Original Article

Muscle Calcium Regulation from Reducing Hypokinetic and Gravity Effects using Chronic Antiorthostatic Sleeping and Chronic Periodic Fluid Redistribution

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Abstract

Background and Aim: Chronic periodic fluid redistribution (CPFR) and chronic antiorthostatic sleeping (CAOS) counteracting diminished muscular activity (hypokinesia [HK]) and earth gravity effects contribute to vascular volume and electrolyte regulation. We hypothesized that CPFR and CAOS counteracting gravity compression effects and HK could affect muscle calcium (Ca^{++}). We therefore studied the potential clinical benefits of CPFR and CAOS on muscle calcium. **Methods:** Studies were conducted on 40 male volunteers. They were equally divided into four groups: active control subjects (ACS), hypokinetic subjects (HKS), CAOS control subjects (CAOSCS), and CAOS hypokinetic subjects (CAOSHS). We measured Ca^{++} in the muscle, plasma, urine, and feces during a preexperimental period of 390 days and an experimental period of 364 days. **Results:** In the CAOSHS group, muscle Ca^{++} increased ($P < 0.05$) and plasma Ca^{++} and Ca^{++} losses decreased ($P < 0.05$) compared to the HKS group. Muscle Ca^{++} increased more and Ca^{++} losses decrease more in a higher than a lower degree of CAOS position or higher than lower fluid shift to the head. In the HKS group without treatment of CPFR and CAOS, muscle Ca^{++} decreased ($P < 0.05$) and plasma Ca^{++} and Ca^{++} losses increased ($P < 0.05$) compared to the ACS, CAOSHS, and CAOSCS groups and the values at the preexperimental period. In the CAOSCS group, muscle Ca^{++} and plasma Ca^{++} and Ca^{++} losses did not change compared to the ACS group, and muscle Ca^{++} did not increase and plasma Ca^{++} and Ca^{++} losses did not decrease as in the CAOSHS group. Muscle Ca^{++}, plasma Ca^{++}, and Ca^{++} losses did not alter in the ACS group compared to their values at preexperimental period. All treated participants experience the need to urinate during the early preorthostatic and orthostatic position. Excretion of urine was ($P < 0.05$) higher in orthostatic position than in CAOS position and ($P < 0.05$) higher with lower fluid shift to the head than with higher fluid shift to the head. The participants were not experience blood pressure and heart rate changes in orthostatic position. **Conclusion:** The study provides evidence that muscle Ca^{++} increases from CPFR and CAOS, suggesting a potential benefit of muscle Ca^{++} regulation with treatment of CPFR and CAOS via chronically applied periodic and progressive fluid volume expansion.

Keywords: Calcium adaptation, calcium metabolism, clinical benefits, fluid volume, preventive measures

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Introduction

The treatment of an ailment with antiorthostatic position (AOP) is nothing new. AOP was used in ancient times for obtaining therapeutic benefits that come from counteracting earth gravity compression effect. Chronic antiorthostatic sleeping (CAOS) and chronic periodic fluid redistribution (CPFR) are applied to counteract earth gravity compression effects and diminished muscular activity (hypokinesia [HK]) to prevent or treat a variety of ailments and diseases.[1] The treatment with CAOS and CPFR is called antiorthostatic sleeping therapy. CAOS moves fluid away from lower part of the body to upper part of the body periodically and progressively and AOP moves fluid shifts from lower part of the body in upper part of the body acutely and continuously.[1]

Decreased fluid volume is most detrimental to the body because it forces organs and systems, particularly vital organs

