Phosphate balance in phosphate supplemented and unsupplemented healthy subjects during and after hypokinesia

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Abstract

Objective: To demonstrate the effect of hypokinesia (HK) and post-HK on phosphate (Pi) imbalance and use of Pi with different Pi imbalance and different Pi consumption: were measured Pi balance, plasma Pi level and Pi loss during HK and post-HK.

Methods: Experiments were conducted during the pre-experimental period of 30 days, and the HK period of 364-days and post-HK period of 30-days. Forty healthy male volunteers 24.2 ± 2.0 yr, were divided into four groups: unsupplemented active control subjects (UACS), unsupplemented hypokinetic subjects (UHKS), supplemented active control subjects (SACS), and supplemented hypokinetic subjects (SHKS). All SACS and SHKS were supplemented with 0.6 mmol dicalcium-phosphate per kg body weight daily.

Results: During HK, Pi imbalance, serum Pi and calcium (Ca^{2+}) levels, fecal Pi loss, and urine Ca^{2+} and Pi loss increased ($P < 0.05$) in SHKS and UHKS compared with pre-experimental values and the values in their respective active controls (SACS and UACS). The measured parameters were changed ($P < 0.05$) more in SHKS than in UHKS. During the initial 20-days of post-HK, serum Pi and Ca^{2+} levels, fecal P loss, and urine Pi and Ca^{2+} losses decreased ($P < 0.05$), while Pi imbalance remained ($P < 0.05$) depressed

in SHKS and UHKS compared with UACS and SACS. The measured parameters were changed ($P < 0.05$) more in SHKS than in UHKS.

Conclusion: The greater Pi imbalance with than without Pi supplementation shows that the risk of higher Pi imbalance is directly related to the magnitude of Pi intake. The higher Pi loss with higher than lower Pi imbalance shows that the risk of greater Pi loss is directly related to the magnitude of Pi imbalance. It is concluded that Pi imbalance increases more when the Pi consumption is higher and that Pi loss increases more with higher than lower Pi imbalance indicating that during HK Pi imbalance is due to the inability of the body to use Pi but not to the Pi shortage in the diet.

The animal kingdom has been formed in a highly muscular activity environment that left its imprint on evolution, structure, function and behaviour of animals and humans while its absence results in induction of catabolism.¹⁻⁴ However, this has not led to the attention required to establish the effect of hypokinesia (diminished muscular activity) on electrolyte deposition which in its absence results in excessive electrolyte loss with tissue electrolyte depletion.⁵⁻⁸

During hypokinesia (HK), physiological systems which regulate the content of each electrolyte in plasma, and other endogenous fluids, and the balance between electrolyte intake and electrolyte loss, and thus total electrolyte content of the body are drastically affected.⁵⁻⁸ Some information has been accumulated on the effect of prolonged HK on electrolyte loss which shows to increase as the duration of HK increases⁷⁻¹² and that electrolyte loss to increase more with higher than lower electrolyte depletion and that electrolyte depletion to increase more with than without electrolyte supplementation.⁵⁻⁸ Failure to prevent electrolyte depletion with a daily electrolyte supplementation indicates that electrolytes are neither entering nor being retained by the body suggesting changes in the electrolyte deposition during prolonged HK.⁵⁻⁸

The mechanisms of phosphate (P_i) loss with P_i depletion are unknown while few studies have been published^{7, 8} on the effect of HK on P_i loss with P_i depletion. During normal muscular activity P_i depletion is usually accompanied by a lower plasma P_i level and P_i loss, while during HK P_i depletion is always accompanied by a higher plasma P_i level and P_i loss.⁷⁻¹² Higher plasma P_i and bodily P_i loss with P_i depletion during HK indicates a different kind of mechanisms than those involved in the lowered plasma P_i and P_i loss with P_i depletion during normal muscular activity. The change in skeletal muscle cell⁸ is considered as one of the main mechanisms of electrolyte loss with tissue electrolyte depletion and consequently on the decreased electrolyte deposition.⁷⁻¹²

