Physical Inactivity of Murine Retrovirus Infected C57BL/6 Mice is Prevented by Melatonin and Dehydroepiandrosterone

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Abstract. **LP-BM5 retrovirus infection and hormone synthesis modulate many physiological systems that could affect physical activity. Infection induced oxidative damage and immunodeficiency of female mice which hormone supplement prevented. Therefore, the effects of retrovirus infection and hormone supplementation were assessed on physical activity using a computerized video system. Retroviral infection increased activity when stationary while lowering average speed and resting time. Hormone supplementation partially modified changes due to murine AIDS. Dehydroepiandrosterone (DHEA) or melatonin (MLT) supplementation restored the average speed; ambulatory time and distance traveled of retrovirus infected mice. MLT as well as the combination of DHEA + MLT increased body movement, but decreased average speed and distance traveled. Thus, retrovirus infection had significant effects on physical activity. Further studies into the relationship between the DHEA and/or MLT and physical activity will assert the contribution of these hormones to the treatment of murine AIDS.** [P.S.E.B.M. 1998, Vol 219]

Human Immunodeficiency Virus (HIV) infection induces a variety of alterations resulting in opportunistic infections, inactivity, and cognitive dysfunction (1, 2). LP-BM5 murine leukemia virus infection causes an AIDS-like syndrome (3). Murine acquired immunodeficiency syndrome in C57BL/6 mice impairs spatial learning without gross-motor impairment (4). In the process of HIV infection, cells undergo physiological changes that result in a decreased use of energy sources (5). AIDS patients develop immune dysfunction with an increased risk of prolonged infection (3). Lower body weight and physical activity due to a loss of lean muscle mass occur whereas the adipose tissue remains unchanged.

Adrenal hormones, cortisol, and DHEA modulate the immune system and physical activity (2, 3, 6). Cortisol decreases T-cell proliferation and function by inhibiting the production of Th1 cell cytokines (3). DHEA supplementation stimulates Th1 cell function by enhancing T cells that produce IL-2 (3). These steroid hormones also affect amino acid metabolism in muscle, implicated in HIV-cachexia (7). Glucocorticoids stimulate amino acid catabolism by influencing the enzymes of amino acid catabolism, transaminase and tryptophan pyrolase. Androgens like DHEA increase amino acid anabolism, principally in bone muscles, stimulating protein synthesis (8). Decreased DHEA production in HIV patients may be a significant cause of muscular atrophy, reduced physical activity, cardiovascular morbidity (9), cancer (10), progression to AIDS, and immunosenescences (11–12). Thus, reduced DHEA may induce the physiological imbalance promoting inactivity (3). A DHEA supplement restored lymphokine levels in older people or animals to the levels found in younger ones (2), elevated serum insulin growth factor-1 (IGF-1) (3), and improved antioxidant activity (13).

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DHEA supplementation improved immune function in AIDS patients (14) and retrovirus-infected mice (12, 15–16). Dehydroepiandrosterone (DHEA) or melatonin (MLT) supplementation prevented the development of immune dysfunction in retrovirally infected mice (8, 17). Therefore we examined the effects of supplementation with DHEA, MLT, or both on the physical activity of retrovirus infected female C57BL/6 mice. We measured physical activity using a computerized video-data acquisition system. Retroviral infection reduced average speed and resting time; DHEA and MLT supplement improved physical activity.

