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IIIII Microgravity Experiments by Aircraft Parabolic Flights Ⅰ IIIII
(Review)

Utilization of Parabolic Flight for the Investigation of Neuromuscular Responses to Altered Gravity Levels in Rats

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Abstract

The microgravity-induced deterioration of skeletal muscle properties is one of the health problems, which are the serious medical concerns for the manned long-duration spaceflight mission. Responses of the activities of electromyogram (EMG) in hindlimb muscles and afferent and efferent neurograms at the L_5 segmental level of spinal cord in conscious rats to altered levels of gravity were studied using parabolic flight of jet airplane. In soleus, EMG was increased in response to elevation of G during the ascending phase, but was eliminated in microgravity (μ-G) environment. Similar response of the level of afferent, not efferent, neurogram was also noted. These phenomena were related to the shortening of muscle due to plantarflexion of ankle joints. As for adductor longus, region-specific responses were observed. The EMG activity in the caudal, not rostral, region decreased and activity patterns were changed from tonic to phasic during μ-G exposure. These phenomena were related to shortening of fibers, caused by abduction of hip joints and extension toward backward. These results clearly indicated that unloading-related undesirable adaptation of antigravity muscles is closely related to inhibition of mechanical and/or neural activities.

Keyword(s): Microgravity, parabolic flight, electromyogram and neurogram, rat soleus and adductor longus

1. Introduction

It has been well-reported that atrophy, associated with shift of myosin heavy chain profiles toward fast-twitch type, occurs in skeletal muscle, such as soleus and adductor longus (AL), following long-term inhibition of antigravity activities caused by spaceflight[1-3,13,16]. These physiological adaptations to microgravity (μ-G) environment are serious medical concerns, since the normal antigravity activities are inhibited on the Earth especially after long-term spaceflight. Therefore, it is essential to investigate the mechanism responsible for such physiological responses to gravitational unloading and develop suitable countermeasures.

Similar responses in various physiological properties are also induced in response to simulation models, such as bedrest in human[14,15,17] and hindlimb suspension of rodents[4,8,9,12,13]. Antigravity activities of skeletal muscles and bones can be inhibited by these models. But, effects of gravity (1-G) on each cell cannot be inhibited. Therefore, it is essential to expose human and/or animals to μ-G environment for investigation of the precise mechanism responsible for the negative adaptation of physiological properties. But it is not easy to perform spaceflight experiment often for such investigation. Thus, experiments using the μ-G created by parabolic flights have been often performed. In this review, we will introduce some of our previous experiments performed utilizing the parabolic flight of jet airplanes.

2. Experiments

All experimental procedures were conducted in accordance with the Japanese Physiological Society Guide and National Institute of Health Guide for the Care and Use of Laboratory Animals. This study was also approved by the Committee on the Animal Care and Use at Osaka University and National Space Development Agency of Japan. All efforts were made to minimize animal suffering and to use only the number of animals necessary to produce reliable scientific data.

3. Responses of Soleus Muscle and Motoneuron

Seven weeks old male Wistar rats were utilized [5]. Two rats were used in each flight experiment. In one rat, electromyogram (EMG) of soleus and afferent and efferent neurograms were recorded. Under anesthesia with *i.p.* injection of sodium pentobarbital, bipolar recording electrodes for EMG and neurogram were implanted into the left soleus (a predominantly slow plantarflexor) and around the left dorsal root and ventral root at the L_5 segmental level of the spinal cord, respectively. Responses of the EMGs in the left soleus, lateral portion of gastrocnemius (LG) and tibialis anterior (TA) to changes in the gravity levels were analyzed in another rat. Detailed descriptions of the intramuscular implantation of electrodes for EMG and neurogram recordings were published elsewhere[5,12].