The P_i is most abundant intracellular element and a critical component for membrane structure, energy storage and transport and bone mineralization. Until now, there has been limited material on the effect of HK on membrane permeability, P_i flux across cell membrane and the effect on P_i deposition and thus P_i loss with tissue P_i depletion. It is not known how total bodily loss of P_i is related to P_i depletion or whether P_i depletion comes from P_i shortage in the food consumed or from an inability of the body to use this electrolyte.⁷⁻¹² Each electrolyte has a well-defined and separate homeostatic mechanism to regulate that electrolyte's content in a tissue at both cell membrane level and higher organ level. Being P_i a major electro-

lyte that works with calcium to build and maintain bone it would be important to study P_i loss with P_i depletion. It is important to investigate the effect of prolonged HK on P_i balance in order to determine the mechanisms of alterations of P_i deposition and P_i loss with P_i depletion.

Methods

Forty healthy male volunteers were subjects. They gave informed consent for taking part in the study after a verbal and written explanation of methods and risks involved were given. There were no medical problems among the subjects and none was receiving therapy that interfered with phosphate or calcium metabolism. All procedures were previously reviewed and approved by the Committee for the Protection of Human Subjects. Financial incentives were given to encourage compliance with the protocol of the study. Subjects were students and were running average distances of 9.5 ± 1.2 km.day⁻¹ at a speed 7.1 ± 1.2 km.h⁻¹ for the last 2 - 3 yr, at an average rate of 5.0 times per week at 37.5 ± 2.2 km.week⁻¹. Hypokinetic subjects during the pre-experimental period of 30-days and post-HK period of 30-days were running average distances of 9.5 ± 1.2 km.day⁻¹ and during HK period of 364 days they were walking average distances of 0.9 ± 0.1 km.day⁻¹. Subjects were 24.2 ± 2.0 of age and had a body weight of 73.7 ± 5.5 kg and peak oxygen uptake of 47.8 ± 7.0 mL.kg⁻¹.min⁻¹.

Assignment of the subjects into three groups was conducted randomly:

Group one: Ten healthy subjects running average distances of 8.5 ± 1.2 km.day⁻¹ for 364 days. They served as unsupplemented active control subjects (UACS).

Group two: Ten healthy subjects walking average distances of 0.9 ± 0.1 km.day⁻¹ for 364 days. They served as unsupplemented hypokinetic subjects (UHKS)

Group three: Ten healthy subjects running average distances of 8.5 ± 1.2 km.day⁻¹ for 364 days supplemented with 0.6 mmol dicalcium phosphate. They served as supplemented active control subjects (SACS).

Group four: Ten healthy subjects walking average distances of 0.9 ± 0.1 km.day⁻¹ for 364 days supplemented with 0.5 mmol dicalcium phosphate. They served as supplemented hypokinetic subjects (SHKS).

Protocol

The study consisted of a 30-day pre-experimental period, a 364 day HK period and a 30 day post-HK period. The diets were served as a 6-day menu rotation. Meals were prepared under standard conditions in a research kitchen. Mean daily energy intakes of the diets were 3370 ± 187 , 2865 ± 210 , 3381 ± 190 and 2880 ± 215 Kcal and mean daily phosphate consumption was 40.3 ± 1.7 , 40.3 ± 1.8 , 41.0 ± 1.6 and 41.0 ± 1.5 mmol and mean daily Ca²⁺ consumption was 43.7 ± 2.2 , 37.8 ± 2.1 , 43.6 ± 2.1 and 43.7 ± 1.4 mmol for UACS, UHKS, SACS and SHKS, respectively.

Hypokinesia

For the simulation of the hypokinetic effect, the number of km walking/day was restricted to an average of 0.9 ± 0.1 km.day⁻¹ and monitored by an accelerometer. Activities allowed were those that approximate the routines of sedentary individuals. Subjects were allowed to walk to dining tables and laboratories where the tests were given. Climbing stairs and activities that required greater efforts were not allowed. Subjects were mobile and were not allowed outside the hospital.

