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短　報（査読あり）

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身体活動量が重要テーマである動物実験における活動量計使用の提言：パイロット研究

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Key Words：physical activity, calorimeter, circadian rhythm, lifestyle diseases, rodents

Introduction

Since the beginning in the 21st century, not only in developed but also in developing countries, the importance of physical exercise in preventing the occurrence of lifestyle diseases including disuse syndrome which is followed by bedridden state accompanying dementia has been loudly advocated [1-6]. Through such campaigns, varied forms of pedometers have been developed and included in numerous medical researches [7,8]. Recently, by taking advantage of the accumulated know-how on the pedometer, especially in Japan, a variety of calorimeters have been marketed and clinical studies using them have started [9].

Contrary to clinical studies, until a kind of pedometer for the human was applied to the rats (Sasaki, 2015) [10], neither pedometer nor calorimeter has been introduced into animal experiments using rodents. Currently reliable method for measuring physical activity in rodents is the use of an indirect calorimetry specific for them [11,12] which is very expensive. Instruments other than the calorimetry cannot be used in conventional cages without video systems and/or implanted sensors [13,14]. Moreover, even with such devices it is impossible to examine activity patterns of more than one animal at a time.

Generally in animals, as in humans, total amount of energy expenditure (TEE) is composed of the basal metabolic rate (BMR), physical activity related energy expenditure (AEE), the thermic effect of food (TEF) and the energy expenditure due to thermoregulation (TE) [11]. Concerning physical exercise, in laboratory rats confined to cages at markedly restricted physical activity, resting energy expenditure (REE)-- a substitution for BMR [12]--, AEE, and TEE was measured, with the calorimetry, by Ichikawa et al [15,16] : the ratio of REE to TEE and that of AEE to TEE were 90% and 10 %, respectively. From these results, it was explained that this profile of energy expenditure in laboratory rats in cages is virtually identical to that of very sedentary elderly persons living in nursing homes [17].

The present study was planned to form the basis for the development of calorimeter specific for rodents and to alert researchers to study with such devices. And expecting that they would finally find any clue to prevent lifestyle diseases, it was challenged to measure the daily activities of rats with one type of calorimeter designed for humans.

Materials and Methods

The protocols adopted in this experiment were approved by the Committee on the Use of Animals for Teaching and Research of Ehime Prefectural University of Health Sciences (Approval No : 2015-006). Six male Wistar rats aged five to six months weighing about 400g were used. These rats were born from some pairs obtained periodically from Japan SLC Co., Ltd. (Hamamatsu, Japan). All rats were housed in a light-

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cycled (07:00, on ; 19:00, off) and air-conditioned room (20℃, 50-60%), and permitted free access to food (MF ; Oriental Yeast Co., Ltd., Tokyo, Japan) and water. Usually the rats were kept in pairs in usual cages (CL-108-1 ; CLEA Japan Co., Ltd., Tokyo) of size 276㎜×445㎜×204㎜. To avoid conflicts between host and new roommate or cagemates, pairings were not changed throughout the experiment [18,19].

As the calorimeter, EW-NK32 (Panasonic Co., Ltd., Osaka, Japan) was chosen: its size and weight including a battery was 50.0㎜×29.5㎜×13.2㎜ and about 20g, respectively (Fig. 1, left). Although EW-NK32 was available in multiple body colors, worrying that other than white would be too conspicuous to the cagemate, white was selected as the color was same as that of rat hair.

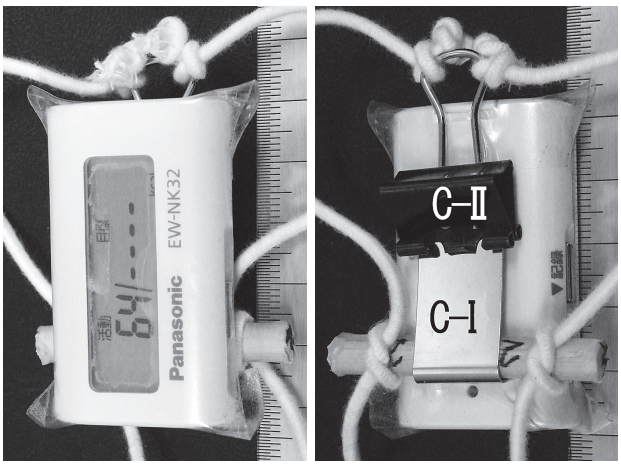

Fig.1 : (left) Front view of the EW-NK32 whose surface was sealed with a piece of clear vinyl tape to protect both its display and buttons from destruction by rats.
(right) Rear view of remodeled EW-NK32 which took a backpack-like appearance.
C-I : The clip which had been prepared for in advance. C-II : The lateral handle-free binder clip whose claw end was tucked into the claw end of C-I.

