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ORIGINAL ARTICLE

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Exercise- and methylcholine-induced sweating responses in older and younger men: effect of heat acclimation and aerobic fitness

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Abstract The purpose of this investigation was to examine the effects of aging and aerobic fitness on exercise- and methylcholine-induced sweating responses during heat acclimation. Five younger [Y group – age: 23±1 (SEM) years; maximal oxygen consumption ($\dot{V}O_{2max}$): 47±3 ml·kg^{-1}·min^{-1}], four highly fit older (HO group – 63±3 years; 48±4 ml·kg^{-1}·min^{-1}) and five normally fit older men (NO group – 67±3 years; 30±1 ml·kg^{-1}·min^{-1}) who were matched for height, body mass and percentage fat, were heat acclimated by daily cycle exercise (≈35% $\dot{V}O_{2max}$ for 90 min) in a hot (43°C, 30% RH) environment for 8 days. The heat acclimation regimen increased performance time, lowered final rectal temperature (T_{re}) and percentage maximal heart rate (%HR$_{max}$), improved thermal comfort and decreased sweat sodium concentration similarly in all groups. Although total body sweating rates ($\dot{M}_{sw}$) during acclimation were significantly greater in the Y and HO groups than in the NO group ($P<0.01$) (because of the lower absolute workload in the NO group), the $\dot{M}_{sw}$ did not change in all groups with the acclimation sessions. Neither were local sweating rates ($\dot{m}_{sw}$) on chest, back, forearm and thigh changed in all groups by the acclimation. The HO group presented greater forearm $\dot{m}_{sw}$ (30–90 min) values and the Y group had greater back and thigh $\dot{m}_{sw}$ (early in exercise) values, compared to the other groups ($P<0.001$). In a methylcholine injection test on days immediately before and after the acclimation, the order of sweat output per gland (SGO) on chest, back and thigh was Y>HO>NO, and on the forearm Y=HO>NO. No group differences were observed for activated sweat gland density at any site. The SGO at the respective sites increased in the post-acclimation test regardless of group ($P<0.01$), but on the thigh the magnitude of the increase was lower in the NO ($P<0.02$) and HO ($P=0.07$) groups than in the Y group. These findings suggest that heat tolerance and the improvement with acclimation are little impaired not only in highly fit older but also normally fit older men, when the subjects exercised at the same relative exercise intensity. Furthermore, the changes induced by acclimation appear associated with an age-related decrease in $\dot{V}O_{2max}$. However methylcholine-activated SGO and the magnitude of improvement of SGO with acclimation are related not only to $\dot{V}O_{2max}$ but also to aging, suggesting that sensitivity to cholinergic stimulation decreases with aging.

Key words Aging · Thermoregulation · Cholinergic stimulation · Sweat gland output

Introduction

The main effector response for dissipating heat when exposed to environmental heat is sweating. Many have reported that sweating declines with increasing age (Wagner et al. 1972; Fennel and Moore 1973; Anderson and Kenney 1987; Kenney and Fowler 1988; Sato and Timm 1988; Tankersley et al. 1991; Armstrong and Kenney 1993; Inoue et al. 1991, 1995; Inoue 1996; Inoue and Shibasaki 1996). We have suggested that the lower sweat gland output with aging may reflect age-related changes in the sweat gland itself (atrophy of some sweat glands) and/or in its pharmacological sensitivity (Anderson and Kenney 1987; Kenney and Fowler 1988; Inoue et al. 1991; Inoue 1996; Inoue and Shibasaki 1996). Several investigators (Drinkwater et al. 1982; Pandolf et al. 1988; Smolander et al. 1990; Havenith et al. 1995) have suggested that aging per se has no effect on sweating

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during exercise in a hot environment. Instead, the decrement in sweating may be related to the declines of maximal oxygen uptake ($\dot{V}O_{2max}$) and/or habitual exercise rather than aging. Therefore, additional experiments are needed to establish whether sweating declines with increasing age independent of $\dot{V}O_{2max}$ or physical fitness indices.