Phosphate balance measurements

Dicalcium-phosphate of 0.7 mmol per kg body weight was given during pre-experimental and HK periods. This P_i amount was designed to facilitate maximal P_i absorption by staying just below renal tubular maximum for P_i absorption (8). Because foods may interfere with P_i absorption, P_i was given separately with orange juice. To minimize diurnal variations, all blood samples for each subject were drawn after the peak in serum P_i level which appears between the 120 and 140 min after P_i consumption (8). Balance of P_i was carried out under strict surveillance and in a controlled condition so that the precise amount of P_i consumed

with diet and excreted in urine and feces could precisely be calculated. P_i content in the diet was calculated by keeping a duplicate of food consumed by each subject and the total P_i loss in 24-hr-urine and fecal samples were measured. Due to individual differences in bowel patterns, collection period for P_i balance calculations were used a multiple day period, i.e., 18-day, to measure a representative P_i balance. Phosphate balance was calculated as the difference between P_i intake minus P_i loss on a per day basis, i.e., P_i balance is equal to total P_i intake minus P_i loss.

Serum, urine and fecal sample collection

To accommodate inter-individual differences in bowel habits, urine and feces were collected daily and pooled to form 7-day composites, while serum samples were collected every other 7-days. Seven day (consecutive days) pool samples were collected. Blood samples were collected with disposable polypropylene syringes. Blood samples were taken at rest and before any meals. Subjects were fasted overnight for 7-8 hr. Blood samples were drawn under identical conditions between 9 and 10 a.m, without venous stasis and after subjects had been sitting for 30 min. The sample volume of blood was 7 - 9 mL. Blood was clotted for 60 min at room temperature and, after rimming, was centrifuged at $3,000 \times g$ for 10 min and stored at -20 °C until analyses were conducted for P_i and calcium (Ca²⁺). An aliquot of serum and urine was acidified to pH<2.0 by adding 6M HCl (to prevent precipitation of Ca²⁺). Twenty-four hr urine collections were obtained daily. Urine samples were stored at 4 °C until needed for P_i and Ca²⁺ analyses. To ensure 24-hr urine collections creatinine was measured by a colorimetric method. Fecal samples were collected in plastic bags, dry-ashed in a muffle furnace at 600° overnight. Ashed samples dissolved in 5% nitric acid. To ensure complete recovery of feces polyethylene glycol was used as a marker.

Electrolyte measurements

Samples were analyzed in duplicate, and appropriate standards were used for all measurements: Serum, urine and fecal P_i levels were measured by spectro-

photometry on a Perkin-Elmer Model, Norwalk, CT, USA. Acidified urine and serum Ca^{2+} levels were measured by atomic absorption spectrophotometry on a Perkin-Elmer model, Norwalk, CT, USA. Urine and fecal samples aspirated directly in atomic absorption spectrophotometer and diluted as necessary.

Data analyses

Results were analyzed with a three-way analysis of variance (hypokinetic vs. active controls) X 2 (supplemented versus unsupplemented) X 2 (pre-intervention vs, post-intervention) ANOVA with repeated measures on the last factor. ANOVAS for each time point measurements were used. A Tukey-Kramer post-hoc test was used to establish which means were significantly different from each other. The predetermined level of significance was set at $p < 0.05$. The results obtained were reported as mean $\pm$ SD (Standard Deviation)

Results

Pre-experimental electrolyte values

During the pre-experimental period fecal P_i loss, urine Ca^{2+} and P_i excretion, P_i balance, serum P_i and serum Ca^{2+} levels (Tables 1 and 2) did not change in hypokinetic and active control groups of subjects. With daily P_i supplementation the measured parameters were not affected in active control and hypokinetic groups of subjects (Tables 1 and 2).

Experimental electrolyte changes

Serum P_i level and Ca^{2+} level, fecal P_i loss and urine P_i and Ca^{2+} loss, and P_i balance did not show any changes in SACS and UACS compared with their pre-experimental values (Tables 1 and 2). The P_i imbalance and serum P_i and Ca^{2+} concentrations, fecal P_i loss, and urine P_i and Ca^{2+} excretion increased ($P < 0.05$) in SHKS and UHKS compared with their pre-experimental values and the values in their respective active controls (SACS and UACS) (Tables 1 and 2). The P_i imbalance and serum P_i and Ca^{2+} concentrations, fecal P_i loss, and urine P_i and Ca^{2+} excretion in-

creased more ($P < 0.05$) in SHKS than in UHKS (Tables 1 and 2).

