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Original Article

Effect of daily short-duration weight-bearing on disuse-induced deterioration of musculoskeletal system

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Abstract

Objectives: To investigate deterioration of musculoskeletal system due to prolonged disuse and the potential of daily short-duration weight-bearing as countermeasures. **Methods:** Twenty-four adult male Sprague-Dawley rats were divided into Control Group (CG, no intervention), Tail-suspension Group (TG, tail-suspension without treatment), and Weight-Bearing Group (WBG, tail-suspension with 20 min/day, 5 days/week body weight loading). After four weeks of treatment, femur and tibia, soleus and extensor digitorum longus were evaluated for bone and muscle quality respectively. Tensile properties of bone-tendon insertion (BTI) were evaluated using patella-patellar tendon complex. **Results:** Disuse induced deterioration on bone, muscle, and BTI after four weeks. Compared with CG, TG and WBG showed significant decrease in bone mineral density (BMD) of trabecular bone in distal femur (4.3-15.2%), muscle mass (31.3-52.3%), muscle cross-sectional area (29.1-35%), and failure strength of BTI (23.9-29.4%). Tensile test showed that the failure mode was avulsion of bone at the BTI. No significant difference was detected between TG and WBG for all assessments on bone, muscle, and BTI. **Conclusions:** Disuse caused deterioration of bone, muscle, and BTI while daily short-duration of weight-bearing did not prevent this deterioration. Mechanical stimulation with higher intensity and longer duration may be necessary to prevent musculoskeletal deterioration resulted from prolonged disuse.

Keywords: Disuse-Induced Deterioration, Bone Mineral Density, Muscle, Bone-Tendon Insertion, pQCT

Introduction

Rest is important for natural repair of damaged tissue and has many potential benefits for ill humans, such as conserve metabolic resource for healing and recovery, reduce oxygen consumption by muscles and reduce harmful falls[1]. However, for patients in the intensive care unit (ICU), prolonged bed rest would lead to disuse of musculoskeletal system and cause systemic problems to other body systems[2-4]. The lack of mechanical stimulation caused decrease in bone mass and strength[4], and induced muscle atrophy[3]. Prolonged bed rest demonstrated to decrease bone mineral density (BMD) and increase calcium excretion[5-7]. The lack of mechanical loading decreased the tendon stiffness[8,9] and affected the development of the bone-tendon insertion (BTI)[10], a unique structure that serves as an interface for force transmission from tendon to bone and acts as a stress absorber during mechanical loading[11]. Therefore, prolonged disuse or unloading might also weaken the BTI complex. Furthermore, the available individual studies only focused on the deterioration of a single specific tissue type in the musculoskeletal system, either bone loss[12,13], or muscle atrophy[14,15], instead of investigating on the systemic adaptation of the whole musculoskeletal system during the period of disuse. This study was performed to investigate whether disuse has a systemic effect on all involved musculoskeletal tissues and whether there are differences in sensitivity within a single type of tissues.

Patients in the intensive care unit (ICU) or patients who require prolonged bed rest, such as those who suffer from hip

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The authors have no conflict of interest.

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fracture, are encouraged to have early mobilization and weight-bearing[16,17]. Burtin et al. reported that cycling for 20 min/day enhanced recovery of functional exercise capacity, self-perceived functional status, and muscle force at hospital discharge of ICU patients with a cardiorespiratory condition[18]. Compare with non-weight-bearing exercise, weight-bearing exercise has further improved hip extensor strength in elder inpatients[19]. Therefore, weight-bearing exercises might provide greater mechanical stimulation on the body and protect the musculoskeletal system from disuse-induced musculoskeletal deterioration. Daily short-duration of weight-bearing (WB) can be easily applied clinically because the duration of the therapy is relatively short and does not require extra equipment. This treatment only requires the patient to be standing upright. We hypothesized that daily short-duration of WB would be sufficient to prevent disuse-induced bone loss, muscle atrophy, and BTI deterioration. To simulate the effect of disuse on the musculoskeletal system during prolonged bed rest, a well-established tail-suspension model in rats was used because this model is well tolerated by the animal with minimal evidence of stress[20,21]. The current study evaluated systemic deterioration of musculoskeletal system, including bone, muscle, and BTI complex, and investigated the effect of WB on prevention of musculoskeletal system deterioration in an established tail-suspension model in rats.

