Vanadium Supplementation Effect on Vanadium Metabolism During Hypokinesia in Rats

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(Received October 21, 2009; Accepted January 14, 2010)

Objectives: Microelement supplementation during Hypokinesia (HK; diminished movement) affects differently microelement metabolism from that of normal muscular activity. In view of the effect of trace element supplementation and HK upon microelement metabolism we investigated the effect of vanadium (V) supplements on tissue V content and V loss during HK.
Methods: Studies were performed on 240 male Wistar rats during a pre-experimental period of 9 days and an experimental period of 98 days. Rats were equally divided into four groups: unsupplemented control rats (UCR), unsupplemented experimental rats (UER), supplemented control rats (SCR) and supplemented experimental rats (SER). A daily supplementation of 0.8 μmol vanadium sulfate was given to the rats in the SCR and SER groups. Muscle V content, plasma V level and V loss was measured in the experimental and control groups of rats.
Results: The gastrocnemius muscle and right femur bone V content decreased ($p < 0.05$), and plasma V level and urinary and fecal V loss increased ($p < 0.05$) in the SER and UER groups compared to their pre-experimental values and their respective control groups (SCR) and UCR). However, the tissue V content decreased more ($p < 0.05$) and plasma V level and V loss increased more ($p < 0.05$) in the SER group than in the UER group. The tissue V content and plasma V level and V loss did not change in the control groups of rats compared to the pre-experimental values.
Conclusions: It is concluded that during HK V supplementation decreases more tissue V content and increases more V loss and plasma V level in V deficient tissue indicating lower V utilization.

Key words: vanadium utilization, vanadium deficiency, tissue vanadium, cell injury, energy deficiency, nutrition

INTRODUCTION

Hypokinesia (diminished movement) is defined as a situation of physical inactivity beyond that associated with a daily functioning. It is known that the entire animal kingdom has been formed in a highly muscular activity environment which left its imprint on evolution, structure, function and behavior of animals and humans while its absence results in energy deficiency, body weight loss, [1–4], microelement disturbances [5–7] and total cell mass loss [8–9]. There few studies have been published on the effect of hypokinesia (HK) on trace element supplementation [10–12] and no other information was retrieved other than the information from non-hypokinetic studies.

Trace element changes in different clinical conditions had received considerable attention and the importance of trace elements in diseases has been established [5–7]. The effect of HK on the microelement supplementation had received little attention and the importance of HK in the microelement metabolism is unclear. It is remarkable, that there few studies have been conducted on the effect of HK on microelement supplementation, though the hypokinetic conditions are a common factor in the urban population. Therefore few studies are available on the effect of HK on trace element supplementation [10–12]. During normal muscular activity the inadequate consumption of microelements and the increase of trace element loss may result in microelement deficiency. During prolonged HK, however, the consumption of microelement supplementation and the excessive microelement loss may result to the microelement deficiency [10–12].

Vanadium (V) is an essential trace element and it is thought to play an important role in the metabolism of carbohydrates and may affect the cholesterol and lipid metabolism [13–14]. In diabetics, V supplementation may regulate blood glucose level [15–16]. Supplementation of V may decrease blood sugar level [15–16], and increase the muscular vascularity and blood flow ("pumped feeling"), mimic insulin action and decreases glycogen synthesis and storage. The supplementation of V may enhance the body weight, skeletal growth and muscle mass [17–18]. The higher V consumption may inhibit ATPase, alkaline phosphatase and the related enzymes [13–14]. For this reason the V supplementation may serve as a regulator of these enzymes and play an important part in the intermediatery metabolism [19].

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There has been limited information on the effect of vanadium supplementation during HK on the carbohydrate, cholesterol and lipid metabolism, blood glucose level, glycogen synthesis, ATPase activity, enzymes, intermediatery metabolism, V deposition and V utilization. Thus we do not know how the V metabolism is affected or if the V metabolic changes come from the V shortage in the food consumed and/or from the impossibility of the body to deposit and utilize V [10–12]. Because V is a trace element which plays an important role in the higher biological activity, in the different biochemical processes and many enzymes and in the intermediary metabolism it would be important to investigate the effect of V supplementation on V metabolism during HK.

