Modification of Physical Movement in Old C57BL/6 Mice by DHEA and Melatonin Supplementation (44404)

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Abstract. **As old age results in reduced physical activity as well as less dehydroepiandrosterone (DHEA) and melatonin (MLT) production, low hormone levels may be a component of inactivity. Therefore, we studied the effects of DHEA and/or MLT supplementation on movement and resting in young and old female C57 black mice. Our results showed for the first time that old female C56BL/6 mice have significantly decreased physical activity. Their average speed and resting time were significantly higher than in young mice, whereas ambulatory time, distance traveled, and body movements when stationary (stereo time) were lower. DHEA supplementation significantly increased stereo time in old mice, while decreasing ambulatory time and distance traveled. MLT supplementation of old mice decreased average speed, resting time, and stereo time compared to untreated, old mice. Supplementation with MLT or DHEA, whose production is reduced in aging, restored physical activity levels in old mice. MLT also increased ambulatory time and distance traveled while reducing body movements of young mice.** [P.S.E.B.M. 1999, Vol 221]

Aging is associated with lower metabolism; physical activity; DHEA and MLT (1) secretion; antioxidants such as vitamin E, beta-carotene, and MLT (2); immune function, and protein synthesis. MLT is synthesized under adrenergic control of the pineal hormone (1). DHEA, the principal adrenal hormone, attenuates the stress response and has immunomodulatory activity in humans and animals (2–5). In humans as well as animals, physical activity declines concomitantly with T-cell immune functions. As DHEA and MLT supplementation corrected immunological dysfunction in old mice (5–9), consumption of these hormones may modulate age-associated inactivity and reduced movement.

Oxygen-free radicals are increased in old age where their presence should contribute to impairment of cell functions (5). The age-related decrease in vitamin E and hormone antioxidants (DHEA and MLT) (2) lowers mechanisms that could prevent oxidative cell damage and thus dysfunction. Therefore, we tested the effects of antioxidant hormone supplementation on age-reduced physical activity. Antioxidants immunomodulatory hormones, DHEA (6–7) and MLT (10), should induce homeostasis for normal cell function at rest and during oxidative stress. Our current study found that old C57BL6 mice had reduced ambulatory time while increasing body movement when stationary. DHEA + MLT and MLT supplementation resulted in higher body movement when stationary and average speed in old mice compared to untreated old mice. DHEA or DHEA + MLT intake decreased ambulatory time and distance traveled. MLT supplementation increased whereas DHEA + MLT jointly reduced distance traveled in old mice.

Materials and Methods

Animals and Treatments. Young and old female C57BL/6 mice, aged 1.5 and 16 months, were obtained from the Charles River Laboratories Inc. (Wilmington, DE). MLT was purchased from Sigma (St. Louis, MO). Diets, Inc. (Bethlehem, PA) prepared synthetic AIN 93A diet color-coded with DHEA or without. The animals were housed in the facilities of Animal Resources at University Medical Center in groups of four mice per cage. Temperature was maintained at 20°–22°C, and the relative humidity

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was 60%–80%. Water and diet were freely available, and the mice were subjected to a 12-h light:dark cycle. The animals were cared for as required by the University of Arizona Committee on Animal Research. The animals were housed and fed the AIN 93A diet for 2 weeks preceding the experiment. The entire treatment period was 12 weeks. Edenland Inc. (Baybush, Kidlare, Ireland) graciously donated the DHEA. Aged mice were divided into four groups of four mice/cage. The groups of old and young mice were labeled A through D and subjected to the following diets: Group A was given AIN 93A diet with 0.005% ethanol in their drinking water for 12 weeks. Group B received 0.02% DHEA supplemented AIN 93A diet (1.2 mg/mouse/day) for the first 3 weeks. For the following 9 weeks, the mice were given a 0.06% DHEA-supplemented diet with 0.005% ethanol in drinking water administered throughout the 12 weeks. Aged mice in our experience took time to adjust to the taste of large amounts of DHEA in solid food, so we gave them 3 weeks to adjust to a low dose. The higher dose of DHEA used was selected because it was the lowest level that modified immune functions in old mice (6). Group C received the unsupplemented AIN 93A diet and 0.005% ethanol in drinking water with 10 μg/ml MLT dissolved (49.8 μg/mouse/day) for 12 weeks. Ethanol was required to dissolve the MLT. Group D was given 0.02% DHEA-supplemented AIN 93A diet with 0.005% ethanol drinking water for the first 3 weeks. During the last 9 weeks, the mice received 0.06% DHEA-supplemented AIN 93A diet and 0.005%-ethanol drinking water with 10 μg/ml MLT added.

