Effect of an Intermittent Eight-hour Joint Fixation Period on Joint Contractures in Rats

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Abstract. [Purpose] It has been shown that an eight-hour joint fixation daily (i.e. for the remaining 16 hours, rats were set free in the cage without joint fixation) for one week induces a joint contracture. The purpose of this study was to investigate the difference between an 8 hours continuous joint fixation and an 8 hours intermittent joint fixation per day (two 4 hours joint fixations) in the development of joint contractures in rats. [Subjects and Methods] The subjects of this study were 34 Wistar rats. On the first day, all of the rats’ right ankle dorsiflexion angles were measured. The right hind limbs of all the rats were fixed in plantar flexion, and then the rats were divided into three groups as follows: Group 1 was in continuous fixation for 24 hours a day; Group 2 was in continuous fixation for 8 hours a day; Group 3 was in intermittent fixation for 8 hours a day. The fixation was performed daily for seven consecutive days. On the last day, ankle dorsiflexion angles of all the rats were measured after the casts had been removed. [Results] The development of joint contractures was statistically significant for Groups 1 and 2. [Conclusion] Our results indicate that 8 hours a day of continuous fixation induces a joint contracture, but 8 hours a day of intermittent fixation does not.

Key words: Joint contracture, Immobilization, Animal study

INTRODUCTION

Restriction of joint motion is one of the symptoms of disuse syndrome. We previously reported that joint fixation for 12 hours per day (i.e. for the remaining 12 hours, rats were free to move in the cage without joint fixation) initiated the development of a joint contracture during an experimental period lasting 7 days, and an 8 hours joint fixation daily (i.e. for the remaining 16 hours, rats were free to move in the cage without joint fixation) induced a joint contracture in a week, but a 4 hours joint fixation daily (i.e. for the remaining 20 hours, rats were free to move in the cage without joint fixation) did not induce a joint contracture in one week. The following question arose from our results: that is, whether 4 hours joint fixation twice daily would induce a joint contracture or not.

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The purpose of this study was to investigate the difference between an 8 hours continuous joint fixation and 8 hours intermittent joint fixation per day (two 4 hours joint fixations) in the development of joint contractures in rats.

SUBJECTS AND METHODS

Thirty-four female 8-week-old Wistar rats with an initial body weight of 192 g to 250 g were used. This study was performed according to the Regulations on Animal Experiments of the Prefectural University of Hiroshima and was approved by the Animal Experiments Committee (Approval Number: 12 MA 003). The animals were housed in a temperature-controlled room at 23°C on a 12 hour light-dark cycle. The rats had provided free access to standard rat food and water.

On the first day of the experiment, all of the rat’s right ankle dorsiflexion angles were measured under anesthesia (sodium pentobarbital, 40 mg/kg). The right hind limbs of all the rats were fixed in plantar flexion with white tape. In this position, the soleus muscle was maintained in a shortened position. The fixation schedule of the groups is shown in Fig. 1. The rats were divided into three groups as fol-
lows: Group 1 was maintained in continuous fixation for 24 hours a day; Group 2 was maintained in continuous fixation for 8 hours a day; Group 3 was maintained in intermittent fixation for 8 hours a day. Except for the duration of joint fixation, the rats in Groups 2 and 3 were able to move freely in their cages without joint fixation. The rats in Groups 2 and 3 were anesthetized with diethyl ether for the removal of the fixation. We observed the movements of right ankle joints when these rats were moving freely in the cage. The fixation was performed daily for seven consecutive days with the right hind limbs fixed as indicated. On the last day, the ankle dorsiflexion angles of all the rats were measured after the casts had been removed under anesthesia (sodium pentobarbital, 40 mg/kg). No free movement was allowed before the ROM measurements were performed. In addition, the rats of Group 1 were anesthetized with diethyl ether every day in order to replicate the physical conditions of the other two groups.

The ROM of ankle joint dorsiflexion was measured as follows. First, the rat was positioned on its side, and a force of 0.3 N was applied perpendicularly to the sole of the foot by a tension meter (Kyowa Co., LTS-1KA)\(^5\). Then, a digital photograph was taken from directly above the hind limb, and the angle of dorsiflexion was measured with computer software (Scion image beta 4.03). To eliminate forefoot movement from the measurement, dorsiflexion ROM was defined as the angle obtained from a line parallel to the longitudinal axis of the fibula and a line parallel to the bottom of the heel. During the ROM measurement, the knee joint was flexed.