The objective of this investigation was to determine the effect of V supplementation and HK on tissue V and V loss which aimed to investigate the capability of the body to utilize V under such conditions. Measurements of tissue V content, plasma V concentration and V loss in urine and feces of rats with and without V supplementation were performed during prolonged HK.

MATERIALS AND METHODS

Four hundred eight 13-week-old Wistar male rats were obtained from a Local Animal Breeding Laboratory. On arrival they were given an adaptational dietary period of 9-days during which they were fed a commercial laboratory diet. During this pre-experimental period of 9 days all rats were kept under vivarium control conditions. At the start of this study, rats were about 90-days old and weighed from 375–395 g. Animals were housed in individual metabolic cages where the light (07:00 to 19:00 h), temperature (25 ± 1°C) and relative humidity (65%) were automatically controlled. All cages were cleaned daily in the morning before feeding. Studies were approved by the Committee for the Protection of Animals.

Assignment of animals into four groups was performed randomly and their conditions were as follows: Group 1: sixty unrestrained rats housed in individual cages for 98-days under vivarium control conditions. They were assigned to the unsupplemented control rats (UCR) group. Group 2: sixty restrained rats kept in small individual cages for 98-days under HK. They were assigned to the unsupplemented experimental rats (UER) group. Group 3: sixty unrestrained rats housed in individual cages for 98-days under vivarium control conditions and were supplemented with 0.8 µmol vanadium sulfate per day. They were assigned to the supplemented control rats (SCR) group. Group 4: sixty restrained rats kept in small individual cages for 98-days under HK and were supplemented with 0.8 µmol vanadium sulfate per day. They were assigned to the supplemented experimental rats (SER) group.

Protocol

Studies during the pre-experimental period of 9-days and an experimental period of 98 days involved a series of biochemical examinations and conditioning of rats to their laboratory conditions [2]. During the pre-experimental period were collecting pre-experimental values with regard to bone and muscle V content, plasma V level, and urine and fecal V loss. This period was also aimed at minimizing the hypokinetic stress inherent to prolonged diminished muscular activity [2]. The mean dietary vanadium intake was 1.7 ± 1.0, 1.7 ± 1.1, 1.9 ± 1.0, and 1.9 ± 1.1 µmol per day for the UCR, UER, SCR and SER groups, respectively.

Simulation of hypokinesia

Hypokinetic rats were kept for 98-days in small individual wooden cages. Cages dimensions of 195 × 80 × 95 mm allowed movement restriction in all directions without hindering food and water intake. All experimental rats could still assume a natural position that allowed them to groom different parts of their body. When necessary, the conditions of the individual cages could be change using special wood inserts. The cages were constructed so that their size could be changed in accordance with size of each rat, thus the degree of restriction of muscular activity throughout the hypokinetic period could be maintained at a relatively constant level.

Food and water consumption

Daily food consumption was measured and 90% of a daily consumption (12 g) was mixed with deionized distilled water (1 : 2 wt/vol) to form slurry which was divided into two meals. All rats were pair-fed and daily food consumption was measured during the pre-experimental and the experimental periods. The control group of rats was allowed to eat approximately the same amount of food as the hypokinetic group of rats. Food was placed daily in individual feeders formed by the little trough and wood partitions. Food was from the same production lots that contained all essential nutrients: 19% protein, 4% fat, 38% carbohydrates, 16% cellulose, vitamins, A, D, E, 0.5% sodium chloride, 0.9% calcium, 0.8% phosphorus, and 0.5% magnesium per one g diet and kept in a cold chamber (-4 °C). The food intake was measured daily by weighing (Mettler PL 200 top loading balance) the slurry food containers. All animals were received daily deionized-distilled water *ad libitum*. The water dispensers (120 to 150 mL) were secured onto a wooden plate installed on the front cage panels and filled daily.