Methods

Tail-suspension model in rats

Twenty-four, three months old, male Sprague-Dawley rats were obtained from the China Astronaut Research and Training Center, Beijing, PR China. The rats were divided into three groups: Control Group (CG), Tail-suspension Group (TG), and Weight-bearing Group (WBG). CG did not receive any intervention while TG and WBG underwent standard tail-suspension to simulate the effect of disuse on the hindlimbs according to the established protocol[21-23]. Briefly, the tail of the rats was wrapped with a plaster. Then surgical tapes were wrapped on top of the plaster. The rats were suspended in a head-down position and formed a 30° angle between the floor and the torso of the rat. The hindlimbs of the rats were not allowed to touch the side of the cage and the floor. The rats were also allowed free-cage movement and access to water and standard rat chow *ad libitum* with their forelimbs. On the second day of tail-suspension, daily cage activity was set for 20 min/day, 5 days/week in WBG while TG did not receive any treatment. The body weights of the rats were measured and recorded. All the rats were euthanized after four weeks of treatment. Both hind limbs of the rats were harvested for isolating relevant tissues, including bone, muscle, and BTI complex for various evaluations (Table 1). Soleus muscle, which contained predominantly slow twitch fibers, and Extensor Digitorum Longus muscle (EDL), which contained predominantly fast twitch fibers, were harvested and weighted before specified evaluations.

Musculoskeletal Systems for Evaluation	pQCT Measurements	Other Assessments
Overall	N/A	Body weight
Bone	iBMD (F, T) tBMD (F, T) Bone CSA (F, T) Cortical CSA (F, T) CSMI (F, T)	N/A
Muscle	Mid-tibia CSA	Muscle mass (EDL, Sol)
Bone-tendon insertion	N/A	Tensile strength (PPT)

The brackets indicate the targeted tissue for the corresponding assessments. F: Femur; T: Tibia; EDL: Extensor Digitorum Longus; Sol: Soleus muscle; PPT: Patella-patellar tendon; iBMD: Integral bone mineral density; tBMD: Trabecular bone mineral density; CSA: Cross-section area; CSMI: Cross-sectional moment of inertia; N/A: Not Applicable.

Table 1. Harvesting and isolation of musculoskeletal tissues for systemic evaluations.

peripheral Quantitative Computed Tomography (pQCT)

The femur and tibia were isolated and placed on a custom-made plastic holder for pQCT measurement (Densiscan 2000, Scanco Medical, Brüttisellen, Switzerland). The integral BMD (iBMD) and trabecular BMD (tBMD) of the femur and tibia were measured according to our established protocol[24,25]. iBMD is the BMD of the whole bone within the region of interest (ROI), which includes both trabecular bone and cortical bone[26]. The geometrical parameters of the femur and tibia, including the cross-sectional area (CSA) of the bony tissue and cross-sectional moment of inertia (CSMI), were also measured. CSMI is calculated as $CSMI=\sum(Pi-C)^2 \times area_p$ where Pi is the position of the i^{th} pixel, C is the position of the center pixel (the centric pixel of the identified bone), and $area_p$ is the area of a pixel. This summation was taken for all pixels inside the measured contour of cortical CSA[24]. The CSA of the muscles at mid tibia, an indicator of muscle strength[27], was measured (Table 1).

