Head-down tilt posture elicits transient lymphocyte mobilization from the iliac, but not mesenteric, lymph nodes of rats

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Suzuki S, Mizuno R, Ikomi F, Ohhashi T. Head-down tilt posture elicits transient lymphocyte mobilization from the iliac, but not mesenteric, lymph nodes of rats. J Appl Physiol 105: 1595–1601, 2008. First published September 25, 2008; doi:10.1152/japplphysiol.90415.2008.—The effects of short-term simulated microgravity on the lymph dynamics of rat lymph nodes were investigated using a combination of Bollman’s cage and head-down tilt (HDT). Efferent lymphatics of the iliac and mesenteric lymph nodes were cannulated for the collection of lymph. There was no significant difference in lymph flow rate from the iliac lymph nodes between non-HDT (control) and HDT rats. Lymph flow rate from the mesenteric lymph nodes in HDT rats was slightly higher than that obtained with the control. The cell count obtained from the iliac lymph nodes in HDT rats was significantly larger than those of the controls, while no significant difference in the number of cells from the mesenteric lymph nodes was observed between the control and HDT groups. The cells from the iliac lymph nodes in the control and HDT rats were mostly lymphocytes. The distribution of subsets of lymphocytes (CD3+, CD4+, CD8a+, and CD45R+) from the iliac lymph nodes in HDT rats was not significantly different from the subsets of lymphocytes in the control. Immunization did not affect the distribution of lymphocyte subsets from the iliac lymph nodes in the control and HDT groups. There was no significant difference in the concentrations of lymph albumin in iliac afferent or efferent lymphatics between the control and HDT groups. These findings suggest that HDT posture in Bollman’s cage induces transient output of lymphocytes from the iliac lymph nodes of rats in vivo without changing the flow rate, lymphocyte subsets, or concentration of albumin.

IT IS WELL KNOWN that microgravity alters human physiology during spaceflight (38). Regarding the cardiovascular system, astronauts often suffer notable facial edema. This facial puffiness is due to a body fluid shift toward the upper extremities from the legs (7, 14, 20, 32, 37). In the upper regions, elevated capillary blood pressure and reduced capillary colloid osmotic pressure lead to an increase in net transvascular fluid filtration, which is associated with the edema (26, 37). In mammals, the lymphatic system plays a significant role in regulating the transport of extracellular fluids and macromolecular substances in tissues, indicating that lymphatic function serves as the critical safety factor preventing edema (29). The lymphatic system also contributes to the regulation and maintenance of immunity, because lymphocytes and albumin circulate continuously between blood and lymph.

Another impact of microgravity in space medical concerns is immune dysfunction during spaceflight (38), which results from multiple factors, including changes in the endocrine stress hormone system and exposure to radiation (30). Reduction in the activity of human T lymphocytes in response to mitogenic stimuli and cytotoxicity of natural killer cells during spaceflight has been reported (5, 6, 16). Also, spaceflight and simulated microgravity cause a significant alternation in the lymphocyte function of rats (10, 23, 24).

Although we recognize the important issues regarding the immunological dysfunction of lymphocytes, changes in lymph dynamics in response to microgravity have not been fully elucidated. Gashev et al. (8) reported that simulated microgravity (head-down tail suspension) of rats caused an inhibition in the active pump activity of isolated lymph vessels, suggesting that microgravity may affect lymph circulation in animals as well as humans.

