, 14, 204-9.
- Nishiyama, T., Sugeno, J., Matsumoto, T., Iwase, S., & Mano, T. (2001). Irregular activation of individual sweat glands in human sole observed by a videomicroscopy. *Autonomic Neuroscience*, 88(1-2), 117-126.

- Notley S.R., Lamarche D.T., Meade R.D., Flouris A.D., Kenny G.P. (2019). Revisiting the influence of individual factors on heat exchange during exercise in dry heat using direct calorimetry. *Experimental Physiology*, 104, 1038-50.
- Ojikutu, R. (1965). Die rolle von hautpigment und schweissdrusen in der klimaannpassung des menschen. *Homo.*, 16, 77-95.
- Okuda N., Kanai M., Watari N., Ohara K. (1981). Morphological changes of the eccrine sweat glands of japanese monkey after heat acclimation the mechanism of peripheral adaptation. In *Contributions to Thermal Physiology*. Pergamon. 293-295.
- Périard, J. D., Racinais, S., & Sawka, M. N. (2015). Adaptations and mechanisms of human heat acclimation: Applications for competitive athletes and sports. *Scandinavian Journal of Medicine & Science in Sports*, 25, 20-38.
- Peterson, A., Abella, E. F., Grine, F. E., Teafor, M. F., & Ungar, P. S. (2018). Microwear textures of *Australopithecus africanus* and *Paranthropus robustus* molars in relation to paleoenvironment and diet. *Journal of Human Evolution*, 119, 42-63.
- Pilbeam, D. R., & Lieberman, D. E. (2017). Reconstructing the last common ancestor of chimpanzees and humans. *Chimpanzees and Human Evolution*, , 22-141.
- Poblet, E., Jimenez, F., Escario-Travesedo, E., Hardman, J. A., Hernández-Hernández, I., Agudo-Mena, J. L., Cabrera-Galvan, J.J., Nicu, C., & Paus, R. (2018). Eccrine sweat glands associate with the human hair follicle within a defined compartment of dermal white adipose tissue. *British Journal of Dermatology*, 178(5), 1163-1172.
- Quinn, R. L. (2019). Isotopic equifinality and rethinking the diet of *Australopithecus anamensis*. *American Journal of Physical Anthropology*, 1– 19. <https://doi.org/10.1002/ajpa.23846>
- Randall, W. C. (1946). Quantitation and regional distribution of sweat glands in man. *The Journal of Clinical Investigation*, 25(5), 761-767.
- Raichlen, D. A., Pontzer, H., & Sockol, M. D. (2008). The laetoli footprints and early hominin locomotor kinematics. *Journal of Human Evolution*, 54(1), 112-117.
- Reed, D. L., Light, J. E., Allen, J. M., & Kirchman, J. J. (2007). Pair of lice lost or parasites regained: The evolutionary history of anthropoid primate lice. *Bmc Biology*, 5(1), 7.
- Rittié, L., Sachs, D. L., Orringer, J. S., Voorhees, J. J., & Fisher, G. J. (2013). Eccrine sweat glands are major contributors to reepithelialization of human wounds. *The American Journal of Pathology*, 182(1), 163-171.

- Ruxton, G. D., & Wilkinson, D. M. (2011). Thermoregulation and endurance running in extinct hominins: Wheeler's models revisited. *Journal of Human Evolution*, 61(2), 169-175.
- Sahnouni, M., Parés, J. M., Duval, M., Cáceres, I., Harichane, Z., Van der Made, J., . . . Derradji, A. (2018). 1.9-million-and 2.4-million-year-old artifacts and stone tool-cutmarked bones from ainoucherit, algeria. *Science*, 362(6420), 1297-1301.
- Samueloff, S. (1987). Thermoregulatory responses in genetically different ethnic groups. *Man in Stressful Environments: Thermal and Work Physiology*. Springfield (Illinois): Charles C Thomas, 23-34.
- Sato, F., Owen, M., Matthes, R., Sato, K., & Gisolfi, C. V. (1990). Functional and morphological changes in the eccrine sweat gland with heat acclimation. *Journal of Applied Physiology*, 69(1), 232-236.
- Sato, K., Nishiyama, A., & Kobayashi, M. (1979). Mechanical properties and functions of the myoepithelium in the eccrine sweat gland. *American Journal of Physiology-Cell Physiology*, 237(3), C184.
- Sato, K. (1977). The physiology, pharmacology, and biochemistry of the eccrine sweat gland. *Reviews of Physiology, Biochemistry and Pharmacology*, 79, (51-131) Springer.
- Sato, K., & Dobson, R. L. (1970). Regional and individual variations in the function of the human eccrine sweat gland. *Journal of Investigative Dermatology*, 54(6), 443-449.
- Sato, K., & Dobson, R. L. (1973). Glucose metabolism of the isolated eccrine sweat gland: II. the relation between glucose metabolism and sodium transport. *The Journal of Clinical Investigation*, 52(9), 2166-2174.
- Sato, K., Feibleman, C., & Dobson, R. L. (1970). The electrolyte composition of pharmacologically and thermally stimulated sweat: A comparative study. *Journal of Investigative Dermatology*, 55(6), 433-438.
- Sato, K., Kang, W. H., Saga, K., & Sato, K. T. (1989). Biology of sweat glands and their disorders. I. normal sweat gland function. *Journal of the American Academy of Dermatology*, 20(4), 537-563.
- Savastano D.M., Gorbach A.M., Eden H.S., Brady S.M., Reynolds J.C., Yanovski J.A. (2009). Adiposity and human regional body temperature. *American Journal of Clinical Nutrition*, 90, 1124-31.
- Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., . . . Schmid, B. (2012). Fiji: An open-source platform for biological-image analysis. *Nature Methods*, 9(7), 676-682.