Post-experimental electrolyte change

Serum Ca^{2+} and P_i levels, urine Ca^{2+} and P_i loss, fecal P_i loss, and P_i balance did not change in SACS and UACS compared with their pre-experimental values (Tables 1 and 2). During the initial 20-days post-HK, serum P_i and Ca^{2+} levels, and urine P_i and Ca^{2+} loss and fecal P_i loss decreased ($P < 0.05$) while P_i imbalance remained lower in SHKS and UHKS compared with their respective active controls (Tables 1 and 2). Serum P_i and Ca^{2+} level, urine P_i and Ca^{2+} loss, fecal P_i loss, P_i imbalance were changed more ($P < 0.05$) in SHKS than in UHKS (Tables 1 and 2). After the 20th day, P_i imbalance decreased, and serum P_i and Ca^{2+} level, urine and fecal P_i loss, and urine Ca^{2+} excretion increased and by the 30th day of the post-HK period the measured indices approached the control values (Table 1 and 2).

Discussion

Our study showed no changes in P_i balance, serum P_i concentration and urine and fecal P_i losses among the supplemented or unsupplemented hypokinetic and control subjects. This is consistent with previous reports^{7, 8} where in pre experimental state, electrolytes balance was relatively stable regardless of the P_i supplementation. This may suggest that in pre experimental state, consumed P_i during supplementation may have been used up for deposition and was consumed by the body which in turn protected the net P_i balance showing any gross changes.

Because SHKS show higher P_i imbalance and higher serum P_i and P_i loss than UHKS indicate that the more P_i is consumed, less P_i is deposited and more P_i is shown in serum and more P_i is lost. This resembles a vicious circle, i.e., the higher P_i intake, the lower P_i deposition and the higher serum P_i level and P_i loss. As a result, the more efficiently P_i is lost the less likely it is to prevent P_i imbalance. The higher serum P_i level and higher P_i loss with greater P_i imbalance in SHKS than in UHKS is attributable to lower P_i deposition in SHKS than in UHKS, because the

TABLE 1. Phosphate Balance and Serum Electrolyte Concentration in Physically Healthy Subjects Measured at Pre-experimental, and During Ambulatory, and Experimental and Post-Experimental Period