Bollman’s cage is a useful method for the collection of lymph from rats in vivo, enabling us to measure lymph flow rate and to analyze lymph contents (2, 17, 18) by cannulation of tubing into appropriate parts of the body such as the thoracic ducts or mesenteric lymph vessels. Also, head-down tail suspension of rats is an established method for investigating the effects of simulated microgravity on the cardiovascular system (8, 22) as well as on immunity (10, 23, 24). We hypothesized that short-term simulated microgravity affects the lymph circulation in the lower extremities of rats. To examine the hypothesis, in the present study, we utilized a combination of Bollman’s cage (for collection of lymph) and head-down tilt (HDT) (for applying simulated microgravity) in vivo to rats on Earth. First we investigated the lymph flow rate and lymphocyte mobilization from iliac lymph nodes in non-HDT (control) and HDT rats, and to compare with the lower extremities, the lymph dynamics of the mesenteric lymph nodes were also examined in the control and HDT rats because it is well known that there are clear, heterogeneous distributions of lymphocytes or lymph content between the lower extremity and mesenteric lymph circulation. Second, to determine whether or not HDT-induced changes in the lymph dynamics take place through selective immunological responses, we examined the effects of immunization on changes in the cellular components of lymphocytes from the iliac lymph nodes in the control and HDT rats using flow cytometry. Finally, we investigated changes in the concentration of lymph albumin in afferent or efferent lymph vessels of the iliac lymph nodes in the control and HDT rats because it is well known that the lymph flow rate and movement of lymphocytes in lymph nodes depend on the concentration of albumin in lymph (1, 27).
**MATERIALS AND METHODS**

**Animals.** Five- to 8-wk-old male Wistar rats \((n = 98; \text{Japan SLC})\) were housed in an environmentally controlled vivarium and fed a standard pellet diet and water ad libitum. All experimental protocols were approved by the Animal Ethics Committee of Shinshu University School of Medicine, in accordance with the principles and guidelines on animal care of the Physiological Society of Japan.

**Surgical procedures.** The rats were anesthetized with a subcutaneous injection of pentobarbital sodium \((50 \text{ mg/kg body wt})\), and additional anesthetics were administered when the rats showed body movement or hyperventilation during surgical procedures. A warm heat pad was used to maintain the body temperature of the rats during the procedures. Figure 1, A–C, shows a schema of cannulation sites for lymph drainage in the present study. All surgical procedures were performed under a microscope \((\text{MTX, Olympus})\). After incision of the abdomen, connective tissues around the lymph vessels were carefully removed using microsurgical instruments and cotton swabs. For collection of lymph from the efferent lymph vessels of the iliac lymph nodes, we cannulated elongated polyethylene tubing \((\text{SP55, Natsume})\) into the efferent \((\text{Fig. 1A})\;\text{tip diameter} \, 500–600 \mu\text{m}\) lymph vessels. In this case, we chose a single efferent lymph vessel for cannulation; the others were tied with a silk \((8-0)\) suture, and the afferent vessels were free. For collection of lymph from the afferent lymph vessels of the iliac lymph nodes, we cannulated elongated polyethylene tubing \((\text{SP55, Natsume})\) into the afferent \((\text{Fig. 1B})\;\text{tip diameter} \, 250–350 \mu\text{m}\) lymph vessels. In this case, we chose a single afferent lymph vessel for cannulation; the others were tied with a silk \((8-0)\) suture, and the efferent vessels were free. For collection of lymph from the efferent lymph vessels of the mesenteric lymph nodes, a single efferent lymph vessel \((\text{Fig. 1C})\;\text{tip diameter} \, 600–700 \mu\text{m}\) was cannulated by elongated polyethylene tubing \((\text{SP55, Natsume})\). The mesenteric lymph nodes usually receive many afferent lymph vessels, and one efferent lymph vessel can drain mesenteric lymph nodal flow as shown in Fig. 1C. In this case, we chose a single efferent lymph vessel for cannulation; the others were tied with a silk \((8-0)\) suture, and the efferent vessels were free. The cannulated vessels were carefully fixed to prevent them from pulling out or kinking. After confirmation of lymph drainage, the abdomen was closed with a suture.