- Schwartz, G. G., & Rosenblum, L. A. (1981). Allometry of primate hair density and the evolution of human hairlessness. *American Journal of Physical Anthropology*, 55(1), 9-12.
- Sellers, W. I., Cain, G. M., Wang, W., & Crompton, R. H. (2005). Stride lengths, speed and energy costs in walking of *Australopithecus afarensis*: Using evolutionary robotics to predict locomotion of early human ancestors. *Journal of the Royal Society Interface*, 2(5), 431-441.
- Smith, C. J., Alexander, L. M., & Kenney, W. L. (2013). Nonuniform, age-related decrements in regional sweating and skin blood flow. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, 305(8), R877-R885.
- Smith, A. A., & Dobson, R. L. (1966). Sweating and glycogenolysis in the palmar eccrine sweat glands of the rhesus monkey. *Journal of Investigative Dermatology*, 47(4), 313-316.
- Speakman J.R., Król E. (2010). Maximal heat dissipation capacity and hyperthermia risk: Neglected key factors in the ecology of endotherms. *Journal of Animal Ecology*, 79, 726-46.
- Stapleton J.M., Poirier M.P., Flouris A.D., et al (2015). Aging impairs heat loss, but when does it matter? *Journal of Applied Physiology*, 118, 299-309.
- Szabo, G. (1962). The number of eccrine sweat glands in human skin. *Eccrine Sweat Glands*. 1-6.
- Szabo, G. (1967). The regional anatomy of the human integument with special reference to the distribution of hair follicles, sweat glands and melanocytes. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, 252(779), 447-485.
- Taylor, N. A. (2006). Ethnic differences in thermoregulation: Genotypic versus phenotypic heat adaptation. *Journal of Thermal Biology*, 31(1), 90-104.
- Taylor, N. A. (2014). Human heat adaptation. *Comprehensive Physiology*,
- Taylor, N. A., & Machado-Moreira, C. A. (2013). Regional variations in transepidermal water loss, eccrine sweat gland density, sweat secretion rates and electrolyte composition in resting and exercising humans. *Extreme Physiology & Medicine*, 2(1), 4.
- Team, R. C. (2013). R: A language and environment for statistical computing.

- Thomson, M. L. (1953). The identification and enumeration of active sweat glands in man from plastic impressions of the skin. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 47(5), 412-417.
- Thomson, M. L. (1954). A comparison between the number and distribution of functioning eccrine sweat glands in Europeans and Africans. *The Journal of Physiology*, 123(2), 225-233.
- Toda, Y. (1967). Measurement and regional distribution of active sweat glands in indonesians. *The Kobe Journal of Medical Sciences*, 13(3), 157-164.
- Vilches, J. J., & Navarro, X. (2002). New silicones for the evaluation of sudomotor function with the impression mold technique. *Clinical Autonomic Research*, 12(1), 20-23.
- Wang, Z., Ji, Y., & Su, X. (2018). Influence of outdoor and indoor microclimate on human thermal adaptation in winter in the severe cold area, china. *Building and Environment*, 133, 91-102.
- Ward, C. V. (2013). Postural and locomotor adaptations of Australopithecus species. *The Paleobiology of Australopithecus* (pp. 235-245) Springer.
- Webster, H. L., & Rundell, C. A. (1982). Laboratory diagnosis of cystic fibrosis. *CRC Critical Reviews in Clinical Laboratory Sciences*, 18(4), 313-338.
- Weibel, E. R., Taylor, C. R., & Hoppeler, H. (1991). The concept of symmorphosis: A testable hypothesis of structure-function relationship. *Proceedings of the National Academy of Sciences*, 88(22), 10357-10361.
- West-Eberhard, M. J. (1989). Phenotypic plasticity and the origins of diversity. *Annual Review of Ecology and Systematics*, 20(1), 249-278.
- Wheeler, P. E. (1991). The thermoregulatory advantages of hominid bipedalism in open equatorial environments: The contribution of increased convective heat loss and cutaneous evaporative cooling. *Journal of Human Evolution*, 21(2), 107-115.
- Whitford, W. G. (1976). Sweating responses in the chimpanzee (*Pan troglodytes*). *Comparative Biochemistry and Physiology Part A: Physiology*, 53(4), 333-336.
- Whittow, G. C. (1971). Comparative physiology of thermoregulation. vol. II. mammals.
- Willis, I., Harris, D. R., & Moretz, W. (1973). Normal and abnormal variations in eccrine sweat gland distribution. *Journal of Investigative Dermatology*, 60(2), 98-103.

Yamamoto, O. (1990). Three-dimensional architecture of the microvasculature in the rat foot-pad, with special reference to vasculature around the eccrine sweat glands. *Cell and Tissue Research*, 262(2), 225-232.