It is well known that for younger individuals sweating increases with acclimation or acclimatization to exercise in the heat, and that heart rate (HR) and core temperature are lowered (Wenger 1988). Whether older persons have a reduced capacity for acclimation or acclimatization to heat has not been resolved (Robinson et al. 1965; Wagner et al. 1972; Pandolf et al. 1988; Armstrong and Kenney 1993; Inoue et al. 1995). Pandolf et al. (1988) performed a heat acclimation study using middle-aged and younger men of similar aerobic fitness and other physical characteristics and found little impairment of the thermoregulatory system, at least through the fifth decade of life for physically well-trained middle-aged men. On the other hand, Wagner et al. (1972) have observed that, when subjects were acclimated to work in the heat, improvements in sweating capacity and sweating sensitivity were more limited in older men and preadolescent boys, compared to young men and older boys. Armstrong and Kenney (1993) found that during passive heating older men respond with a lower skin blood flow at a given mean body temperature and a lower maximal sweat rate on the chest than young subjects matched for aerobic fitness and other physical characteristics. These relatively decreased effector responses in the older men were still evident after heat acclimation. It has also been found that in older men enhancement of sweating during the summer occurred later and its reduction during winter occurred earlier (Inoue et al. 1995). However, little is known about the effect of heat acclimation on the sweating responses of those over 60 years old (Armstrong and Kenney 1993; Inoue et al. 1995) or the mechanisms involved.

Our purpose was to examine: (1) thermoregulatory responses during exercise over 8 days of heat acclimation, and (2) methylcholine-activated sweat gland density and output per gland before and after heat acclimation. Age and physical fitness effects were ascertained. We studied three groups, younger, highly fit older and normally fit older groups, who matched well for height, mass, surface-area-to-mass ratio and skinfold thickness. $\dot{V}O_{2max}$ was also well matched between the younger and highly fit older men, but the normally fit older men had a lower $\dot{V}O_{2max}$.

Methods

Subjects and screening procedures

Nine older (56–73 years old) and five younger (20–26 years old) white men served as volunteer subjects after explanation of the testing procedures and procurement of written consent. All of the older subjects were natives of State College (Pa.), and the younger subjects were students at the Pennsylvania State University. The older men were divided into two groups classified as normally fit and highly fit on the basis of $\dot{V}O_{2max}$ and their exercise habits. The four highly fit older men (HO) were more active (cycling, aerobic dance and jogging etc.), compared to the normally fit older (NO) and younger (Y) men. Before participating in the study, each subject was examined by a physician and given a graded exercise test to determine $\dot{V}O_{2max}$ in the room maintained at an air temperature of 24°C. The protocol for the graded exercise test began with 5 min of walking at 3 or 3.5 mph (1.34 or 1.56 $m \cdot s^{-1}$) at a 5% grade. After a 1-min rest period, the subject resumed walking at the same work load. Thereafter, the grade was raised 2.5% every 2 min to a maximum of 20%. If the subject progressed past this level, speed was increased by 0.5 mph (0.22 $m \cdot s^{-1}$) at 2-min intervals until termination of the test. The test was concluded when the subject felt exhausted or when they could not keep up the given walking speed. The criteria for detecting $\dot{V}O_{2max}$ included levelling of oxygen uptake ($\dot{V}O_2$) and a respiratory gas-exchange ratio greater than 1.05. DuBois surface area was determined from height and mass, and percentage body fatness was estimated from skinfold thicknesses measured at ten sites using a subcutaneous adipose tissue caliper (Allen et al. 1956). The physical characteristics of Y, HO and NO groups are presented in Table 1. No subject was taking medication, and none had a history of cardiovascular or respiratory complications. All experiments were conducted between the beginning of December and the end of April. Thus the subjects were not naturally heat acclimated.

Protocol

Subjects were heat acclimated by cycling on a cycle ergometer at an intensity of 35% $\dot{V}O_{2max}$ for 90 min per day for 8 days, separated by 2 days of rest between the 4th and 5th acclimation days, in a hot, dry environment (43°C ambient temperature, 30% RH). During each session subjects wore gym shorts, socks and jogging shoes. Each subject performed the cycle exercise at the same time of day to avoid potential effects of circadian variations. Subjects were asked to refrain from other physical activity for the heat acclimation session. No food or water was ingested from 2 h before arrival at the laboratory until the end of each exposure. In order to minimize the risk, all heat acclimation sessions were individually terminated, if necessary, by predetermined endpoints, i.e. a rectal

Table 1 Subject characteristics. Values are means ±SEM. (*Y* Younger group, *HO* highly fit older group, *NO* normally fit older group, *n* number of subjects, *A_D/Mass* surface-area-to-mass ratio, %*BF* percentage body fatness, $\dot{V}O_{2max}$ maximal oxygen uptake)