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3.1 Parabolic Flight

Changes in the environmental gravity levels were created by the parabolic flight performed by a jet airplane (Mitsubishi MU-300, Diamond Air Service, Nagoya, Japan)[5]. A total of 9 experiments were performed. The period of each experiment was 1 h per day. Within each experimental period, 13-15 parabolic flights were repeated. As is shown in **Fig. 1**, various levels of gravity between 0 and 2-G were created during the ascending and descending periods. The conscious rat, kept in a plexiglass box of 40 x 30 x 30 (height) cm, was exposed to μ-G for approximately 20 sec in each parabolic flight 2-3 days after complete recovery from the surgery. The recordings of neuromuscular activities, as well as video filming, were performed continuously.

3.2 Recordings and Analyses of Data

The recordings were performed using a telemetry system[5]. The neural signals were amplified and recorded on a digital audio tape recorder. The amplified raw signals, stored in the cassette tape recorder, were processed by a PowerLab/ 16sp, an analog-to-digital converter, digitized at 2 kHz per channel, and stored on disk. The integrated areas of the bursts were determined using a computer software package. The total mean integrated neural activity per second at a specific gravity level was calculated. Mean levels of 13-15 parabolic flights were also calculated for the integrated EMG and neurograms for each rat and values from 9 rats were used for statistical analyses.

In a pilot study, effects of acute change in the angle of the ankle joint on the soleus EMG and neurograms were investigated in conscious adult rats in 1-G environment. The integrated levels of EMG and afferent and efferent neurograms were decreased in response to plantarflexion of the ankle joint caused by tail suspension (unpublished observation). These activities were immediately restored, when the ankle joint was dorsiflexed after termination of tail suspension. It is suggested that the levels of both soleus EMG and neurogram are closely associated with the ankle joint angles.

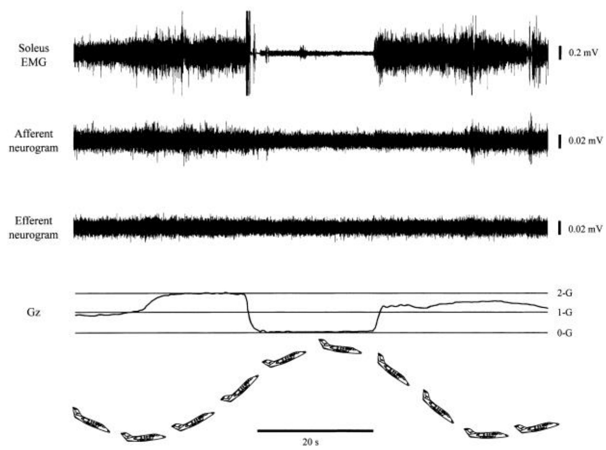

Fig. 1 Typical patterns of soleus electromyogram (EMG) and afferent and efferent neurograms at the L5 segmental level of the spinal cord of a rat during parabolic flight. The time-course changes of gravity (Gz) levels and the patterns of flight are also indicated. Cited from ref. No. 5.

The effect of ankle joint angle on the soleus muscle length was also analyzed in anesthetized rats with body size similar to the rats used in this study. The soleus was exposed and the length from tendon to tendon was measured at various angles of the ankle joint. Since the knee angle did not influence the soleus length, all measurements were performed keeping the knee angle at ~90^o. In the rats utilized for the flight experiment, the knee, ankle, and metatarsophalangeal joints were marked using black ink to analyze the ankle joint angle. The posture-maintenance patterns of rats were continuously video-filmed throughout the 13-15 parabolic flights at 60 frames per seconds. The ranges of motion of the ankle joints were analyzed using frame-by-frame analysis and the angles of ankle joints were measured to estimate the length of the soleus muscle using the results obtained from the pilot study. Synchronized data analyses were performed for the joint angles, EMG, and neurogram levels in each rat.