Materials and Methods

Animals and Treatments. Young C57BL/6 mice, aged 1.5 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 diets, pelleted and color-coded with DHEA added to some. The animals were housed in the sterile facilities of Animal Resources at University Medical Center of Arizona in groups of three to four mice per cage. Temperature was maintained at 20°–22°C; the relative humidity 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 control diet AIN 93A for 2 weeks preceding the experiment. The entire treatment period was 12 weeks. DHEA was graciously donated by Edenland Inc. (Baybush, Kidlare, Ireland). LP-BM5 Murine leukemia retrovirus mixture was administered intraperitoneally to the mice in 0.1 of ml minimum essential medium (MEM) with an esotropic titer (XC) of 4.5 $\log_{10}$ plaque-forming units $\times$ 10^{-3}/ml. This induced disease (15) as previously published (12). Infection of adult female C57BL/6 mouse with the retrovirus leads to the rapid induction of clinical symptoms without any latent phase (16). Uninfected mice were injected with 0.1 ml of complete culture medium used for LP-BM5 virus growth as controls. Administration of ML, DHEA, or DHEA + MLT diets was begun 2 weeks after LP-BM5 infection and continued until the mice were sacrificed. The groups of uninfected and retrovirus-infected mice consisted of eight mice each. The groups were labeled A through D and subjected to the following diets. Group A was given unsupplemented control diet AIN 93A with 0.005% ethanol drinking water for 12 weeks. As all treatment groups consumed ethanol in drinking water, this group was the primary control. Ethanol was required to dissolve the MLT. Group B received 0.02% DHEA-supplemented diet (1.2 mg/mouse/day) for the first 3 weeks. After aclimitization, the amount was increased to 0.06% DHEA. For the following 9 weeks, the mice were given 0.06% DHEA-supplemented diet (3.6 mg/mouse/day), and 0.005% ethanol in drinking water was administered throughout the 12 weeks. Group C received unsupplemented diet and 0.005% ethanol in drinking water with 10 μg/ml MLT dissolved (49.8 μg/mouse/day) for 12 weeks. Group D was given 0.02% DHEA-supplemented diet with 0.005% ethanol drinking water for the first 3 weeks. During the last 9 weeks, the mice received 0.06% DHEA-supplemented diet and 0.005% ethanol drinking water with 10 μg/ml MLT added. Other mice, the untreated controls, were age- and sex-matched. They were given no treatment, with four mice in these groups.

Activity Measurements. Using a Videomex-V video data acquisition system (Columbus International Corporation, Columbus, OH) components of physical activity were measured. Then they were statistically evaluated for each of five parameters: A) ambulatory time, the amount of time the mouse spent moving; (B) activity when stationary, the amount of body movement that the animal performed while stationary; C) average speed of movement while the animals were moving; D) distance traveled while animals moved in their cages; and E) 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 from the same group (A–D). 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 (18–19) during an 8-hr dark cycle. The video tracking apparatus monitored each mouse for 8 hr/day from 8:00 PM until 4:00 AM, which is during their peak activity period. This was repeated for each animal for 8 hr, and results of both measurements were combined.

Statistics. All results are expressed as the mean ± S.E. Data of physical activity were analyzed by Duncan multiple range test. $P < 0.05$ was considered to indicate a significant difference between groups (20).

Results

The data collected were analyzed for each uninfected or infected mouse. Each treatment (DHEA, MET, DHEA, and MLT) was compared to the untreated control as well as to the ethanol control with all five physical activity parameters examined (Fig. 1–5). Ethanol was necessary to dissolve MLT into the drinking water. As ethanol consumption modified some physical activity parameters, it was the control used in the following description of the results. However, the statistical analysis on the figures identified each group in comparison to all other groups. Hormone supplementation retrovirus and retrovirus infection did not significantly change body weight or food consumption, as shown previously (15).

Effect of Retrovirus Infection on Activity in Young Mice. Infected mice had lower average speed, distance traveled, and resting time (Fig. 2, 3, 4) with significantly ($P < 0.05$) more stereo time, body movements while stationary (Fig. 5), than uninfected mice. However, ethanol-treated, uninfected and retrovirus infected mice showed that

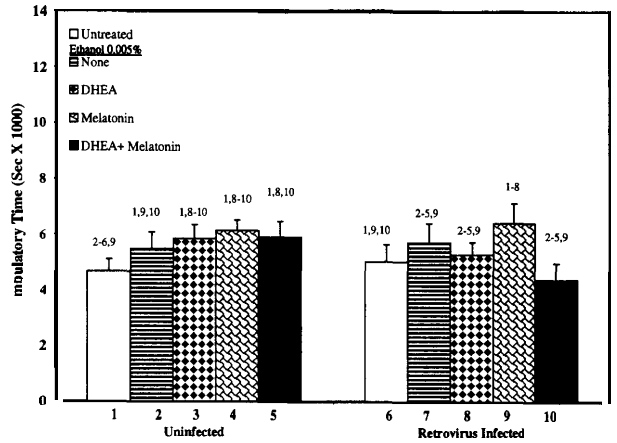

Figure 1. The effects of DHEA and MLT administration on ambulatory time (sec) in uninfected and retrovirus-infected mice are displayed. The results are mean ± SE. Monitoring of ambulatory times of the eight mice was done twice for 8 hr, totaling 16 hr/mouse. Ambulatory times (sec) were significantly different ($P < 0.05$) from the groups whose numbers are above the bars.