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and systems to work harder. Diminished muscular activity and gravity affect fluid inside the body by pulling the various body fluids to lower extremities.[1] Reduction of fluid volume contributes to higher plasma electrolyte level and electrolytes losses.[2-9] To reduce electrolyte losses, different measures have been used;[10-15] however, CPFR and CAOS that counteract hypokinetic (HK) and gravity effects and move fluid away from the lower part of the body into upper part of the body could be the best solution for regulation of electrolytes.[16,17] It seems very difficult to believe that despite from time of Hippocrates were known the therapeutic benefits of counteracting gravity compression effects, the role of earth gravity on healthy and diseased humans has been investigated extensively,[18] and the effects of diminished muscular activity and fluid migration to lower extremities have been studied for many years.[2-15] Only very few studies have been published on the measures of counteracting the gravity compression effects[16,17] and other conditions on different organ systems including electrolyte metabolism. As bizarre as it seems in retrospect, scientists have managed to ignore one of the biggest challenges of investigating the therapeutic benefits of reducing gravity compression effect and HK effect and fluid shift to lower extremities on electrolytes and other conditions in decades. It is now a recognized therapeutic benefit the adaptation to CPFR and CAOS environment. We uncover something that has been right under our nose forever.

CPFR and CAOS that counteract the compressive effects of earth gravity and diminished muscular activity and fluid shift to lower extremities determine total fluid volume, hypervolemia and hypovolemia, and electrolyte metabolism. The treatment with CPFR and CAOS is a vital cornerstone of regulating fluid volume, electrolyte metabolism, and functions of organs and systems in physiologic and pathologic conditions and treating healthy and diseased humans. The intensity and duration of CPFR and CAOS treatment increase fluid volume and make electrolyte metabolism to work better than any other measures endeavor to date. We hypothesize that counteracting fluid shift to lower parts of the body and diminished muscular activity and earth gravity compression effects could contribute to electrolyte regulation. To demonstrate the clinical benefits of muscle Ca^{++} and to determine a potential effect of regulating muscle Ca from CPFR and CAOS, via chronically applied periodic and progressive fluid volume redistribution (FVR), we measured muscle Ca^{++}, plasma Ca^{++}, and Ca^{++} losses in the urine and feces of physically healthy participants during fluid migration from lower half of the body into upper half of the body and diminished muscular activity and reduced gravity effects.

Materials and Methods

The study was conducted as 390-day preexperimental period and 364-day experimental period. The study was confirmed 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. All participants received verbal and written explanations of the experimental and test protocols before providing written informed consent. There was no history of any medical problems, and none of the participants was under any drug therapy which could have interfered with metabolism of calcium. In the course of the study were not drop-outs. Financial incentives relative to average monthly earnings were used to encourage compliance with the protocol of the study. Forty physically healthy male volunteers of 24.5 ± 7.7, 25.8 ± 6.6, 26.3 ± 4.0, and 25.6 ± 5.5 years of age for the active control subjects (ACS), HK subjects (HKS), CAOS control subjects (CAOSCS), and CAOS hypokinetic subjects (CAOSHS) groups, respectively, were chosen as participants. All subjects run average distances of 9.2 ± 1.3 km/day at a speed of 9.1 ± 1.3 km/h for 3–5 years. Participants had a mean body weight of 75.6 ± 8.4, 77.0 ± 6.4, 74.8 ± 5.5, and 76.6 ± 7.4 kg for ACS, HKS, CAOSCS, and CAOSHS groups, respectively. Subjects had a mean peak oxygen uptake of 48.0 ± 7.5 mL/kg/min. In preexperimental period of 390 days, participants run average distances of 9.2 ± 1.4 km/day at a speed of 9.1 ± 1.4 km/h.

Assignment of participants 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 participants run average distances of 9.2 ± 1.3 km/day. They were assigned to the ACS group. Group 2: Ten participants walked average distances of 2.8 ± 0.3 km/day. They were assigned to the HKS group. Group 3: Ten participants run average distances of 9.2 ± 1.4 km/day and were submitted to CPFR and CAOS. The participants were assigned to CAOSCS group. Group 4: Ten participants walked average distances of 2.8 ± 0.4 km/day and were submitted to CPFR and CAOS. These participants were assigned to CAOSHS group.

Protocol

The investigation consisted of a 390-day preexperimental 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 3630 ± 350, 3095 ± 257, 3650 ± 420, and 3131 ± 260 standard deviation (SD) kcal, and mean daily consumption of Ca^{++} was 43.3 ± 1.3, 43.2 ± 1.1, 43.1 ± 1.5, and 43 ± 1.2 SD mmol for the ACS, HKS, CAOSCS, and CAOSHS groups, respectively. All participants were housed in a facility in which temperature, humidity, activities, and dietary intakes were monitored 24 h per day and 7 days per week.