**HDT animals.** We performed the surgical procedures with cannulation within 1 h after induction of anesthesia. Rats were allowed to recover from the anesthesia for 1.5 h. After movement and eyelash reflex were confirmed, the recovered (conscious) animals were held in Bollman’s cages \((\text{KN-326, Natsume})\) for the measurement of lymph flow rate and collection of lymph \((2)\). Thus we needed 2.5 h before the onset of measurements. Control rats were kept at 0° \((\text{Fig. 1D, n = 40})\) for 3 h, whereas the HDT rats in Bollman’s cage were angled at 45° from horizontal \((\text{Fig. 1E, n = 44})\) for 3 h. Bollman’s cage \((\text{KN-326, Natsume})\) has a frame that can keep animals at 0° or 45° in the cage. Gashev et al. \((8)\) reported that head-down tail suspension of rats has been useful for investigating effects of simulated microgravity on lymph circulation in vivo. For purpose of the present study, we mimicked and modified Gashev’s methods for the collection of lymph by using a combination of Bollman’s cage and HDT. During observation for 3 h, there were no remarkable differences in the movements of rats, especially in leg muscle mechanical activities, between the control and HDT animals. To obtain stable lymph flow rates and collection of lymph from the mesenteric lymph nodes, the rats were given vegetable oil \((2 \text{ ml})\) orally 2 h before onset of lymph drainage because mesenteric lymph flow strongly depends on lipid absorption from the small intestines \((17, 18)\). Additionally, a physiological salt solution \((5 \text{ ml})\) was subcutaneously injected into the back of rats; this is used in mesenteric lymph studies to prevent excess loss of body fluids. Each tubing end was set at negative pressure \((-10 \text{ cmH}_2\text{O})\) throughout the measurement of lymph flow rate and collection of lymph. Lymph from the tubing was collected into a plastic tube and used for subsequent analyses. To prevent vaporization of the collected lymph, we covered the tube with a plastic film.

**Measurement of lymph flow rate and number of cells.** In the first protocol, we measured the efferent lymph flow rate \((\mu\text{l/h})\) and the number of cells from the iliac or mesenteric lymph nodes in the control \((\text{for 3 h})\) and HDT \((\text{for 3 h})\) rats at intervals of 1 h during 3 h of lymph collection. The flow rate \((\mu\text{l/h})\) was measured using a microsyringe \((\text{LF-050, Kusano Kagaku})\). The number of cells in lymph was counted by a hemocytometer \((03-303-1, \text{Erma})\).

**Flow cytometric analyses.** In the second protocol, we analyzed the cellular components of collected lymph from the iliac lymph nodes in
the control and HDT rats using flow cytometry. We measured the cellular components of lymph at 1 h after the collection because the numbers of cells at later times was too small to perform an analysis. The collected cells were suspended in cold PBS, centrifuged at 3,300 rpm for 3 min, and then washed twice with PBS. Single color direct fluorescence staining was performed, using the following monoclonal antibodies: R-phycocerythrin (R-PE)-conjugated mouse anti-rat CD3, CD4, CD8a, and CD45R (BD Biosciences Pharmingen). Cell suspensions (10^6 cells/100 µl) were incubated with each antibody at 4°C for 15 min in a dark-room environment, after which the cells were washed once with PBS and resuspended in PBS. The cells were analyzed using flow cytometry (FACScan, Becton-Dickinson). The data were measured and analyzed using Lyssys II software and BD Cell Quest, respectively (Becton Dickinson); 10^4 cells were examined for each sample. In addition, we analyzed the cellular components of collected lymph from the iliac lymph nodes in the control and HDT rats after immunization with a mixture of a keyhole limpet hemocyanin (KLH, Thermo Scientific) and an adjuvant (TiterMax Gold, TiterMax) (9, 21, 28, 35). The KLH (100 µg/part) and adjuvant (100 µl/part) were subcutaneously injected into both sides of the groin in 5-wk-old male Wistar rats. A sham nonimmunized group without administration of drugs received subcutaneous injections of saline into both sides of the groin. After 2 wk of immunization, lymph from efferent lymph vessels of the iliac lymph nodes was collected for the subsequent analyses. We confirmed that after injection of Evans-blue dye into the groin the dye had reached the iliac lymph nodes, indicating that KLH causes immunization of cells located within the iliac lymph nodes. Also we measured the size (short and long length, and thickness) and wet weight of the iliac lymph nodes with and without immunization. Hematoxylin and eosin (H-E) staining of the section preparation of the lymph nodes was performed for the histological studies to confirm successful immunization of the iliac lymph nodes, indicating increases in the number of lymphocytes in the nodes.