Plasma, urine and fecal sample collection

Urine and feces were collected from each rat every day and pooled to form 6-days composites, while plasma samples were collected every 6-days. A 6-day (consecutive day) pooled samples were collected. Blood sample were collected with disposable polypropylene syringes. Blood samples of 1.5–2.5 mL were obtained via a cardiac puncture from ether-anaesthetized rats. The potential of contamination of V was reduced by using microelement free gloves, and needles and vacuum containers. To obtain plasma, blood samples were transferred into polypropylene tubes containing sodium heparin. Samples were centrifuged immediately at 10,000 x g for 3 min at room temperature and were separated using glass capillary pipets which had been washed in the hydrochloric acid and deionized water. Aliquots for plasma V analysis were stored at

-20°C. A stainless steel urine-feces separating funnels (Hoeltge, model HB/SS) was placed beneath each rat to collect uncontaminated twenty-four hours urine samples. Twenty-four hour urine samples uncontaminated by stools were obtained. Creatinine excretion was measured by a colorimetric method using Jaffe's reaction to ensure twenty-four hour urine sample collection. The urine was collected in a beaker with layer of electrolyte oil to prevent evaporation. The beakers were replaced daily. Urine for each twenty-four period was collected in acidified acid-wash containers and was stored at -4°C until needed for V analysis. Fecal samples were collected in plastic bags, dried-ashed in a muffle furnace at 600°C overnight. Ashed samples were dissolved in 5% nitric acid. To ensure complete recovery of feces polyethylene glycol was used as a marker.

Tissue preparations, electrolyte extraction and analysis

Tissue samples were collected during pre-experimental and experimental period mean ± SD of muscle and bone water and electrolyte content was presented. The muscle and bone water and electrolyte content was reported in the average of six rats. Six animals from each group were exsanguinated under ether anesthesia by cardiac puncture. Six hypokinetic and six control rats from each group were decapitated on the 1st, 5th and 9th day of pre-experimental period, and on the 3rd, 7th, 15th, 30th, 50th, 70th and 98th of experimental period. Gastrocnemius muscle and right femur bone V content is given as the average of 6-rats. We have examined the right femur because during HK it is affected more than left femur [20–22]. Bones were cleaned of soft tissues, dried to a constant weight, weighed, reduced to ash in a muffle furnace at 600°C for 144 minutes, then ash was weighed and dissolved in 0.05 N HCl (hydrochloric acid) and, as a chloride solution, was analyzed for V. The muscles were excised immediately after decapitating the animals. The muscles were thoroughly cleaned of connective tissues, fatty inclusions and large vessels, weighed on Teflon liners and placed in a drying chamber at 110°C. After drying to a constant weight tissue transferred to quartz tubes for mineralization by means of concentrated HNO_3 (nitric acid), distilled off in a quartz apparatus. After ashing, the residue was dissolved in 0.05 M HCl and, as a chloride solution, was analyzed for vanadium in muscle and bone from the supplemented and unsupplemented hypokinetic and control groups of animals.

Tissue, plasma, urine and fecal vanadium measurements

Samples were analyzed in duplicate and appropriate standards concentrations were used for the vanadium measurements. The polypropylene syringes and test tubes were considered trace-element-free. Glassware was acid washed in a 1 : 1 hydrochloric acid and deionized-distilled water mixture for a minimum period of four hours and rinsed five to six times in deionized distilled water. The V content in gastrocnemius muscle and right femur bone and V levels in plasma, urine and feces were measured by an atomic absorption spectrophotometer. Samples of urine and feces were diluted as necessary and aspirated directly into an atomic absorption spectrophotometer, Perkin-Elmer 420 model, Perkin-Elmer Corp., Norwalk, CT. USA.

Data analyses

The V values in muscle, bone, plasma, urine, and feces were subjected to a 3-way analysis of variance (ANOVA) to determine whether V supplementation during HK affects V metabolism; ANOVA with repeated measures of the 3-way interaction (pre-experimental/experimental values, experimental/control groups, and supplemented/unsupplemented groups) was used. 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 alpha < 0.05. The results obtained were reported as mean ± SD.

RESULTS

During the pre-experimental period, muscle and bone V content, plasma V concentration and urinary and fecal V loss did not change in the control and the experimental groups of rats with and without vanadium supplementation (Table 1).