Mechanical testing for bone tendon insertion (BTI) complex

The tensile properties of the patella-patellar tendon (PPT) insertion were evaluated according to our published protocol[28,29]. Briefly, the quadriceps-patella-patellar tendon-tibia (QPPTT) complex was isolated from the right hindlimbs. All periarticular connective soft tissues and soft tissues around the knee were removed. The QPPTT complex was mounted onto a custom-made fixator and attached to a mechanical testing machine (H25K-S, Hounsfield Test Equipment Ltd, Surrey, UK) with a 250 N load cell (Figure 1). A constant tensile load of 1 N was applied to the QPPTT complex. The thickness and

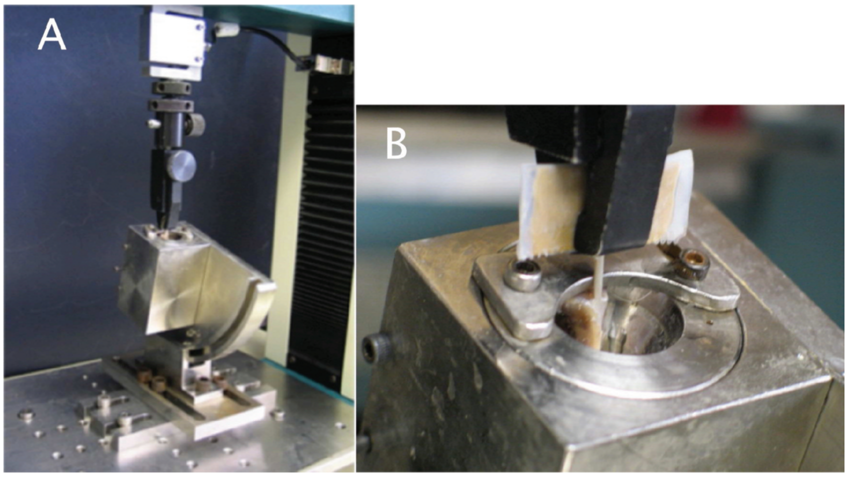

Figure 1. Setup for mechanical testing for obtaining maximal tensile strength. A: A custom-made jig for tensile test for the quadriceps-patella-patellar tendon-tibia (QPPTT) complex. B: The testing jig consisted of an upper clamp that secured the patella together with the distal quadriceps, and a lower clamp that gripped the proximal tibia. A piece of sand paper was placed between the clamps to increase the friction between the clamp and the patella.

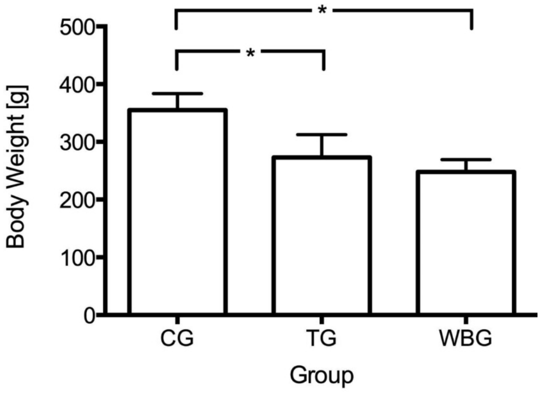

Figure 2. Body weight of rats from different groups after four weeks of treatment. CG showed significantly higher tensile strength than that of TG and WBG. No significant difference was detected between TG and WBG. *: $p<0.05$.

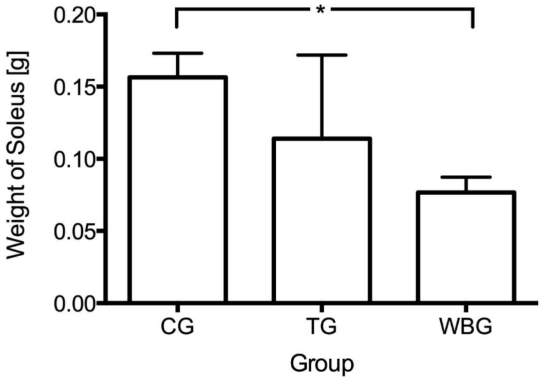

Figure 3. Weight of soleus muscle in different groups. CG showed significantly higher value than that of WBG. *: $p<0.05$.

width of the PPT insertion were measured with a fine caliper and the cross-sectional area of the insertion was calculated. This value was used to normalize the failure load for calculating failure stress. Saline was applied to the QPPTT complex frequently to prevent dehydration of the tissue. A testing speed of 100 mm/min up to failure of the QPPTT complex was used based on our previous published work[28,29].

