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Original Article

Bone density benefits with periodic fluid redistribution during diminished muscular activity in humans

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Abstract

Objective: Bone loss is an established reaction to diminished muscular activity (Hypokinesia; HK). It has been assumed that periodic fluid redistribution (PFR) could contribute to vascular volume. The fluid volume expansion would then increase tissue perfusion. We hypothesized that chronic PFR could contribute to or increase bone density during HK. Therefore we investigated the potential benefits of bone density with chronic PFR during HK.

Methods: Studies were conducted on 40 male volunteers. They were equally divided into four groups: active control subjects (ACS), hypokinetic subjects (HKS), periodic fluid redistribution control subjects (PFRCS) and periodic fluid redistribution hypokinetic subjects (PFRHS). The density of lumbar vertebrae (L1-L4), ulna and radius, tarsal and metatarsal, tibia and fibula were measured during pre-experimental period of 390 days and experimental period of 360 days.

Results: Density of lumbar vertebrae (L1-L4), ulna and radius, tarsal and metatarsal, tibia and fibula increased ($p<0.05$) in the PFRHS group compared to the HKS group. Density of lumbar vertebrae (L1-L4), ulna and radius, tarsal and metatarsal, tibia and fibula decreased ($p<0.05$) in the HKS group compared to their pre-experimental levels and the values in the other groups. In the PFRCS group lumbar vertebrae (L1-L4), ulna and radius, tarsal and metatarsal, tibia and fibula density were improve much less than in the PFRHS group. Bone density was not affected in the ACS group compared to their pre-experimental levels.

Conclusion: The current study shows that bone density increases with chronic PFR suggesting potential benefits of bone density with chronic PFR during diminished muscular activity.

Introduction

Periodic head down (PHD) position, and head down (HD) position (in humans) and hindlimb suspension (in rats) share a significant shift in thoracic fluid volume; however, the intensity of fluid redistribution (FR) with PHD position and intensity of FR with HD position are different as are many other features specific to FR with PHD position (1, 2). With PHD position fluid volume is intravascular and intracellular and contributes to vascular volume. The PHD position creates an expanded and sustainable vascular volume. The plasma aldosterone, renin, angiotensin II, catecholamines and antidiuretic hormone adapt to

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PHD position. The PHD position produces adaptation of fluid shifting to upper body part tissues and induces a hydrostatic gradient to increase venous return and improve cardiac output and total fluid volume. PHD tilt regulates cardiovascular and endocrine system and increases physical capacity, power and strength.

Earth gravity and diminished muscular activity (Hypokinesia; HK) affects redistribution of fluids inside the body by pulling the various body fluids downwards in the lower part of the body. Because of fluid volume migration to lower extremities more fluid shifts to the pelvic region and lower half part of the body. Retention of large fluid volume in the lower extremities than what is the norm for the lower part of the body leads to lower blood volume and lower filling with blood of central vascular bed (3). Fluid volume which can fit into venous system of lower extremities can determine the severity in delivery of fluid volume to the upper part of the body and thus extracellular and interstitial fluid volume. The decreased total fluid volume is most detrimental to body because it forces the body, particularly the vital organs, to work much harder than they normally should. The reduction of fluid volume may contribute to higher plasma electrolyte level which potentially may lead to electrolyte losses and electrolyte deficiency (4-10). To counteract the consequences of diminished muscular activity different preventive measures including that of physical exercises have been used that unfortunately have shown to have limited effect (11-16).

Bone atrophy is a well-established adaptation of skeleton to the diminished muscular activity. A calcaneus electrolyte loss (17, 18, 19) and an increase electrolyte excretion (4-10) in hypokinetic animals and humans characterize skeleton reaction to reduced mechanical function. Some studies have shown how bone loss in regions of skeleton experiencing the greatest reduction of physical stress (20-24). Bone mass loss is driven primarily by the inability of body to use electrolytes (4-10) and by the reduced muscular forces as muscle atrophy is developed (20-22). It is the severe bone loss and the deterioration of its microarchitecture which highlights premature osteoporosis. Bone loss may increase the risk of skeletal fractures, while uncoupled bone cell reaction during the remodeling could affect the healing of bone fractures. Skeletal injuries could adversely affect man's ability to carry out physical activities, slow performance due to further risk or pain, and/or increase the risk for fractures because of decreased skeletal integrity. Moreover, the incomplete recovery of bone density and the irreversible changes in the geometry and microarchitecture of the skeleton may contribute to or increase the premature onset of age-related osteoporosis.