Simulation of hypokinetic conditions

To simulate a lower degree of HK, the number of kilometers walking per day was restricted to an average of 2.8 ± 0.5 km/day and was monitored daily by an accelerometer. The activities allowed were those that approximated the normal routines of HK individuals. Participants were allowed to walk to the dining rooms, lavatories, and different laboratories where the tests were

administered. Climbing stairs and other activities that required greater efforts were not allowed. Participants were mobile and were not allowed outside the installation grounds so that the level of diminished muscular activity could remain relatively constant and easily monitoring.

Simulation of reduced earth gravity effects

To simulate reduced gravity effects, the participants were slept without a pillow at −6° to −50° of AOP. The participants were slept for at least 8 h at night and for at least 2 h at mid-day. The actual tests at different degrees of CAOS were performed when the adaptability of the participants was achieved. The degree of CAOS increased progressively by −2° each time. The degree of CAOS increased after the ability of participants to adapt to the specific degree of CAOS established. The degree of CAOS increased approximately every 24–31 days or more and after the ability of volunteers to adapt to that degree of CAOS was determined. At each degree of CAOS, participants were kept about the same duration of time to secure the adaptability of participants to that degree of CAOS. The individual differences of biochemical, physiological, metabolic, cardiovascular, endocrine, and renal reactions of the participants and their symptoms and reactions to CAOS were taken into account. The experimental schedule changed periodically to conform to the ability of volunteers to adapt progressively to CAOS. To reduce stress and to ensure the comfort of volunteers, the intensity and duration of CAOS was modified as required.

Blood, urinary, and fecal sample collection

To accommodate inter-individual differences in bowel habits, urine and feces were analyzed daily and were pooled to form 6-day composites, while blood samples were measured every 6 days during the preexperimental and the experimental period. The 6-day (consecutive days) pooled samples were collected. Blood samples were collected with disposable polypropylene syringes. Following overnight fasting for about 6–7 h, venous samples of the blood were taken at rest and before each meal. Blood samples were drawn under the same conditions between 8.00 and 9.00 a.m. without a venous stasis and after participants had been sitting for about 30 min. The sample volume was 6–8 mL. To obtain plasma, blood samples were collected in heparinized ice-chilled tubes and were centrifuged immediately at 10.000 × *g* for 3 min at room temperature and separated using glass capillary pipettes which were washed in hydrochloric acid (HCl) and deionized distilled water. Immediately after centrifugation, plasma samples were frozen on dry ice and were stored at −20°C until analyses were conducted for plasma Ca^{++} level. An aliquot of the plasma and urine was acidified to pH >2.0 by adding 6M HCl (to prevent Ca^{2+} precipitation). Twenty-four-hour urine samples were stored at −4°C until needed for Ca^{++} analysis. To ensure 24 h urine collections, creatinine loss was measured by a colorimetric method using the Jaffe's reaction. Feces were collected in plastic bags, weighed, and stored at −20°C for Ca^{++} analysis. Fecal samples were dried-ashed in a muffle furnace at 600°C overnight. Ashed samples were dissolved in 5% nitric acid. To ensure complete feces, recovery polyethene glycol was used as a marker.

Muscle preparations, calcium extraction, and analysis

Muscle biopsies were performed by a percutaneous needle technique[19] under local anesthesia. Specimens were taken from the lateral portion of the quadriceps femoris muscle, 15–20 cm proximal to the knee. The muscle (mean weight 13.3 mg) was placed on a piece of quartz glass and with nonmetal tweezers carefully dissected free from all visible fat and connective tissue. Traces of the blood were wiped off by rolling the specimens on the piece of quartz glass. Muscle was then placed on a platinum hook and dried in an oven at 100°C to constant weight, extracted in 1 mL of petroleum ether for 2 h, and dried to constant weight, and fat-free dry solids (FFDSs) weight was calculated. The calcium extracted from muscle by treatment with 250 μL 2.5 M HNO_3 for 24 h. From each sample, 100 μL of supernatant was diluted to 10 mL with 0.25% $SrCl_2$, and analysis for calcium in muscle was performed by atomic absorption spectrophotometry on a Perkin-Elmer 420 Model, Perkin-Elmer Corp., Norwalk, CT, USA. The results obtained on muscle calcium content throughout the investigation were calculated in mmol/100 g FFDS.