Activity Measurements. Using a Videomex-V video data acquisition system (Columbus International Corporation, Columbus, OH) physical activity was measured. Then the physical activity was evaluated statistically for each of five parameters: 1) *ambulatory time,* the amount of time the mouse spent moving; 2) *stereo time,* the amount of movement that the animal performed when stationary; 3) *average speed* of movement while the animals were moving; 4) *distance traveled* while animals moved in their cages; and 5) *resting time* when mice were not traveling. The video camera was focused on eight clear plastic cages against a white background. During each video session, each cage contained one mouse and all were from the same group. An IBM computer analyzed the video image in real time (30 frames/sec). From the video image, the computer compared pixel change with a calibrated standard to determine the distance traveled. The computer also calculated the amount of time each mouse moved, the average speed, and the resting time (11–13) during an 8-hr dark cycle. The video tracking apparatus monitored each mouse for 8 hr per day from 8:00 PM until 4:00 AM, their peak activity period. This was repeated for each animal for 8 hr another night, and results of both measurements were combined.

Statistics. All results are expressed as the mean ± SE. Duncan's multiple range test was used to analyze data of physical activity. $P < 0.05$ was considered to be significantly different between groups (14).

Results

When we reported the immune changes due to DHEA and MLT in these mice, we noted that hormone supplementation had not changed their food or water consumption (8). We now report on the effects of hormone supplementation on physical movement in the same mice. Each treatment (DHEA, MLT, and DHEA + MLT) was compared to the ethanol control with all five physical activity parameters being examined. No effects of age or hormone treatment was detected on body weight, food, or water intake (unpublished data), confirming previous studies.

Effect of Age on Physical Activity. Video assessment of physical activity in old mice during their active (dark) time was investigated. Ethanol (0.005%) in drinking water was added to solubilize MLT. The level of ethanol consumption was very low, much below the 0.5%–1.0% needed to produce significant blood alcohol levels or immune changes (15). Ethanol consumption did not change physical activity in young mice. Ethanol-fed old mice (controls) showed significant differences ($P < 0.05$) in physical activity from ethanol-consuming young mice. Average speed and resting time were significantly ($P < 0.05$) higher whereas ambulatory time and the body movement when stationary were lower in old mice (Figs. 1 and 5). However, distance traveled (Fig. 3) showed no significant ($P < 0.05$) change.

Hormone Supplementation and Physical Activity in Young Mice. MLT-supplemented young mice significantly ($P < 0.05$) increased their ambulatory time and distance traveled compared to untreated young ones (Fig. 3). DHEA, MLT, and MLT + DHEA consumption significantly ($P < 0.05$) reduced body movement when stationary (Fig. 5). DHEA supplementation of young mice caused no significant change in average speed whereas MLT and DHEA + MLT significantly increased average speed and lowered resting time (Fig. 2).