Group	*n*	Age (years)	Ht (cm)	Mass (kg)	A_D (m^2)	A_D/Mass ($cm^2 \cdot kg^{-1}$)	%BF (%)	$\dot{V}O_{2max}$	
								($l \cdot min^{-1}$)	($ml \cdot kg^{-1} \cdot min^{-1}$)
Y	5	23±1*	177±2	73.9±4.3	1.91±0.05	260±8	17±2	3.47±0.25	47.1±2.5
HO	4	63±3	176±3	71.1±2.0	1.87±0.05	263±3	17±2	3.34±0.30	47.5±4.1
NO	5	67±3	172±2	73.0±3.0	1.85±0.04	254±5	20±2	2.17±0.15*	30.1±1.4*

* $P<0.01$ from the other groups

temperature (T_{re}) >39.0°C, HR >90%HR_{max}, excessive physical fatigue, dizziness, nausea, an absence of sweating, or subject request.

On the day before and the day after the heat acclimation regimen, each subject also participated in the methylcholine-activated sweating test as described subsequently in detail.

Measurements during the heat acclimation sessions

During each heat acclimation session, T_{re}, skin temperatures, HR, total body sweating rate and thermal comfort were measured. Local sweating rate ($\dot{m}_{sw}$) was assessed on days 1, 2, 4, 6 and 8, $\dot{V}O_2$ on days 1, 3, 5, 7 and 8 and sweat sodium concentration ($[Na^+]$) on days 1 and 8.

T_{re} was measured with a calibrated Yellow Springs Instruments thermistor inserted 10 cm past the anal sphincter. Skin temperature was measured at four sites (chest, back, forearm and thigh) with uncovered thermocouples. Unweighted mean skin temperature ($\bar{T}_{sk}$) was calculated as: $\bar{T}_{sk}=(T_{chest}+T_{back}+T_{forearm}+T_{thigh})/4$. HR was monitored from a single-lead (CM_5 position) electrocardiogram. T_{re}, $\bar{T}_{sk}$ and HR were recorded and stored on a computer and displayed every 5 min during the experiment.

$\dot{V}O_2$ was measured twice, 35–37 and 65–67 min after starting the exercise, by an open-circuit technique. Expired gas was collected in Douglas bags and O_2 and CO_2 concentrations were determined using a Beckman E-2 paragnetic O_2 analyser and a Lira model 864 infrated CO_2 analyser. The volume of expired gas was measured with a dry gas meter and corrected to standard temperature and pressure (dry) (STPD) conditions. Mean $\dot{V}O_2$ was calculated from the two measurements. Thermal comfort (rating scale – 7: very comfortable; 9: comfortable; 11: slightly uncomfortable; 13: somewhat uncomfortable; 15: uncomfortable; 17: very uncomfortable; 19: very, very uncomfortable) was evaluated every 15 min.

Total body sweating rate was estimated from the change in nude body mass using a scale accurate to ±10 g. Local capsule (12.6 cm^2) sweat samples were collected successively on the chest (midsternum at the level of the third rib), back (below the tip of the scapula), forearm (5 cm below the antecubital fossa) and thigh (midanterior) at 15-min intervals. The capsules were placed on the same areas identified by marking points. The amount of sweat was obtained from weighed filter paper (Inoue et al. 1991). An electronic balance accurate to ±0.1 mg was used for weighing and results are expressed in $mg \cdot cm^{-2} \cdot min^{-1}$. $[Na^+]$ was determined on elution of Na^+ from the paper. The $[Na^+]$ in the eluted solution was determined by flame photometry.

Methylcholine-induced sweating test

On the days immediately before and after the heat acclimation sessions, the methylcholine injection test was performed on the respective subjects, after they rested quietly for 20 min at an ambient temperature of 24°C. Small areas on the chest, back, forearm and thigh of each subject were shaved and cleaned. A thin layer of petroleum jelly was applied to promote beading of sweat droplets. The jelly was mixed by kneading with bromphenol blue to dye the sweat droplets blue. The blue sweat droplets were used to determine sweat gland density and gland output by a photographic method (Bell 1981; Kenney and Fowler 1988). A 0.05-ml aliquot (5×10^{-3} M) of acetyl-β-methylcholine chloride in saline (0.9%) was injected just under the skin surface of the chest, back, forearm and thigh. A 26-gauge needle fitted to a 1-ml syringe was used after its preparation and sterilization. The injected solution created a weal about 1 cm in diameter. Photographs were taken of each area surrounding the injection site at 1, 2, 3, 4 and 5 min post-injection to count the number of methylcholine-activated sweat glands and to estimate sweat gland output.