Calcium measurements

Samples were analyzed in duplicate and appropriate standards were used for measurements. The Ca^{++} levels in the muscle and feces and in the acidified plasma and urine were measured. The urine and fecal samples were diluted as necessary and aspirated directly into an atomic absorption spectrophotometer of Perkin-Elmer430 model, Perkin-Elmer Corp., Norwalk, CT, USA.

Statistical analysis of data

A two-way interaction (treatment [3 levels] × days [6 levels]) analysis of variance (ANOVA) was used to determine muscle Ca^{++} changes from CPFR and CAOS and a potential clinical benefit of treating Ca^{++} losses. ANOVAs with repeated measures of 2-way interaction, that is, treatment and days, and preexperimental and experimental values, and HK and antiorthostatic sleeping HK groups, and the KH and control groups, were used. ANOVAs for each time point measurements were used. The statistical analysis of the results was made with GraphPad Prism statistical software (GraphPad Software Inc., La Jolla, California). The level of significance was set to < 0.05.

Results

At the initial stages, the participants reported clinical symptoms some of which were typical to AOP [Table 1]. The participants of the CAOSCS group and the CAOSHS group have shown various symptoms in the right organs and in the right arms and legs. The symptoms were greater in the CAOSCS than in the CAOSHS groups of participants. The CAOSH group and less the CAOSCS group of participants have shown benefits in their health and well-being; they experience significant lessening of tiredness, weight, and sleep needs, and they have gained significant height, energy, power, and strength. The CAOSHS and the CAOSCS groups have remarked that

Table 1: Symptoms from chronic antiorthostatic sleeping and chronic periodic fluid redistribution

Frequent urination
Puffiness in the face
Tachycardia
Ventricular extrasystoles
Arrhythmias
Loud heart sounds
Tinnitus in the right ear
Feeling of fullness (pressure) or stuffiness in the right ear
Near- and far-sightedness in the right eye
Visual acuity problems in the right eye
Eyelid ptosis
Muscle spasms in the right leg and the right hand
Pain in the right side of trapezius muscle
Pain in the right knee
Pain in the right leg and in the right hand
Cold sensation in the right food and in the right hand
Urticaria in the right arm and leg
Sputum production clearance

they have benefited from CPFR and CAOS and continued the treatment after the study has been completed. The HKS group and the ACS group of participants have adapted the treatment of CPFR and CAOS when they resume their everyday life activities. Significant physiological, biochemical, and muscle changes developed from CAOS and CPFR and reduced gravity compression effect.

At the preexperimental period, muscle Ca^{++} decreased and plasma Ca^{++} and Ca^{++} losses in the urine and feces increased in the CAOSHS and the CAOSCS groups; however, as the duration of preexperimental period increased and the participants were adjusted to CPFR and CAOS, muscle Ca^{++} increased and plasma Ca^{++} and Ca^{++} losses in the urine and feces decreased [Table 2]. In the HKS group and the ACS group, muscle Ca^{++}, plasma Ca^{++}, and Ca^{++} losses did not change [Table 2].