We also analyzed the cellular components of lymph collected from the mesenteric lymph nodes in the control and HDT rats using flow cytometry as well as the iliac lymph node studies.

**Measurement of albumin concentration in lymph.** In the final protocols, we measured the concentration of albumin in afferent and efferent lymph of the iliac lymph nodes in the control and HDT rats at intervals of 1 h for 3 h. The concentration of albumin (g/100 ml) in lymph was measured using nephelometry analysis.

**Data analyses.** The data are presented as means ± SE; n indicates the number of animals. Significant differences (P < 0.05) were determined through ANOVA, followed by Student-Newman-Keuls post hoc test and paired and unpaired Student’s t-test, as appropriate.

**RESULTS**

**Flow rate and number of cells in efferent lymph vessels of iliac or mesenteric lymph nodes in control and HDT rats.** Efferent lymph flow rates of the iliac lymph nodes in the control (Fig. 2A, n = 11) and HDT rats (Fig. 2A, n = 13) were constant during lymph drainage for 3 h. There was no significant difference in the flow rate between the control (66 ± 6 µl/h at 1 h, n = 11) and HDT rats (51 ± 5 µl/h at 1 h, n = 13). The number of cells from the iliac lymph nodes in the control (Fig. 2B, n = 7) and HDT rats (Fig. 2B, n = 9) decreased time dependently during 3 h of lymph drainage. There was a significant difference in the number of cells between the control (0.33 ± 0.15 × 10^7/ml at 1 h, n = 7) and HDT rats (3.76 ± 0.62 × 10^7/ml at 1 h, n = 9, P < 0.05 vs. control) in each corresponding period. The total number of cells from the iliac lymph nodes in HDT rats (5.46 ± 0.35 × 10^7/ml, n = 9, P < 0.05 vs. control) for 3 h was significantly larger than that of the control (0.41 ± 0.06 × 10^7/ml, n = 7).

Efferent lymph flow rate of the mesenteric lymph nodes in the control rats (Fig. 2C, n = 5) was constant during 3 h of lymph drainage, while a slight increase in the efferent lymph flow rate in HDT rats (Fig. 2C, n = 6) was observed during the 3 h of lymph drainage. There was no significant difference in the flow rate between the control (132 ± 41 µl/h at 1 h, n = 5) and HDT rats (146 ± 27 µl/h at 1 h, n = 6). The numbers of cells from the mesenteric lymph nodes in the control (Fig.
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Length (short and long), thickness, and wet weight of iliac lymph nodes with and without immunization. A lot of hematoxylin-positive cells were present in the lymph nodes with immunization. These findings demonstrate that the methods used in the present study could immunize the iliac lymph node of rats.

Effects of immunization on changes in the cell number or cellular subsets from efferent lymph vessels of iliac lymph nodes in control and HDT rats. The number of cells from the iliac lymph nodes in the control rats with immunization (0.75 ± 0.18 × 10⁷/ml, n = 5) was not significantly different from that obtained with immunization. The number of cells from the iliac lymph nodes in the HDT rats with immunization (3.69 ± 0.13 × 10⁷/ml, n = 5) was not significantly different from that obtained with nonimmunized ones (3.76 ± 0.62 × 10⁷/ml, n = 9).