Bone density decreases during diminished muscular activity leading to more age-related changes analogous to osteoporosis. Reduced bone density and strength is more apparent in some skeletal regions, such as the pelvis. Extracellular fluid volume expansion through a daily intake of fluid-salt supplementation in very small divided doses induces osteotropic and anabolic effect (19-24). Fluid volume expansion through chronic periodic fluid redistribution (PFR) may contribute to or increase bone density. Thus, PFR which moves fluid volume away from lower part of the body into regional areas of the body may be one solution for normal fluid volume and bone density. It is remarkable however; that thus far no studies have been published on the effect of chronic PFR on bone density during diminished muscular activity. Therefore to determine the potential benefits of bone density with periodic addition of fluid volume into regional areas of the body during diminished muscular activity we measured bone mineral density in the lumbar vertebrae (L1-L4), tibia and fibula, ulna and radius, tarsal and metatarsal of healthy subjects during diminished muscular activity.

Methods and Materials

The studies conducted during a 390-day pre-experimental period and a 364-day experimental period. The studies had conformed to the principles of the Declaration of Helsinki. All study protocols were reviewed and approved by the Committee for the Protection of Human Subjects of the Institutional Review Board (IRB). Subjects received verbal and written explanations of all experimental and test protocols prior to providing written informed consent. Among the subjects were no medical problems and

none of the subjects were under any drug therapy that could have interfered with bone density. Forty physically healthy male subjects 25.0±6.0 years of age were chosen as subjects. All subjects run average distances of 9.1±1.4 km.day-^{l} at a speed of 9.5±1.3 km.h-^{l} for three to five years. Subjects had a body weight of 73.8±7.0 kg and peak oxygen uptake of 47.7±6.0 mL.kg-^{l}. min-^{l}. In the pre-experimental period all subjects run average distances of 9.1±1.3 km.day-^{1} at a speed of 9.5±1.4 km.h-^{l}.

Assignment of subjects into four groups was done randomly by an assistant blinded from the recruitment and treatment procedures and a concealed method was used.

Group 1: Ten subjects run average distances of 9.1±1.5 km.day-^{l} for 364-days. They were assigned to the active control subjects (ACS) group. Group 2: Ten subjects walked average distances of 3.2±0.7 km.day-^{l} for 364-days. They were assigned to the hypokinetic subjects (HKS) group. Group 3: Ten healthy subjects run average distances of 9.1±1.6 km.day-^{l} and were subjected to PFR for 8 to 10 hrs per day for 364 days. They were assigned to the periodic fluid redistribution control subjects (PFRCS) group. Group 4: Ten healthy subjects walked average distances of 3.2±0.6 km.day-^{l}and were subjected to PFR for 8 to 10 hrs per day for 364 days. They were assigned to periodic fluid redistribution hypokinetic subjects (PFRHS) group.

Protocol

The investigation consisted of a 390-day pre-experimental period and a 364-day experimental period. The diets were served as a 7-day menu rotation. The meals were all prepared under standard conditions in a research kitchen. Mean daily energy consumption of the metabolic diet was 3530±453, 3045±460, 3570±511 and 3151±463 SD Kcal, and the mean daily calcium consumption was 43.1±1.5, 43.0±1.7, 43.0±1.3 and 43.5±1.5 SD mmol for the ACS, HKS, PFRCS and PFRHS groups, respectively. Subjects were housed in a facility in which humidity, temperature, activities, and dietary intakes were monitored 24 hrs per day and 7 days per week.