Statistics

All assessments were reported as mean ± standard deviation. Coefficient of variation (CV) was calculated by dividing the standard deviation by the mean. Statistical analysis was performed using SPSS 16.0 for Windows (IBM, NY, USA). One-way Analysis of Variance (One-way ANOVA) with Bonferroni post hoc test was performed to evaluate the differences among different groups. Statistical significance was set at $p<0.05$.

Results

Body weight

After four weeks of tail-suspension and different treatments, TS (76.89% of CG, $p<0.05$), and WBG (69.85% of CG, $p<0.05$) showed significant decrease in weight compared with the CG. However, no significant difference was detected among TS and WBG (Figure 2).

	Femur			Tibia		
pQCT Variables	**CG**	**TG (%)**	**WBG (%)**	**CG**	**TG (%)**	**WBG (%)**
iBMD (g/cm^3)	730.6 ± 35.9^{ab}	660.0±51.9 (90.3)	601.2±41.4 (82.2)	717.1 ± 40.4^{ab}	668.5±69.2 (93.2)	655.4±28.6 (91.4)
tBMD (g/cm^3)	495.94 ± 24.6^{ab}	420.39±44.2 (84.8)	424.14±10.3 (85.5)	402.2 ± 33.6^{ab}	385.0±11.4 (95.7)	354.1±51.0 (88.0)
Bone CSA (mm^2)	16.58 ± 2.03^{ab}	11.60±0.61 (70.0)	11.35±1.12 (68.5)	9.29 ± 0.53^{ab}	7.93±0.40 (85.4)	7.43±0.52 (80.0)
Cortical CSA (mm^2)	9.73 ± 1.13^{ab}	6.27±0.53 (64.4)	5.47±0.49 (56.2)	5.29 ± 0.30^{ab}	4.50±0.44 (85.1)	3.99±0.19 (75.4)
$CSMI_x$ (mm^4)	12.23 ± 3.59^{ab}	5.86±1.13 (47.9)	5.54±1.15 (45.3)	4.71 ± 1.22^{ab}	3.59±0.40 (76.2)	3.00±0.29 (63.7)
$CSMI_y$ (mm^4)	14.54 ± 2.91^{ab}	7.42±0.92 (51.0)	5.85±2.35 (40.2)	3.47 ± 0.53^{ab}	2.20±0.31 (63.4)	1.96±0.19 (56.5)
$CSMI_{polar}$ (mm^4)	26.77 ± 6.24^{ab}	13.28±1.05 (49.6)	11.39±1.94 (42.5)	8.17 ± 1.09^{ab}	5.79±0.61 (70.9)	4.97±0.43 (60.8)

Note: the percentage in the brackets represent the percentage of current assessment corresponds to CG. pQCT: peripheral quantitative computed tomography; iBMD: integral bone mineral density (BMD); tBMD: trabecular BMD; CSA: cross-sectional area; CSMI: cross-sectional moment of inertia; Number in brackets represent the percentage of the value compared to that of CG; ^{a}: $p<0.05$ vs. TG; ^{b}: $p<0.05$ vs. WBG.

Table 2. Comparison among different treatment groups with respect to bone quality of femur and tibia.

Evaluations on bone mineral status

For both femur and tibia, CG showed significantly higher iBMD, tBMD, bone CSA, cortical CSA, and CSMI than TG and WBG ($p<0.05$) (Table 2). All pQCT-measured parameters did not show significant difference between TG and WBG in either femur or tibia ($p>0.05$). iBMD of femur and tibia in TG and WBG showed the least percentage of decrease (82.2% and 90.3% of CG) while CSMI showed the greatest percentage decrease (43.0-76.2% of CGs).

