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SWEAT GLAND ACTIVITY FOLLOWING THERMAL AND CHOLINERGIC TRAINING

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Abstract. Heat acclimation is an important counter-mechanism to minimize heat distress. Acclimation can occur through several mechanisms including warm-water immersion. Iontophoresis of pilocarpine has been shown to cause an acute sweat response, but has not been studied to assess its sustaining effects. The purpose of this investigation was to compare the physiological responses of repeated exposure to local heat vs. cholinergic agents on sweat gland function. Ten healthy, males (25.5±4.3 yrs; 178.8±7.5 cm; 85±10.5 kg) served as their own controls. Sweat gland training consisted of 2-weeks of treatment, including 7-days of repeated exposure to pilocarpine to the right upper extremity (RUE), followed by 7-days of localized heat acclimation training (43ºC moist heating packs) to the left upper extremity (LUE). Sweat rate (SR), sweat gland density (SGD), and sweat rate per gland (S/G) were determined following pilocarpine stimulation on day 1 (T1), day 7 (T2), and day 14 (T3) on the RUE and LUE. Baseline readings of SR (6.59±0.69 $g \cdot m^2 \cdot min^{-1}$ and 6.17±0.67) and SGD (118±3 glands/cm^2 and 113±2) for the RUE and LUE were not significantly different on day 1 ($p>0.05$). Following repeated pilocarpine iontophoresis, the RUE exhibited a 4% decrease in SGD (114±3 glands/cm^2) and a 50% reduction in SR (3.28±0.36 $g \cdot m^2 \cdot min^{-1}$). Following heat acclimation the LUE showed an increase in SGD of 7% (121±3 glands/cm^2) and a 36% increase in sweat rate (SR=8.42±0.93 $g \cdot m^2 \cdot min^{-1}$) ($p<0.05$). These data indicate that sweat glands are more productive following local heat acclimation and are less responsive following repeated exposure to cholinergic-agonists.

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Key words: Heat acclimation – Thermoregulation – Sweat gland

Introduction

It is well documented that sweat rate in humans is directly related to heat load. Humans also have an intrinsic ability to adapt following repeated exposures to heat

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stress. Previous research has demonstrated that acclimatization to heat stress is associated with a greater maximal sweat rate and an increased sweating response to a given increase in core temperature [4,7,10]. The physiological factors involved with acclimatization involve both central and peripheral adaptations. The central factors, primarily the autonomic nervous system, govern the involuntary responses in thermoregulation and the reflex ability to initiate sweat production. Peripheral mechanisms involve modifications at the level of the eccrine sweat gland.

It has been shown that repeated thermoregulatory stimulation will progressively increase peripheral sweat production. Kuno suggested that changes in the excitability or sensitivity of the central nervous system to thermal stimuli was the most important factor accounting for increased sweating seen with heat acclimation [5]. Conversely, it has been suggested that the increase in sweat rate is due more to peripheral adaptation than any of the gradual central changes.

For example, Nadel, Pandolf, Roberts, and Stolwijk [6] reported that a peripheral mechanism is responsible for the potentation of sweating after physical training. Additionally, Sato and Sato [10] found similar results when they performed skin biopsies on a heterogeneous group of 12 males. Of these subjects, 3 described themselves as non-athletic and "poor" sweaters, the other nine were athletic and "good" sweaters. The results demonstrated that fit individuals' eccrine sweat glands were larger than those of their unfit counterparts. It was also observed that the hypertrophied sweat glands of the fit individuals had other physiological and anatomical adaptations [10]. Furthermore, Buono and Sjoholm [1] found that in the absence of any known central thermoregulatory drive, sweat production in trained individuals is significantly greater than that of the untrained. The effects of gland training were also evident under local anesthesia and nerve blockade, indicating that central factors need not be involved in the improvement of peripheral sweat production [2]. It would follow that physical training increases peripheral sweat production by increasing the size of the gland, increasing periglandular acetylcholine concentrations, and/or increasing the cholinergic sensitivity of the gland.