Simulation of hypokinesia through diminished muscular activity

To simulate a certain degree of hypokinesia the number of km walking per day was restricted to an average of 3.2±0.7 km.day-^{l} and was monitored daily by an accelerometer. The activities allowed were those that approximated the normal routines of hypokinetic individuals. Subjects were allowed to walk to the dining rooms, lavatories and different laboratories where the tests were administered. Climbing stairs and other activities which required greater efforts were not allowed. Subjects were mobile and were not allowed outside the experimental facility grounds so that the level of hypokinesia could remain constant and their movements could easily monitor.

Simulation of periodic fluid redistribution through periodic head down tilt

To produce PFR the volunteers were submitted without a pillow to PHD position for 8 to 10 hrs per day during sleeping in the pre-experimental period of 390 days and experimental period of 364 days. During pre-experimental period the volunteers were submitted to PFR by increasing progressively PHD position to -2, -4, -6, -8, -10 and -12 degrees every 64 to 71 days. For the rest pre-experimental and actual experimental period volunteers were submitted to PFR by increasing progressively PHD tilt to -4, -6, -8, -10 -12 and -14 degrees for 8 to 10 hrs per day. Procedures selection was determined from a preliminary study in establishing volunteers' adaptation ability to PHD tilt. To ensure all volunteers comfort the PHD position of -4 to -14 degrees was modified as required. The individual differences of biochemical and physiological reactions, that is, cardiovascular, renal/endocrine and metabolic reactions of volunteers and their clinical symptoms and sensitivity to different PHD positions were taken into consideration. The schedule of PHD position was alternated from time to time to conform to the requirements of adaptational ability of volunteers.

Bone density measurements

Samples were analyzed in duplicate, and appropriate standards were used for all measurements:

Bone density values (g/cm^2) of the lumbar vertebrae (L1-L4), on the boundary between the median and distal thirds of the tibia and fibula diaphysis, the length of the radius and ulna (calculated from the styloid process) and in the foot (tarsal and metatarsal bones) were measured using the Lunar of dual-energy, X-ray absorptionmetry (DXA; GE-Lunar DPX-L) with DPX-L software (version 1.6; GE Lunar). (Lunar Radiation Corp., Madison, WI). The values calculated from the analyses of the whole body scan. We calibrated the machine daily and performed daily and weekly quality-assurance tests as recommended by the manufacturers. The precision errors (% CV) for whole body and radius shaft BMD measurements were 0.5% and 0.8%, respectively. The two-person inter observer error for DXA analysis was 0.1%. Daily phantom measurements on DXA indicated a steady but extremely slow machine drift; BMD was adjusted accordingly.

Data analyses

A 2-way interaction [treatment (4 levels) by days (6 levels)] analysis of variance (ANOVA) was used to determine the potential benefit of bone density regulation with chronic PFR. The ANOVAs with repeated measures of 2-way interaction (treatment/days, pre-experimental/experimental values, hypokinetic/periodic fluid redistribution hypokinetic groups, hypokinetic/control groups) was used. The ANOVAs for each time point measurements were used. The statistical analysis of the results was performed with GraphPad Prism statistical software (GraphPad Software Inc., La Jolla, Ca). All data are expressed as mean±SD Standard Deviation), and a p value $p<0.05$ was considered statistically significant.

Results

The PFRCS group and PFRHS group were reported symptoms analogous to those of HD tilt. Most common complaints were headache, dizziness, muscle aches and pains. With PHD position the subjects were manifested symptoms most of which were typical to PHD position; symptoms were more pronounced in the PFRCS group than the PFRHS group. However, as the duration of PHD position increased and the subjects were adapted to PHD position symptoms disappeared and none of the subjects were complained of any symptoms. The PFRHS group and much less the PFRCS group had gained significant height, power and strength. After completion of the study the subjects with PFR treatment decided to continue sleeping at PHD position.