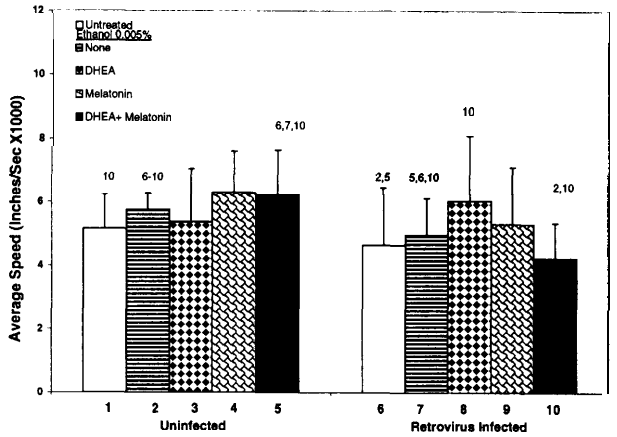

Figure 2. The effects of DHEA and MLT administration on average speed (inch/sec) in uninfected and retrovirus-infected mice are displayed. The results are mean + SE. Monitoring of average speeds of the eight mice was done twice for 8 hr, totaling 16 hr/mouse. Average speeds (inch/sec) were significantly different ($P < 0.05$) from the groups whose numbers are above the bars.

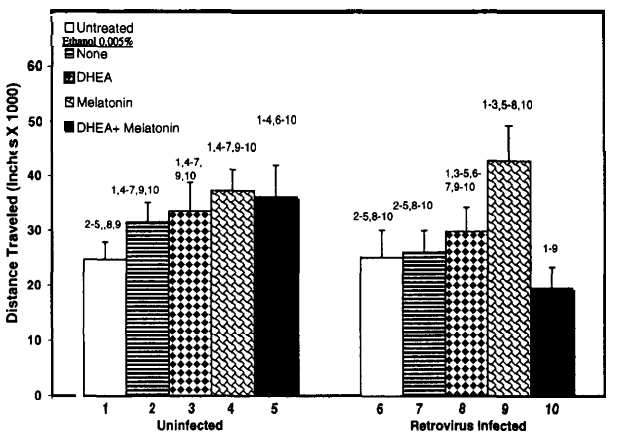

Figure 3. The effects of DHEA and MLT administration on distance traveled (inches) in uninfected and retrovirus-infected mice are displayed. The results are mean ± SE. Monitoring of distance traveled of the eight mice was done twice for 8 hr, totaling 16 hr/mouse. Distances traveled (inches) were significantly different ($P < 0.05$) from the groups whose numbers are above the bars.

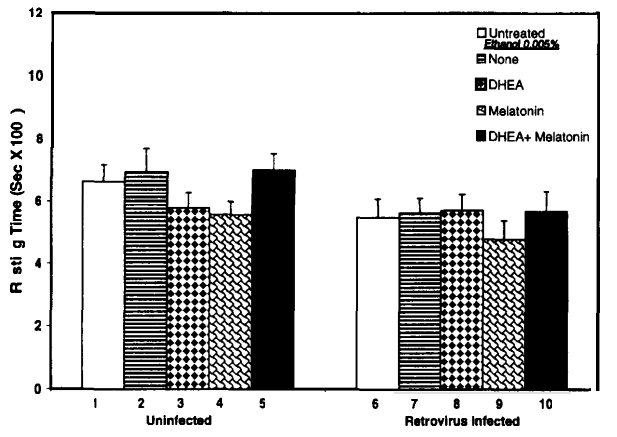

Figure 4. The effects of DHEA and MLT administration on resting time (sec) in uninfected and retrovirus-infected mice are displayed. The results are mean ± SE. Monitoring of resting times of the eight mice done twice for 8 hr, totaling 16 hr/mouse. Resting times (sec) were significantly different ($P < 0.05$) from the groups whose numbers are above the bars.

average speed and body movement while stationary were significantly ($P < 0.05$) different (Fig. 2, 5) whereas no changes were observed in other movements.

Hormone Supplementation and Physical Activity in Young Mice. Retrovirally infected mice that received DHEA maintained significantly ($P < 0.05$) greater ambulatory time and distance traveled than unsupplemented infected mice (Fig. 1, 3). MLT treatment of retrovirally infected mice significantly ($P < 0.05$) increased distance traveled above that of uninfected mice (Fig. 3). DHEA but not MLT supplementation produced similar resting times in retrovirus-infected and uninfected mice (Fig. 4). Although DHEA and MLT each lowered resting time (Fig. 4), DHEA + MLT-supplemented, infected mice had significantly ($P < 0.05$) higher body movement while stationary than uninfected controls (Fig. 5). MLT supplemented, young mice showed significant ($P < 0.05$) increased ambulatory time and distance traveled compared to controls (Fig. 3). DHEA, MLT, or MLT + DHEA significantly ($P < 0.05$) reduced their body movement while stationary (Fig. 5). MLT or MLT + DHEA significantly ($P < 0.05$) increased distance traveled whereas DHEA, MLT or MLT + DHEA had no significant effect on other movements of uninfected mice (Fig. 3).

