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EFFECTS OF VOLUNTARY AND FORCED EXERCISE ON THERMOREGULATION AND SURVIVAL IN AGED C57BL/6J MICE

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SUMMARY

To evaluate the effect of exercise on thermoregulation in senescent animals, three groups of male C57BL/6J mice aged 28–30 months were tested for cold tolerance, defined as the rate of decline in colonic temperature during 3 h exposure to 10°C ambient temperature. Following this test, the mice were exposed to one of the following exercise conditions: (1) forced exercise on a treadmill for 60 min daily at a rate of 5 m/min; (2) continuous access to voluntary exercise in wheel-activity cages, which resulted in a mean rate of 1.1 m/min; or (3) no expressed exercise with 60 min daily placement on the non-activated treadmill. After 3 weeks, assessment of cold tolerance was repeated. A combined mortality rate of 36% was observed in the exercise groups for this period, while there were no deaths in the non-exercised group. The high mortality rate among exercised animals indicated that these regimens were hazardous for aged mice. Moreover, between tests the non-exercised group exhibited a 0.6°C increase in body temperature and 38% improvement in cold tolerance which could be interpreted as a normal adaptation for repeated cold exposure. In contrast, no significant change in either of these variables was observed among survivors in the exercise groups. Thus, introduction of these exercise regimens in senescent mice decreased survival and did not improve the age-related impairment in thermoregulation.

Key words: Exercise; Mice; Survival; Lifespan; Thermoregulation; Body temperature; Cold tolerance

INTRODUCTION

Although the positive role of exercise for general fitness and health in the later years of life is a popular perception, experimental support for the relationship is not well

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established [17]. In support of a beneficial effect for exercise, experimental results in laboratory rodents suggest that various regimens of exercise initiated early in life can increase survival [5,6,13,21] as well as improve certain aspects of physiological function in aged animals [7,9,23,24,26,29–31].

The results of studies in which exercise is implemented later in life in laboratory rodents are inconsistent. There are reports of improved survival [11], no effects on survival [14,20,27], and decreased survival [6]. Survival effects may depend upon what age the exercise regimen is introduced. Edington *et al.* [6] reported a "threshold effect," whereby, daily forced exercise on a treadmill increased survival in rats when implemented prior to 300 days of age, but reduced survival when implemented beyond this age. Regarding physiological effects, there are reports of improved function [7,29] but also reports of paradoxical, potentially adverse effects when forced exercise is implemented in aged animals [19]. Studies of forced exercise begun at different ages in mice [23,24, 30,31] reported increases in muscle mass and a number of enzymatic variables including muscle aldolase, creatinine kinase, and superoxide dismutase of young (6 month) mice resulted from exercise; whereas, decreases in many of these parameters were observed among aged (27 month) exercised mice. When voluntary exercise was initiated in male C57BL/6J mice at 20–24 months, significantly improved memory performance in a step-through passive avoidance task was observed which was not noted when this treatment was begun at either 10–14 or 28–30 months [27]. Thus, findings regarding the benefits of exercise for aged animals as well as for aged humans are equivocal [17].

The capacity for thermoregulation represents a physiological function that exhibits a marked age-related decline in laboratory rodents as well as in humans. Body temperature is usually lower among aged subjects than among young and adult counterparts. This observation has been reported for man [10,25,28,36], rat [1], and mouse [15,32]. A relationship between lower body temperature and mortality among aged C57BL/6J mice has been demonstrated [22]. Results of cold stress tests (short-term exposure to low ambient temperature) have also revealed an age-related decline in cold tolerance in this mouse strain [8,16,33,35].

Previous research in our laboratory demonstrated that a 3-week exposure to electrical stimulation of hypothalamic areas supporting self-stimulation (rewarding areas) retarded the age-related deterioration of cold tolerance among aged male C57BL/6J mice [34]. However, what remained unclear was the extent to which increased physical activity might have contributed to this effect. Specifically, the effect on cold tolerance might have resulted from increased physical activity accompanying brain stimulation during the procedure or in the home cage as a consequence of this procedure. Thus, the present study was designed to assess the effects of exercise on the thermoregulatory abilities of aged mice. In particular, we tested the hypothesis that introduction of a daily routine of physical activity would affect cold tolerance in aged mice as did hypothalamic stimulation.