To measure the degree of muscle contracture, the length-tension curve of the soleus was determined using an Autograph (AG-50KNG, Shimadzu Co., Japan)\(^5\). Under anesthesia (sodium pentobarbital, 40 mg/kg), the ankle was positioned in full plantar flexion, and the calcaneus and tibia were fixed with a 0.7 mm Kirschner wire. Then, skin and the gastrocnemius were removed surgically. After sacrificing the rat, the femur was cut above the origin of the gastrocnemius and was mounted on the Autograph with the upper clamp. The tarsal bone was mounted with the lower clamp. The tibia, fibula, and all lower leg muscles, except for the soleus, were cut, and the tension torques were measured at the 10 mm lengthened position of the soleus muscles.

Data are expressed as means ± standard deviations. Two-way analysis of variance was used for the analysis of the ankle joint dorsiflexion ROM. The independent variables were the day of the ROM measurement (the first experiment day × the last experiment day) and the groups (group 1 × group 2 × group 3). The dependent variable was the angle of dorsiflexion. If a significant interaction was found, the simple main effect was calculated for each independent variable. Also, the significance of differences between the

### Table 1. The ROM of ankle joint dorsiflexion (in degrees)

|            | Group 1\(^*\) | Group 2\(^*\) | Group 3\(^*\) |
|------------|---------------|---------------|---------------|
| first day  | 42.7±3.5      | 43.0±3.4      | 42.7±3.7      |
| last day   | 76.8±11.1     | 49.0±4.1      | 44.7±4.4      |

Values are means ± SD, * p<0.05; between first and last experimental day

### Table 2. The degrees of muscle contractures shown in torque tension (in Newton)

|         | Group 1\(^*\) | Group 2\(^*\) | Group 3\(^*\) |
|---------|---------------|---------------|---------------|
| right fixed side | 0.47±0.19    | 0.09±0.03    | 0.13±0.04    |
| left non fixed side | 0.11±0.06   | 0.12±0.05    | 0.07±0.05    |

Values are means ± SD, * p<0.05; between right and left soleus muscles

The movement of right ankle joint was observed in Groups 2 and 3 when the rats were freely walking and resting in the cages.

The average and standard deviation of the dorsiflexion angles of the first and last days of the experiment of each group are shown in Table 1. Statistically, it was shown that there was interaction between the independent variables. Therefore, the simple main effect was calculated for each independent variable. First, the independent variable for the day of the ROM measurement in the first experimental day showed no significant difference between the groups; but on the last day of the experiment, there was a significant difference among the groups. Second, the independent variable the groups showed a significant difference between the first and last experimental days in Group 1 and Group 2, but not in Group 3. These results indicate that, statistically, the development of joint contractures was seen in Groups 1 and 2.

The average and standard deviation of the right and left tension torques at the 10 mm lengthened point of the soleus muscle of each group are shown in Table 2. Statistically, there was a significant difference between the right and left soleus muscles in Group 1.

### DISCUSSION

A few studies have commented on the tissues that are believed to be responsible for the limitation of ROM of joints after immobility. The relationships between the changes of tissue elasticity and periods of joint immobilization were as
follows: the skin and skeletal muscle elasticity decreases in the first week\(^6\); joint capsule elasticity decreases over four weeks\(^7\); ligament elasticity increases after eight-weeks\(^8\). These reports indicate that the cause of the joint limitations in Groups 1 and 2 were the skin and soleus muscle. There was a significant difference between the right and left tension torques in Group 1, indicating that skin and muscle caused the joint contractures. In the test for muscle contracture using the length-tension curve of the soleus muscle, there was no significant difference between the right and left tension torque in Group 2. There was no relationship between the joint contracture and tension torque of the soleus. Therefore, these results suggest that the cause of the joint contracture in Group 2 was the skin.

We wanted to clarify whether there is a difference in the development of a joint contracture between 8 hours intermittent joint fixation and 8 hours continuous joint fixation. Such an experiment has not been conducted in the past. The results of Group 2 showed that an 8 hours continuous joint fixation induced some kind of changes in the skin. The skin had not recovered its normal elasticity after the rats had completed 16 hours free joint movement in the cage. The decreased skin elasticity induced the development of joint contractures. We suggest that the important difference between Group 2 and Group 3 was the 4 hours free joint movement in the cage after 4 hours of joint fixation. Therefore, the results for Group 3 indicate that 4 hours of free joint movement in the cage after 4 hours of joint fixation preserved the normal elasticity the skin.

We conclude that 8 hours continuous joint fixation per day induces joint contracture in one week, but 8 hours intermittent joint fixations per day prevents the development of joint contractures in rats. Further investigation is necessary to clarify how many hours is the minimum exercise time necessary for 8 hours intermittent joint fixations.

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