From the 2-min photograph, a slide was made and projected onto a measured grid. The diameter of each bead was measured using a Craftsman calipered micrometer (Kenney and Fowler 1988). By treating each bead as a hemisphere, an output could be estimated. Although this methodology may not provide an accurate absolute measurement, it correlates well with evaporimeter measurements (Kennedy et al. 1984), and it allowed us to make intergroup comparisons of sweat gland output per gland (SGO).

Statistical analysis

All data are expressed as means±SEM. All of the measurements taken during a heat acclimation session, and sweat gland density and SGO during the methylcholine injection test were analysed using a one-way, two-way or three-way analysis of variance and Tukey's post-hoc comparisons. The significance level was set at $P<0.05$.

Results

No significant differences were observed among the Y, HO and NO groups for height, mass, surface area, surface-area-to-mass ratio, and percentage body fat (Table 1). During the heat acclimation sessions there were no group differences in the relative exercise intensity (approximately 35% $\dot{V}O_{2max}$), but $\dot{V}O_2$ during the 90-min exercise test was significantly lower in the NO group than in the Y and HO groups ($P<0.006$; day 1: 17.7±1.3 (SEM), 17.4±1.2 and 11.6±0.5 $ml \cdot kg^{-1} \cdot min^{-1}$ in Y, HO, and NO groups, respectively; day 8: 16.9±1.1, 16.4±1.1 and 11.0±0.5 $ml \cdot kg^{-1} \cdot min^{-1}$), since the absolute work-

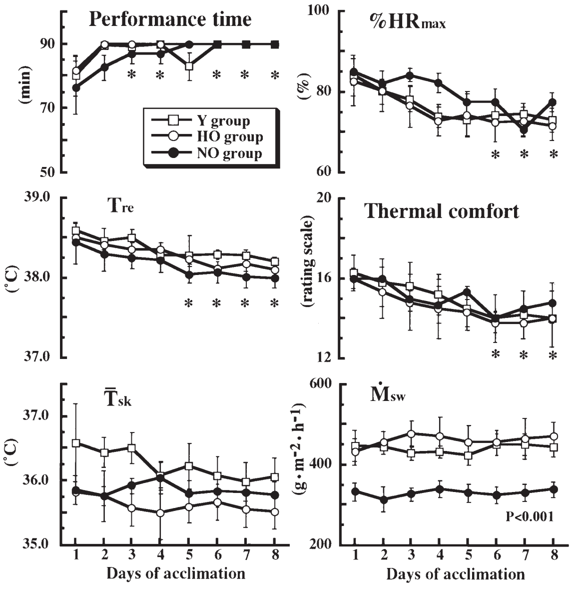

Fig. 1 Performance time, final rectal temperature (T_{re}), final mean skin temperature ($\bar{T}_{sk}$), final percentage heart rate to maximal heart rate ($\%HR_{max}$), final thermal comfort and total body sweating rate ($\dot{M}_{sw}$) for younger (*Y*), highly fit older (*HO*) and normally fit older (*NO*) groups over 8 days of exercise-heat acclimation. Maximal heart rate was obtained during the maximal oxygen consumption ($\dot{V}O_{2max}$) test. A 2-day rest period was instituted between days 4 and 5. Values are means±SEM. The *P* value presented for $\dot{M}_{sw}$ is for the overall group effect (NO versus Y and HO groups). *Significant differences from the value at day 1 in each group