It has also been observed that sweat gland training need not involve physical activity. Ogawa and Asayma [8] have demonstrated the changes in sweat gland activity in natural heat acclimatization may be mimicked by exposing the body to localized exogenous stimuli (hot air or water immersion). Ogawa *et al.* [9] demonstrated that immersion of an arm in 43ºC water for two hours per day for 15-consecutive days resulted in increases in sweat rate in the trained limb when compared to the untrained limb. It is also interesting to note that the effect of local heat training appears to develop quickly. Increases in secretory capacity of the

sweat gland may occur within a few days of the introduction of local heat acclimation [8,9].

The majority of the literature examining thermoregulation and sweat rate uses exercise or heat as a means of increasing sweat production [2,5,6,7,8,9]. It has also been demonstrated that maximal sweat gland activity may be elicited with the use of cholinergic-agonists [12]. The methods of cholinergic stimulation are more commonly used for assessment of sweat capacity and in the diagnosis of various pathological conditions [12]. No data appears to exist regarding the effects of repeated exposure of these cholinergic agents on the sweat gland. Although cholinergic agents (i.e. pilocarpine) have been shown to elicit an increased sweating response, anecdotal evidence from our laboratory has indicated that repeated exposures to pilocarpine may decrease sensitivity of the sweat gland.

The purpose of this investigation was to compare the physiological response following repeated exposure of heat and exogenous cholinergic agents. It is hypothesized that seven consecutive days of applying heat to an extremity will cause an increase in sweat capacity due to localized "training". Conversely, seven days of repeated exposure to pilocarpine will cause a decrease in the sensitivity of sweat gland receptors, resulting in less sweat production in the treated area.

Material and Methods

Experimental design: A 2x3 repeated measures design was utilized with dependent measures of sweat rate (SR), sweat gland density (SGD) and sweat per gland (S/G) for the right (R) and left (L) upper (UE) extremities. Sweat measures were collected on day 1 (T1), day 7 (T2), and day 14 (T3).

Subjects: Approval of the protocol was granted by the institution's committee for the protection of human subjects. Prior to participation, all subjects completed an informed consent form. Ten healthy, recreationally active males (25.5±4.3 yrs; 178.8±7.5 cm; 85.0±10.5 kg) consented to participate.

Procedures: The design indicated that the right and left upper extremities undergo 7-days of repeated exposure to pilocarpine iontophoresis (Pc) or heat (HA), respectively. On days 1 (T1), 7 (T2), and 14 (T3) assessment took place on both limbs. Pharmacological stimulation was accomplished via pilocarpine iontophoresis, using a Wescor Sweat Inducer 3700 (Logan, UT). Pilocarpine was delivered via reagent-impregnated (0.5% pilocarpine) solid agar gel discs (Pilogel, Wescor). Upon the termination of stimulation, collection of sweat took place for 15-minutes using a Macroduct Sweat Collector (Wescor). Sweat collection procedures were performed as previously described by Yaggie *et al.* [13]. The

sample was weighed to the nearest one-hundredth of a gram to obtain sweat rate (SR) ($g \cdot m^2 \cdot min^{-1}$). Sweat gland density (SGD) ($glands/cm^2$) was determined by placing iodinated paper on the sample site for approximately 10-seconds immediately following sweat collection. This produced an imprint of the stimulated area. Sweat rate per gland (S/G) was calculated as a function of SR and SGD. The location of stimulation on the upper extremities was over the belly of the flexor carpi radialis. The area of each stimulation was carefully marked as to allow repeated exposure to the identical site.