In order to fit these calorimeters into the rats, each was remodeled as follows. First of all, a wooden rod (Diameter, 6㎜ ; Length, 45㎜ ; Weight, 0.6g) was inserted into a gap between the back cover of EW-NK32 and the handle of the clip (C-I) which was prepared for in advance (Fig. 1, right). Next, from a binder clip (C-II, Width ; 19㎜) one handle was removed, and the claw end of lateral handle-free C-II (Weight ; 2.6g) was tucked into the claw end of C-I. Then, two rubber ropes (Thickness ; 2㎜, Elongation percentage which seems to be an important factor both in wearing and putting off the device ; 260%, Color ; white, Clover Co., Ltd., Osaka, Japan) whose length of about 15㎝ (Weight ; 0.6g) were tied to the handle left on C-II. Finally, other end of each rope was tied to right and left end of the rod, respectively. At this time, EW-NK32 became about 24g in weight, took a backpack-like appearance.

Before making rats to wear the remodeled EW-NK32, its surface was sealed with a piece of clear vinyl tape to protect both its display and buttons from destruction by rats' bites if it was dislodged (Fig. 1, left). Prior to measurement, five kinds of the host's physical data which the device could set up as the minimum were input : weight, 10Kg ; height, 100㎝ ; age, six year-old ; gender, male ; step length, 30㎝. For measurable items, as many as eight items including steps were prepared in EW-N K32, however, like one device used in the previous study [10], as, instead of a pendulum, a type of "3D Accelerometer Sensor Filter" was installed to be unresponsive to a few steps, adoption of steps was abandoned. Finally, only two items that is AEE/day and TEE/day were adopted.

Although most of animal experiments utilizing indirect calorimetric technologies were started after 24-h fasting, in consideration for daily use as in humans, both food and drinking water were not restricted throughout experiment. The remodeled EW-NK32 could be worn by a rat under slight anesthesia (Fig. 2).

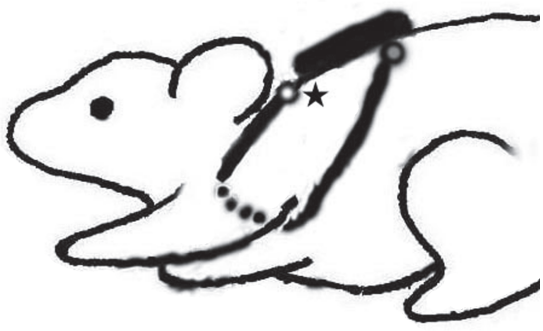

Fig.2 : Illustration of the remodeled EW-NK32 worn by a sitting rat in a side view. ★ : The remaining handle of C-II which was tied by shoulder straps of both sides and positioned towards the rat head.

As to the position of the device on the rat, the claw end of C-I was positioned towards its head. Measurement continuing for 24-h was done every second or third day. Because at 02:00 am, the value recorded by EW-NK32 was automatically reset and new measurement was resumed, starting time of the measurement was set on 14:00 ± 60min. AEE/24h and TEE/24h was divided into two parts : those of the first day (AEE-YD/TEE-YD)

and of the second day (AEE-YD/TEE-TD). AEE/24h and TEE/24h was divided into two parts : those of the first day (AEE-YD/TEE-YD) and of the second day (AEE-YD/TEE-TD). Experiment was executed eight times at least per animal ; meanwhile, both water and food were not exchanged and replenished. At the end of each examination, the remodeled EW-NK32 could be put off quickly without anesthesia. Although as the unit of the amount of energy expenditure, Cal or kilocalorie was prepared in EW-NK32, dividing by 4.184, each value was expressed as mean ± SD KJ. Obtained data were analyzed using the t-test.

Results

Except for a few slipping off-accidents of the remodeled EW-NK32, the experiment was executed well. Results of energy expenditures measured with the remodeled EW-NK32 in six male rats were shown in Fig. 3. AEE-YD and AEE-TD was 40.4 ± 4.0KJ and 33.8 ± 2.5KJ, respectively. The ratio of AEE-YD to AEE-TD was 119.7 ± 9.0%. There was a significant difference between AEE-YD and AEE-TD ($p<0.05$). The sum of AEE-YD and AEE-TD or AEE/day was 74.2 ± 5.8KJ.