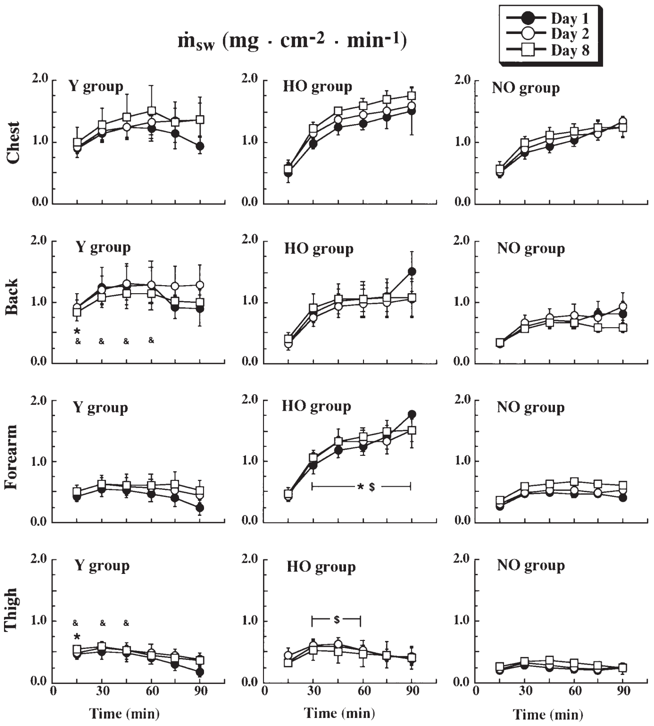

Fig. 2 Time course of local sweating rate ($\dot{m}_{sw}$) on chest, back, forearm and thigh for younger (*Y*), highly fit older (*HO*) and normally fit older (*NO*) groups during days 1, 2 and 8 of exercise-heat acclimation. Values are means±SEM. $^{*, \&, \$}$ $P<0.05$ between Y and HO, Y and NO, and HO and NO groups, respectively

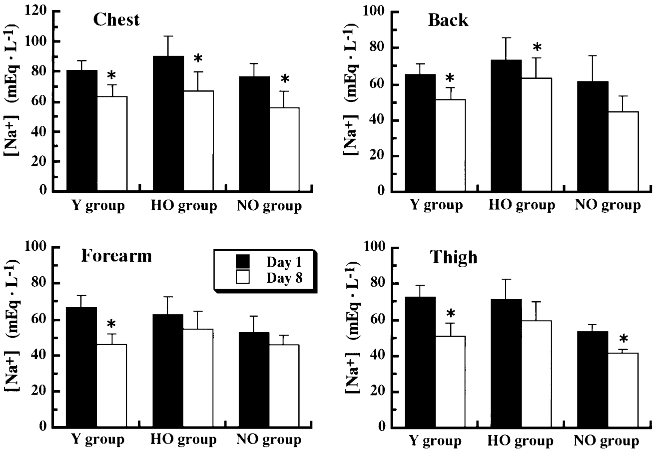

Fig. 3 Mean sweat sodium concentration on chest, back, forearm and thigh for younger (*Y*), highly fit older (*HO*) and normally fit older (*NO*) groups for day 1 and day 8 of exercise-heat acclimation. Values are means±SEM. $^{*}P<0.05$ between day 1 and day 8

loads were significantly lower in the NO group than the other groups ($P<0.001$; 6.5±0.4, 6.5±0.6 and 3.4±0.2 kgm·min^{-1}·kg^{-1}). No day effect was observed for the $\dot{V}O_2$.

Figure 1 shows performance time, final T_{re}, final $\bar{T}_{sk}$, final %HR$_{max}$, final thermal comfort and total body sweating rate during the 8 days of heat acclimation. With the progression of heat acclimation, performance time improved similarly in all groups ($P<0.05$), although the 90-min exercise was performed completely on the second day of the heat acclimation in the HO group and on the 6th day in the Y and NO groups. Final T_{re} did not differ among the groups during heat acclimation, but T_{re} decreased with heat acclimation in all groups and the drop was significant on days 5–8 compared to day 1 ($P<0.05$). There were no group and day effects in final $\bar{T}_{sk}$, although the $\bar{T}_{sk}$ in the Y group tended to decrease from day 1 to day 4. Final %HR$_{max}$ and final thermal comfort decreased over the days of heat acclimation in all groups. The decrements in both measurements were significant on days 6, 7 and 8 compared to day 1 ($P<0.05$). No group differences were observed for either %HR$_{max}$ or thermal comfort. Total body sweating rate was significantly greater ($P<0.01$) in the Y (447±38 and 445±25 g·m^{-2}·h^{-1} in day 1 and day 8, respectively) and HO (434±33 and 472±34 g·m^{-2}·h^{-1}) groups than in the NO group (333±22 and 339±9 g·m^{-2}·h^{-1}). No day-related differences were observed for the total sweating rate in any group.