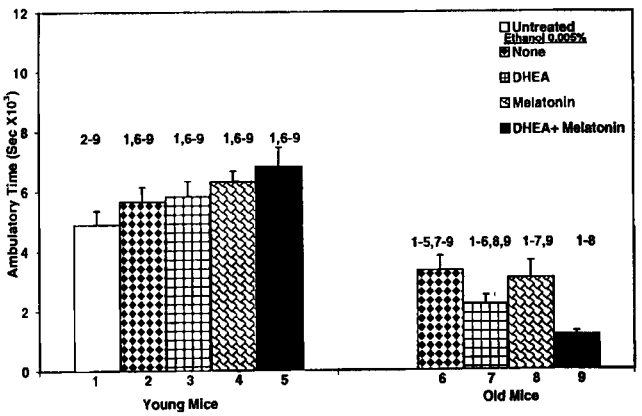

Figure 1. The effects of DHEA and MLT administration on ambulatory time (Sec) in young and old mice are displayed. The results are mean ± SE; $n = 8$ from 8 hr monitoring of the physical activity of each mouse done twice, totaling 16 hr/mouse. Ambulatory times (Sec) were significantly different ($P < 0.05$) from groups with numbers above the particular bar. Untreated mice received no supplementation whereas those given ethanol but no hormones were entitled, but none was given.

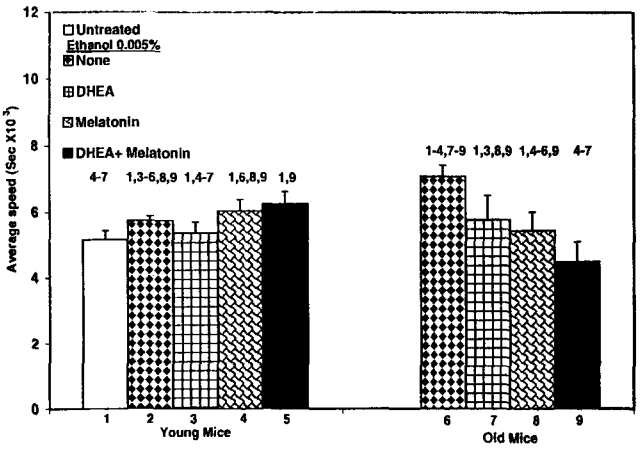

Figure 2. The effects of DHEA and MLT administration on average speed (Inch/Sec) in young and old mice are displayed. The results are mean ± SE; $n = 8$ from 8 hr monitoring the physical activity of each mouse done twice, totaling 16 hr/mouse. Average speeds (Inch/Sec) were significantly different ($P < 0.05$) from groups with numbers above the particular bar. Untreated mice received no supplementation whereas those given ethanol but no hormones were entitled, but none was given.

Hormone Supplementation and Physical Activity in Old Mice. MLT and DHEA secretion decline significantly in old people (5, 16) who usually have lower physical activity than young adults with maximum production of these hormones. DHEA + MLT and MLT given to old mice induced a significantly ($P < 0.05$) higher body movement when stationary and average speed (Figs. 2 and 5) compared to untreated old mice. MLT alone increased distance traveled in old mice whereas DHEA + MLT together decreased it (Fig. 5) compared to old controls (Figs. 2 and 3). DHEA supplementation significantly ($P < 0.05$) lowered resting time more than did MLT or DHEA + MLT (Fig. 4). Old mice treated with MLT significantly ($P < 0.05$)