The EMG activity in the antigravity soleus muscle gradually increased, when the gravity was elevated from 1-G to 1.5-G (+23%) and to 2-G (+67%) during the ascending phase of parabolic flight (**Figs. 1** and **2a**). However, it was decreased about 72% from the 1-G level immediately, when the rat was

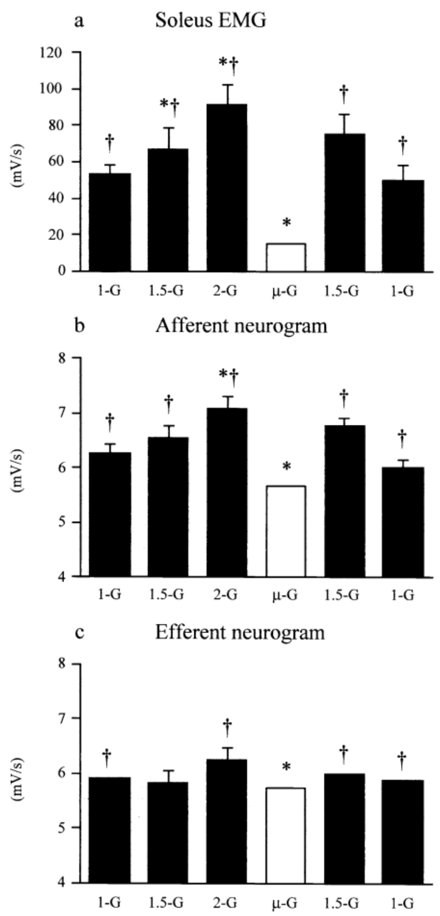

Fig. 2 Integrated levels of soleus EMG and afferent and efferent neurograms during parabolic flight. Mean±SEM. * and †: $p<0.05$ vs. 1-G (Pre μ-G) and μ-G, respectively. See **Fig. 1** for the abbreviation. Cited from ref. No. 5.

exposed to μ-G. The low EMG level that persisted during the 20-sec μ-G was restored immediately, once the gravity level increased to 1.5-G and then 1-G. The responses of afferent neurogram levels were similar to those of the soleus EMG, even though the magnitude of the reduction of integrated neurogram level in response to μ-G exposure was smaller but statistically significant (326% vs. 1-G level, **Fig. 2b**). The level of efferent neurogram was also significantly decreased, but only 39% vs. 1-G level, during the μ-G exposure (**Fig. 2c**). Both afferent and efferent neurogram levels were normalized to 1-G levels during the descending and recovery phase.

The EMG level in LG increased gradually insignificantly when the gravity level was elevated, and then decreased, when the rat was exposed to microgravity (data not shown). However, the activity level during the 20-sec μ-G was identical to that obtained at 1-G. The EMG level of TA even increased insignificantly in response to the exposure to microgravity (data not shown).

The mean angle of ankle joints was approximately 30^{o} at 1-2-G (**Fig. 3**). The ankle joints were immediately extended, when the rat was exposed to μ-G and the mean angle was approximately 160^{o}. The estimated muscle length at 30^{o} was

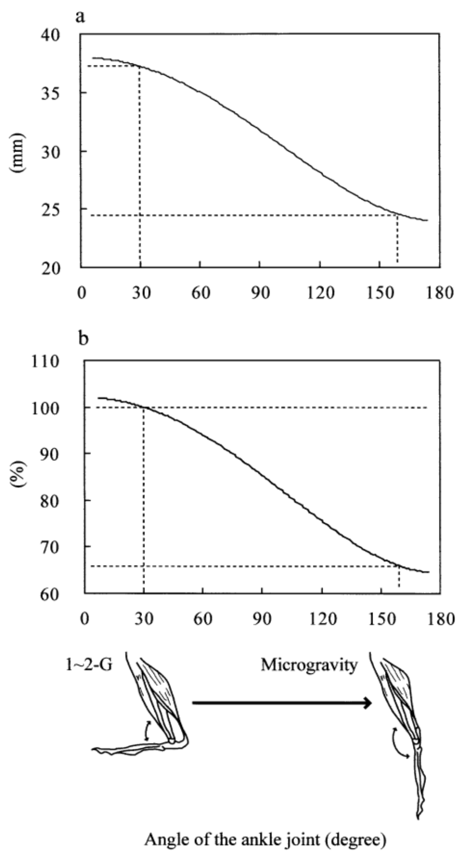

Fig. 3 The relationship between the anterior angle of ankle joint and the length of rat soleus (panel a, absolute; panel b, relative to the gravity-loaded state). The mean angle of ankle joints was ~30^{o} between 1- and 2-G. The ankle joints were plantarflexed in response to microgravity exposure and the mean angle was ~160^{o}. Cited from ref. No. 5.

