

## Dietary Magnesium Depletion Affects Metabolic Responses during Submaximal Exercise in Postmenopausal Women<sup>1,2</sup>

Henry C. Lukaski<sup>3</sup> and Forrest H. Nielsen

U.S. Department of Agriculture, Agricultural Research Service, Grand Forks Human Nutrition Research Center, Grand Forks, ND 58202

**ABSTRACT** Magnesium is an essential mineral that is required for optimal biological function including energy metabolism. Although national nutritional surveys indicate that usual magnesium intakes do not meet recommendations, particularly among older women, diet-induced magnesium depletion is considered rare among humans without concurrent illness. We examined the effects of dietary magnesium restriction on biochemical measures of magnesium nutriture and physiologic responses during submaximal exercise in 10 postmenopausal women, 45–71 y old, not receiving hormone replacement therapy. The women consumed diets containing conventional foods with varying magnesium content totaling 112 mg/8.4 MJ (2000 kcal) supplemented with 200 mg magnesium daily for 35d (control), then 112 mg/8.4 MJ for 93d (depletion) followed by 112 mg/8.4 MJ supplemented with 200 mg magnesium/d for 49d (repletion) in a depletion-repletion experiment. RBC magnesium concentration ( $P < 0.05$ ), magnesium retention ( $P < 0.05$ ) and skeletal muscle magnesium concentration ( $P < 0.05$ ) decreased when dietary magnesium was restricted. Peak oxygen uptake, total and cumulative net oxygen uptake determined by using indirect calorimetry and peak heart rate increased ( $P < 0.05$ ) during standardized submaximal work with restricted compared with adequate dietary magnesium. These findings indicate that dietary magnesium depletion can be induced in otherwise healthy women; it results in increased energy needs and adversely affects cardiovascular function during submaximal work. This may also explain previous observations of increased energy cost during standardized exercise in physically active men and women considered to have reduced magnesium nutriture. *J. Nutr.* 132: 930–935, 2002.

**KEY WORDS:** • cellular magnesium • magnesium retention • oxygen uptake • heart rate • humans

Magnesium is an intracellular cation that is essential for the optimal function of a diversity of life-sustaining processes (1). It serves as a cofactor for >300 enzymatic reactions in which food is catabolized and new chemical products are formed; it is required for both aerobic and anaerobic energy production and for glycolysis as part of the Mg-ATP complex. Magnesium also serves as a regulator of many physiologic functions including neuromuscular, cardiovascular, immune and hormonal functions, as well as the maintenance of cellular membrane stability.

Despite our knowledge of the fundamental and integrative roles that magnesium plays in the maintenance of biological function, attempts to identify functional consequences of dietary magnesium deficiency in humans are limited. In normal

adults, experimental magnesium deficiency results in altered cardiovascular function, including hypertension and electrocardiographic abnormalities (2,3), and impaired carbohydrate metabolism with insulin resistance and decreased insulin secretion (2,4). Epidemiologic data tend to confirm these initial findings (5,6). Conflicting data, however, emerge from magnesium intervention trials of patients with documented cardiovascular disease (7). Some of the inconsistencies may be explained by uncontrolled variables such as predisposing conditions that limit magnesium intake or absorption, or promote excretion.

Dietary magnesium generally does not meet recommended intakes for adults. Results of a recent national survey in the United States indicate that a substantial proportion of women do not consume the recommended daily intake of magnesium; this problem increases among women >50 y old (8). The average magnesium intake for women is 228 mg/d compared with the recommendation of 320 mg/d (8). The average intake estimate is derived from 1-d diet recall and thus may overestimate actual magnesium intake (9).

Magnesium also has been proposed as a limiting nutrient for exercise and physical performance (10–13). Surveys of physically active individuals indicate that magnesium intakes among certain groups of athletes do not meet recommendations for adults (14). A few reports indicate that magnesium

<sup>1</sup> Preliminary results were presented at Experimental Biology '97, New Orleans, LA. [Lukaski, H. C., Milne, D. B. & Nielsen, F. H. (1997) Work inefficiency in diet-induced magnesium deficiency in postmenopausal women. *FASEB J.* 11: A147 (abs.)].

