Chronic periodic fluid redistribution effect on muscle calcium in healthy subjects during prolonged hypokinesia

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Abstract Studies have shown that chronic periodic fluid shifting upwards is not sensed as excessive fluid volume and excretion mechanisms are not activated. To determine if chronic periodic fluid and volume shifting upwards can affect muscle calcium (Ca\(^{2+}\)) during hypokinesia (HK) we measured muscle Ca\(^{2+}\) content, plasma Ca\(^{2+}\) concentration, and Ca\(^{2+}\) losses in urine and feces. Studies were conducted on 40 healthy male volunteers. They were divided into four equal groups: active control subjects (ACS), hypokinetic subjects (HKS), periodic fluid redistribution control subjects (PFRCS), and periodic fluid redistribution hypokinetic subjects (PFRHS). Plasma Ca\(^{2+}\) level decreased (p < 0.05) in Ca\(^{2+}\) repleted muscle, muscle Ca\(^{2+}\) level increased (p < 0.05), and Ca\(^{2+}\) losses in urine and feces decreased (p < 0.05) in the PFRHS group compared with the HKS group. Plasma Ca\(^{2+}\) level increased (p < 0.05) in Ca\(^{2+}\) deficient muscle, muscle Ca\(^{2+}\) level decreased (p < 0.05), and Ca\(^{2+}\) losses in urine and feces increased (p < 0.05) in the HKS group compared with their pre-experimental levels and the values in their respective control groups (ACS and PFRCS). This study shows that the muscle Ca\(^{2+}\) content increases and Ca\(^{2+}\) excretion decreases, suggesting the clinical potential of chronic periodic fluid and volume redistribution in treatment of muscle Ca\(^{2+}\) deficiency.

Keywords Calcium repletion · Cell mass preservation · Hypervolemia · Oxygen supply · Aerobic glycolysis · Mitochondrial density · Oxidative phosphorylation · Adenosine triphosphate synthesis

Abbreviations

HK Hypokinesia (HK; diminished movement)
CPFR Chronic periodic fluid redistribution
ACS Active control subjects
HKS Hypokinetic subjects
PFRCS Periodic fluid redistribution control subjects
PFRHS Periodic fluid redistribution hypokinetic subjects
Ca\(^{2+}\) Calcium
ATP Adenosine triphosphate
OP Oxidative phosphorylation

Introduction

Periodic fluid redistribution (PFR) is a condition in which fluid shifting upwards is not sensed as an excessive volume of fluid and the excretion mechanisms are not activated. Periodic redistribution of fluid is a factor of higher vascular and interstitial fluid volume and lower interstitial fluid pressure; this enables appropriate management of diseases in which interstitial fluid pressure increases, improving access to nutrients, oxygen, and drugs. Periodic redistribution of fluid can affect electrolyte metabolism enabling appropriate regulation of electrolyte deposition [1] in which tissue electrolyte level is altered. However its identification requires specific knowledge of biochemical indicators to differentiate it from other forms of fluid and volume redistribution, for example fluid and volume redistribution during postoperative periods and/or during postural manipulations.

Hypokinesia (diminished movement) is defined as a condition of physical inactivity beyond that associated with daily functioning [2–4], fluid and volume shifting to the...
lower extremities, and deconditioning of vessels of the lower part of the body. Hypokinesia (HK) is a consequence of catabolism, low energy production, body weight loss [2–4], hypovolemia, cell injury, and hormonal and electrolyte changes [5, 6]. Deposition of electrolytes, for example magnesium, phosphate, calcium, potassium, sodium, and chloride, is affected during HK [7–13]. Long term exposure to HK also impact muscle. There is loss of muscle mass, strength and endurance, especially in the lower extremities. Changes in muscle performance, coupled with the effects of HK on connective tissues and the demands of activities of varying intensities, place hypokinetic population at risk of fatigue and injury. To counteract the consequences of prolonged HK on electrolytes, including calcium (Ca$^{2+}$), deposition, different measures have been used [14–19].