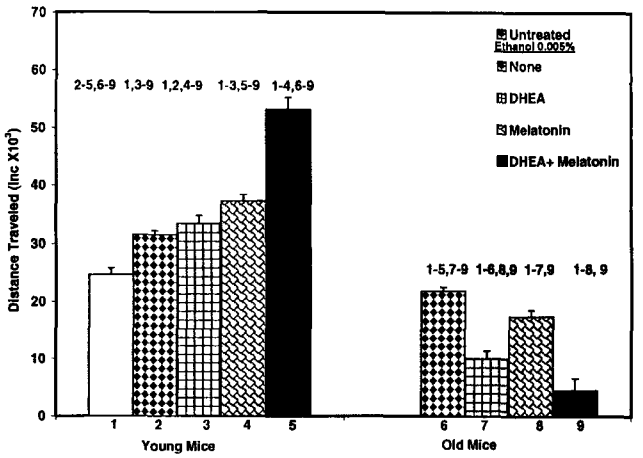

Figure 3. The effects of DHEA and MLT administration on distance traveled (Inch) in young and old mice are displayed. The results are mean ± SE; $n = 8$ from 8 hr monitoring the physical activity of each mouse done twice, totaling 16 hr/mouse. Distance traveled (Inch) were significantly different ($P < 0.05$) from groups with numbers above the particular bar. Untreated mice received no supplementation whereas those given ethanol but no hormones were entitled, but none was given.

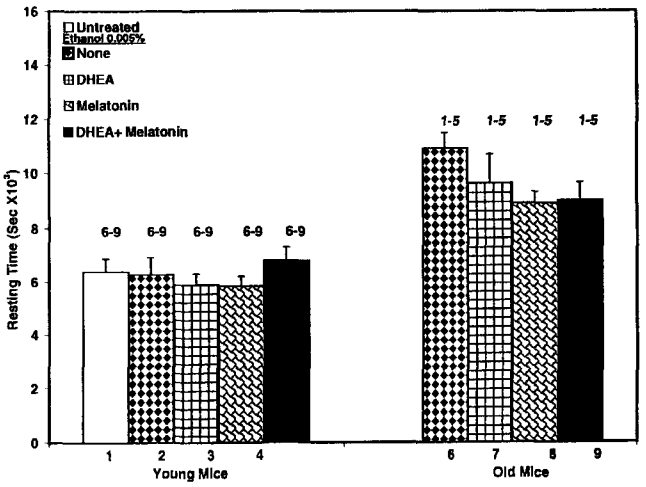

Figure 4. The effects of DHEA and MLT administration on resting time (Sec) in young and old mice are displayed. The results are mean ± SE; $n = 8$ from 8 hr monitoring the physical activity of each mouse done twice, totaling 16 hr/mouse. Resting time (Sec) were significantly different ($P < 0.05$) from groups with numbers above the particular bar. Untreated mice received no supplementation whereas those given ethanol but no hormones were entitled, but none was given.

increased body movement when stationary (Fig. 5) and decreased average speed (Fig. 2) ($P < 0.05$). Old mice treated with DHEA significantly ($P < 0.05$) increased body movement when stationary (Fig. 5). DHEA or DHEA + MLT significantly ($P < 0.05$) decreased ambulatory time and distance traveled (Figs. 1 and 3). MLT + DHEA lowered ambulatory time, average speed, and distance traveled to that of young mice (Figs. 1–3). In addition, DHEA and MLT treatments each separately restored average speed to those young controls (Fig. 2).

Discussion

Restoration of physical activity by DHEA and MLT supplementation in this study provides a biological picture

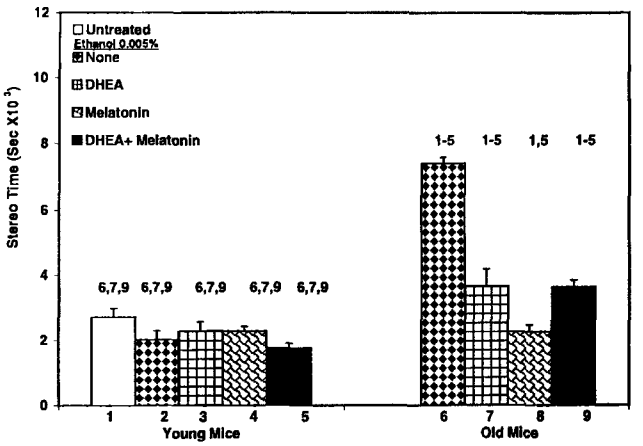

Figure 5. The effects of DHEA and MLT administration on stereo time (Sec) in young and old mice are displayed. The results are mean ± SE; $n = 8$ from 8 hr monitoring the physical activity of each mouse done twice, totaling 16 hr/mouse. Stereo times (Sec) were significantly different ($P < 0.05$) from groups with numbers above the particular bar. Untreated mice received no supplementation whereas those given ethanol but no hormones were entitled, but none was given.