At the experimental period, muscle Ca^{++} increased ($P < 0.05$) and plasma Ca^{++} and Ca^{++} losses in the urine and feces decreased ($P < 0.05$) in the CAOSHS group as compared to the HKS group [Table 2]. Muscle Ca^{++} increased ($P < 0.05$) more and Ca^{++} losses decrease ($P < 0.05$) more in a higher than a lower degree of CAOS position and higher than lower fluid shift to the head. In the HKS group without the treatment of CPFR and CAOS, muscle Ca^{++} decreased ($P < 0.05$) and plasma Ca^{++} and Ca^{++} losses in the urine and feces increased ($P < 0.05$) compared to the ACS group, CAOSHS group, and CAOSCS group and the values at the preexperimental period [Table 2]. In the CAOSCS group, muscle Ca^{++} and plasma Ca^{++} and Ca^{++} losses did not change compared to the ACS group, and muscle Ca^{++} did not increase and plasma Ca^{++} and Ca^{++} losses did not decrease in the CAOSCS group as in the CAOSHS group [Table 2]. In the ACS group, muscle Ca^{++}, plasma Ca^{++}, and Ca^{++} losses in the urine and feces did not change compared to their values at the preexperimental period [Table 2]. All treated participants experience the need to urinate during the early preorthostatic position and orthostatic position. Excretion of urine was ($P < 0.05$) higher in orthostatic position than in CAOS position and ($P < 0.05$) higher with lower fluid shift to the head than with higher fluid shift to the head. The participants did not experience blood pressure and heart rate changes in orthostatic position.

Discussion

In the present study, symptoms of CPFR and CAOS disappeared as the duration of experimental period and adaptation process to CPFR and CAOS increased. The symptoms did not affect the ability of the CAOSHS group and the CAOSCS group to adapt to CPFR and CAOS conditions. The severity of symptoms of the CAOSCS group may be attributable to the higher physical activity, suggesting that physical exercise aggravate symptoms with the treatment of CPFR and CAOS. The reason why the symptoms were shown only in the right organs and in the right arms and legs is not known and the results cannot explain why the right organs and the right arms and legs are affected. The expectation is that over longer time, symptoms will not appear in the left organs and in the left arms and legs and the symptoms are readjustment and harmonization signs following body's adaptation to new environmental conditions. The health and well-being benefits may be signs of adaptation of the different body functions to come when the body adapts completely to the new environmental conditions of CAOS and CPFR with reduced HK and gravity compression effects. As the human body seeks a new equilibrium to CAOS and CPFR, the circulating fluid volume increases.

Under CPFR and CAOS conditions, the human body goes through a multitude of biochemical and physiological changes. CAOS and CPFR are the potent stimuli for muscle Ca^{++} regulation. CAOS and CPFR contributed to higher muscle Ca^{++} in the CAOSHS group compared to the HKS group. The significant differences between the CAOSHS group and HKS group suggest that CAOS and CPFR act more as stimulus than as stressor of muscle Ca^{++} regulation. Evidently, muscle Ca^{++} increased from CAOS and CPFR than from HK alone. Muscle Ca^{++} increased because is taken up for deposition and used by the body which in turn protects or increases the net muscle Ca^{++}.[2-9] The lower Ca^{++} losses in the CAOSHS group compared to the HKS group may be attributable to Ca^{++} losses regulation because Ca^{++} losses cannot decrease the muscle Ca^{++} repletion, except when Ca^{++} losses are regulated.[20-25] The reduced Ca^{++} losses suggest that muscle Ca^{++} repletion is not sensed as excessive because Ca^{++} losses cannot reduce in Ca^{++}-repleted muscle unless the surpluses of muscle Ca^{++} is sensed as chronic redistribution. The lower plasma Ca^{++} in the CAOSHS group compared to the HKS group may be attributable to Ca^{++} regulation because plasma Ca^{++} cannot decrease in Ca^{++}-repleted muscle unless is sensed as simple Ca^{++} redistribution. The lower plasma Ca^{++} may be attributable to Ca^{++} deposition since plasma Ca^{++} cannot reduce in

Table 2: Muscle calcium, plasma calcium, and urinary and fecal calcium measured in the control group and hypokinetic group of subjects and in the chronic antiorthostatic sleeping control Group and chronic antiorthostatic sleeping hypokinetic group of subjects During the preexperimental and the experimental period