~37 mm, but it was reduced to ~24 mm during μ-G exposure. The percent decrease in muscle length during μ-G exposure was ~35% relative to the gravity-loaded state.

4. Responses of Adductor Longus Muscle

The AL muscle plays an important role for adduction of hip joint in gravitational environment and is composed of mainly slow-twitch fibers. Pronounced atrophy and shift of fiber phenotype in AL muscle of Wistar Hannover rats were observed following gravitational unloading by hindlimb suspension[9,12]. However, the mechanism responsible for such adaptation was not clear. Therefore, the responses of EMG activities in rat AL to altered levels of gravity were studied in order to investigate the roles of neural and mechanical factors in the adaptation[11]. Since the characteristics of fibers in AL are region-specific, the responses of EMG and fiber length in the rostral and caudal regions, with different fiber phenotypes, to altered gravity were studied in conscious male Wistar Hannover rats. Electrode implantation was performed in both rostral and caudal regions longitudinally, as was explained for soleus muscle above.

Changes in the environmental gravity levels were created by the parabolic flight performed by a jet airplane (Mitsubishi MU-300, Diamond Air Service, Nagoya, Japan). A total of 6 experiments were performed using one rat for each experiment. Within each experimental period, ~12 parabolic, or level, flights were repeated. The level flights were performed to avoid the effects of hypergravity prior to the exposure to μ-G. Various levels of gravity between 0- and 2.5-G were created during the ascending and descending phases of flight. In 4 experiments, the time course change of the gravity level was 1→1.5→2→μ→1.5→1-G. And one flight, in which the highest G level reached to ~2.5-G, was also added at the end of each experiment. Further, μ-G was created after the level flight in the remaining 2 experiments.

4.1 Electromyogram Activity

The EMG activity of AL was tonic during quadrupedal resting position on the floor at 1-G and the EMG activity level was greater in the caudal than the rostral region ($p<0.05$, **Fig. 4**). The EMG activity in the rostral, but not the caudal, region tended to increase, when the rat was exposed to 2-G ($p>0.05$). But, the increase of EMG level was not significant, when the gravity was increased to 2.5-G (data not shown). The integrated level of EMG in the caudal region decreased significantly vs. that at 1- and 2-G, when the rat was exposed to μ-G ($p<0.05$, **Fig. 4**). But that in the rostral region at μ-G was similar to the 1-G level, although the mean level was significantly less than the 2-G level ($p<0.05$). The EMG level in the rostral region increased, when the gravity level was increased to 1.5-G during the descending phase ($p<0.05$). The increase of EMG level at 1.5-G in the caudal region was insignificant ($p>0.05$) by unknown reason, but returned to normal at 1-G ($p<0.05$).

The integrated levels of EMG in both regions were stable, when the gravity was slightly increased to 1.2-G before the entry to μ-G during the level flight (data not shown). But the EMG activity in both regions decreased, when the rat was exposed to μ-G. The reduction in the caudal region was