Experimental Period				Post-experimental period			
Days	Phosph bal mmol/day	Phosphate mmol/L	Calcium mmol/L	Days	Phosph bal mmol/day	Phosphate mmol/L	Calcium mmol/L
Unsupplemented Active Control Subjects (UACS), n=10							
Pre-HK	+0.03±0.05	0.87±0.01	2.15±0.03				
60 th	+0.03±0.03	0.86±0.04	2.13±0.04	2 nd	+0.04±0.04	0.87±0.03	2.15±0.04
120 th	+0.02±0.04	0.85±0.03	2.14±0.01	3 rd	+0.03±0.03	0.86±0.02	2.14±0.03
180 th	+0.03±0.02	0.86±0.04	2.13±0.03	5 th	+0.04±0.04	0.87±0.03	2.15±0.03
240 th	+0.02±0.05	0.85±0.03	2.14±0.04	7 th	+0.03±0.05	0.87±0.01	2.14±0.04
300 th	+0.03±0.04	0.86±0.04	2.13±0.02	15 th	_.04±0.04	0.860.03	2.15±0.04
364 th	-0.02±0.05	0.86±0.02	2.14±0.03	30 th	+0.03±0.03	0.87±0.02	2.14±0.05
Unsupplemented Hypokinetic Subjects (UHKS), n=10							
Pre-HK	+0.03±0.04	0.88±0.02	2.14±0.05				
60 th	-2.92±0.05*	0.98±0.05*	2.36±0.03*	2 nd	-2.43±0.04*	0.66±0.03*	1.89±0.0*
120 th	-2.47±0.03*	0.96±0.03*	2.34±0.05*	3 rd	-2.61±0.05*	0.68±0.04*	1.93±0.05*
180 th	-3.15±0.04•	0.97±0.03*	2.39±0.06*	5 th	-2.15±0.04*	0.63±0.03*	1.87±0.03*
240 th	-2.61±0.05*	0.97±0.03*	2.36±0.04*	7 th	-2.32±0.03*	0.69±0.04*	1.95±0.05*
300 th	-3.27±0.03*	1.01±0.04*	2.43±0.05*	15 th	-1.74±0.04*	0.65±0.03*	1.89±0.04*
364 th	-2.75±0.05•	0.98±0.03*	2.39±0.04*	30 th	0.10±0.05	0.87±0.05	2.13±0.05
Supplemented Active Control Subjects (SACS), n=10							
Pre-HK	+0.06±0.03	0.90±0.05	2.11±0.05				
60 th	+0.05±0.05	0.92±0.04	2.03±0.03	2 nd	+0.05±0.04	+0.92±0.04	2.03±0.03
120 th	+0.04±0.04	0.93±0.04	2.02±0.05	3 rd	+0.06±0.05	+0.91±0.03	2.04±0.03
180 th	+0.03±0.03	0.93±0.03	2.00±0.03	5 th	_.05±0.03	+0.92±0.03	2.06±0.04
240 th	+0.05±0.05	0.92±0.04	2.01±0.05	7 th	+0.06±0.04	+0.91±0.04	2.07±0.03
300 th	+0.03±0.04	0.93±0.04	2.00±0.04	15 th	+0.04±0.03	+0.89±0.01	2.09±0.02
364 th	+0.04±0.05	0.92±0.03	2.01±0.05	30 th	+0.05±0.05	+0.87±0.3	2.13±0.03
Supplemented Hypokinetic Subjects (SHKS), n=10							
Pre-HK	+0.06±0.03	0.90±0.06	2.11±0.04				
60 th	-5.01±0.03**	1.08±0.03**	2.58±0.05**	2 nd	-5.38±0.05**	0.53±0.04**	1.66±0.03**
120 th	-4.85±0.04**	1.07±0.04**	2.56±0.03**	3 rd	-5.55±0.04**	0.55±0.02**	1.67±0.04**
180 th	-6.33±0.03**	1.10±0.03**	2.61±0.04**	5 th	-4.73±0.03**	0.50±0.05**	1.62±0.03**
240 th	-5.73±0.05**	1.08±0.05**	2.58±0.03**	7 th	-4.15±0.05**	0.57±0.03**	1.68±0.04**
300 th	-7.05±0.03**	1.13±0.07**	2.63±0.05**	15 th	-3.66±0.04**	0.52±0.05**	1.64±0.03**
364 th	-6.39±0.04**	.111±0.05**	2.61±0.04**	30 th	+0.11±0.05	0.91±0.06	2.17±0.05

All values are expressed as mean ± SD.

* $P<0.05$ significant differences between active control and hypokinetic groups of subjects. Each of the hypokinetic groups was compared with their respective active controls (UACS vs UHKS and SACS vs SHKS).

+ $P<0.05$ significant differences between supplemented and unsupplemented hypokinetic group

TABLE 2. Fecal Phosphate Loss and Electrolyte Loss in Urine of Physically Healthy Subjects Measured at Pre-experimental, and During Ambulatory, and Experimental and Post-Experimental Period