Evaluations on muscle mass and CSA

WBG (47.7% of CG, $p<0.05$) showed significantly lower muscle mass in the soleus muscle than that of CG (Figure 3). TG (78.7% of CG, $p<0.05$) and WBG (72.4% of CG, $p<0.05$) showed significantly lower muscle mass in EDL than that in CG (Figure 4). Moreover, TG (63.9% of CG, $p<0.05$) and WBG (62.4% of CG, $p<0.05$) showed significantly lower CSA of tibia muscle than that of CG (Figure 5).

Evaluations on bone-tendon insertion tensile property

The PPT samples failed with an avulsion of bone at the BTI complex during mechanical testing. The tensile failure strength of PPT insertion was the highest in CG (Figure 6). TG (76.1% of CG, $p<0.05$) and WBG (70.6% of CG, $p<0.05$) showed significantly lower failure strength than that of CG. However, no significant difference in failure strength was detected between TG and WBG.

Discussion

The tail-suspension disuse model demonstrated systemic deterioration of the musculoskeletal system, including bone, muscle, and BTI complex after 4 weeks of tail-suspension. There were variations in musculoskeletal tissue deterioration, which depended on type of tissues for evaluation. The current study was the first to report the deterioration of the BTI in the

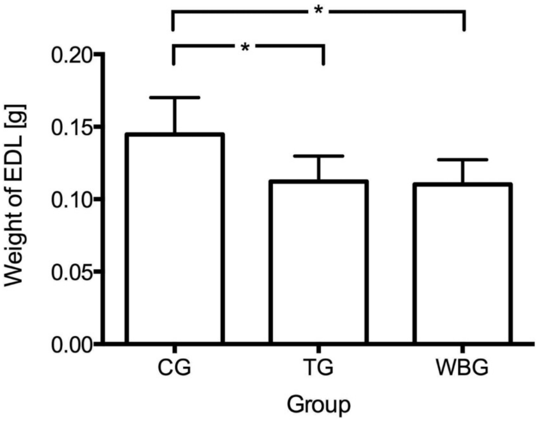

Figure 4. Weight of extensor digitorum longus muscle (EDL) in different groups. CG showed significantly higher weight than those of TG and WBG. *: $p<0.05$.

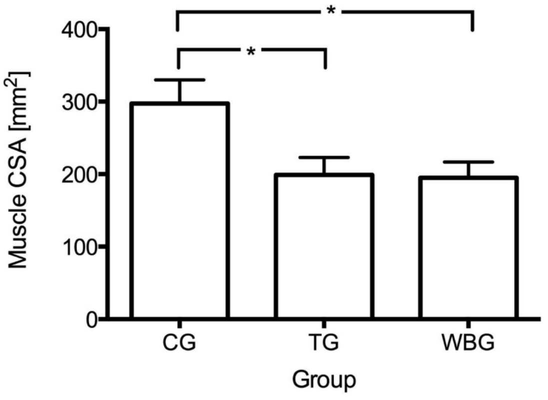

Figure 5. The cross-sectional area (CSA) of muscle at the mid tibia. CG showed significantly higher CSA than that of WBG. *: $p<0.05$.

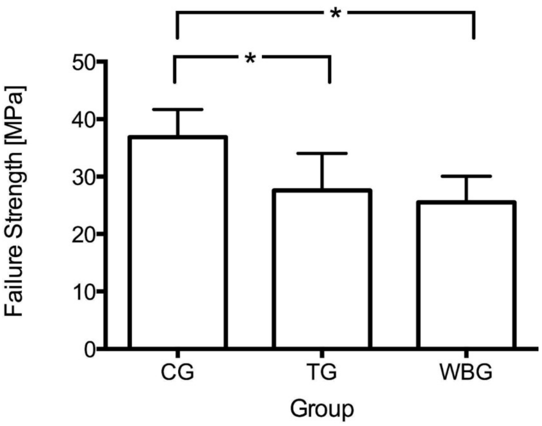

Figure 6. Tensile strength of the patella - patellar tendon (PPT) complex of different groups. CG showed significantly higher failure strength than that of TG and WBG. *: $p<0.05$.

tail-suspension model.