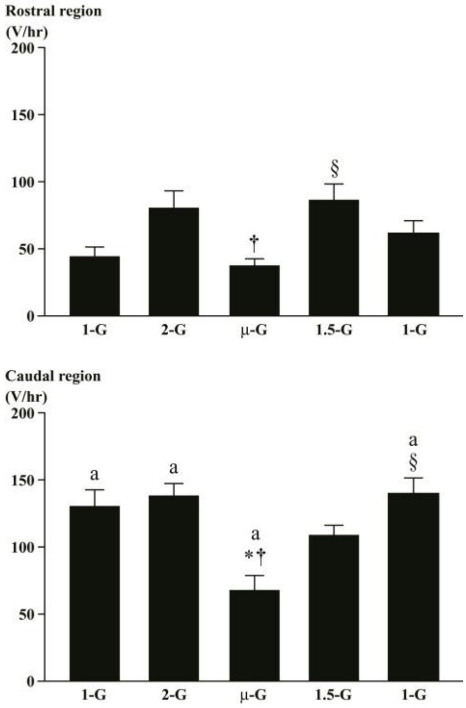

Fig. 4 Responses of the integrated electromyogram activities in the rostral and the caudal region of adductor longus muscle. Various gravity levels between ~0 (μ)-G and ~2-G were created by a parabolic flight. Mean±SEM. The mean values were obtained from 40 parabolic flights. *, †, and §: p<0.05 vs. 1-G, 2-G, and μ-G, respectively. a: p<0.05 vs. the respective G level in the rostral region. Cited from ref. No. 11.

statistically significant (p<0.05). Significant increase in EMG level was observed in both regions at 1.5-G during the descending phase (p<0.05). The patterns of EMG in both regions became phasic, when the rat was exposed to μ-G environment.

Significant decrease in EMG level was induced only in the caudal region, when the rats were exposed to μ-G (**Fig. 4**). Even though the magnitude of the unloading-related decrease of EMG tended to be greater when the prior gravity level was 2.5-G, no statistical significance was noted between three different G levels (data not shown). The EMG level in the rostral region tended to be even increased (p>0.05) in μ-G environment.

5. Responses of Joint Angles and Shape of Muscle

The responses of hip, knee, and ankle joints to changes of the gravity levels were video filmed throughout the 1-hr experiment[11]. And the joint angles were analyzed later. These angles were compared with those during a sedentary quadrupedal prone position on the floor (n=5). The rat was sacrificed by over-dose injection of sodium pentobarbital (15 mg/100 g body weight) and AL muscles were exposed. The rat was placed on a styrene form board and the hip, knee, and ankle joints in each limb were fixed at the specific angles equivalent to

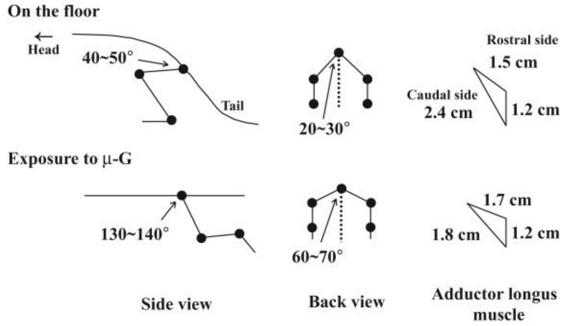

Fig.5 The mean angles of hip joint of rat and perimeters of adductor longus muscle during quadrupedal resting position on the floor and exposure to μ-G. Cited from ref. No. 11.

those either during exposure to μ-G or a sedentary quadrupedal prone position on the floor, bilaterally. The AL muscles were covered with absorbent cottons containing 4% buffered-formaldehyde and were removed after 2 hrs of fixation. Subsequently, each muscle was sampled and the shape of muscle was analyzed by measuring the lengths of outer three sides.

The mean hip joint angle from back view was approximately 20-30^{o} and that from side view was approximately 40-50^{o} during the sedentary quadrupedal position on the floor (**Fig. 5**). Pronounced change was observed in the shape of AL muscle, when the rat was exposed to μ-G environment. The hip joints were abducted to 60~70^{o} and extended toward backward to 130~140^{o} during the exposure to μ-G. Due to the changes in posture and joint angles, the length of the outer side of caudal region decreased by ~25%. But that of the rostral region was even increased (~13%).