On days 2 through 6 stimulation and collection took place only on the right upper extremity. On day 7 both limbs were again tested again, as previously described. The second portion of the procedure occurred on days 7-13 after stimulation. On these days, heated pads were wrapped in two towels and placed on the subject's left forearm for 45-minutes per session. The pads were heated using a Hydrocollator Heating Unit M-2 (Chattanooga, TN). The temperature of the heat pad at the level of the skin was held at a mean temperature of 42°C, while not exceeding 45°C. This was accomplished by placing a thermometer between the skin and the heat pad and monitoring the temperature every minute. If the temperature exceeded 45°C, or it was uncomfortable for the subject, additional layers of towels were placed between the heat pad and the skin. If the temperature fell below 42°C, layers of towel were removed or a newly heated pad replaced the old. Other than redness, there were no adverse reactions to the localized heat acclimation. This activity continued for 7 consecutive days (days 8-13). Pilocarpine iontophoresis was again performed on day 14 on both upper extremities. A summary of the sweat gland training and testing schedule can be found on Table 1.

Table 1
Chart of sweat gland training schedule

	Day of training													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
	T1						T2							T3
RUE	Pc#	Pc	Pc	Pc	Pc	Pc	Pc#							Pc#
LUE	Pc#						Pc# HA	HA	HA	HA	HA	HA	HA	Pc#

Pc=pilocarpine stimulation of the limb; HA=heat acclimation of the limb (42°C);
#=indicates sweat activity measures (day of testing)

Results

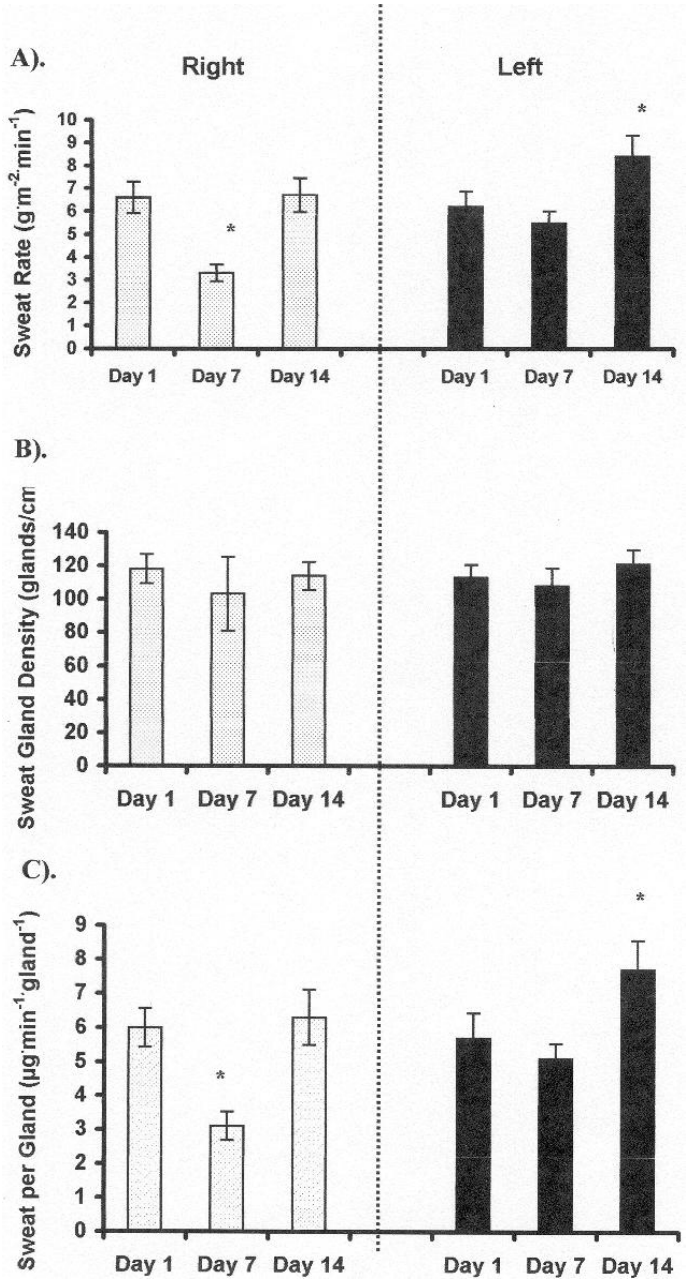

Fig. 1
Sweat gland characteristics; right and left extremity sweat gland data (mean ±SE) of subjects. (A) SR, (B) SGD, (C) SPG. Bonferroni multiple comparisons were used to scrutinize data within groups for each variable ($p<0.05$); *denotes significance