In the PFRHS group and PFRCS group lumbar vertebrae (L1-L4), tibia and fibula, ulna and radius, tarsal and metatarsal density decreased some what at the initial stages of pre-experimental period, however, as the duration of pre-experimental period increased bone density increased (Table I). In the HKS and the ACS groups the lumbar vertebrae, tibia and fibula, ulna and radius, metatarsal and tarsal density remained stable at the pre-experimental period (Table I).

In the experimental period lumbar vertebrae (L1-L4), tibia and fibula, ulna and radius, metatarsal and tarsal density increased ($p<0.05$) in the PFRHS group compared to the HKS group (Table I). With chronic PFR minerals are been taken up for deposition and been used by the body which in turn protected the net bone density. The lumbar vertebrae, tibia and fibula, ulna and radius, metatarsal and tarsal density decreased ($p<0.05$) in the HKS group compared to the pre-experimental period levels and the values in the other groups (Table I). In the PFRCS group tibia and fibula, lumbar vertebrae, ulna and radius, metatarsal and tarsal density was not improved as much as in the PFRHS group (Table I). In the ACS group lumbar vertebrae, tibia and fibula, ulna and radius, metatarsal and tarsal density was not affected compared to their pre-experimental period (Table I).

Discussion

Periodic fluid shifting to upper part of the body is not sensed by baroreceptors as excessive fluid volume but rather as simple fluid redistribution and the excretion mechanisms are not activated. Periodic fluid shifting to upper part of the body provides stimulation for sustainable fluid volume. In a normally functioning body, any means through which fluid

TABLE I: Mineral Density (g/cm^2) of Long Bones, Foot Bones, Arm Bone and Lumbar Vertebrae Measured in the Control and the Hypokinetic Groups and the Periodic Fluid Redistribution Control and the Hypokinetic Groups During the Pre-experimental and the Experimental Period.

Duration of experimental in days	*Tibia and fibula, g/cm^2*	*Tarsal and metatarsal, g/cm^2*	*Ulna and radius, g/cm^2*	*Lumbar vertebrae (L1-L4), g/cm^2*
		Active control subjects (ACS), n=10		
Pre-experimental Average values	2.055±0.15	1.13±0.05	0.78±0.06	1.04±0.05
60th	2.057±0.10	1.14±0.03	0.79±0.01	1.05±0.04
120th	2.055±0.11	1.13±0.05	0.80±0.04	1.04±0.02
180th	2.057±0.13	1.14±0.02	0.79±0.05	1.05±0.05
240th	2.055±0.10	1.13±0.04	0.80±0.02	1.04±0.04
300th	2.058±0.11	1.14±5.05	0.79±0.04	1.06±0.05
364th	2.057±0.12	1.15±0.02	0.80±0.02	1.05±0.05
		Hypokinetic subjects (HKS), n=10		
Pre-experimental Average values	2.056±0.10	1.14±0.02	0.78±0.03	1.05±0.02
60th	1.850±0.14*†	1.00±0.05*†	0.73±0.02*†	0.96±0.04*†
120th	1.861±0.15*†	1.03±0.02*†	0.74±0.04*†	0.97±0.05*†
180th	1.797±0.13*†	0.98±0.03*†	0.68±0.03*†	0.92±0.02*†
240th	1.808±0.12*†	1.00±0.04*†	0.70±0.02*†	0.93±0.03*†
300th	1.743±0.14*†	0.96±0.02*†	0.66±0.05*†	0.90±0.05*†
364th	1.756±0.11*†	0.98±0.03*†	0.68±0.03*†	0.91±0.04*†
		Periodic fluid redistribution control subjects (PFRCS), n=10		
Pre-experimental Average Values	2.070±0.10	1.23±0.02	0.86±0.01	1.12±0.02
60th	2.116±0.11	1.24±0.03	0.87±0.02	1.13±0.05
120th	2.127±0.10	1.25±0.04	0.87±0.05	1.14±0.02
180th	2.155±0.13	1.26±0.02	0.88±0.04	1.15±0.04
240th	2.166±0.11	1.27±0.05	0.89±0.02	1.17±0.05
300th	2.185±0.15	1.28±0.03	0.90±0.03	1.18±0.03
364th	2.207±0.14	1.30±0.05	0.91±0.04	1.19±0.04
		Periodic fluid redistribution hypokinetic subjects (PFRHS), n=10		
Pre-experimental Average Values	2.070±0.16	1.22±0.06	0.85±0.05	1.11±0.06
60th	2.203±0.16+	1.25±0.04+	0.86±0.04+	1.14±0.05+
120th	2.209±0.14+	1.26±0.05+	0.87±0.02+	1.15±0.04+
180th	2.221±0.16+	1.28±0.04+	0.89±0.05+	1.17±0.05+
240th	2.237±.13+	1.30±0.06+	0.91±0.03+	1.19±0.06+
300th	2.253±0.15+	1.32±0.04+	0.93±0.04+	1.22±0.05+
364th	2.274±0.16+	1.35±0.05+	0.95±0.06+	1.25±0.03+