6. Other Studies

6.1 Hoffman-Reflex in Human

Responses of Hoffman (H)-reflex in the soleus muscle to changes of gravity levels created by parabolic flight of a jet airplane were investigated in 4 healthy male subjects[7]. The subjects maintained a sitting position with seat belts fastened, keeping the anterior ankle and posterior knee angles at ~135^{o}. The gravity levels were altered from 1- to 2-G, and then μ-G was created for ~20 sec. The levels were recovered from 1.5- to 1-G during the descending phase. The time interval between the stimulation and either M- or H-wave was not influenced by the changes in gravity levels. The amplitude of the M-wave during hyper- and μ-G was identical to that obtained at 1-G. However, the H-wave amplitude was increased, when the subjects were exposed to μ-G (~4 times vs. 1-G level). The H/M ratio was also elevated during μ-G. Further, such a phenomenon was maintained throughout the 20-sec of μ-G exposure. Hypergravity at 1.5- or 2-G had no effect on the H-wave amplitude. It is suggested that an acute exposure to μ-G increases the excitability of the soleus motor pool and the increased excitability is restored immediately, when the gravity level is elevated.

6.2 Ocular Characteristics in Rats

Changes of intraocular pressure and retinal vasculars during transient exposure to μ-G in adult rats were investigated using 13 adult rats[6]. Nine rats, anesthetized by *i.p.* injection of sodium pentobarbital, were exposed to μ-G created by parabolic flight of Glufstream-II (G-II) aircraft, and 4 served as the ground controls. Intraocular pressures (IOPs) were measured by a tonometer before, during, and after the exposure to μ-G. The changes of retinal vasculars were also monitored with a fundus camera, before, during, and after the exposure to μ-G. The ratio of artery/vein (A/V) was calculated to compare. The mean IOP at 1 minute before the exposure to μ-G was 12.6±1.6 mmHg, which was identical to the ground controls (12.3±1.3 mmHg). But the mean IOP significantly increased within 20 sec during exposure to μ-G (25.8±2.0 mmHg; $p<0.001$). And the mean IOP decreased to the baseline values (14.9±1.7 mmHg) after the exposure to μ-G. Reduction in the caliber of retinal arteries was also noted during μ-G. The mean ratio of A/V during μ-G was 0.50±0.08, which was significantly less than that of control values (0.75±0.11, $p<0.05$). The increase in IOP and trend of arteries to constrict during μ-G could be related to the disproportion between intravascular and extravascular body fluids as a result of the absence of the 1-G hydrostatic gradient. Results of the study indicated that fluid shift and its effect on the eye occur rapidly, within 20 sec during exposure to μ-G.

6.3 Posture Change Due to Light and/or Gravity in Carp Fishes

It was investigated how carp fishes, *cyprinus carpio*, change the posture in response to gravity and/or light[10]. The changes of gravity levels were created by the parabolic flight of a jet airplane (G-II, Diamond Air Service, Japan). EMGs of the flexor and levator muscle, which regulate pectoral fins, and the middle portion of the lateral red muscle were recorded throughout one hour experimental period. The behavior of fins and body posture was also recorded by video filming simultaneously. Fishes clearly tilted their body posture toward the light in μ-G, not in 1~2-G, environment. The postural changes due to the dorsal light response were induced by changing the direction of light to right or left during exposure to μ-G. EMG activities of all of these muscles tended to be increased during μ-G may be due to sudden exposure to unusual gravity levels. However, such responses were not observed in hyper-G, compared to 1-G, environment. The study was also conducted at 1-G environment using otoliths-removed fishes. During body tilting toward the right in response to changes of the direction of light from the top to the right, EMG activity in the right lateral muscle, which is closely associated with the movement of tail fin, was increased. That in the left muscle was inhibited, on the contrary. The data suggested that tail fin plays the major role for body tilting and pectoral fins serve for maintenance of posture in carp fishes.

7. Conclusion

Responses of the activities of EMG in hindlimb muscles and afferent and efferent neurograms at the L_5 segmental level of spinal cord in conscious rats to altered levels of gravity were studied using parabolic flight of jet airplane. In soleus, EMG was increased in response to elevation of gravity during the ascending phase, but was eliminated in μ-G environment. Similar response of the level of afferent, not efferent, neurogram was also noted. Reduction of EMG was related to the shortening of muscle due to plantarflexion of ankle joints. As for AL, region-specific responses were observed. The EMG activity in the caudal, not rostral, region decreased and activity patterns were changed from tonic to phasic during μ-G exposure. These phenomena were also related to shortening of fibers, caused by abduction of hip joints and extension toward backward. These results clearly indicated that unloading-related undesirable adaptation of antigravity muscles is closely related to inhibition of mechanical and/or neural activities.