Figure 2 shows time courses for $\dot{m}_{sw}$ on the chest, back, forearm and thigh on days 1, 2 and 8 in the Y, HO and NO groups. No day differences were observed for the $\dot{m}_{sw}$ regardless of group or site, suggesting that regional distribution of $\dot{m}_{sw}$ did not change with 8 days of heat acclimation in any group. Back and thigh $\dot{m}_{sw}$ was significantly greater ($P<0.05$) in the Y group than in HO (from start to 15 min of exercise) and NO (from start to 45–60 min) groups. Thigh $\dot{m}_{sw}$ in the HO group was significantly greater than in the NO group from 30 to 60 min of exercise ($P<0.05$). The HO group had greater forearm $\dot{m}_{sw}$ than the Y and NO groups from 30 min to the end of exercise ($P<0.05$).

Figure 3 shows mean sweat [Na^{+}] on the chest, back, forearm and thigh in the Y, HO and NO groups on days 1 and 8 of heat acclimation. The [Na^{+}] from the respective sites decreased from day 1 to day 8 in all groups, but was not significant on the forearm and thigh in the HO group or on the back and forearm in the NO group. By site there were no intergroup differences for [Na^{+}] on either day.

Table 2 shows sweat gland density associated with methylcholine stimulation before and after acclimation.

Table 2 Sweat gland density (glands · cm^{-2}) during methylcholine injection test in pre- and post-acclimation. Values are means±SEM. (*Y* Younger group, *HO* highly fit older group, *NO* normally fit older group, *n* number of subjects)

Group	*n*	Chest		Back		Forearm		Thigh	
		Pre	Post	Pre	Post	Pre	Post	Pre	Post
Y	5	60±5	60±7	70±8	61±3	116±10	106±8	80±6	77±6
HO	4	59±3	60±3	62±6	65±4	114±8	116±9	71±2	76±4
NO	4	60±4	59±4	61±9	58±9	121±11	112±10	86±7	87±11

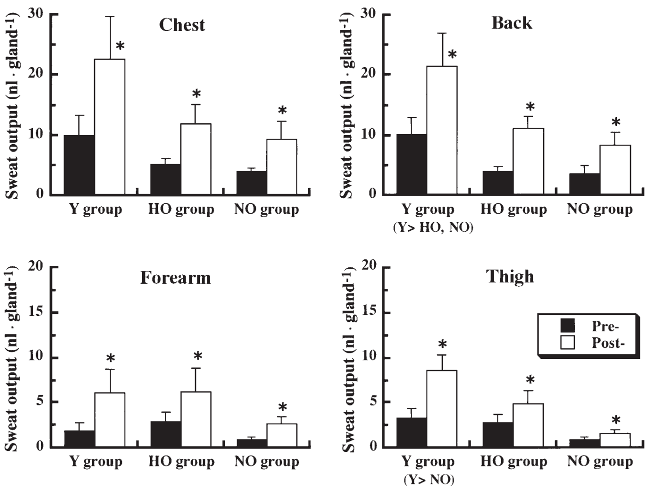

Fig. 4 Sweat gland output after 2 min of methylcholine injection for younger (*Y*), highly fit older (*HO*) and normally fit older (*NO*) groups during pre- and post-acclimation. Values are means±SEM. *$P<0.05$ between pre- and post-acclimation

There were no time effects for the density after 2 min of methylcholine injection in any groups. The number of active sweat glands at any sites did not differ significantly by heat acclimation or group. The sweat gland density was significantly greater on the forearm than the other sites in any group ($P<0.05$) and ordered forearm >thigh>chest=back. Methylcholine-activated SGO increased significantly after heat acclimation regardless of group or site ($P<0.01$; Fig. 4), but on the thigh the magnitude of the increase in the SGO with heat acclimation was lower in the NO ($P<0.02$) and HO ($P=0.07$) groups than in the Y group. The SGO on chest, back and thigh was ordered Y>HO>NO, and on the forearm it was Y=HO>NO (but the HO group had a remarkably higher forearm $\dot{m}_{sw}$ during heat acclimation session), but it was not significant on the chest and forearm.

Discussion

Effect of aging and aerobic fitness

Three groups were selected, i.e. younger (Y), highly fit older (HO) and normally fit older (NO) groups, in order to examine the effects of aging or fitness on thermoregulatory responses, especially exercise- and methylcholine-induced sweating responses, by comparing groups Y and HO (with same $\dot{V}O_{2max}$ and different ages) or HO and NO (with different $\dot{V}O_{2max}$ and same ages) groups. Some authors have suggested that physical characteristics such as body fatness and surface-area-to-mass ratio are closely related to an individual's ability to thermoregulate during heat and/or exercise exposures (Bar-Or et al. 1969; Havenith et al. 1995). By matching three groups for height, mass, surface area, surface-area-to-mass ratio, percentage body fatness, we attempted to nullify any impact of these variables.