TEE-YD and TEE-TD was 197.0 ± 2.2KJ and 117.2 ± 7.8KJ, respectively (Fig. 3).

The ratio of TEE-YD to TEE-TD was 168.7 ± 12.1%. There was a significant difference between TEE-YD and TEE-TD ($p<0.05$). The sum of TEE-YD and TEE-TD or TEE/day was 314.2 ± 7.8KJ. Proportion of AEE/day to TEE/day was 23.6 ± 1.9% (Fig. 4).

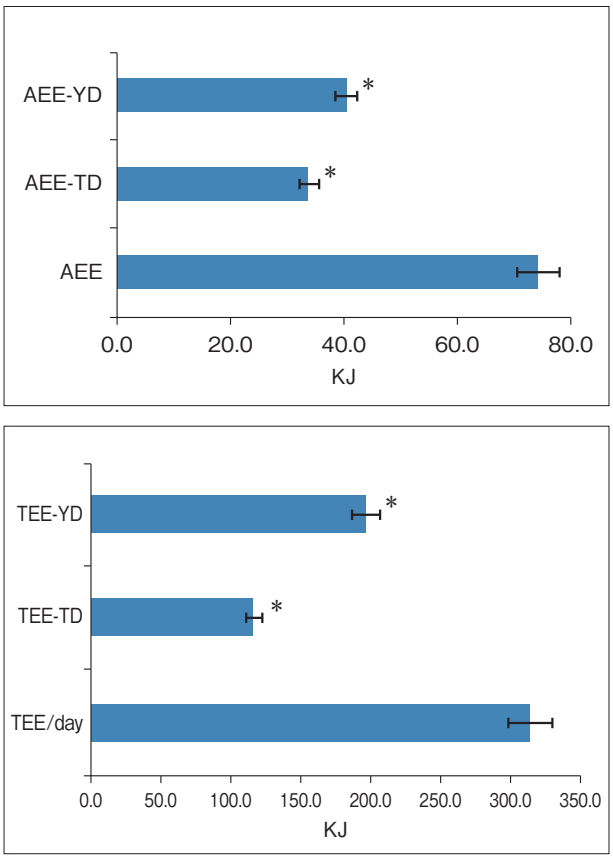

Fig.3 : Bar graphs showing AEE/day (upper) and TEE/day (lower) in six rats.
AEE-YD/TEE-YD : AEE/TEE recorded between 14:00 and 02:00, AEE-TD/TEE-TD : AEE/TEE recorded between 02:00 and 14:00, * : $p< 0.05$.

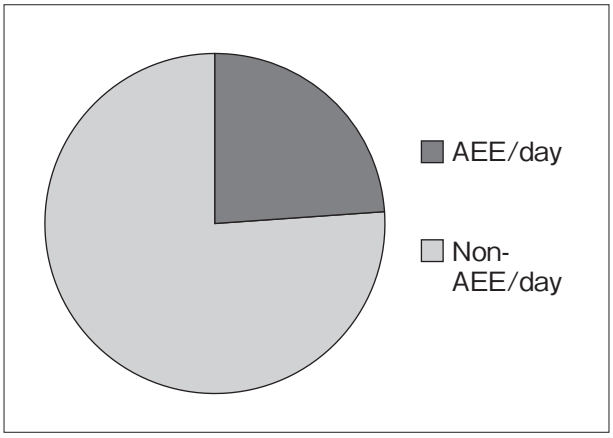

Fig.4 : The circle graph showing the proportion of AEE/day to TEE/day in six rats.

Discussion

In the present study, for the first time in more than one rat living in usual cages, 24h-AEEs were measured with one type of calorimeter designed for humans or EW-NK32. In other words, physical activities in their daily lives could be recorded with neither large scale instrument nor experimenter intervention.

TEE could be recorded as about 300KJ in the present study. However, the item includes BMR which was calculated from data of six year-old boys (e.g. 234.2KJ)[20]. Therefore, the value of TEE in the preset study is much higher than that of the rats (7.5-9 KJ) which was measured with a full-fledged instrument or a calorimetry [11,12]. In other words, real value of the rat's TEE other than BMR, that is, those of AEE, TEF, and TE had been buried into BMR, so results especially concerning TEE may be called into question.