Experimental period (days)	Calcium			
	Muscle (mmol/100g FFDS)	Plasma (mmol/L)	Urinary (mmol/days)	Fecal (mmol/days)
ACS, (*n*=10)				
Preexperimental	41.10±2.31	2.20±0.03	3.40±1.10	14.2±2.2
60th	41.11±3.24	2.20±0.03	3.42±1.11	14.3±2.3
120th	41.12±2.30	2.21±0.01	3.40±1.10	14.0±2.2
180th	41.13±3.22	2.20±0.02	3.42±1.13	13.9±2.4
240th	41.12±2.31	2.21±0.03	3.43±1.10	14.0±2.3
300th	41.13±3.34	2.20±0.01	3.40±1.15	14.2±2.5
364th	41.14±2.25	2.19±0.02	3.41±1.11	14.1±2.3
HKS, (*n*=10)				
Preexperimental	41.12±4.32	2.20±0.02	3.42±1.12	14.3±2.5
60th	37.58±3.40*†	2.38±0.03*†	4.30±1.13*†	18.2±2.4*†
120th	37.88±4.30*†	2.36±0.02*†	4.26±1.15*†	17.7±2.4*†
180th	36.97±3.38*†	2.42±0.03*†	4.46±1.10*†	19.3±2.5*†
240th	37.15±3.33*†	2.37±0.04*†	4.40±1.12*†	18.7±2.6*†
300th	34.12±4.42*†	2.50±0.02*†	5.06±1.13*†	22.3±2.4*†
364th	34.30±3.34*†	2.46±0.04*†	4.88±1.14*†	21.6±2.5*†
CAOSCS, (*n*=10)				
Preexperimental	40.33±2.35	2.25±0.03	3.70±1.11	15.8±2.2
60th	41.89±3.42	2.22±0.02	3.42±1.12	14.8±2.3
120th	41.78±2.50	2.23±0.01	3.46±1.10	15.0±2.2
180th	42.57±4.27	2.20±0.03	3.31±1.12	14.1±2.0
240th	42.43±3.31	2.21±0.02	3.35±1.11	14.3±2.2
300th	43.30±4.28	2.18±0.03	3.24±1.12	13.6±2.3
364th	43.24±3.30	2.19±0.01	3.30±1.13	13.8±2.0
CAOSHS, (*n*=10)				
Preexperimental	40.35±2.41	2.25±0.03	3.71±1.12	16.0±2.1
60th	42.78±3.50+	2.18±0.02+	3.25±1.12+	13.4±2.5+
120th	42.73±2.53+	2.20±0.01+	3.23±1.13+	13.6±2.3+
180th	43.01±3.35+	2.15±0.03+	3.20±1.10+	13.3±2.2+
240th	42.92±4.43+	2.17±0.02+	3.23±1.13+	13.5±2.4+
300th	45.46±3.28+	2.10±0.03+	3.00±1.11+	12.6±2.0+
364th	45.38±3.44+	2.12±0.02+	3.05±1.12+	13.0±2.2+

FFDS. The values were expressed as mean±SD. †P<0.05 significant differences between the preexperimental and experimental period values. *P<0.05 significant differences between the hypokinetic group and the control group. +P<0.05 significant differences between the chronic antiorthostatic sleeping hypokinetic group and the hypokinetic group. FFDS: Fat-free dry solids, ACS: Active control subjects, HKS: Hypokinetic subjects, CAOSCS: Chronic antiorthostatic sleeping control subjects, CAOSHS: Chronic antiorthostatic sleeping hypokinetic subjects

Ca^{++}-repleted muscle except when Ca^{++} is deposited.[20-25] The higher muscle Ca^{++} and the lower Ca^{++} losses from higher CAOS degree and higher fluid shift to the head may be attributable to different Ca^{++} regulation mechanisms because higher CAOS degree is not perceived as stressor and fluid shift to the head is not sensed as excessive volume, but rather as simple FVR and the excretion mechanisms are not activated.