<sup>2</sup> U.S. Department of Agriculture, Agricultural Research Service, Northern Plains Area is an equal opportunity/affirmative action employer and all agency services are available without discrimination. Mention of a trademark or proprietary product does not constitute a guarantee of warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.

<sup>3</sup> To whom correspondence should be addressed.  
E-mail: hlukaski@gfhnrc.ars.usda.gov.

supplementation enhances strength gain (15) and improves exercise performance (16,17). However, it is unclear whether these improvements in performance are related to remediation of an existing magnesium deficiency or a pharmacologic effect (18).

The present study was designed to test the hypothesis that restricted dietary magnesium elicits alterations in biochemical indicators of magnesium status, magnesium retention and functional responses to controlled exercise in postmenopausal women.

## SUBJECTS AND METHODS

**Experimental Design.** Postmenopausal women ( $n = 12$ ) participated in a magnesium depletion-repletion study. Two of the women did not complete all of the nutritional, biochemical and physiologic tests; data are reported only for the 10 women who accomplished all tests. Upon arrival at the Grand Forks Human Nutrition Research Center, the women began a 35-d equilibration or control period during which they consumed a basal diet supplemented with 200 mg magnesium/d. A magnesium-depletion period of 93 d followed in which only the basal, low magnesium diet was fed. This low magnesium diet supplied 112 mg of magnesium when an 8.4 MJ (2000 kcal) intake was consumed. The study concluded with a magnesium repletion period of 49 d in which the low magnesium diet was supplemented with 200 mg of magnesium daily. Phlebotomy and collection of excreta for determination of magnesium retention were performed at regular intervals during the study. Physiologic testing and skeletal muscle biopsies were performed during the last 12 d of each experimental period to permit adaptation to the dietary treatment.

**Subjects.** Postmenopausal women ( $n = 10$ ) aged (mean  $\pm$  SEM)  $59.7 \pm 2.7$  y (range, 44.9–71.0 y), with a mean body weight of  $69.1 \pm 3.5$  kg (range, 51.9–91.5 kg) and a body mass index of  $26.3 \pm 0.8$  kg/m<sup>2</sup> (range, 22.2–32.7 kg/m<sup>2</sup>) completed all aspects of the study. The women were recruited through public advertisements and selected after medical, nutritional and psychological evaluations established that they had no underlying disease, and were emotionally suited to participate in the clinical research project. They neither received hormone replacement therapy nor participated in physical training, but did participate irregularly in recreational activities. The participants gave their written informed consent to participate in this

study, which was approved for human subjects by the Institutional Review Board of the University of North Dakota and by the Human Studies Review Committee of the USDA. The volunteers resided in the metabolic unit of the Grand Forks Human Nutrition Research Center under close supervision 24 h/d, 7 d/wk; they consumed only food and beverages provided by the dietary staff and collected all excreta. When outside of the Unit, the women were chaperoned by trained staff members.

**Diets.** Registered dietitians planned the basal low magnesium diet that was based on ordinary Western foods presented in a 3-d rotating menu cycle (Table 1). The energy distribution of the diet was 10% protein, 35% fat and 55% carbohydrate, which is consistent with the diet composition of the average adult in the United States (19). This basal, low magnesium diet was calculated (20) to supply ~700 mg calcium, 11.25  $\mu$ g cholecalciferol, 2.4 mg copper, and 94 mg magnesium when a 8.48 MJ (2000 kcal) intake was consumed. The calcium content of the diet also was consistent with normal calcium intakes of women in the United States (8) and has been shown to maintain calcium balance in postmenopausal women (21). The diet was adequate in other essential nutrients.

During the control and repletion periods, the women received magnesium supplements. The supplement was magnesium gluconate (500 mg/capsule, providing 28.5 mg of magnesium; Willner Chemists, New York, NY); it was given as two capsules with breakfast, lunch and supper, and one capsule with the evening snack. Total supplemental magnesium was 200 mg/d.