In the present study, 81–95% of cells from the iliac lymph nodes in the control rats without (n = 6) and with (n = 5) immunization or HDT rats without (n = 7) or with (n = 5) immunization were lymphocytes. There was no significant difference in the percentage of lymphocytes between the control and HDT rats without immunization (Fig. 4B). There was no significant difference in the percentage of CD3+, CD4+, CD8a+, or CD45R+ lymphocytes from the iliac lymph nodes between the control and HDT rats with immunization (Fig. 4C). In addition, there was no significant difference in the percentage of lymphocytes expressing the same antigen without and with immunization. These findings indicate that neither HDT nor immunization caused a significant difference in lymphocyte components drained from the iliac lymph nodes.

We also studied the effects of HDT on lymphocyte subsets from the mesenteric lymph nodes in the control and HDT rats without immunization. There was no significant difference in the percentage of CD3+ (control, 82 ± 2%; HDT, 78 ± 4%), CD4+ (control, 54 ± 3%; HDT, 54 ± 2%), CD8a+ (control, 18 ± 1%; HDT, 16 ± 1%), or CD45R+ (control, 10 ± 2%; HDT, 13 ± 3%) lymphocytes from the mesenteric lymph nodes between the control (n = 5) and HDT (n = 5) rats without immunization.

Concentration of lymph albumin in afferent or efferent lymph vessels of iliac lymph nodes between control and HDT rats. There was no significant change in the concentration of lymph albumin in afferent lymph vessels of the iliac lymph nodes between the control and HDT rats during lymph drainage for 3 h (Fig. 5A). In a corresponding period, no significant difference in the concentration of albumin was observed between the control (1.8 ± 0.2 g/100 ml at 1 h, n = 4) and HDT (1.7 ± 0.1 g/100 ml at 1 h, n = 4) rats.

There was no significant change in the concentration of lymph albumin in efferent lymph vessels of the iliac lymph nodes between the control and HDT rats during lymph drainage for 3 h (Fig. 5B). In each corresponding period, no significant difference in concentration of albumin was observed between the control (1.9 ± 0.1 g/100 ml at 1 h, n = 4) and HDT (1.8 ± 0.3 g/100 ml at 1 h, n = 4) rats.

DISCUSSION

Effects of short-term HDT on flow rate and number of cells in efferent lymph vessels of iliac or mesenteric lymph nodes. Larger animals such as dogs, sheep, cows, and rabbits are useful for collecting lymph from popliteal and mesenteric lymph nodes as well as the thoracic ducts. In contrast, in small animals, mainly rats, the collection of lymph is limited by the smaller size of the lymph vessels that permit cannulation of tubing. In the present study, like other investigators (36), we successfully collected lymph from the iliac lymph nodes of Wistar rats, and the procedure and HDT treatments enabled us to evaluate the effects of simulated microgravity on lymph flow rate and cellular output from the iliac lymph nodes, which could drain the interstitial fluids of the lower extremities.

Hargens et al. (11) reported that HDT influenced interstitial fluid pressures in subcutaneous and muscular regions of the lower legs in humans, while colloid osmotic pressures of blood and interstitial fluid were unchanged in response to HDT. An increase in calf circumference and decreases in lower leg volume and soleus muscle water content also occurred after HDT. They therefore concluded that leg tissues dehydrate after an initial loss of venous volume. Even though it is clear that the reduction of venous volume in the lower extremities initially leads to changes in the fluid dynamics between the capillary and interstitial space, there are only a few studies on the effects

Table 1. Length (short and long), thickness, and wet weight of the iliac lymph nodes with and without immunization