During the experimental period, the muscle and bone V content decreased ($p < 0.05$), and plasma V concentration and urinary and fecal V loss increased ($p < 0.05$) in the supplemented and the unsupplemented experimental groups of rats compared to their pre-experimental values and the values in their respective supplemented and unsupplemented control groups of rats (Table 1). However, the muscle and bone V content decreased more ($p < 0.05$) and plasma V concentration and urinary and fecal V loss increased more ($p < 0.05$) in the supplemented than in the unsupplemented experimental groups of rats (Table 1). The bone and muscle V content, plasma V level and urine and fecal V loss did not change in the supplemented and unsupplemented control groups of rats compared to their pre-experimental values (Table 1).

DISCUSSION

During the pre-experimental period, no changes were observed in tissue V content, plasma V level and V loss of the supplemented or unsupplemented experimental and control groups of rats. This is consistent with prior publications [5–7] where in the pre-experimental period, the tissue V content, plasma V level and V loss remained relatively stable regardless of V supplementation. This suggests that in the pre-experimental period, the consumed V during supplementation has been taken up for deposition and was used by the body which in turn protected the net tissue V content, plasma V level and urinary and fecal V loss without showing any gross differences among the examined groups of animals with and without vanadium supplementation.

The V content decreased more in the gastrocnemius muscle with less weight-bearing supporting morphology and function than in the right femur

Table 1 Urinary and Fecal Vanadium Loss, Plasma Vanadium Level and Tissue Vanadium Content Measured in the Control and the Experimental Groups of Animals With and Without Vanadium Supplementation at the Pre-experimental Period and During the Experimental Period.

	Vanadium				
Days	Urinary nmol/days	Fecal nmol/days	Plasma nmol/L	Gastrocnemius Muscle, ng/L (wet tissue wt)	Right Femur Bone, ng/L (100 μg ash)
			Unsupplemented Control Rats (UCR), n = 60		
Pre-experimental	0.12 ± 0.03	4.12 ± 1.13	0.74 ± 0.01	12.5 ± 0.3	25.7 ± 0.7
3rd	0.11 ± 0.01	4.07 ± 1.11	0.73 ± 0.02	12.7 ± 0.2	25.9 ± 0.5
7th	0.11 ± 0.02	4.02 ± 1.12	0.72 ± 0.01	12.9 ± 0.3	26.3 ± 0.6
15th	0.11 ± 0.01	4.03 ± 1.11	0.71 ± 0.01	12.7 ± 0.4	26.7 ± 0.4
30th	0.10 ± 0.02	4.00 ± 1.13	0.72 ± 0.02	13.3 ± 0.3	27.0± 0.7
50th	0.11 ± 0.03	4.01 ± 1.08	0.73 ± 0.01	12.9 ± 0.5	26.7 ± 0.6
70th	0.11 ± 0.04	4.03 ± 1.14	0.72 ± 0.02	13.5 ± 0.2	26.9 ± 0.6
98th	0.11 ± 0.03	4.01 ± 1.07	0.73 ± 0.03	13.3 ± 0.4	27.3 ± 0.5
			Unsupplemented Experimental Rats (UER), n = 60		
Pre-experimental	0.12 ± 0.03	4.11 ± 1.15	0.74 ± 0.04	12.3 ± 0.5	25.8 ± 0.6
3rd	0.16 ± 0.04*†	5.45 ± 1.13*†	0.81 ± 0.05*†	11.0 ± 0.3*†	23.5 ± 0.5*†
7th	0.15 ± 0.02*†	5.35 ± 1.14*†	0.80 ± 0.03*†	11.3 ± 0.5*†	23.8 ± 0.7*†
15th	0.17 ± 0.02*†	5.83 ± 1.15*†	0.82 ± 0.04*†	10.7 ± 0.3*†	22.7 ± 0.5*†
30th	0.16 ± 0.03*†	5.63 ± 1.13*†	0.81 ± 0.03*†	11.0 ± 0.4*†	23.0 ± 0.6*†
50th	0.17 ± 0.04*†	6.07 ± 1.14*†	0.80 ± 0.05*†	10.5 ± 0.3*†	22.3 ± 0.5*†
70th	0.17 ± 0.02*†	5.87 ± 1.15*†	0.82 ± 0.04*†	10.7 ± 0.4*†	22.7 ± 0.7*†
98th	0.20 ± 0.05*†	7.31 ± 1.12*†	0.85 ± 0.02*†	10.1 ± 0.5*†	22.0 ± 0.5*†
			Supplemented Control Rats (SCR), n = 60		
Pre-experimental	0.13 ± 0.04	4.62 ± 1.16	0.75 ± 0.03	13.2 ± 0.4	26.1 ± 0.5
3rd	0.14 ± 0.03	5.05 ± 1.15	0.75 ± 0.04	13.6 ± 0.4	26.3 ± 0.4
7th	0.14 ± 0.04	5.03 ± 1.17	0.76 ± 0.03	13.9 ± 0.5	26.6 ± 0.6
15th	0.14 ± 0.05	5.07 ± 1.19	0.75 ± 0.04	13.8 ± 0.5	26.9 ± 0.7
30th	0.14 ± 0.04	5.05 ± 1.15	0.75 ± 0.03	14.2 ± 0.4	26.8 ± 0.5
50th	0.14 ± 0.05	5.07 ± 1.18	0.76 ± 0.04	14.4 ± 0.5	27.0 ± 0.6
70th	0.14 ± 0.03	5.03 ± 1.17	0.76 ± 0.03	14.5 ± 0.4	27.3 ± 0.5
98th	0.14 ± 0.05	5.07 ± 1.14	0.75 ± 0.05	14.6 ± 0.5	27.5 ± 0.3
			Supplemented Experimental Rats (SER), n = 60		
Pre-experimental	0.13 ± 0.03	4.62 ± 1.13	0.75 ± 0.03	13.0 ± 0.3	26.1 ± 0.6
3rd	0.21 ± 0.05*+†	7.35 ± 1.17*+†	0.88 ± 0.06*+†	10.3 ± 0.5*+†	21.7 ± 0.5*+†
7th	0.19 ± 0.04*+†	7.05 ± 1.15*+†	0.87 ± 0.05*+†	10.5 ± 0.4*+†	22.1 ± 0.4*+†
15th	0.23 ± 0.06*+†	7.73 ± 1.16*+†	0.90 ± 0.04*+†	9.9 ± 0.5*+†	21.2 ± 0.7*+†
30th	0.21 ± 0.04*+†	7.31 ± 1.15*+†	0.89 ± 0.05*+†	10.0 ± 0.3*+†	21.5 ± 0.6*+†
50th	0.23 ± 0.05*+†	8.17 ± 1.17*+†	0.90 ± 0.07*+†	9.7 ± 0.4*+†	20.7 ± 0.5*+†
70th	0.23 ± 0.06*+†	7.87 ± 1.19*+†	0.91 ± 0.06*+†	9.9 ± 0.5*+†	20.9 ± 0.5*+†
98th	0.27 ± 0.05*+†	9.65 ± 1.16*+†	0.94 ± 0.03*+†	9.5 ± 0.3*+†	20.5 ± 0.7*+†