All values were expressed as mean±SD.
*p<0.05 significant differences between the control and the hypokinetic groups of subjects.
†p<0.05 significant differences between the pre-experimental and experimental period values.
+p<0.05 significant differences between the hypokinetic and the periodic fluid redistribution hypokinetic groups of subjects.

volume is stimulated would lead to more circulating fluid volume and, therefore increase total fluid volume and extracellular fluid volume. The intuitive concept behind the potential benefits of fluid volume expansion with chronic PFR is grounded on the assumption that chronic PFR is a potent stimulus for sustainable fluid volume. It is further supposed that sustainable fluid volume with chronic PFR could contribute to or increase bone density during diminished muscular activity.

In this study significant differences were found among the hypokinetic subjects who were treated with PFR and those who were not suggesting potential benefits of bone density with PFR. Chronic PFR is a potent stimulus for the protection and/or increase of bone density as was shown by the significant differences between the groups. Once subjects have adjusted to chronic PFR they continue to show a progressive bone density increase. The higher bone density with chronic PFR and the lower bone density with HK

alone suggest that they are under different control mechanisms. With large fluid shifting to the head bone density cannot increase unless fluid shifting to the head is sensed as simple fluid redistribution and the excretion mechanisms are not activated. This shows that fluid volume shifting to regional areas of the body is not sensed as stressor but rather as stimulus. Some studies have shown that a daily intake of fluid and salt supplementation in small divided doses may contribute to bone density because fluid volume expansion is not sensed as excessive fluid volume but rather as simple fluid volume redistribution (19-24). Other studies have shown that a sustainable fluid volume through a daily intake of fluid and salt supplementation in small divided doses may counteract diminished activity and increase tissue electrolytes (25-30). It is generally believed that renal and endocrine systems adjust bone mineral density regulating hormones to reduce electrolytes losses. Later of periodic fluid shifting to upper part of the body, kidneys and endocrine glands establish new “normal” electrolyte and hormone level appropriate for chronic PFR and/or more fluid volume. Evidently, periodic fluid shifting to upper part of the body may increase bone density during diminished muscular activity.

Bone density in the control subjects with and without PFR did not show significant differences; this may be attributable to physical activity. Physical activity may counteract PFR mechanisms. Physical activity that moves fluid volume to lower part of the body can determines the severity in delivery of fluid volume to the upper part of the body. Some studies (31-35) have shown that physical activity may not contribute to more fluid volume. Fluid volume is neither intravascular nor intracellular fluid and therefore does not contribute to vascular volume. Physical activity may determine PFR efficacy because the higher physical activity the lower PFR efficacy. Physical activity may act more as stressor rather than as stimulus of bone mineral density as was shown by the minor changes of bone density in the PFRCS group compared to the other groups. A much less adaptability to chronic PFR was shown in the PFRCS group than PFRHS group. Thus one would not observe bone density improvements in the PFRCS group as in the PFRHS group. With physical activity PFR did not play any significant part in bone density. However, even with physical activity PFR is a potent stimulus of bone density when it is used over longer time than the time required by the hypokinetic subjects. Physical activity was involved in the protection of bone density as was shown by the no changes in the ACS group compared to the HKS group.