The dietary intake of each woman was based on her energy needs as calculated by using the Harris-Benedict equation (22) plus an additional 50% of basal energy expenditure for normal physical activity. Body weight was maintained within  $\pm 2\%$  of individual values determined at the end of the control period by use of individualized exercise prescriptions and modest changes in food intake in 0.85-MJ (200-kcal) increments. Changes in food intake resulted in proportional changes in nutrient intakes.

**Chemical analyses.** Venous blood was taken by phlebotomy and limited to  $\leq 250$  mL/mo for routine health assessment and determination of magnesium status. Blood was drawn into plastic syringes from an antecubital vein, which had been made visible by temporary use of a tourniquet, after the volunteers had fasted for 10 h. Serum was processed within 90 min of the time the blood was drawn. Serum magnesium was determined by flame atomic absorption spectrometry

TABLE 1

Food items in the 3-d menu rotation diet

	Day 1	Day 2	Day 3
Breakfast	Cornflakes Non-dairy creamer White bread Butter Blueberries Cranapple juice	Pork sausage White bread Butter Grape jelly Orange drink	Coffee cake Butter Milk, 2%
Lunch	Beef stew Lettuce French dressing Rusks Ice cream Lemon-lime Carbonated beverage	Chicken rice casserole Cauliflower Rusks Carrot cake Cream cheese frosting Apple juice	Crispy chicken Buttered ditalini Green beans Vanilla wafers Cranapple juice
Supper	Chicken cheese casserole Crackers Cream cheese Cherry gelatin Tropical punch	Minestrone soup Rusks Lettuce Russian dressing Applesauce Lemonade	Barbequed pork Vegetables Steamed rice Shortbread cookies Lemon fruit drink Lemon-lime Carbonated beverage
Snack	Shortbread cookies Orange fruit drink	Pound cake Milk, 2%	Lime gelatin Pears Whipped topping

(AAS)<sup>4</sup> after dilution with a 0.5% solution of lanthanum oxide in deionized water (23). Concurrent analysis of Sera Chem controls (Fisher Scientific, Orangeberg, NY) yielded values of  $0.93 \pm 0.05$  (mean  $\pm$  SD) mmol/L ( $2.26 \pm 0.11$  mg/dL;  $n = 55$ ) and  $1.73 \pm 0.11$  mmol/L ( $4.20 \pm 0.27$  mg/d;  $n = 60$ ) compared with certified values of  $0.97 \pm 0.34$  mmol/L ( $2.36 \pm 0.83$  mg/dL) and  $1.81 \pm 0.49$  mmol/L ( $4.40 \pm 1.2$  mg/dL), respectively. RBC magnesium was determined by using AAS after removal of platelet-rich plasma and digestion of erythrocytes with nitric acid and peroxide (24).

All food was weighed with an accuracy of 0.1 g during preparation in the metabolic kitchen and was consumed quantitatively by all volunteers. Urine and feces were collected carefully to avoid trace mineral contamination. Duplicate diets at the 10.6 MJ (2500 kcal) intake were prepared daily for analysis and were blended in a plastic blender with stainless steel blades. Adjustments for differences in individual energy intakes were calculated proportionately.

The magnesium content of 3-d composites of diets and feces was determined by inductively coupled plasma emission spectroscopy [ICAP; Jarrell-Ash, Waltham, MA; (25)] after wet digestion of aliquots of freeze-dried, blended material with nitric and perchloric acids (26). Urinary magnesium was determined by ICAP analysis of a diluted aliquot (25). Concurrent replicate analysis of a Total Diet SRM #1548 ( $n = 9$ ) (National Institute of Standards and Technology, Gaithersburg, MD) yielded a value of  $533 \pm 10$  (mean  $\pm$  SD)  $\mu\text{g/g}$  compared with a certified value of  $556 \pm 27$   $\mu\text{g/g}$ . Replicate analysis of standard bovine liver SRM #1577a ( $n = 14$ ) yielded a value of  $612 \pm 18$   $\mu\text{g/g}$  compared with a certified value of  $600 \pm 14$   $\mu\text{g/g}$ . Magnesium balance or retention was calculated as the difference between intake and excretion (feces plus urine) for 3-d periods continuously throughout the study.