Experimental Period				Post-experimental period			
Days	Urinary Phosphate mmol/day	Fecal Phosphate mmol/ay	Urinary Calcium mmol/day	Days	Urinary Phosphate mmol/day	Fecal Phosphate mmol/day	Urinary Calcium mmol/day
Unsupplemented Active Control Subjects (UACS), n=10							
Pre-HK	27.7±1.5	113.8±10.5	4.37±0.3				
60 th	24.8±1.4	112.1±11.2	4.35±0.2	2 nd	28.7±1.4	115.3±10.3	4.38±0.6
120 th	26.3±1.3	111.7±13.4	4.36±0.3	3 rd	28.5±1.6	114.4±11.0	4.36±0.3
180 th	24.5±1.5	112.5±12.3	4.34±0.4	5 th	27.6±1.4	115.7±10.6	4.38±0.5
240 th	25.8±1.5	110.4±11.6	4.36±0.3	7 th	28.7±1.5	114.2±12.1	4.37±0.4
300 th	26.7±1.3	112.3±10.5	4.35±0.5	15 th	28.0±1.6	115.6±11.2	4.38±0.3
364 th	25.6±1.4	111.8±12.3	4.36±0.4	30 th	27.5±1.3	114.7±12.4	4.37±0.7
Unsupplemented Hypokinetic Subjects (UHKS), n=10							
Pre-HK	7.4±1.4	115.1±12.6	4.35±0.4				
60 th	37.7±2.7*	171.5±15.4*	5.97±0.5*	2 nd	19.0±1.2*	75.5±11.0*	3.04±0.3*
120 th	35.2±3.9*	151.3±13.6*	5.67±0.7*	3 rd	20.2±1.4*	77.1±12.3*	3.08±0.5*
180 th	37.3±2.6*	188.8±14.6*	6.18±0.4*	5 th	18.7±1.3*	73.0±12.4*	3.01±0.4*
240 th	36.0±2.5*	163.4±15.4*	5.86±0.4*	7 th	19.7±1.5*	78.5±13.2*	3.07±0.5*
300 th	40.1±3.3*	198.2±14.1*	6.31±0.6*	15 th	18.9±1.4*	74.3±10.3*	3.03±0.3*
364 th	38.5±2.8*	182.4±13.2*	6.15±0.8*	30 th	24.6±2.2	112.0±12.4	4.31±0.5
Supplemented Active Control Subjects (SACS), n=10							
Pre-HK	30.5±1.5	130.5±13.0	4.81±0.3				
60 th	33.0±1.4	133.4±12.6	4.76±0.4	2 nd	33.8±1.3	130.6±10.3	4.70±0.3
120 th	32.7±1.5	132.6±13.0	4.74±0.3	3 rd	34.3±1.2	127.0±13.2	4.67±0.4
180 th	34.1±2.2	134.7±13.4	4.73±0.5	5 th	33.6±1.5	125.7±11.4	4.60±0.5
240 th	33.5±1.5	133.6±12.5	4.74±0.4	7 th	32.4±1.3	126.8±12.6	4.55±0.4
300 th	34.0±2.5	134.8±12.0	4.75±0.3	15 th	31.7±1.4	123.2±11.0	4.50±0.5
364 th	33.5±2.2	133.3±11.4	4.74±0.4	30 th	27.5±1.5	115.6±11.3	4.36±0.4
Supplemented Hypokinetic Subjects (SHKS), n=10							
Pre-HK	30.4±1.7	130.5±13.5	4.82±0.6				
60 th	54.2±2.8**	246.9±14.3**	8.84±0.5**	2 nd	13.5±1.5**	50.2±11.0**	2.1. ±0.4**
120 th	45.7±3.5**	215.3±12.4**	8.15±0.6**	3 rd	14.0±1.3**	52.0±10.3**	2.14±0.5**
180 th	61.4±2.7**	282.1±15.7**	9.25±0.5**	5 th	12.6±1.5**	47.8±12.0**	2.10±0.3**
240 th	50.5±2.8**	251.5±14.8**	8.24±0.4**	7 th	14.2±1.6**	53.7±11.5**	2.15±0.5**
300 th	65.0±3.7**	330.6±12.4**	10.53±0.5**	15 th	13.0±1.4**	49.1±10.4**	2.11±0.3**
364 th	55.3±2.4**	291.8±16.5**	9.37±0.5**	30 th	35.7±2.2	135.7±13.5	4.45±0.6

All values are expressed as mean ± SD.

* $P < 0.05$ significant differences between active control and hypokinetic groups of subjects. Each of the hypokinetic groups was compared with their respective active controls (UACS vs UHKS and SACS vs SHKS).