Hypokinesia is accompanied by fluid and volume shifting to the lower extremities and retention in them of a larger volume of fluid than that which is normal for the lower part of the body, resulting in lower fluid volume and diminished filling with blood of the central vascular bed [20]. As a result of fluid shifting to the lower extremities more fluid migrates to the pelvic region and the lower half part of the body. The fluid that can fit into the venous system of the lower part of the body can determine the severity of changes in the delivery of fluid to the upper part of the body, and thus extracellular and interstitial fluid volume. The lower blood volume may lead to a higher plasma electrolyte level, which can potentially result in greater electrolyte losses [7–13]. Use of such measures as periodic fluid redistribution, which is accompanied by periodic fluid shifting upwards and fluid volume expansion, may be important to Ca$^{2+}$ deposition during prolonged hypokinesia.

Fluid redistribution, which is accompanied by periodic fluid and volume shifting upwards and downwards, has been shown to regulate muscle sodium content when it occurs regularly over a long period of time [1]. Therefore we investigated the effect of periodic redistribution of fluid and volume over 1 year for at least 8 h per day on muscle Ca$^{2+}$ content, plasma Ca$^{2+}$ concentration, and Ca$^{2+}$ losses.

Materials and methods

Forty physically healthy male subjects 23.8 ± 6.6 years of age gave informed consent to take part in the study after a verbal and written explanation of the procedures and risks involved were given. There were no medical problems among the volunteers and none of the subjects was under any drug therapy or had any medical condition which could have interfered with Ca$^{2+}$ metabolism. During the pre-experimental and experimental period there were no drop-outs. The procedures were previously reviewed and approved by the Committee for the Protection of Human Subjects. Financial incentives were used to encourage compliance with the protocol of the study. All subjects were students who had run average distances of 9.1 ± 1.4 km day$^{-1}$ at a speed of 9.5 ± 1.3 km h$^{-1}$ for 3–5 years. Subjects had a body weight of 73.8 ± 7.0 kg and peak oxygen uptake of 47.7 ± 6.0 mL kg$^{-1}$ min$^{-1}$. During the pre-experimental period of 290 days all subjects had run an average distance of 9.1 ± 1.3 km day$^{-1}$ at a speed of 9.5 ± 1.4 km h$^{-1}$.

Assignment of subjects to four groups was done randomly; randomization was conducted by someone independent of recruitment and periodic fluid redistribution training, and a concealed method was used.

- **Group 1**: ten subjects had run an average distance of 9.1 ± 1.5 km day$^{-1}$ for 364 days. They were assigned to the active control subjects (ACS) group.
- **Group 2**: ten subjects had walked average distances of 3.7 ± 0.7 km day$^{-1}$ for 364 days. They were assigned to the hypokinetic subjects (HKS) group.
- **Group 3**: ten healthy subjects had run an average distance of 9.1 ± 1.6 km day$^{-1}$ and were subjected to a −6° to −8° head down tilt position for 8–10 h per day for 364 days. They were assigned to the periodic fluid redistribution control subjects (PFRCS) group.
- **Group 4**: ten healthy subjects had walked an average distance of 3.7 ± 0.6 km day$^{-1}$ and were subjected to a −6° to −8° head down tilt position for 8–10 h per day for 364 days. They were assigned to the periodic fluid redistribution hypokinetic subjects (PFRHS) group.

Protocol

The investigation consisted of a 290-day pre-experimental period and a 364-day experimental period. 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 in the metabolic diet was 3530 ± 453, 3045 ± 260, 3570 ± 511, and 3151 ± 263 kcal, and mean daily Ca$^{2+}$ consumption was 43.1 ± 1.5, 43.0 ± 1.7, 43.0 ± 1.3, and 43.5 ± 1.5 mmol for the ACS, HKS, PFRCS, and PFRHS groups, respectively.

Simulation of hypokinesia

To simulate a specific degree of hypokinesia the number of kilometers walking per day was restricted to an average of 3.7 ± 0.7 km day$^{-1}$ and was monitored daily by use of an accelerometer. 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 that required greater effort were not allowed. Subjects were
mobile and were not allowed outside the hospital grounds, so the level of diminished muscular activity could remain relatively constant and easily monitored.

Simulation of periodic fluid redistribution

To simulate the effect of chronic periodic fluid redistribution, the subjects were submitted to −6° to −8° head down tilt for 8–10 h per day during the pre-experimental period of 290 days and the experimental period of 364 days. In the pre-experimental period the subjects were progressively subjected to periodic fluid redistribution by increasing the level of head down tilt to −2°, −4°, −6°, and −8° every 34–61 days. The subjects were then subjected to the −6° to −8° periodic head down tilt position for 8–10 h per day for the remainder of the pre-experimental period and all the experimental period. Selection of the pre-experimental period of 290 days and subjection of the subjects every 34–61 days to the head down tilt position of −2°, −4°, −6°, and −8°, and their exposure to the head down tilt position of −6° to −8° for 364 days was the result of preliminary experimentation. The −6° to −8° periodic head down tilt position was alternated from time to time according to the responses of subjects at the position at that time. Personal differences and the sensitivity of the biochemical and physiological reactions, that is, renal, hormonal, cardiovascular, and metabolic reactions of the subjects to the chronic periodic fluid redistribution, were taken into consideration.