MATERIALS AND METHODS

Subjects

A total of 54 male C57BL/6J mice between 28 and 30 months of age were obtained

from the colony maintained at the Gerontology Research Center. The conditions in this colony and the procedures for health monitoring have been described elsewhere [35]. The mean lifespan of this mouse strain has been estimated to be 26–27 months in our facility [12].

The mice had been housed in plastic cages (4–5/cage) with woodshavings for bedding. For the present experiment, they were housed individually in metal cages with wire mesh floors (Wahmann) that were located in a vivarium with an ambient temperature of 22° ± 1°C and a 12-h light/12-h dark photocycle. The animals were provided *ad libitum* access to food (NIH-07 formula, 24% protein, 4.2 kcal/g) from stainless steel hoppers located in the cages and to water from an automated, filtered system.

Procedure

After 2 weeks of adaptation to the vivarium, all mice were tested for cold tolerance with a procedure described in detail elsewhere [33]. Briefly, this procedure involved weighing the animal and then confining it in a plastic restrainer which prevented gross motor activity but not shivering. Baseline colonic temperature (T_{co}) was recorded at room temperature using a thermoprobe (Yellow Springs Series 400) inserted 2–3 cm rectally and a telethermometer (Yellow Springs Model 49TA). Then the restrained mouse was placed into a temperature-controlled room with ambient temperature at 10° ± 0.5°C. T_{co} was recorded every 30 min. This exposure continued for 180 min or until T_{co} dropped below 24°C, at which time the animal was removed from the cold room. All animals were warmed for 1 h under a heat lamp before being returned to the vivarium.

The linear rate of temperature decline was calculated for each individual. These slopes were estimates of cold tolerance. On the next day, the mice were assigned quasi-randomly to one of three treatment groups with the stipulation that the mean estimates of cold tolerance among groups were not statistically significantly different ($P > 0.05$). The treatment groups were as follows: (1) Voluntary Exercise Group (VE; $n = 20$); (2) Forced Exercise Group (FE; $n = 19$); and (3) Non-Exercised Group (NE; $n = 15$). The VE group was housed individually in wheel-activity cages (Wahmann) described previously [18] which permitted voluntary exercise. These cages were located in the same vivarium as before. The number of wheel revolutions were recorded daily from mechanical counters located on the cages. The FE group was maintained in the original housing; however, the mice were placed daily for 60 min on a treadmill (Collins) with speed set at 5 m/min. Pilot studies indicated that this speed permitted aged mice to run adequately without shock reinforcement, i.e. continuous running during sessions. The NE group also continued to be maintained in original housing and were placed daily for 60 min on the treadmill that was not activated. Following 3 weeks of treatment, all surviving mice were weighed, and a second test of cold tolerance was administered. All tests of cold tolerance were conducted during diurnal hours, 1000–1300 h.

Statistical analysis

For assessing the effects of exercise, data on body weight, baseline T_{co}, and cold

tolerance were submitted to separate 3 (exercise groups) by 2 (tests) analyses of variance (ANOVA) with repeated measures on the last factor [2]. Only data of survivors were analyzed. Comparisons of body weight, baseline T_{co}, and cold tolerance between survivors and non-survivors in the FE and VE groups were made with two-tailed t-tests for each variable within each group. Fisher exact probability tests were used to assess group differences in mortality. For the VE group, Pearson product-moment correlation coefficients were used to assess the linear relationships of wheel activity to cold tolerance and baseline T_{co}.

RESULTS

Mortality

From the sample of 54 mice initially tested for cold tolerance, 40 survived to take the second test. Dead animals were found during daily inspections. There were no deaths in the NE group, six in the FE group, and eight in the VE group. Comparing the mortality rates of the three groups (Table I), there was a significant difference between NE and FE groups ($P = 0.05$), as well as between NE and VE groups ($P = 0.05$), but there was no significant difference in mortality between the two exercise groups ($P = 0.20$).

Body weight

The mean body weight of all groups showed a decline between tests (Table I). The results of the ANOVA revealed a significant main effect of test, $F(1, 37) = 5.13, P = 0.03$. However, further analysis of the simple main effect of test [37] revealed that the decline in body weight was statistically significant only for the VE group, $F(1,37) = 5.08, P = 0.02$.