Muscle Ca^{++} repletion was accompanied by lower plasma Ca^{++} and Ca^{++} losses and muscle Ca^{++} depletion by higher plasma Ca^{++} and Ca^{++} losses. This could be attributable to different Ca^{++} regulation mechanisms in the CAOSHS from those in the HKS. In the CAOSHS group, muscle Ca^{++} repletion was shown by lower than higher plasma Ca^{++} and Ca^{++} losses, and in the HKS group, muscle Ca^{++} depletion by higher than lower plasma Ca^{++} and Ca^{++} losses. Thus, with treatment, muscle Ca^{++} repletion accompanied by lower plasma Ca^{++} and Ca^{++} losses, and without treatment, muscle Ca^{++} depletion accompanied by higher plasma Ca^{++} and Ca^{++} losses. This may be due to FVR and fluid volume expansion and different Ca^{++} regulation mechanisms.[2-9] The CAOS and CPFR make electrolyte regulation to work more efficient from what normally would. The kidneys and the endocrine systems adjust electrolyte-regulating hormones to reduce electrolyte losses. Later adaptation to CAOS and CPFR conditions, the endocrine glands and the kidneys show a new normal level of electrolytes and hormones. CAOS and CPFR preconditions the renal and the endocrine system to shift the fluid to the head

and reduce stress on renal and endocrine system and helps in overall adaptation of organs and systems to shift the fluid to the head. Chronic fluid and salt supplementation that expands fluid volume decrease fluid and electrolyte losses because fluid volume expansion is sensed as FVR and the excretion mechanisms are not activated. Some studies have shown that chronic fluid and salt supplementation increases tissue electrolytes and reduces electrolyte losses.[26-31] It is generally believed that fluid migration to thoracic region triggers a chain of events which restores the regulation of electrolytes to its initial status, increasing muscle electrolytes, and decreasing plasma electrolytes and electrolyte losses.

In the CAOSHS group, the capacity of the body to regulate muscle Ca^{++} was higher than in the HKS group. The HKSs with CAOS and CPFR have shown a less labile and more responsive muscle Ca^{++} regulation than the HKSs without. This shows a common conception that CAOS and CPFR are vital for muscle Ca^{++} regulation. Adaptation to CAOS and CPFR determines the ability of the body to regulate muscle Ca^{++}. Thus, regulation of muscle Ca^{++} depends on the capacity of the body to adapt to CAOS and CPFR environment. This adds a vital contribution to regulation of muscle Ca^{++} because the higher the ability of the body to adapt to CAOS and CPFR the greater the capacity of the body to regulate muscle Ca^{++}. This shows that muscle Ca^{++} regulation depends on the ability of the body to adapt to CAOS and CPFR. This can be reached when the adaptation to CAOS and CPFR determines the ability of the body to regulate muscle Ca^{++}. Adaptation is achieved when muscle Ca^{++} is deposited more efficient than normally would and muscle Ca^{++} regulated better than before. Regulation of muscle Ca^{++} also depends on the magnitude and duration of CAOS and CPFR and the capacity of the body to adapt to higher degree of CAOS and longer duration of CPFR. In CAOS position, the participants experience lesser earth gravity as the pull of gravity and fluid shift to the head is diminished. In CAOS position, fluid moves away from lower half part of the body into upper part of the body easier while in orthostatic position harder, thereby affecting differently fluid shift to thoracic region, fluid redistribution, and total fluid volume.

The different Ca^{++} adaptation process, different Ca^{++} regulation, different vascular volume, different blood and oxygen delivery to tissues, different fluid redistribution, and different antiorthostatic condition in CAOS from that in AOP could have contributed to differences in muscle Ca^{++} in CAOS from those of AOP. Fluid migrates to upper half part of body tissues periodically and progressively, preconditions the body tissues to fluid volume expansion and hydrostatic gradients which reduces stress effect on those body tissues and help in the overall Ca^{++} adaptation to fluid volume expansion and circulatory gradients, contributing to differences of muscle Ca^{++} in CAOS condition from that of AOP. In case of CAOS condition, fluid volume is intravascular and intracellular and therefore contributes to vascular volume which is important to deposition of electrolytes. Some studies have shown that chronic fluid volume expansion from chronic supplementation of fluid and salt contributes to electrolytes deposition.[26-31] CAOS pulling the various body fluids to upper part of the body produces periodic and progressive fluid redistribution, while AOP produces acute and continuous fluid redistribution inside the body. Moving the various body fluids away from lower part of the body, periodically, CAOS determines the delivery of fluids to upper part of the body tissues progressively, while shifting the various body fluids acutely AOP determines fluid shift to upper half part of the body continuously. This determines the differences between periodic and progressive fluid movement to thoracic region of the body and acute and continuous fluid migration to body's thoracic region that could have contributed to higher muscle Ca^{++} in CAOS and lower muscle Ca^{++} in AOP.