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References

1) L. Allen, W. Yasui, T. Tanaka, Y. Ohira, S. Nagaoka, C. Sekiguchi, W.E. Hinds, R.R. Roy and V.R. Edgerton: J. Appl. Physiol., **81** (1996) 145.
2) V.J. Caiozzo, M.J. Baker, R.E. Herrick, M. Tao and K.M. Baldwin: J. Appl. Physiol., **76** (1994) 1764.
3) V.R. Edgerton and R.R. Roy: Handbook of Physiol., Sect. 4, Environmental Physiology III, The Gravitational Environment, Ed. by M.J. Fregly and C.M. Blatteis, Oxford Univ. Press, New York, 1996, p. 721.
4) F. Kawano, A. Ishihara, J.L. Stevens, X.D. Wang, S. Ohshima, M. Horisaka, Y. Maeda, I. Nonaka and Y. Ohira: Am. J. Physiol., Regul. Integr. Comp. Physiol., **287** (2004) R76.
5) F. Kawano, T. Nomura, A. Ishihara, I. Nonaka and Y. Ohira: Neuroscience, **114** (2002) 1133.
6) T. Morimoto, F. Kawano, T. Izawa, L. Ohira, K. Nishida and Y. Ohira: 34th Annual Meeting Int'l. Gravit. Physiol., Abstract Book, Toyohashi, Japan, Jun., 2013, p. 55.
7) T. Nomura, F. Kawano, A. Ishihara, Y. Sato, G. Mitarai, S. Iwase, A. Kamiya, T. Mano and Y. Ohira: Neurosci. Let., **316** (2001) 55.
8) M. Ohira, H. Hanada, F. Kawano, A. Ishihara, I. Nonaka and Y. Ohira: Jpn. J. Physiol., **52** (2002) 235.
9) T. Ohira: Master's thesis, Graduate School of Biosciences, Osaka Univ., 2009.
10) Tak. Ohira, T. Shibaguchi, Tomo Ohira, M. Yamada, F. Kawano, A. Takabayashi and Y. Ohira: The 7th Congress of the Federation of Asian and Oceanian Physiological Societies, Taipei, Taiwan, Sept., 2011, p11.
11) T. Ohira, M. Terada, F. Kawano, N. Nakai, T. Ochiai, J. Gyotoku, A. Ogura and Y. Ohira: Jpn. J. Aerosp. Environ. Med., **46** (2009) 21.
12) T. Ohira, M. Terada, F. Kawano, N. Nakai, A. Ogura and Y. Ohira: PLoS ONE 6, e21044, 2011.
13) Y. Ohira, B. Jiang, R.R. Roy, V. Oganov, E. Ilyina-Kakueva, J.F. Martin and V.R. Edgerton: J. Appl. Physiol., **73** (1992) 51S.
14) Y. Ohira, T. Yoshinaga, I. Nonaka, M. Ohara, T. Yoshioka, K. Yamashita-Goto, R. Izumi, K. Yasukawa, C. Sekiguchi, B.S. Shenkman and I.B. Kozlovskaya: Jpn. J. Physiol., **50** (2000) 41.
15) Y. Ohira, T. Yoshinaga, M. Ohara, I. Nonaka, T. Yoshioka, K. Yamashita-Goto, B.S. Shenkman, I.B. Kozlovskaya, R.R. Roy and V.R. Edgerton: J. Appl. Physiol., **87** (1999) 1776.
16) D.A. Riley, E.I. Ilyina-Kakueva, S. Ellis, J.L.W. Bain, G.R. Slocum and F.R. Sedlak: FASEB. J., **4** (1990) 84.
17) K. Yamashita-Goto, R. Okuyama, K. Kawasaki, K. Fujita, T. Yamada, I. Nonaka, Y. Ohira and T. Yoshioka: J. Appl. Physiol., **91** (2001) 417.

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