Baseline T_{co}

The mean baseline T_{co} of all groups during the second test was higher than during the first test (Table I). The results of the ANOVA yielded a significant main effect of test, $F(1,37) = 11.65, P = 0.002$. However, further analysis of the simple main effect of test revealed that the only significant increase in baseline T_{co} was among mice in the NE group, $F(1,37) = 13.84, P = 0.0007$.

Cold tolerance

The mean rate (°C/min) of temperature decline of all groups decreased between the first and second tests which indicated improved cold tolerance (Table I and Fig. 1). The results of the ANOVA revealed a significant main effect of test, $F(1,37) = 10.01, P = 0.003$. However, further analysis of the simple main effect of test indicated that the only significant improvement in cold tolerance was observed in the NE group, $F(1,37) = 10.30, P < 0.001$.

Comparison of survivors and non-survivors

Figure 2 provides comparisons of mean estimates of body weight, T_{co}, and cold

TABLE I

EFFECTS OF DIFFERENT EXERCISE REGIMENS ON MORTALITY, BODY WEIGHT, COLONIC TEMPERATURE (T_{co}), AND COLD TOLERANCE AMONG AGED MALE C57BL/6J MICE (MEAN ± STANDARD DEVIATION)

Variables	*Groups*					
	Non-exercised (n = 15)		*Forced exercise (n = 19)*		*Voluntary exercise (n = 20)*	
	Test 1	*Test 2*	*Test 1*	*Test 2*	*Test 1*	*Test 2*
Mortality rate (percent)		0		32		40
Body weight (grams)	32.1 ± 2.5	31.4 ± 4.0	34.3 ± 3.0	33.4 ± 3.1	34.4 ± 3.6	32.0 ± 4.4*
Baseline T_{co} (°C)	37.0 ± 0.5	37.6 ± 0.8*	37.2 ± 0.4	37.5 ± 0.6	37.1 ± 0.4	37.3 ± 0.7
Cold tolerance (°C/min)	0.0932 ± 0.0325	0.0575 ± 0.0372*	0.0980 ± 0.0444	0.0747 ± 0.0681	0.0915 ± 0.0295	0.0854 ± 0.0477

*$P < 0.05$, between tests according to results of F-test for simple main effects.

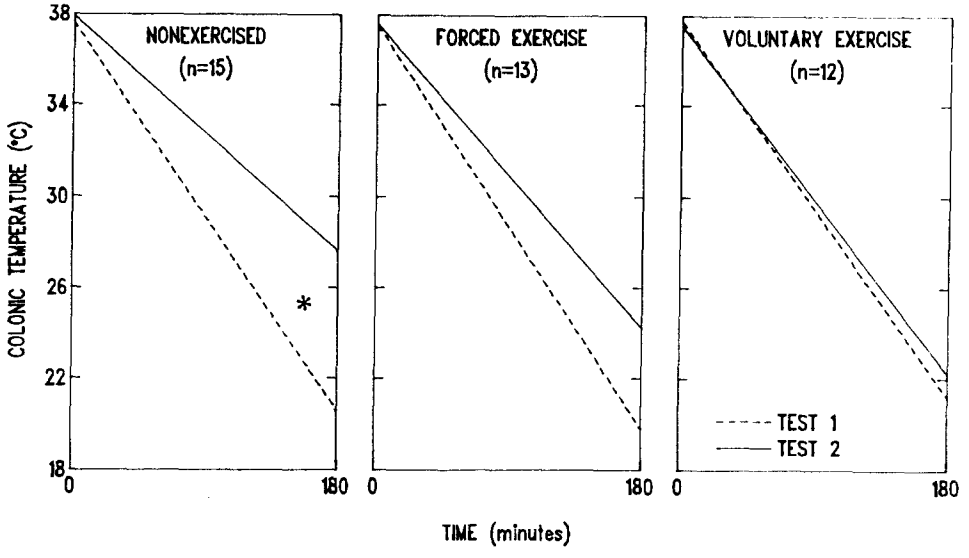

Fig. 1. Cold tolerance (estimated linear decline of T_{co} over 3 h at 10°C) in 28–30-month-old male C57BL/6J mice according to exercise treatment. *$P < 0.05$ according to results of F-test for simple main effects.