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Short Length, mm</th>
<th>Long Length, mm</th>
<th>Thickness, mm</th>
<th>Wet Weight, μg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Without immunization</td>
<td>3/6</td>
<td>2.6 ± 0.2</td>
<td>5.1 ± 0.5</td>
<td>1.1 ± 0.1</td>
</tr>
<tr>
<td>With immunization</td>
<td>3/6</td>
<td>3.0 ± 0.2</td>
<td>6.6 ± 0.6</td>
<td>1.4 ± 0.1*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n and N indicate nos. of animals and tissues, respectively. *Significant difference (P < 0.05) from the nodes without immunization.
of simulated microgravity on lymph dynamics. Bulekbaeva et al. (3, 4) demonstrated the short-term (30 min) effects of HDT on lymph flow of the dog thoracic ducts. In their investigations, lymph flow in the thoracic ducts increased after stimulation at 1 min and returned to control values nearly 10 min later. On the other hand, lymph flow in the cervical lymph vessels was reduced after stimulation at 1 min and remained depressed during the course of 30-min HDT. They therefore suggested that changes in the simulated microgravity by HDT significantly influence transient changes in central lymph flow due to the direct redistribution of body fluids to the cranial part of the body. In the present study, we first demonstrated the effects of HDT posture in Bollman’s cage on lymph flow rate from the lower extremities and mesentery of rats. HDT for 3 h in the present study did not cause significant changes in the lymph flow rate from the iliac lymph nodes in the control and HDT rats. There was no significant difference in flow rate of the iliac lymph nodes between the control and HDT rats. HDT slightly increased the lymph flow rate from the mesentery of rats. These findings suggest that short-term HDT posture did not affect the lymph flow rate in the lower extremities of rats. We suppose that during HDT the blood volume shift from the lower to upper extremities will reduce capillary filtration, and then the maintenance of lymph drainage may accelerate a decrease in fluid volume in the interstitial space, explaining why HDT induces leg tissue dehydration (11).

In comparing the dynamics of lymph flow rate from the lymph nodes, an interesting observation was made regarding lymphocyte mobilization. The numbers of cells from the iliac lymph nodes in the control and HDT rats were $0.33 \pm 0.15 \times 10^7$/ml and $3.76 \pm 0.62 \times 10^7$/ml, respectively, after the stimulation at 1 h, indicating that HDT caused ~10-fold mobilization of lymphocytes from the iliac lymph nodes. In contrast, the numbers of cells from the mesenteric lymph nodes in the control and HDT rats were $3-5.4 \times 10^7$/ml, and there was no significant difference between the control and HDT rats. Changes in lymph flow in response to HDT have been investigated in vivo (3, 4). In addition, the effects of short-duration spaceflight (2–11 days) on leukocyte subpopulations in blood were measured before and immediately after landing (12, 31). The present study is the first to offer information on the effects of HDT posture on cell output from lymphoid tissues, suggesting that the HDT elicits transient cell mobilization from the iliac but not mesenteric lymph nodes of rats. We speculate that short-term HDT may affect the lower extremities of rats rather than the mesenteries and that adhesion of lymphocytes in the iliac lymph nodes may be released by the HDT posture. The precise mechanisms that produced the heterogeneity of the short-term HDT-mediated lymph flow rate and lymphocyte mobilization between the iliac and mesenteric lymph nodes remain unclear. However, morphological differences in the distribution of the lymph nodes as shown in Fig. 1C, and physiological differences in absolute value of lymph flow rates and lymph content between the iliac and mesenteric lymph nodes may be, in part, related to such mechanisms. In addition, leg muscle contractions during Bollman’s cage and HDT posture of our rats may also contribute to the observed results. Further investigations will be needed to clarify the precise mechanisms.

Changes in cardiovascular function in response to microgravity occur frequently in astronauts who stay in space for days or months. At an early stage (minutes to hour), circulatory alternation was already observed in the study using HDT. The duration of simulated microgravity in rats in the present study was very short due to the experimental conditions. Thus the changes in lymph flow rate and cells from the iliac lymph nodes in the present study may occur quite soon after stimulation of simulated microgravity. The present study was limited, to studying only transient (<60 min) HDT-mediated changes in the lymph circulation, because we needed at least 1 h for collection of a sufficient volume of lymph for the analyses. Another limitation related to the investigation period, i.e., that we could not control the animals for long-term HDT (>3 h), should be noted. In the future, further methodological improvements will be needed to investigate the effects of HDT on lymph circulation in rats in vivo experiments.