Nonetheless, being apart from the real values of AEE and TEE, the ratio of AEE-YD to AEE-TD is thought not only to be meaningful but also worthy of claiming researcher's attention. In experiment of Kurogochi et

al [21], locomotion activity in male Wistar rats declined abruptly from dawn to morning. From their data, the ratio of sum of the locomotive activities in the first day to that in the second day was calculated by the author ; the ratio was 61 : 39 or locomotive activities in the first day was about 1.5 times higher than that in the second day. In the present study, the proportion of AEE-YD to AEE-YD and that of TEE-YD to TEE-TD was 120 and 169 %, respectively (Fig. 3). Both the results of Kurogochi et al [21] and those of the present study may reflect the circadian rhythm of physical activity in rats [22]. Contrary to above-mentioned results, the ratio of AEE to TEE was 1:3 (Fig. 4) ; which was different from that obtained by Ichikawa et al [15,16], that is, 1:10. This discrepancy may be due to the difference in the used instrument ; they used the indirect calorimetry.

From the viewpoint of the animal welfare, the some doubt that the weight of the remodeled EW-NK32 or about 24g becomes an overload for the rat is left. However, such a doubt was proved to be groundless as in the case of remodeled pedometers [10] ; it is thought that the 24g load which was nearly equal to the remodeled pedometer was never an overload for the host rat.

In conclusion, although some doubts about precision with the use of human calorimeters exist, the devices enabled 24-h measuring of physical activity in six rats. The present study would form the basis for the development of the pedometer or calorimeter specific for rodents. If pedometers and/or calorimeters are adopted in animal experiments, knowledge obtained from subsequent researchers would contribute to control not only lifestyle diseases but also dementia following bedridden state.

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Abstract

As in clinical studies concerning the exercise that is effective to control life-style diseases, the energy expenditure is a pivotal item, so experiments using calorimeter have been started in Japan. It had been found by Ichikawa et al (1987) that the profile of energy expenditure in laboratory rats in cages is virtually identical to that of very sedentary elderly persons living in nursing homes. However, into animal experiments especially in rodents, neither pedometer nor calorimeter has been introduced. Recently, the usefulness of a pedometer designed for humans was proven in rats (Sasaki, 2015). In the present study, with Panasonic EW-NK32, in rats living in usual cages, 24-h measurements of both physical activity related energy expenditure (AEE) and total amount of energy expenditure (TEE) were executed. AEE and TEE was divided into two portions; that of the 1st day (AEE-YD) : that of the 2nd day (AEE-TD) and TEE-YD : TEE-TD, respectively. AEE-YD and AEE-TD was 40.4 and 33.8KJ, respectively. AEE-YD／AEE-TD was 119.7% ($p<0.05$). TEE-YD and TEE-TD was 197.0 and 117.2KJ, respectively. TEE-YD／TEE-TD was 168.7% ($p<0.05$). AEE/day／TEE/day was 23.6%. Although, the accuracy of real values may be called into question, ratios between items were thought to be meaningful; AEE-YD／AEE-TD and TEE-YD／TEE-TD may reflect the circadian rhythm in physical activity in rats. The present study would form the basis for the development of the pedometer or calorimeter specific for rodents, and knowledge obtained from experiments using such devices would contribute to control not only life style diseases but also dementia following bedridden state.

生活習慣病の研究ではエネルギー消費量(EE)が重要テーマである。著者(2015)はヒト用歩数計でケージ内ラットの24-h歩数を測り齧歯類専用機器開発を提唱した。今回はヒト用活動量計で24時間，ケージ内ラットの身体活動時EE (AEE) と総EE (TEE)を測定した。AEE／TEEは，装着時(14:00)～翌日02:00迄のAEE(AEE-YD)／TEE(TEE-YD)と，02:00～終了時(翌日14:00)のAEE(AEE-TD)／TEE(TEE-TD)に分けた。AEE-YD／AEE-TDは，40.4KJ／33.8KJ，合計(AEE/day)は74.2 KJ，AEE-YD／AEE-TDは119.7%($p<0.05$)であった。TEE-YD／TEE-TDは，197.0KJ／117.2 KJ，TEE/dayは314.2KJ，TEE-YD／TEE-TDは168.7%($p<0.05$)，AEE/day／TEE/dayは23.6%であった。結果は身体活動の日内リズムを反映しヒトの健康に貢献すると考えられる。

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