tolerance obtained during the first test for those mice that died between tests and those that survived to take the second test. In the FE group, there were no significant differences between survivors and non-survivors in initial T_{co}, t (17) = 1.18, $P > 0.05$, or in cold tolerance, t (17) < 1.0. In the VE group, T_{co} recorded during the first test was significantly higher among survivors compared to non-survivors, t (18) = 2.21, $P < 0.05$. The mean slope of temperature decline of survivors in this group was about 25% lower than that of non-survivors, but this difference was not significant, t (18) = 1.74, $P = 0.10$. In both the FE and VE groups, the mean body weight of survivors was higher than that of non-survivors. This difference was significant in the FE group, t (17) = 2.14 $P < 0.05$, but not in the VE group, t (18) = 1.55, $P > 0.05$.

Correlation between wheel activity and cold tolerance

The mean (± S.E.M.) daily wheel revolutions among mice in the VE group was 2918 (±777). By multiplying mean revolutions times the circumference of the activity-wheels, this estimate converted to an average rate of 1.1 m/min. The correlation between mean daily wheel revolutions and cold tolerance as measured during the second test indicated that higher levels of activity corresponded to higher cold tolerance (lower rate of decline), but the coefficient was not significant, r (10) = 0.44, $P = 0.15$. Similarly, wheel activity was also correlated with higher baseline T_{co} observed during the second test, but again the coefficient was not significant r (10) = 0.46, $P = 0.10$.

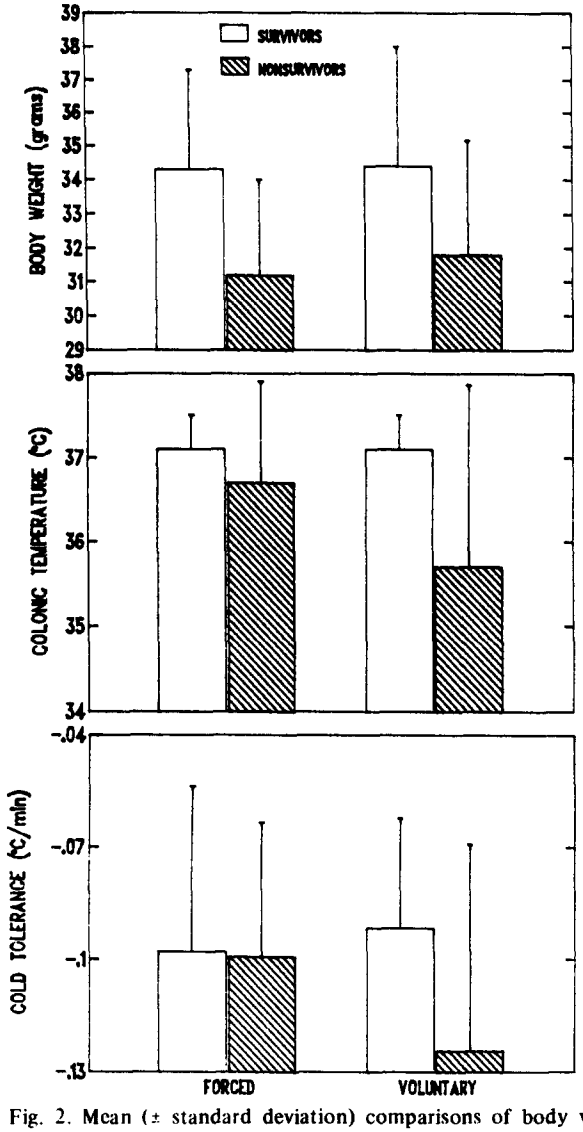

Fig. 2. Mean (± standard deviation) comparisons of body weight, T_{co}, and cold tolerance between surviving and non-surviving 28–30-month-old male C57BL/6J mice in forced and voluntary exercise groups.

DISCUSSION

Neither baseline T_{co} nor cold tolerance among aged mice were improved significantly following 3 weeks of daily exercise, whether of a forced or voluntary type. In fact, only mice in the control group that did not receive any specific exercise treatment exhibited significantly increased T_{co} and improved cold tolerance.