+ $P < 0.05$ significant differences between supplemented and unsupplemented hypokinetic group

higher P_i imbalance, the lower P_i deposition and the higher serum P_i level and P_i loss.⁷⁻¹² The higher serum P_i level and P_i loss could have been attributable to the decreased P_i deposition because serum P_i and P_i loss cannot increase with P_i imbalance unless P_i deposition decreases.⁷⁻¹² The decreased P_i deposition promotes P_i shifting in plasma leading to the higher plasma P_i level and P_i loss with P_i imbalance.⁷⁻¹² The higher serum P_i level and bodily P_i loss with P_i imbalance during HK indicates a different kind of mechanisms than those involved in lowered serum P_i level and P_i loss with P_i imbalance during normal muscular activity. There are grounds to indicate that serum P_i concentration and P_i loss increases more with higher than lower tissue P_i depletion^{7, 8} and that P_i depletion increases more in bone and muscles with lower than higher weight bearing supporting morphology and function.¹⁵⁻¹⁷ This is ensured by many factors and primarily by the decreased deposition due to the muscle cell changes which makes impossible for the body to use electrolytes even in the face of tissue electrolyte depletion.^{7, 8}

Decreased P_i use could also have been attributable to the dissociation between bone formation and bone resorption with resorption exceeding that of bone formation^{13,14}, during which bone cell mass and electrolyte deposition decreases⁸, leading to the higher serum P_i level and P_i loss. The findings strongly indicate this because the serum P_i level and P_i loss increased more in hypokinetic subjects than in active control subjects. Decreased P_i deposition may be attributable to decreased mechanical load imposed upon the bone structures^{13, 14}, bone undergoes remodeling and bone cell mass decreases⁸ leading to the higher serum P_i level and P_i loss. The higher serum P_i level and P_i loss and the higher still with P_i supplementation indicates that the more P_i is consumed, and the more P_i is shifted into serum with versus without P_i supplementation. This leads to the higher serum P_i level and P_i loss with P_i imbalance or P_i supplementation still results to the higher serum P_i level and P_i loss. Thus, higher serum P_i level and P_i loss with P_i imbalance is attributable to the decreased P_i deposition due to many factors and primarily to cell changes.^{7, 8} The mechanism by which serum P_i concentration and P_i loss in-

crease with P_i imbalance has not been established; however, the basic analysis that as a consequence of decreased P_i deposition serum P_i level and P_i loss increases with P_i imbalance has been seen to be valid.⁷⁻¹²

Irrespective of the mechanisms involved with regard to the P_i loss with P_i imbalance, there is a final common pathway, namely during HK tissue P_i depletion increases^{7,8} which, in turn, shut off the synthesis of adenosine triphosphate (ATP).²⁰ The ATP depletion causes a series of cell injuries, including plasma-membrane permeabilization, lipid degradation and phospholipase.¹⁸ The ATP becomes energetically less efficient and, for the production of ATP, more substrates are used, which causes even greater shortage of already sparse reserves of ATP^{1,2} and thus more cell injury. ATP depletion is responsible for muscle cell-membrane injury¹⁹ and a rise in intracellular electrolyte level.^{7, 8} Reduction of ATP, with concurrent intensification of the energy-producing process, may be indicative of increase in the rate of ATP-consuming process, in particular the intensification of transport ATPase due to possible changes on the membrane level¹⁹ The reduction of ATP probably reflects the functional state of the entire organism, because HK is characterized by a decreased energy metabolism.^{1,2} During HK occurs a reduction of the nucleotide pool due to the general catabolic orientation processes of the body.^{1,2} The changes in energy metabolism are related to the triggering of compensatory mechanisms in response to the hypokinetic effect, and they are directed toward preserving the structural integrity of cell which is eventually affected because of ATP depletion and Na^+ , K^+ -ATPase changes. The muscle cell injury would result in diminished holding capacity for P_i which leads during the phase of development of this condition to the P_i loss with tissue P_i depletion.^{7, 8} The decrease of P_i deposition in this later situation may exacerbate the P_i loss with P_i imbalance further. Thus P_i supplementation would fail to prevent the P_i imbalance when muscular activity restricted allowing the cell mass to swell further and P_i loss to increase more