Blood, urine, and fecal sample collection

To accommodate inter-individual differences in bowel habits, urine and feces were analyzed daily and were pooled to form 6-days composites. Blood samples were measured every 6 days during the pre-experimental and the experimental periods. Six-day (consecutive days) pooled samples were collected. Blood samples were collected with disposable polypropylene syringes. After overnight fasting for approximately 6–7 h, venous samples of 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 venous stasis and after subjects had been sitting for approximately 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 10000×g for 3 min at room temperature and separated using glass capillary pipettes which were washed in hydrochloric acid and deionized distilled water. Immediately after centrifugation plasma samples were frozen on dry ice and were stored at −20°C until analysis was conducted to determine plasma Ca2⁺ level. An aliquot of the plasma and urine was acidified to pH >2.0 by adding 6 M HCl (to prevent Ca2⁺ precipitation). Twenty-four-hour urine samples were stored at −4°C until needed for Ca2⁺ analysis. To ensure 24 h urine collections creatinine loss was measured by a colorimetric method using Jaffe’s reaction. Feces were collected in plastic bags, weighed, and stored at −20°C for Ca2⁺ analysis. Fecal samples were dried and ashed in a muffle furnace at 600°C overnight. Ashed samples were dissolved in 5% nitric acid. To ensure complete feces recovery poly(ethylene glycol) was used as a marker.

Muscle preparations, calcium extraction, and analysis

Muscle biopsies were performed by a percutaneous needle technique [21] 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 15.6 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 blood were wiped off by rolling the specimens on the piece of quartz glass. The muscle was then placed on a platinum hook, dried in an oven at 110°C to constant weight, extracted with 1 mL petroleum ether for 2 h, dried to constant weight, and fat-free dry solids (FFDS) weight was calculated. Calcium was extracted from the muscle by treatment with 250 μL 2.5 M HNO₃ for 24 h. For each sample, 100 μL supernatant was diluted to 10 mL with 0.25% SrCl₂ and analysis of calcium in the muscle was performed by atomic absorption spectrophotometry with a Perkin–Elmer (Norwalk, CT, USA) 420 model. The results obtained for muscle calcium content throughout the investigation were calculated in mmol/100 g⁻¹ FFDS.

Calcium measurements

Samples were analyzed in duplicate and appropriate standards were used for measurements. Ca2⁺ levels in muscle and feces and in acidified plasma and urine were measured. The urine and fecal samples were diluted as necessary and aspirated directly into a Perkin–Elmer 430 model atomic absorption spectrophotometer.

Data analysis

A 2-way interaction (treatment (4 levels) by days (6 levels)) analysis of variance (ANOVA) was used to determine whether chronic periodic fluid redistribution can affect muscle Ca²⁺ content and Ca²⁺ losses during HK. ANOVA 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 level of significance was set at p < 0.05. The results obtained were reported as mean ± standard deviation (SD).
Table 1 Muscle calcium content, plasma calcium level, and calcium losses measured in the control and the hypokinetic groups and in the periodic fluid redistribution control and the hypokinetic groups during the pre-experimental and experimental periods