All values are expressed as mean ± SD.

*$p < 0.05$ significant differences between the control and the experimental groups of rats.

+$p < 0.05$ significant differences between the supplemented and unsupplemented experimental groups of rats.

†$p < 0.05$ significant differences between the groups of subjects and the pre-experimental period values.

Abbreviations

Hypokinesia (HK)
Unsupplemented control rats (UCR)
Unsupplemented experimental rats (UER)
Supplemented control rats (SCR)
Supplemented experimental rats (SER)
Vanadium (V)
Adenosine triphosphate (ATP)
Adenosine diphosphate (ADP)

with more weight bearing supporting function and morphology and tissue V content decreased more in the supplemented than unsupplemented groups. Some studies have shown that during HK electrolyte content decreases more in muscle and bone with less weight-bearing supporting morphology and function [20–22] and the tissue electrolyte content decreases more with than without electrolyte supplementation [8–9]. Plasma V level and V loss increased more in the supplemented than the unsupplemented experimental groups. The fact that tissue V content decreased more and plasma V level and V loss increased more in the supplemented than in the unsupplemented experimental groups demonstrate that the supplemented experimental group experienced a lower V utilization than the unsupplemented experimental group. If supplemented experimental group had not experienced lower V utilization than the unsupplemented experimental group, the supplemented experimental group could not have shown lower tissue V content and higher plasma V level and V loss than unsupplemented experimental group; because the tissue V content cannot decrease more with than without V supplementation and plasma V level and V loss cannot increase more in higher than lower V deficiency unless V utilization decreases more [5–7]. This demonstrates that during HK the lower tissue V content with than without V supplementation and the higher plasma V level and V loss in the higher than lower V deficient tissue is attributable to different mechanisms than those involved in the decreased tissue V content without than with V supplementation and decreased plasma V level and V loss in V deficient tissue of different intensity during normal activity.