The performance time, T_{re}, $\bar{T}_{sk}$, thermal comfort and $\%HR_{max}$ did not differ among the three groups during 90 min of exercise at a low relative intensity (≈35% $\dot{V}O_{2max}$) under hot dry conditions throughout a heat acclimation regimen. These results suggest the possibility that not only highly fit older men but also normally fit older men in their sixth decade have a similar heat tolerance as younger men when performing a low relative workload in a hot dry environment. If the NO group had been exercising at the same absolute workload as the Y and HO groups, the NO groups would show more heat strain, as reported by Havenith et al. (1995).

During the heat acclimation sessions, total body sweating rate was greater in the Y and HO groups than in the NO group, but was not different between Y and HO groups, who had higher $\dot{V}O_{2max}$ values. Based on similar T_{re} responses among the three groups, the greater total sweating rate in the Y and HO groups indicates superior sweating function.

During the heat acclimation sessions, the HO group had greater $\dot{m}_{sw}$ on the forearm than did the Y group, in spite of a similar total body sweating rate between the groups. This suggests that participation in regular physical activity may enhance some local sweat responses. The greater forearm $\dot{m}_{sw}$ may have been because of a greater SGO because no group effects were observed in sweat gland density following cholinergic stimulation. Nevertheless we could not measure the total number of exercise-induced active sweat glands directly. Sato and Sato (1983) have reported that persons with greater SGO, from functionally active sweat glands, have larger glands which are highly sensitive pharmacologically. However, the HO group did not show a greater SGO following cholinergic stimulation even on the forearm compared to the Y group, but they did have a greater SGO than the NO group. The discrepancy between the forearm $\dot{m}_{sw}$ results following methylcholine injection and following exercise in the heat in the present study may suggest that sensitivity to cholinergic stimulation decreases with aging (despite the possibility of the HO group having larger glands). Furthermore the discrepancy may also imply that an age-related decrement in sensitivity to cholinergic stimulation precedes any possible reduction in sweat gland size.

Back and thigh $\dot{m}_{sw}$ were significantly lower in the HO and NO groups than in Y during the early stages of exercise during the heat acclimation sessions, despite similar T_{re} and $\bar{T}_{sk}$. The relatively sluggish responses were consistent with our previous studies (Inoue et al. 1991; Inoue and Shibasaki 1996) in which we reported lower $\dot{m}_{sw}$ during the earlier stages of heat exposure in older men. Several factors may contribute to sluggish $\dot{m}_{sw}$ such as: lower sensitivity to acetylcholine released from sudomotor efferents (Kenney and Fowler 1988; Sato and Timm 1988) and decreased thermal sensitivity (Natsume et al. 1992). In the present study the lower sensitivity to methylcholine was confirmed on the back and thigh in the HO and NO groups, compared to group Y. This lesser responsiveness on the back and thigh supports our previous suggestion that an age-related decrement in sweating function may well spread from the lower limbs to the back of the trunk (Inoue 1996).

The sweat [Na$^+$] was similar among Y, HO and NO groups regardless of site and acclimation. It is well known that there is a positive linear relationship between $\dot{m}_{sw}$ and [Na$^+$] and the slope of the relationship decreases with physical training (suggesting improved sodium reabsorption in the ducts) (Sato and Dobson 1970; Inove et al. 1998). Based on these findings, our results suggest that the NO group, which had a lower $\dot{m}_{sw}$, also reabsorbed less sodium from sweat compared to the Y and HO groups, and perhaps that older men can maintain sodium reabsorption in their sweat gland ducts because of their regular exercise and relatively high $\dot{V}O_{2max}$.

Effect of heat acclimation

The performance time, T_{re}, %HR$_{max}$ and thermal comfort were improved similarly in all groups with heat acclimation. These results suggest that both highly fit older men and normally fit older men in their sixth decade have a similar capacity for heat acclimation as do younger men, when performing low relative workloads in a hot dry environment. These results agree with the findings of Robinson et al. (1965) who demonstrated that four physically fit men aged 44–66 years were heat acclimated nearly as well as they had been at 21 years of age.