| Experimental period in days | Calcium content (mmol/100 g FFDS) | Plasma (mmol/L) | Urinary (mmol/days) | Fecal (mmol/days) |
|-----------------------------|------------------------------------|----------------|--------------------|-----------------|
| Active control subjects (ACS) (n = 10) | | | | |
| Average values | | | | |
| Pre-experimental | 41.10 ± 3.61 | 2.20 ± 0.05 | 3.40 ± 1.12 | 14.2 ± 2.2 |
| 60th | 41.12 ± 4.41 | 2.20 ± 0.01 | 3.38 ± 1.13 | 13.7 ± 2.0 |
| 120th | 41.14 ± 3.50 | 2.19 ± 0.01 | 3.38 ± 1.11 | 13.8 ± 2.2 |
| 180th | 41.13 ± 4.42 | 2.18 ± 0.04 | 3.39 ± 1.12 | 13.9 ± 2.0 |
| 240th | 41.17 ± 5.51 | 2.19 ± 0.05 | 3.37 ± 1.15 | 13.7 ± 2.2 |
| 300th | 41.16 ± 3.41 | 2.18 ± 0.03 | 3.38 ± 1.11 | 13.9 ± 2.1 |
| 364th | 41.19 ± 4.40 | 2.19 ± 0.04 | 3.39 ± 1.10 | 13.8 ± 2.2 |
| Hypokinetic subjects (HKS) (n = 10) | | | | |
| Average values | | | | |
| Pre-experimental | 41.11 ± 4.52 | 2.21 ± 0.04 | 3.42 ± 1.12 | 14.1 ± 2.2 |
| 60th | 37.77 ± 3.30* † | 2.39 ± 0.01* † | 4.30 ± 1.14* † | 18.8 ± 2.4* † |
| 120th | 37.90 ± 4.51* † | 2.37 ± 0.04* † | 4.28 ± 1.11* † | 18.3 ± 2.3* † |
| 180th | 37.07 ± 4.42* † | 2.40 ± 0.02* † | 4.38 ± 1.13* † | 20.0 ± 2.5* † |
| 240th | 37.25 ± 5.40* † | 2.38 ± 0.05* † | 4.33 ± 1.14* † | 18.8 ± 2.4* † |
| 300th | 34.14 ± 4.53* † | 2.53 ± 0.03* † | 5.08 ± 1.13* † | 23.5 ± 2.3* † |
| 364th | 34.33 ± 5.54* † | 2.50 ± 0.01* † | 4.97 ± 1.12* † | 22.3 ± 2.0* † |
| Periodic fluid redistribution control subjects (PFRCS) (n = 10) | | | | |
| Average values | | | | |
| Pre-experimental | 40.63 ± 2.35 | 2.28 ± 0.03 | 3.56 ± 1.12 | 15.5 ± 2.2 |
| 60th | 42.64 ± 3.50 | 2.24 ± 0.05 | 3.22 ± 1.10 | 14.0 ± 2.5 |
| 120th | 42.48 ± 2.38 | 2.25 ± 0.03 | 3.25 ± 1.13 | 14.8 ± 2.0 |
| 180th | 43.01 ± 5.00 | 2.23 ± 0.02 | 3.16 ± 1.11 | 13.7 ± 2.3 |
| 240th | 42.90 ± 3.41 | 2.24 ± 0.03 | 3.18 ± 1.12 | 14.0 ± 2.2 |
| 300th | 45.11 ± 5.47 | 2.22 ± 0.04 | 3.10 ± 1.13 | 13.1 ± 2.2 |
| 364th | 44.97 ± 3.37 | 2.23 ± 0.02 | 3.13 ± 1.12 | 13.5 ± 2.4 |
| Periodic fluid redistribution hypokinetic subjects (PFRHS) (n = 10) | | | | |
| Average values | | | | |
| Pre-experimental | 40.65 ± 3.70 | 2.27 ± 0.03 | 3.56 ± 1.10 | 15.0 ± 2.1 |
| 60th | 42.48 ± 5.47† | 2.21 ± 0.05† | 3.18 ± 1.12† | 13.7 ± 2.0† |
| 120th | 42.43 ± 4.43† | 2.18 ± 0.04† | 3.25 ± 1.10† | 14.0 ± 2.3† |
| 180th | 42.81 ± 4.40† | 2.15 ± 0.05† | 3.04 ± 1.12† | 13.3 ± 2.0† |
| 240th | 42.72 ± 5.61† | 2.18 ± 0.03† | 3.16 ± 1.11† | 13.7 ± 2.2† |
| 300th | 45.26 ± 4.52† | 2.12 ± 0.04† | 2.94 ± 1.10† | 12.3 ± 2.3† |
| 364th | 44.93 ± 5.54† | 2.17 ± 0.05† | 2.98 ± 1.11† | 12.7 ± 2.0† |

All values are expressed as mean ± SD

FFDS fat free dry solids

† p < 0.05 significant differences between the pre-experimental and experimental period values

* p < 0.05 significant differences between the active control and the hypokinetic groups of subjects

† p < 0.05 significant differences between the hypokinetic and the periodic fluid redistribution hypokinetic groups of subjects

Results

During the pre-experimental period, muscle Ca^{2+} content, plasma Ca^{2+} concentration, and Ca^{2+} losses in urine and feces remained relatively stable in the control and the hypokinetic groups of subjects (Table 1). In the periodic fluid redistribution control and hypokinetic groups of subjects muscle Ca^{2+} content, plasma Ca^{2+} level, and
Ca\(^{2+}\) losses in urine and feces were different from those in the hypokinetic and control groups of subjects (Table 1).