Because no significant decrease in thermoregulation was observed, one might conclude that the exercise regimens did not prove to be maladaptive as reported in previous physiological assessments of exercise introduced to aged laboratory rodents [19,23,24,31]. However, the mortality data showed that the treatments were detrimental to survival. During the 3-week interval, a 36% mortality rate (14/39) was observed among exercise groups, while no deaths occurred among NE animals. The underlying causes of mortality in the exercise groups were not investigated. These findings support a previous report of detrimental effects of exercise on survival in adult animals [6]. Moreover, although the exercise treatments did not appear to be detrimental to thermoregulation, this conclusion would be biased by the substantial mortality that occurred in the experimental groups. Thermoregulation may have been impaired in the animals that died.

Based on data obtained during the first test, survival of the exercise experience could be predicted in the FE and VE groups but with different variabiles. For the FE group, lower body weight was predictive of death, but T_{co} and cold tolerance were not. In the VE group, lower T_{co} was predictive of death, but body weight and cold tolerance were not. Whether these findings indicate that implementation of the two different regimens involved different physiological responses to the treatments remains to be determined.

The effects of the exercise regimens on survival were not closely associated with the level of exercise *per se*, or with energy expenditure. Specifically, there was no significant difference in mortality rates between the VE and FE groups although the level of activity during exercise was higher in the former group. For example, in terms of actual distances run daily during periods of exercise, the average rate in the VE group was over 5 times greater than that in the FE group – 1584 m/day *vs.* 300 m/day, respectively. The greater energy expenditure in the VE group was indicated by the significant loss in body weight, which was not observed in the FE group. Thus, while mortality was related to the implementation of the treatments, it was not related to the level of exercise.

As measured by wheel revolutions, the level of exercise in the VE group also was not significantly correlated with T_{co} or cold tolerance recorded during the second test. This observation provides additional evidence that the degree of exercise over the 3-week period was not significantly related to performance in subsequent tests reflecting thermoregulatory abilities.

The fact that neither exercise regimen improved cold tolerance diminishes the argument that increased physical activity might account for the results of the previous experiment demonstrating the beneficial effects of brain stimulation on cold tolerance [34]. Indeed, only the control group in the present study demonstrated significantly improved cold tolerance. In addition, the NE group exhibited an increase in baseline T_{co} between tests. This observation agrees with a previous finding in similarly aged mice in a study of repeated cold exposure [35]. However, the improvement in cold tolerance observed in the NE group was not in concert with other results from our laboratory. Specifically, in the previous brain stimulation study [34], the cold tolerance of a control group of aged mice (30 month) declined after a 3-week interval; and in the longitudinal study [35] the cold tolerance of 30-month-old mice also declined with repeated exposure at 2-week intervals.

These conflicting findings may be due to methodological differences among the studies. In the brain stimulation study [34], as well as in the present study, the control groups received similar treatments. They were removed from their home cages daily and placed in the experimental chambers that were unoperational for periods equivalent to those for the experimental groups. However, in the brain stimulation study, control animals had undergone surgical implantation of electrodes in the hypothalamus, a treatment which might have detrimental effects on cold tolerance. In this light, it is important to note that there was no improvement in cold tolerance among aged mice provided hypothalamic stimulation [34]. Rather the effect was one of preventing further age-related decline observed in the control group. In addition, the type of housing differed between studies – metal cages with no bedding in the present study *vs.* plastic cages with bedding in the previous study. In the longitudinal study [35], the mice were also group-housed in plastic cages and did not receive any treatment or handling during the 2-week interval between tests. Therefore, it appears that some aspect of sensory stimulation, associated with handling and/or introduction to a different environment as represented by the experimental chambers, may provide beneficial effects on cold tolerance in aged mice. This prospective intervention deserves further experimental attention. In this light, studies of the effects of handling that have been shown to increase performance of aged rats in learning tasks are noteworthy [3,4].

It should be emphasized that the current findings do not support a conclusion that exercise of all types is detrimental to aged mice. Specifically, what is concluded is that a 3-week regimen introduced to aged C57BL/6J mice was detrimental to survival and did not significantly improve cold tolerance among survivors. Other regimens, perhaps ones introduced gradually and earlier, may prove beneficial in both regards.

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