The lower serum P_i level and P_i loss during post-HK indicates whole body P_i depletion, because the serum P_i level and P_i loss cannot decrease without

whole body P_i depletion unless some other overwriting factors coexist. The lower serum P_i level and P_i loss is a comparable index of whole body P_i depletion.⁷⁻¹² The fact that decreased serum electrolyte level and electrolyte loss reflect whole body electrolyte depletion has been known for many years.⁸ However, it has been rarely used due to the difficulties of measuring electrolyte content in tissue to confirm electrolyte depletion. Measuring P_i loss and serum P_i level with and without P_i supplementation during post-HK, it was shown that the higher P_i consumption, the lower serum P_i level and P_i loss, and the greater whole body P_i depletion. The measured P_i parameters started to normalize after the 20th day of post-HK period. This shows that during HK P_i imbalance cannot normalize with or without P_i supplementation unless muscular activity is restored. In favor of this is the fact that P_i imbalance and serum P_i level and P_i loss did not normalize with or without daily P_i supplementation until the end of HK and after subjects had resumed their muscular activities.

The whole body P_i depletion differences among SHKS and UHKS, as were seen with the lower serum P_i level and P_i loss during post-HK, show that whole body P_i depletion was greater in SHKS than in UHKS. Because SHKS show lower serum P_i level and P_i loss than UHKS, SHKS experienced greater whole body P_i depletion than UHKS. Lower serum P_i level and P_i loss in SHKS than in UHKS were probably attributable to the more severe whole body P_i depletion in SHKS than in UHKS, because it is known that the higher P_i depletion and the lower serum P_i level and P_i loss the greater whole body P_i depletion.⁷⁻¹² The fact that serum P_i level and P_i loss decreased more in SHKS than in UHKS supports the view that SHKS were experienced more severe whole body depletion than UHKS. It is possible that the higher P_i consumption and or the lower P_i deposition accounts for the greater whole body P_i depletion in SHKS than in UHKS because during HK, SHKS lost more P_i than UHKS. However it is unclear by what mechanisms SHKS would have shown greater whole body P_i depletion than UHKS.

Hypokinetic subjects experienced P_i imbalance which was greater with than without P_i supplementa-

tion. However, the mechanism of P_i imbalance and its severity in SHKS than UHKS is undefined. This is because of the difficulties of routinely measuring tissue P_i to confirm tissue P_i depletion, while the mechanism of lower P_i deposition with P_i depletion has not been established. Even with normal regulation of P_i at the cell level, it is very difficult or impossible for the body to use P_i until P_i deposition is restored to conform to the demands of the body.⁸ Prolonged HK causes a reduction in P_i deposition^{7, 8} which is accompanied by higher serum P_i and P_i loss which cannot be retained in the body even with P_i imbalance. Because P_i is deposited in cells of different tissues, reduction in cell mass will inhibit the P_i deposition and lead to the buildup of extracellular P_i resulting to higher P_i loss with P_i imbalance. Therefore, additional consumption of P_i during HK can be considered as a symptomatic therapy²¹, while the clinical consequences of P_i imbalance remain unknown because few studies have been conducted on the effect of HK on P_i deposition. Consequently, P_i imbalance may be a common clinical problem and more so with than without P_i supplementation, while this should alert the clinicians of P_i imbalance because of more and more people are increasing subjected to sedentary living and working conditions.

Conclusion

The higher P_i imbalance in SHKS with P_i supplementation than in UHKS without P_i supplements shows that P_i imbalance is directly related to the magnitude of P_i consumption. The higher total bodily P_i loss in SHKS than in UHKS in turn shows that the bodily loss of P_i is directly related to the magnitude of P_i imbalance. The dissociation of P_i imbalance and bodily P_i loss shows that lower P_i deposition is the main mechanism of P_i imbalance. With enhanced P_i imbalance, bodily P_i loss would increase during HK unless factors leading to the decreased P_i deposition are reversed. It is concluded that P_i imbalance increases more with than without P_i supplementation and that bodily P_i loss increases more with higher than lower P_i imbalance. In all, during prolonged HK P_i imbalance in physically healthy subjects is not due to the P_i

shortage in foods consumed. Rather, it is due to the inability of the body to take up P_i when subjects are submitted to prolonged HK.

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