The plasma Ca\(^{2+}\) level decreased \((p < 0.05)\) in Ca\(^{2+}\) repleted muscle, and the muscle Ca\(^{2+}\) level increased \((p < 0.05)\) and Ca\(^{2+}\) losses in urine and feces decreased \((p < 0.05)\) in Ca\(^{2+}\) repleted muscle of the PFRHS group compared with the HKS group during the experimental period (Table 1). By contrast the plasma Ca\(^{2+}\) level increased \((p < 0.05)\) in Ca\(^{2+}\)-deficient muscle, and the muscle Ca\(^{2+}\) content decreased \((p < 0.05)\) and Ca\(^{2+}\) losses in urine and feces increased \((p < 0.05)\) in Ca\(^{2+}\) deficient muscle of the HKS group compared with their pre-experimental levels and the values in their respective control groups (ACS and PFRCS) (Table 1). In the ACS group and the PFRCS group the muscle Ca\(^{2+}\) content, plasma Ca\(^{2+}\) level, and Ca\(^{2+}\) losses in urine and feces did not change significantly compared with their pre-experimental values (Table 1).

Discussion

The lower plasma Ca\(^{2+}\) level in Ca\(^{2+}\) repleted muscle may be attributable to Ca\(^{2+}\) deposition, because Ca\(^{2+}\) deposition promotes shifting of Ca\(^{2+}\) into the tissue, resulting in lower plasma Ca\(^{2+}\) level in Ca\(^{2+}\) repleted muscle. The higher muscle Ca\(^{2+}\) level and the lower Ca\(^{2+}\) losses show that the periodic fluid redistribution hypokinetic subjects had experienced Ca\(^{2+}\) deposition because Ca\(^{2+}\) losses cannot decrease in Ca\(^{2+}\) repleted muscle unless Ca\(^{2+}\) deposition increases. Fluid and volume redistribution may be important for Ca\(^{2+}\) deposition when it occurs periodically over 1 year and for at least 8 h per day of head down tilt of \(-8^\circ\). The higher muscle Ca\(^{2+}\) level and the lower Ca\(^{2+}\) losses during chronic periodic fluid redistribution suggest deposition mechanisms different from those in the lower muscle Ca\(^{2+}\) level and higher Ca\(^{2+}\) losses during HK. The decreased Ca\(^{2+}\) losses during chronic periodic fluid redistribution suggest that shifting of fluid to the head is not sensed as excessive fluid volume, because Ca\(^{2+}\) losses cannot decrease during large fluid shifting upwards unless fluid migration to the head increases Ca\(^{2+}\) deposition. Studies [22–27] have shown that fluid volume expansion by a daily intake of fluid and salt supplementation slows electrolyte losses and increases tissue electrolytes, because fluid volume expansion is not sensed as excessive fluid volume but as simple fluid and volume redistribution and fluid and volume excretion mechanisms are not activated.

The progressive changes in muscle Ca\(^{2+}\) content and Ca\(^{2+}\) losses with duration of periodic fluid redistribution show that the longer fluid is redistributed periodically the more Ca\(^{2+}\) is deposited. Periodic fluid shifting upwards and downwards over 1 year and for at least 8 h per day can increase muscle Ca\(^{2+}\) and reduce Ca\(^{2+}\) losses. This confirms the common belief that periodic fluid redistribution over 1 year and for at least 8 h per day is important because it regulates Ca\(^{2+}\) deposition, which in turn increases muscle Ca\(^{2+}\) content and reduces Ca\(^{2+}\) losses. Thus the large shift of fluid from the lower to the upper part of the body during periodic head down tilt of \(-8^\circ\) in populations living and working under hypokinetic conditions may be more of a stimulus than a stressor of Ca\(^{2+}\) deposition. This adds an important contribution to Ca\(^{2+}\) deposition, because populations regularly and/or irregularly experience Ca\(^{2+}\) losses and Ca\(^{2+}\) deficiency for different reasons. It is evident that the hypokinetic subjects who were submitted to periodic redistribution of fluid over 1 year and for at least 8 h per day had less labile and more responsive Ca\(^{2+}\) deposition.