There were no apparent differences between the CAOSCS group who had CPFR and CAOS treatment and the ACS group who did not. The CAOSCS group did not have higher muscle Ca^{++} than the ACS group and as higher muscle Ca^{++} as the CAOSHS group. This may be related to many factors and primarily to physical exercise. Physical exercise can be harmful to the human body because the more exercise the more weight lift and the more pay the toll from earth gravity effect. Physical exercise assumes to be beneficial; however, in reality, it takes a heavy toll on the human body. Physical exercise aggravates gravity compression effect on the human body and minimizes the potential of CAOS and CPFR. Physical exercise greatly affects FVR inside the body by pulling the various body fluids in lower half part of the body. Physical exercise by moving fluid to lower half part of the body determines the severity in delivering fluid volume to upper half part of the body and thus total fluid volume. Some studies have shown that physical exercise acts more as stressor than as stimulus.[10] Fluid volume is neither intravascular nor intracellular and therefore does not contribute to expansion of vascular volume and deposition of electrolytes.[32-36] Physical activity is not associated with more blood volume and electrolyte deposition. Physical exercise determines the ability of the body to adapt to CAOS and CPFR because the higher physical activity the lower the ability of the body to adapt to CAOS and CPFR environment. One would not notice muscle Ca^{++} regulation in the CAOSCS group as in the CAOSHS group; however, physical exercise is a powerful stimulus for the protection of muscle Ca^{++} as was shown by the no changes in the ACS group compared to the HKS group. Physical exercise in CAOS and CPFR environment may not play an important part of muscle Ca^{++} regulation. CAOS and CPFR even with physical exercise are the potent stimuli for muscle Ca^{++} regulation, when used over longer time than the time required without physical exercise. The minor muscle Ca^{++} change in the CAOSCS group compared to the CAOSHS group shows that the HKSs had not probably adapted to CPFR and CAOS condition and physical exercise had completely suppressed the effect of CPFR and CAOS. It is clear why one would not observe the greatest muscle Ca^{++} in the CAOSCS group as in the

CAOSHS group and that CPFR and CAOS treatment without physical activity are important in stimulation of muscle Ca^{++}.

Conclusion

Significant differences were found of muscle Ca^{++} and Ca^{++} losses in the CAOSHS group from those in the HKS group. The high muscle Ca^{++} and low plasma Ca^{++} and Ca^{++} losses in the CAOSHS group from the HKS group may be attributable to different mechanisms of electrolyte regulation. Muscle Ca^{++} increased more and Ca^{++} losses decreased more from higher than a lower degree of CAOS and higher than lower fluid shift to thoracic regional areas of the body, and muscle Ca^{++} increased more from lower than higher physical exercise. However, the underlying mechanisms by which CPFR and CAOS regulate muscle Ca^{++} and Ca^{++} losses have not been established yet. Further studies are required to determine how muscle Ca^{++} increases more and Ca^{++} losses decrease more from higher than lower degree of CAOS position and higher than lower fluid shift to the head, how muscle Ca^{++} increases more from lower than higher physical exercise, and how the adaptation to CPFR and CAOS affect Ca^{++} metabolism. In conclusion, the results show that CPFR and CAOS contribute to muscle Ca^{++} and Ca^{++} losses regulation, showing the potential of CPFR and CAOS to counteract fluid shift to lower part of the body and diminished muscular activity and reduce gravity compression effects on muscle Ca^{++} and Ca^{++} losses through chronically applied periodic and progressive fluid volume expansion. It would probably be the biggest discovery known to humankind if we found out how CPFR and CAOS can restore or reverse electrolytes and prevent or treat physiological and biochemical conditions and diseases by increasing circulating blood volume, reducing earth gravity compression effects, counteracting fluid shift to lower extremities and diminished muscular activity effects.

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Conflicts of interest

There are no conflicts of interest.

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