Means (±SEM) for all sweat parameters (SR, SGD, S/G) are shown in Fig. 1. Repeated measures ANOVA revealed a significant main effect of treatment ($p<0.05$) for SR across treatment levels ($F_{4,34}=17.44$, $p<0.00$). Post-hoc analysis using Bonferroni multiple comparisons indicated the SR of the right extremity (Pilocarpine treated) between day one (T1) and seven (T2) decreased significantly from 6.6±0.7 to 3.3±0.4 $g \cdot m^2 \cdot min^{-1}$ respectively. Between day seven (T2) and 14 (T3) the SR of the left extremity (heat treated) increased significantly from 5.5±0.5 to 8.4±0.9 $g \cdot m^2 \cdot min^{-1}$ respectively. A significant difference in SR was not seen in the limbs between times of non-treatment. Furthermore, there were no significant differences in SR between the left and right limb on the initial assessment on day one. There was an observable main effect on SGD ($F_{4,34}=3.217$, $p=0.024$), however, no discernable differences were depicted through pairwise comparisons.

There was no significant difference in SGD noted across time T1 (118±3, 113±3), T2 (103±7, 108±3), and T3 (114±3, 121±3 glands/cm^2) in the RUE or the LUE, respectively. A significant difference was observed in the S/G measures (Wilks' $\lambda=0.196$, $F_{4,34}=10.716$, $p<0.01$). The RUE displayed a significant decrease ($p=0.001$) following pilocarpine training (T1=6.1±0.6, T2=3.1±0.4 $\mu g \cdot min^{-1} \cdot gland^{-1}$). However, the S/G in the RUE showed a significant increase ($p=0.006$) from T2 (3.1±0.4) to T3 (6.3±0.8 $\mu g \cdot min^{-1} \cdot gland^{-1}$). The LUE displayed no significant differences ($P=0.924$) between T1 and T2 (T1=5.7±0.7, T2=5.5±0.4 $\mu g \cdot min^{-1} \cdot gland^{-1}$). However, the S/G in the LUE showed a significant increase ($p=0.008$) from T2 (5.5±0.4) to T3 (7.7±0.9 $\mu g \cdot min^{-1} \cdot gland^{-1}$).

Discussion

It has been well established that peripheral adaptations of the sweat gland can influence sweat production. [1,2,8,9,10]. Therefore, local manipulation of peripheral factors should modify the output of the sweat gland and exhibit influence on thermoregulation. The results of the present study suggest that the eccrine sweat gland can be trained to increase sweat production through localized heat acclimatization, and exhibit a reduction in sweat gland sensitivity following repetitive use of cholinergic agonists.

Heat acclimatization: The current finding that 7-days of local heat exposure increases sweat gland sensitivity support previous work [2,9]. However, these studies do not indicate whether the differences observed pre- and post-training are related to individual sweat gland activity, responsiveness, and productivity. Through use of alternate methods, the present investigation noted changes in sweat rate with no observable changes in sweat gland density across time (T1, T2 & T3)

or treatment (HA or Pc). There are also differential effects observed with heat training and S/G values. Sweat rate per gland (S/G) measures are a function of SR and SGD. Hence, the increase in sweat rate seen after localized heat acclimation is not due to an increased number of activated glands. Density of sweat glands remained consistent between the pre- and post-heat acclimation measures. Increases in sweat rate appear to be a result of enhanced sensitivity of S/G.

Increased output of the sweat gland may be attributed, in part, to the potentation of sweating. Acclimation [9] and acclimatization [5] have been shown to increase sweat capacity, as well as, enhance temporal responsiveness, which allows for a earlier onset of sweating resulting in an increased ability to cool the body per unit time.