Disuse or unloading induced deterioration of bone might lead to decrease in bone strength. Bone strength is contributed by several factors, including the quality of the bone matrix, which consists of BMD and extracellular proteins, and bone geometry, such as the bone CSA. The product of BMD and bone CSA is also known as the bone strength index, which is a noninvasive measurement for predicting the bone bending strength[30]. Our result demonstrated that there was a decrease in BMDs, including iBMD and tBMD, and bone structural measurements, including bone CSA, cortical CSA, and axial CSMIs. This suggested that the bending strength of the bone in the hindlimbs was weakened after prolonged duration of disuse. There was a difference in the rate of bone loss between cortical bone and trabecular bone due to disuse. Our results showed that tBMD had greater decrease than iBMD, a BMD measurement that includes both tBMD and cortical bone BMD. Our findings were comparable to other similar studies. Cervinka et al. demonstrated that, bone loss occurred in the cortical compartment during the first 60 days of bed rest while trabecular bone loss might be more significantly for longer periods[12]. Vico et al. found that cortical bone loss was less significant than that of trabecular bone at the weight-bearing tibia after six months of spaceflight[13]. In addition to BMD, the bone resorption markers, such as urinary concentration of C-telopeptide of type I collagen (CTX-I) and deoxypridinoline (DPD), were elevated after bed rest and suggested an increase in bone resoprtion[7]. Therefore, after prolonged disuse, there is imbalance of bone formation and bone resorption. Furthermore, the distribution of calcium and phosphorus in bone was affected by prolonged disuse[5], which might also weaken the material properties of bone. Therefore, disuse affects both the structural characteristics and the material properties of bone and hence the skeletal mechanical properties.

Muscle atrophy is characterized by decreased muscle size, such as the muscle fiber CSA and protein content, and muscle functions, such as reduced force, and increased insulin resistance[31]. The current study showed that muscle mass and CSA were decreased in the rats of TG. This muscle atrophy might be caused by the reduction in protein synthesis, which was triggered by unloading and decrease in mechanical strains imposed on the muscles[15,32,33]. Furthermore, disuse and unloading also induced alterations of muscle cell phenotype from slow fibers (myosin heavy chain type I) to fast fiber (myosin heavy chain type II)[3,34]. This changes in muscle fiber type would be most significant in soleus muscle because it was predominately composed of slow-twitch muscle fibers[3]. The atrophic muscles were also reported to show increase in insulin resistance[31,35]. In general, insulin is responsible for stimulation of muscle protein synthesis when intramuscular amino acid availability is sufficient[36]. Therefore, insulin resistance might further affect the performance and change the metabolic adaptations of the muscles.

Tail suspension-induced deterioration of BTI was reflected by significant decrease in tensile failure strength of BTI complex, with typical avulsion of bone at patellar tendon insertion in TG in rats. Results from our current study and previous studies from others demonstrated that hindlimb suspension of rats induced bone and muscle atrophy[3,37]. Comparable BTI deterioration was also found in rats after eleven days of spaceflight[38]. Atrophy of adjacent muscles near the BTI caused decrease in mechanical loading to the corresponding BTI and tendon, which may contribute to the deterioration of the BTI.

Variations in activity level might lead to differences in mechanical stimulation and loading that might also lead to large variation in the measurements. However, from the pQCT result of the current study, the variations of the measurements for TG and WBG were 5-15% and 17% for failure strength, which were comparable to the variations of the measurements for CG (5-15% for pQCT and 13% for failure strength). Therefore, the variation in activity level might be smaller than biological variations among individual rats.