consistent with the positive effects of these hormones on other systems showing senescence (5, 10). DHEA as well as MLT have a unique capacity to modify physical activity as they have often done on the immune system. Our findings showed that there are significant differences in physical activity between young and old mice. The present study clearly demonstrated that hormonal supplementation modified the physical activity in young and old C57BL/6 mice. Age significantly reduced physical activity, whereas DHEA and MLT supplementation improved physical activity in old mice. Aging induces low MLT production with dysregulation of the sleep-wake cycle (16); therefore, MLT supplementation might be expected to help regulate sleep and hence physical activity. As expected, resting time increased whereas ambulatory time, body movement while stationary, and distance traveled all decreased with age. In addition, body movement when stationary and average speeds were higher in older mice than in younger mice.

While lower metabolism, physical activity, DHEA (13) and MLT (8, 10) secretion, and protein synthesis characterize aging, their interrelationship in causing aging needs more definition. DHEA supplementation normalized average speed of old mice to that of young controls and increased their body movement when stationary, while decreasing their resting time and distance traveled. DHEA more than MLT increased the body movement when stationary and decreased average speed of old mice. Intraperitoneal injection of DHEA into young mice at low levels increased motor activity while high levels, 0.5–2.0 mg/kg body weight, reduced motor activity (17) in male C57BL/6 mice by reducing locomotor actvity. However, our dietary doses of DHEA (48 mg/kg body weight) were 100 fold higher and had little effect on activity of young mice, suggesting that the tissue levels were not as elevated by dietary consumption as following intraperitoneal injection. A dose of 1.5 g/kg (0.15%) ethanol decreased activity. This dose of ethanol was 30 times higher than the dose we used which had almost no effect on activity. The combination of ethanol + DHEA was not different from ethanol alone, but it was greater than DHEA alone (17). Supplementation with DHEA + MLT also increased body movement when stationary and resting times but decreased ambulatory time and distance traveled. MLT increased resting time and body movement when stationary, while significantly lowering ambulatory time and average speed in old mice compared to untreated young ones. Older mice supplemented with the MLT also increased average speed and distance traveled similar to results in untreated young mice. MLT at high levels can induce feelings of fatigue and drowsiness, reset circadian rhythms, and enhance sleep quality (18–19). MLT production declines with age although exercise can elevate or decrease MLT levels (19–20). Plasma MLT in young males showed an increase in serum MLT after exercise (21). DHEA + MLT and MLT caused a significantly greater body movement when stationary and average speed. Moreover, MLT alone elevated distance traveled in old mice whereas DHEA and MLT jointly reduced it compared to old controls. DHEA supplementation significantly depressed resting time more than MLT or DHEA + MLT. Other studies have found elevated MLT in women athletes (18). DHEA or DHEA + MLT treatment significantly decreased ambulatory time and distance traveled. MLT + DHEA lowered ambulatory time, average speed, and distance traveled to that of untreated young mice. In addition, DHEA and MLT treatments each separately restored average speed of old mice to that of untreated young ones. Finally, our data are in agreement with those showing that MLT and DHEA helped overcome physical inactivity (22) that occurred simultaneously with immune dysfunction due to murine retrovirus infection, which hormone supplementation retarded (4, 6).

In summary, our intervention strategy overcame some of the age-related loss of physical activity much as it had done previously for immunosenescence (18, 23). Production of reactive oxygen species is severely increased due to aging (5, 23) when tissue and immune damage occur. Since the immune system plays an important role in aging and is directly affected by reduced antioxidant levels, immunoregulatory DHEA, as well as MLT secretion, immune dysfunction may be linked to physical inactivity.

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