During HK, muscle electrolyte deficiency results from lower electrolyte deposition because of many factors and primarily of cell mass reduction [28]. Muscle Ca\(^{2+}\) repletion during periodic redistribution of fluid may be attributable to higher Ca\(^{2+}\) deposition, because of preservation or restoration of cell mass. The principal factors of intact cell structure are: fluid volume expansion, oxygen supplies to the tissues, aerobic glycolysis, mitochondrial density, adenosine triphosphate (ATP) production, and increased oxidative phosphorylation (OP). Preservation or restoration of cell function eventually increases cellular transport, reduces intracellular electrolyte level, and maintains the integrity of cell structure, resulting in the stability of cellular contents that can affect Ca\(^{2+}\) deposition and increase the holding cell capacity for Ca\(^{2+}\), which in turn contributes to less Ca\(^{2+}\) loss and greater muscle Ca\(^{2+}\) content.

Studies have shown that daily intake of fluid and salt supplementation in small divided doses increase circulating fluid volume and reduce electrolyte losses [29–34], because fluid volume expansion is not sensed by the baroreceptors as an excessive volume of fluid but rather as simple fluid volume redistribution, so fluid volume excretion mechanisms are not activated. The chronic fluid volume expansion increases blood volume and oxygen delivery to the tissue and preserves or restores cell structure and normalizes or compensates cell function [5, 6]. Depending on duration of fluid shifting upwards and downwards per day and degree of head down tilt, fluid volume expansion, oxygen supply to the tissue, normalization of cell structure, or compensation of cell function, the response at the cellular level is one of preservation or restoration of cell function and cell mass.

OP by mitochondria and ATP synthesis and production are very susceptible to blood supply and oxygen delivery to the tissues. As blood flow and oxygen tension within the cell increases, OP [35] and ATP synthesis [36] increase and
cell structure is preserved or restored. An increase of mitochondrial density and/or function is the most likely reason for the increase of OP and the synthesis of ATP and cell structure preservation or restoration. The increase of OP and ATP synthesis and production has a widespread effect on cellular function and morphology and preservation or restoration of cell structure. As OP and ATP synthesis and production increase, the cell shifts to aerobic glycolysis which enables ATP synthesis and production from the breakdown of cellular glycogen. Production of the new glycogen is stimulated and glycogen depots are repleted when during HK glycogen supply is depleted [37]. Moreover aerobic degradation of glycolysis becomes more efficient than the less oxygen-dependent mitochondrial pathways, and cell function and cell structure during greater blood delivery and oxygen supplies to the tissue is eventually preserved or restored, thereby stimulating deposition of Ca$^{2+}$ resulting in higher muscle Ca$^{2+}$ content and lower Ca$^{2+}$ losses during HK.

**Conclusion**

The lower plasma Ca$^{2+}$ level in Ca$^{2+}$ repleted muscle is indicative of Ca$^{2+}$ deposition, because Ca$^{2+}$ deposition promotes Ca$^{2+}$ shifting into the muscle and results in lower plasma Ca$^{2+}$ level in Ca$^{2+}$ repleted muscle. The higher muscle Ca$^{2+}$ content and the lower Ca$^{2+}$ losses are indicative of Ca$^{2+}$ deposition because Ca$^{2+}$ losses cannot decrease in Ca$^{2+}$ repleted muscle unless Ca$^{2+}$ deposition increases. The decrease of Ca$^{2+}$ losses during chronic periodic fluid and volume redistribution suggest that fluid shifting to the head is not sensed as an excessive volume of fluid because electrolyte losses cannot decrease during large fluid shifting upwards unless fluid migration to the head results in electrolyte deposition. The mechanism by which chronic periodic fluid and volume redistribution increases muscle Ca$^{2+}$ content and reduces Ca$^{2+}$ losses is not clear. It is evident that chronic periodic redistribution of fluid and volume can increase muscle Ca$^{2+}$ level and slow Ca$^{2+}$ losses, suggesting Ca$^{2+}$ deposition regulation. Further research on the chronic periodic fluid and volume redistribution effect on Ca$^{2+}$ deposition is in order.

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