Soleus muscle, which consists of slow twitch fibers, would suffer more pronounced atrophy than EDL after prolonged period of disuse[39,40]. Based on the result of the current study, soleus muscle did not show more pronounced atrophy than that of EDL muscle. This can be explained by no significant difference was found between CG and TG for soleus muscle while significant difference was detected in EDL muscle. Even though the mean percentage changes of EDL and soleus between CG and TG were comparable (77.6% for EDL and 72.9% for soleus), the variation in the mass of the soleus muscle (CV=50%) was larger than the variation in that of the EDL (CV=16%). This might be due to the fact that the rats would intrinsically contract the EDL for support during hindlimb suspension[40], which might vary the deterioration rate of the EDL.

Reloading after prolonged disuse may cause damage to muscle in addition to atrophy. Riley et al. reported that eccentric contraction-like lesions appeared in hindlimb suspension unloading rats after 15-60 min of reloading[41]. The atrophic fibers were more susceptible to sarcomere reloading damage as the filaments force per filament was found increased[42].

Therefore, this muscle damage might lead to further decrease in muscle weight in WBG but not in TG.

Weight-bearing activities are defined as activities during which the body works against the force of gravity and the feet and legs carry one's body weight. The current treatment protocol for short-duration weight-bearing exercise did not prevent the disuse-induced, systemic deterioration of the musculoskeletal system. TG and WBG did not show significant difference among each other with respect to the measured variables on the musculoskeletal system. Zhang et al. reported that four hours of daily standing only partially relieved the deterioration in femur while prevented mass reduction and histomorphometric changes in the soleus muscle[43]. However, van Der Wiel et al.[44] and Rubin et al.[45] reported that, for anabolic stimulation on bone, the addition of weight-bearing exercise was more effective than extending duration of the exercise. Umemura et al. reported that five jumps per day was sufficient to increase bone mass and breaking force in rats[46]. Therefore, the duration of the weight-bearing exercise might not be the determining factor compared with the inclusion of weight-bearing exercises. In order to minimize the duration of the stimulation therapy, exercises that are higher intensity than weight-bearing standing maybe necessary for maintenance of the musculoskeletal system. However, typical exercises, such as swimming and jumping which are reported to prevent bone loss[47], may not be appropriate for the patients with prolonged bed-rest. The amount of space is limited in the ICU and ICU patients often attached with a wide-ranging instruments and sensors which would also limit their movement. Other exercise modalities that act on the whole body may be applied to stimulate the musculoskeletal system. Low-magnitude high-frequency vibration is an exercise modality[48] that provides mechanical loading and was shown effective on different types of tissues of the musculoskeletal system, including bone[49,50], muscle[51,52], and tendon[53]. Therefore, this exercise modality might have potential for prevention of disuse-induced musculoskeletal deterioration.

There were a few limitations in the current experimental study, including: 1) the function of quadriceps muscle was not assessed that might be related to the tensile properties of QPPTT complex. The quadriceps consists of a mix of muscle fiber types and earlier studies have demonstrated that unloading and disuse caused atrophy of quadriceps muscle in human[54,55] and rats[56]. Therefore, the quadriceps muscles might also be affected and led to decrease in tensile strength PPT complex. 2) As an important component of the joint, articular cartilage might also be affected by the microgravity or disuse[57] that should also be evaluated in the future studies. Future studies would also benefit from examining tissues, including muscle, tendon, and bone within the same region of the body (e.g. tibia, calf muscle and Achilles tendon insertion) to observe the changes in relation to each tissue.

Conclusion

Disuse caused systemic deterioration in the musculoskeletal system, including the bone, muscle, and BTI complex. The present regime for short duration of weight-bearing treatment did not show significant effect on prevention of deterioration of the musculoskeletal system resulted from prolonged disuse.

Acknowledgement

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