The increased productivity of the sweat gland (SR) may also be attributed to its size. Previous work has indicated that individual fitness levels may affect the morphology of sweat glands [10]. Heat acclimatization may be observed in fit individuals as a result of activity and exercise participation. The heat associated with physical activity will increase core temperature that will elicit a thermoregulatory response and adaptation [1]. Comparisons of eccrine sweat gland biopsies of fit and unfit individuals show anatomical and physiological differences. These differences include, 1) increased gland size, 2) a higher sweat rate per gland, 3) a higher sweat rate per unit tubular length of the secretory coil, and 4) a higher sweat rate per unit volume of the secretory coil. However, the subjects' fitness level was determined through self-report. Buono and Sjoholm [1], reported that peripheral sweat productivity was significantly correlated (r=0.73) to fitness level. Trained males (VO_{2max}=65.9±7.1 ml·kg $^{-1}$·min^{-1}) displayed a two-fold increase in sweat rate when compared to untrained males (VO_{2max}=43.8±6.1 ml·kg $^{-1}$·min^{-1}). Furthermore, sweat gland density and sweat per gland also displayed significant effects of training that may result afore mentioned morphological changes. It is likely that heat trained sweat glands displayed an increase in sweat productivity due to glandular hypertrophy and temporal responsiveness.

Cholinergic exposure: There was a significant decrease in SR and calculated S/G following repeated pilocarpine iontophoresis. This desensitization was observed pre- (T1) to post- training (T2), but returned to pre-test responsiveness when cholinergic exposure was removed (T3). There was no significant difference, however, in the number of stimulated glands (SGD) observed across time (T1, T2 & T3). These data would indicate that the glands within the test site are still responsive to cholinergic agents (SGD), however there may have been a diminution of receptor sensitivity that resulted in a reduction in overall sweat productivity (SR). Sweat glands appear to display a typical muscarinic receptor

response resulting in desensitization when exposed to repeated bouts of exogenous stimuli. No evidence appears in the literature describing the prolonged exposure of cholinergic agents on the sweat gland, however, similar effects have been noted in cultured heart cells [3] and neurons [11]. As with other hormone and neurotransmitter receptors, the number of muscarinic acetylcholine receptors on cells decrease after prolonged exposure to muscarinic agonists [14]. It has been observed in other systems that repeated exposure of cholinergic agonists may result in a permanent down regulation of muscarinic receptors. However, the effects of pilocarpine exposure in the current investigation do not exhibit a permanent effect. Following 7-days of no cholinergic training sweat characteristics returned to baseline values (T3). Therefore, this temporary desensitization may be attributed to sequestration of muscarinic receptors from within the cellular membrane. With continual stimulation of muscarinic receptors from exogenous agents, those receptors will display a transient rescission from the membrane surface into the cytoplasm, via receptor-mediated endocytosis. This phenomenon is observed in multiple systems in order to decrease the sensitivity of a particular stimulus in order to prevent glandular hypersensitivity.

In conclusion, peripheral control of sweat production may be manipulated through various exposures and training, in the same subjects. Localized heat training appears to increase sweat productivity and is likely mediated by physiological and morphological peripheral changes. Moreover, these changes may be targeted to specific segments of the body to enhance thermoregulatory responses in able-bodied athletes. Furthermore, this also raises the possibility that local exogenous stimulation could be used in individuals that have deinnervated or decentralized thermoregulatory systems (eg., spinal cord injury) to improve sweat gland function. Exogenous stimulation of sweat glands using pilocarpine on consecutive days results in an overall decrease in sweat productivity possibly resulting from decreased receptor sensitivity or sequestration. Sweat gland density did not appear to change regardless of training or exposure. This phenomenon supports the hypothesis that sweat productivity is not a resultant of sweat gland density, rather, more likely determined by receptor sensitivity or glandular size. The results of this study may have implications for those using pilocarpine testing either for research or clinical applications (i.e. diagnosis of cystic fibrosis). Frequent exposure of pilocarpine to a given test site may result in a diminished response, yielding faulty results.

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