It was found that the longer fluid volume is redistributed periodically into regional areas of the body the greater bone density. This indicates a common conception that the duration of PFR and fluid volume is vital to bone density increase. Duration of fluid volume expansion may determine body’s ability to regulate bone density (18). Evidently bone density may be affected by duration of PFR provided that an optimum fluid volume could be achieved. This adds a vital contribution to bone density because the longer PFR is administered the greater bone density. This shows that bone density may depend on the duration of PFR to which subjects are submitted. PFR duration and fluid volume expansion may act more as stimulus rather than as stressor of bone density. It is clear that fluid volume expansion and PFR duration made the hypokinetic subjects with chronic PFR to be less labile and more responsive to bone density than the hypokinetic subjects without.

There was a relation between PFR and fluid volume and bone density. When fluid volume shifts to upper parts of the body periodically and extracellular fluid volume expands, mineral regulation improves and bone density increases. Evidently, fluid volume expansion and chronic PFR may be important to prevention and/or treatment of bone loss. Studies have shown that increased bone density may be attributable to mineral regulation (19-24) and fluid volume expansion (25-30).

It would appear that a remedial procedure for prevention and/or treatment of bone loss would be the use of chronic PFR. The potential therapeutic implications of bone density with chronic PFR may be attributable to fluid volume expansion and bone mineral regulation. This is because with fluid volume expansion and bone mineral regulation bone density

increases. Some studies have shown that with chronic fluid volume expansion minerals are readily bound into bone tissue (19-24) and more minerals are deposited into the bone structures (25-30). Thus chronic PFR through fluid volume expansion and bone mineral regulation may contribute to or increase bone density.

With chronic PFR blood supply and oxygen delivery to tissue increase significantly. Fluid volume expansion and tissue oxygen delivery compensate or normalize adenosine triphosphate (ATP) and oxidative phosphorylation (OP) synthesis while with pure diminished muscular activity OP (36) and ATP synthesis (37) decrease. Increase of mitochondria density and/or function is considered as the most likely reason for OP and ATP synthesis. Mitochondria density increases with fluid volume expansion when with diminished muscular activity decreases (38). Fluid volume expansion is a potent stimulus for proliferation of mitochondrial enzymes. Mitochondria density and cytochrome c, which are crucially important in production of aerobic energy, increase when with diminished activity decrease (38). The mitochondria density depends on the duration and intensity one can endure fluid volume expansion and body's ability to spare total glycogen. With chronic fluid volume expansion new glycogen synthesis is stimulated and total glycogen depots are repleted while with diminished muscular activity glycogen stores are depleted (39).

As is known ATP is used as the primary source of energy for many metabolic processes and may affect both bone matrix and bone mineral metabolism. ATP plays a vital and complex role in the regulation of bone turnover, exerting a powerful effect on both osteoblasts and osteoclasts. Some studies have reported that ATP is capable of modulating osteoblasts bone formation (40) and osteoblast proliferation (41). Increase of ATP synthesis though fluid volume expansion and PFR could be a stimulus for osteoblasts and an inhibitor for osteoclasts and thus a potent stimulus of bone density. Therefore bone density could be attributable to PFR and fluid volume which could very well turn out to be beneficial to or even increase bone density during diminished activity.

Conclusion

The significant differences in the hypokinetic subjects with and without chronic PFR have shown potential benefits of bone density with chronic PFR. The increase of bone density may be attributable to bone mineral regulation through chronic PFR. Chronic PFR is a potent stimulus for protection and/or increase of bone density. In conclusion chronic PFR may be used to efficient regulate bone mineral deposition and thus contribute to or increase bone density with diminished activity. However, the underling mechanisms by which chronic PFR contributes to or increases bone density have yet to be established. Further studies are required to determine how the body uses the mechanisms of fluid sifting to upper part of the body to counteract the consequences of fluid shifting to lower part of the body to benefit bone density and other body functions.

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