Pandolf et al. (1988) compared thermoregulatory responses between active middle-aged (mean age 46.4 years) and younger men (mean age 21.2 years), who were matched for $\dot{V}O_{2max}$ and physical characteristics, during heat acclimation sessions of two 50-min (separated by 10 min of rest) periods of treadmill walking (1.56 m·min^{-1}, 5% grade) per day for 10 consecutive days in a hot dry environment (49°C, 20% RH). They observed superior performance time, T_{re} and HR responses in the active middle-aged men during early (days 1–3) heat acclimation, compared to the younger men. We did not observe this in our highly fit older men. $\dot{V}O_{2max}$ was similar between our highly fit older men and the middle-aged men studied by Pandolf et al. (1988) (47.5 versus 51.3 ml·kg^{-1}·min^{-1}). The discrepancy in results during the early period of acclimation may be associated with differences in age (6th versus 4–5th decade) of subjects, although more detailed examinations involving larger numbers of subjects are needed in order to discern appropriate mechanisms.

In this study $\dot{m}_{sw}$ responses at the respective sites to the stress of exercising in the heat were little affected by heat acclimation (at an exercise intensity of 35% $\dot{V}O_{2max}$, in an environment of 43°C and 30% RH for 1.5 h per day). The regional distribution of $\dot{m}_{sw}$ did not change with 8 days of heat acclimation. This is inconsistent with previous results which showed that sweat rate increases during the early stages of heat acclimation and the improvement was more marked on the limbs than on the trunk (Hofler 1968; Shvartz et al. 1979). This discrepancy may be because of differences in ambient conditions, mid-acclimation schedule (2 days off) and duration (8 days) rather than differences in exercise habits, aerobic fitness and age among the subjects, since we used subjects with a greater range of them. However, we have observed the decrements of T_{re} during heat-exercise stress with the progression of heat acclimation in all groups, despite no change in $\dot{m}_{sw}$. The decreased T_{re} with heat acclimation was the result of promotion of heat dissipation, since $\dot{V}O_2$ (as an index of metabolic heat production) during heat-exercise stress did not change with heat acclimation. Roberts et al. (1977) found that forearm blood flow increased after short-term heat acclimation. It seems that a greater cutaneous vasodilation may contribute to greater heat transfer from the core to the skin and it may result in the decrements of T_{re} observed in this study. Based on the findings that the $\dot{m}_{sw}$ – core temperature relationship was also improved by heat acclimation (Roberts et al. 1977), no change in $\dot{m}_{sw}$ and the decrements of T_{re} observed in this study suggest that sweating function improves in response to heat-exercise stress with heat acclimation in all groups.

The augmentation of sweating was supported by the increment of methylcholine-activated SGO observed in all groups with acclimation. The improvement on the thigh was lower in the HO and NO than in the Y group. Sato et al. (1990) have examined the effects of long-term heat acclimation on functional and morphological changes in sweat glands using eccrine sweat glands of Pates monkeys and found that heat acclimation increases sweat gland size and sensitivity to cholinergic stimulation in vivo and in vitro. The improvement of sweat function observed here with heat acclimation may also be caused by improved sensitivity to cholinergic stimulation associated with short-term acclimation. Thus, the lower improvement on the thigh observed in the HO and NO groups suggests that the improvement of the sensitivity to cholinergic stimulation with heat acclimation may be associated with differences in age. More detailed examinations involving larger numbers of subjects are needed to discern appropriate mechanisms.

Sweat [Na$^+$] was decreased by heat acclimation in the present study (but the decrease was not significant on the forearm and thigh in the HO group and on the back and forearm in the NO group) despite the similar $\dot{m}_{sw}$ before and after acclimation, supporting previous findings that reabsorption of sweat sodium in the ducts is improved by heat acclimation or acclimatization (Kirby and Convertino 1986; Inoue et al. 1995). Kirby and Convertino (1986) have found a reduced plasma aldosterone level after short-term heat acclimation. Based on their finding, the possible improvement in sodium reabsorption may be because of augmented sweat gland responsiveness to aldosterone through heat acclimation. Such improvement may decrease with increasing age and loss of aerobic capacity.

In summary, heat tolerance and its improvement by acclimation were little impaired not only in highly fit older but also in normally fit older men in their sixth decade, in spite of their lower $\dot{m}_{sw}$ when performing same relative exercise intensity. Furthermore, the changes brought about by acclimation appear to be associated with an age-related decrease in $\dot{V}O_{2max}$. However methylcholine-activated SGO and the magnitude of improvement of SGO with acclimation were affected not only by $\dot{V}O_{2max}$ but also by aging, suggesting that sensitivity to cholinergic stimulation decreases with aging.

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