Discussion

The present study clearly demonstrates that LPBM-5 murine retrovirus infection affects aspects of physical activity, stereo time, and resting time. Similar to HIV infection, MAIDS is accompanied by a host of neurological changes such as increased Quinolic acid levels and platelet activating factor that impairs responses to acquisition in later stages of the disease (4, 21–22). In addition, hormonal supplementation with DHEA, or MLT + DHEA modified

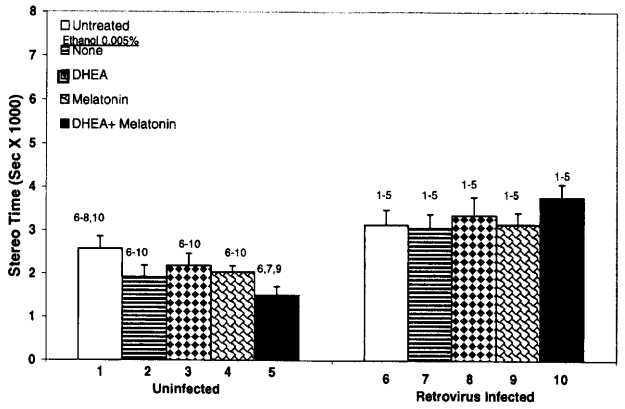

Figure 5. The effects of DHEA and MLT administration on body movement while stationary, stereo time (sec), in uninfected and retrovirus-uninfected mice are displayed. The results are mean ± SE. Monitoring body movement of the eight mice while stationary was done twice for 8 hr, totaling 16 hr/mouse. The body movements while stationary (sec) were significantly different ($P < 0.05$) from the groups whose numbers are above the bars.

the physical activity in retrovirus-infected C57BL/6 mice. DHEA and MLT production is diminished with AIDS patients (10, 14), whereas progression of retroviral disease and immunosenescence (11) are accentuated (12, 15, 17).

MLT is a lipid-soluble hormone that crosses the blood-brain barrier into the brain affecting resting and sleep-related functions. At high levels it can induce feelings of fatigue and drowsiness, reset circadian rhythms, and enhance sleep quality (23–24, 21) MLT is being used extensively to treat sleep disorders which could affect physical activity. In addition, it affects the immune system in subjects with murine (12, 17) or human AIDS (25). However, MLT consumption increased average speed, ambulatory time, and distance traveled in retrovirally infected, young mice in comparison to uninfected controls. Exercise can elevate (26) or decrease MLT levels (27–28). MLT supplementation enhances immunity in AIDS and retroviral infection (29, 25) although exercise can elevate (26) or decrease MLT levels (27–28).

DHEA supplementation increased the low levels of serum DHEAS in HIV-infected people (30). It was immunorestorative in both human (31) and animal (12). Serum DHEA declines during HIV infection and with it declines CD4 cell numbers. This suggests that DHEA regulates immune responses through its effects on cytokine production by Th1 and Th2 cells (32, 15). The loss of body weight and lean muscle mass with lowered physical activity are also correlated with less DHEA synthesis in AIDS patients. DHEA supplementation enhanced immune responses in retrovirus mice (12). It also improved average speed and distance traveled in retrovirus-infected mice.

In retrovirus-infected mice (33), lymphoid cells increase the production of tumor necrosis factor (TNF). TNF levels increase in the late stages of HIV-infected patients who have decreased serum levels of DHEA and MLT (30, 29). TNF may inhibit lipoprotein lipase synthesis (34), as well as fatty acid synthase (35). TNF stimulates muscle proteolysis and decreases protein synthesis during cancer cachexia (36). Prevention of retrovirus-induced oxidation, lipid changes, excessive TNF production, and loss of tissue antioxidants by DHEA supplementation (15, 37) regulates immune function and thus may affect energy sources supporting physical activity. Physical activity declines in AIDS patients with reduced DHEA levels (31). Decline of DHEA production in AIDS may be caused by intra-adrenal block during DHEA-sulfate synthesis, its metabolic clearance, or secretion of a postulated pituitary corticoadrenal stimulating factor (38). The MLT and DHEA serum levels profoundly influence the body's physiology. Retrovirus infection and supplementation with immunoregulatory hormones affect physical activity in mice.

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