Vanadium is thought to mimic the physiological effects of insulin by a mechanism which has not been identified [5–7]. Through this insulin-mimetic effect the V is thought to promote glycogen synthesis, maintain blood glucose level and stimulate muscle anabolism. During HK catabolism [2–4] and hypoglycemia occur, because the glycogen stores are depleted while the synthesis of new glycogen is inhibited [23]. The increase sensitivity to insulin may also be involved in the hypoglycemia [23]. The glycogen synthesis, blood glucose level and muscle anabolism decrease which cannot but affect V utilization and increase V loss. Hypokinesia activates Na^+, K^+-ATPase a process which requires energy when there is energy deficiency [2–4]. This exhausts the supplies of ATP and slows the cellular transport [24]. Studies [2] using indirect methods have shown a deficiency of enzymes as well. Thus V which promotes glycogen synthesis, maintains blood glucose level and stimulates muscle anabolism during normal activity may not play any vital part during HK. Because during HK the glycogen synthesis, blood glucose level and production of energy slows indicating different mechanisms than those in the increase of glycogen synthesis and blood glucose level and energy production with V intake during normal muscular activity.

During HK is no need to split ATP as a source of energy for the muscle contraction [2–4] and because there is no need for splitting of ATP [25] there is no ADP formation [26]. Thus, total amount of creatine phosphate which is the first source of energy for ATP reconstitution reduces. Because there is limited creatine phosphate there is little release of energy to cause bonding for a new phosphate ion to ADP to reconstitute ATP. The ADP is a stimulator of oxygen uptake and formation of new ATP molecules during oxidative phosphorylation; this process slows markedly during HK [27]. The decrease number of mitochondrial and/or function have been suggested as the most likely culprit to explain the decreased oxidative phosphorylation. Thus there are limited reserves of ATP due to inhibition of ADP synthesis and decrease production of ATP. Moreover the ATP becomes energetically less efficient and for the ATP production much more substrates are used, which causes even greater shortage of the already sparse reserves of ATP [25].

The slow ATP production [25] which occurs with an increase of energy-producing processes is indicative of increase in the rate of ATP-consuming processes, in particular an increase of the transport of ATPase due to the possible changes in the membrane structure [28]. There is also a reduction of nucleotide pool due to the general catabolic processes [2–4]. The decrease level of ATP and the increase level of glycolysis constitute a 43 and 32%, respectively. The changes in energy metabolism, i.e. lower ATP level and higher glycolysis, are attributable to compensatory mechanisms in response to the hypokinetic effect, and they are directed toward preserving the structural integrity of cell which is eventually affected due to lower ATP and higher glycolysis. The decline of energy, decrease of ATP synthesis and increase of glycolysis may lead to the cell injury [29] thereby interfering with V utilization resulting in higher V loss in V deficient tissue.

CONCLUSION

The lower tissue V content in the supplemented than in the unsupplemented experimental groups shows that the tissue V content decreases more with than without V supplementation. The higher V loss and plasma V level in the supplemented than the unsupplemented experimental groups shows that V loss and plasma V level increases more in the higher than lower tissue V deficiency. The less efficient V utilization with than without V supplementation is the main cause of lower tissue V content and higher plasma V level and V loss. In all, V supplementation decreases more tissue V content and increases more V loss and plasma V level in V deficient tissue. The tissue V content cannot decrease more with V supplementation and plasma V level and V loss cannot increase more in V deficient tissue unless V utilization decreases more. It is concluded that tissue V deficiency is more evident with than without V supplementation and V loss and plasma V level exacerbated more in higher than lower tissue V deficiency. The mechanisms by which these conditions act to modulate V metabolism and the processes by which the tissue V content decrease more and plasma V level and V loss increase more with than without V supplementation are not clear. It is evident however that V supplementation during prolonged HK

has a significant effect on V metabolism in response to the impaired V utilization.

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