Effects of immunization on lymphocyte components of iliac lymph nodes. In the present study, the studies using flow cytometry demonstrated that >80% of cells from the iliac lymph nodes in the control or HDT rats were lymphocytes and there was no significant difference in the percentage of lymphocytes between the control and HDT rats. Subsequent analyses also showed there were similar distributions of CD3+, CD4+, CD8α+, or CD45R+ cells from efferent lymphatics of the iliac lymph nodes in the control and HDT rats. Both in the control and HDT rats, CD3+ and CD45R+ cells were 91–94% and 4–6%, respectively, indicating that mainly T lymphocytes emerged from the iliac lymph nodes. Wang et al. (36) reported that CD4+ and CD8+ lymphocytes from iliac lymph nodes of Wistar rats were 53 ± 1% and 18 ± 1%, respectively. This distribution is quite similar to our present results (CD4+: 51 ± 4% in control, 40 ± 7% in HDT; CD8a+: 21 ± 3% in control,
 Meanwhile, Miura et al. (17) demonstrated that in mesenteric lymph nodes of rats, CD3+, CD4+, CD8a+, or CD45R+ cells from efferent lymphatics of the immunized rats between the control and HDT. These findings suggest that local immunization may not affect the subsets of lymphocytes collected from the iliac lymph nodes of rats.

Effects of short-term HDT on concentration of lymph albumin in afferent and efferent lymph vessels of iliac lymph nodes. The concentration of lymph albumin in afferent lymph vessels is considered to be one of the potent factors in regulating lymphocyte output from lymph nodes (15, 27). Measured values of albumin concentration of lymph in afferent lymph vessels of the HDT rats were quite similar to those obtained from the controls. These similarities were also observed in efferent lymphatics. Our findings suggest that short-term HDT did not affect the concentration of lymph albumin in pre- and postnodal lymph vessels, and the mechanisms of albumin concentration-dependent lymphocyte mobilization from the lymph node were not involved. Physical stimuli to skin such as massage or vibration significantly increase lymphocyte counts in dogs and rabbits following augmentation of lymph flow rate and concentration of lymph albumin (13, 25). In contrast to these physical factors, it has been revealed that chemical factors including chemokines and cytokines play a significant role in lymphocyte transport in lymph nodes (19, 34). Not only the neuroendocrine system but also cardiovascular function contribute to the regulation of immunity in space medicine (33). The present study could not determine the mechanisms regulating lymphocyte transport in response to the HDT. In addition, it may be noteworthy that there are limitations with study actually activated the iliac lymph nodes of rats. However, there were no significant differences in the distributions of CD3+, CD4+, CD8a+, or CD45R+ cells from efferent lymphatics of the immunized rats between the control and HDT. These findings suggest that local immunization may not affect the subsets of lymphocytes collected from the iliac lymph nodes of rats.

Fig. 4. A: percentage of lymphocytes from the iliac lymph nodes in control rats without (n = 6) and with (n = 5) immunization, or HDT rats without (n = 7) or with (n = 5) immunization. B: percentage of CD3+, CD4+, CD8a+, or CD45R+ lymphocytes from the iliac lymph nodes in control (n = 6) and HDT (n = 7) rats without immunization. C: percentage of CD3+, CD4+, CD8a+, or CD45R+ lymphocytes from the iliac lymph nodes in control (n = 5) and HDT (n = 5) rats with immunization.

Fig. 5. Concentration of albumin (g/100 ml) of lymph in afferent (A; n = 4) and efferent (B; n = 4) lymph vessels of the iliac lymph nodes of control and HDT rats.
our combination of Bollman’s cage and HDT used to simulate microgravity accurately because the rats bear weight and probably contract their leg muscles in this model of HDT. Further investigation will be needed to evaluate neural and/or humoral factors that control simulated microgravity-mediated changes in lymph and lymphocyte dynamics.

**REFERENCES**