**Skeletal muscle biopsy.** Cellular magnesium status was assessed by measurement of the magnesium concentration of skeletal muscle obtained by using percutaneous muscle biopsy of the vastus lateralis muscle of one leg. A total of three biopsies were performed, the first and third on one leg and the second on the other leg. A single biopsy specimen was obtained during the last 12 d of each experimental period according to Bergstrom (27);  $\sim 30$ – $40$  mg of muscle tissue was obtained from each biopsy. After adipose and connective tissue were dissected from each specimen, the specimen was rinsed with distilled-deionized water and blotted dry. The samples were placed in clean teflon tubes and dried to constant weight at  $50^\circ\text{C}$ . The samples were digested in  $250$   $\mu\text{L}$  of Vycor distilled nitric acid (GFS Chemicals, Columbus, OH) in a sand bath until they were nearly dry. The cooled, digested samples were diluted to  $1.0$  mL final volume with 2% Vycor distilled nitric acid. Magnesium was determined in the digests by ICAP analysis. Concurrent analysis of standard bovine liver SRM# 1577a ( $n = 10$ ) (National Institute of Standards and Technology) yielded a value of  $615 \pm 14$   $\mu\text{g/g}$ , compared with a certified value of  $601 \pm 28$   $\mu\text{g/g}$ . Muscle magnesium concentration was expressed as mmol/kg dry weight.

**Metabolic responses at rest and during submaximal work.** Submaximal work capacity was determined by using a cycle ergometer (Monark; Varberg, Sweden) and a continuous, progressive exercise protocol that was terminated when the volunteer reached a heart rate of 80% of age-adjusted peak heart rate [e.g.,  $0.8 (220 - \text{age})$ ]. Exercise tests were performed at the end of each dietary period. After an overnight fast, the women rested for 5 min while seated on the ergocycle, then pedaled at 50 revolutions/min starting with an initial external resistance of 0 W, then increasing by 25-W increments every 3 min until the target heart rate was attained (see below) and the test was terminated. Heart rate was monitored continuously during the preexercise and exercise periods by using standard electrocardiographic leads (II, aVF and CM5), and was recorded during the last 10 s of each minute with a multichannel recorder. Oxygen uptake and carbon dioxide output were determined at rest and during exercise with indirect calorimetry (28) every 2 s with an automated system (Q Plex I; Quinton Instruments, Seattle, WA). The oxygen and carbon dioxide analyzers were calibrated before each test with reference gas

mixtures whose composition was previously determined by standard chemical procedures. The oxygen consumption and carbon dioxide data were presented as an average value per minute.

During the control period, each woman participated in three separate bouts of ergocycle testing on different days to become familiar with the equipment and the test protocol. The peak power output of each volunteer was recorded and was used as the termination point for all subsequent submaximal exercise tests. The test-retest reproducibility of the physiologic responses to this end point was  $\pm 2\%$  for heart rate, oxygen uptake and carbon dioxide output. To preserve familiarity with the ergocycle and to ensure the maintenance of aerobic capacity and body composition, each woman received an individualized ergocycle exercise prescription based on an intensity of 50% peak power output and performed for three 15-min sessions weekly on nonconsecutive days (29).

Some indirect estimates of energy utilization were calculated. Gross or total oxygen uptake was calculated as the sum of minute oxygen uptake during each exercise test. Cumulative net oxygen uptake was determined as the sum of each minute oxygen consumption corrected for resting oxygen uptake (30).

**Statistics.** Data are presented as the mean  $\pm$  SEM. The effect of dietary magnesium on biochemical, nutritional and physiologic responses was assessed by repeated-measures ANOVA (31). When a significant main effect was identified, comparisons among dietary magnesium intakes were made with Tukey-Kramer contrasts with adjustments for multiple comparisons (31). A paired *t* test was used to determine whether retention or balance data were different than 0 (31).

## RESULTS

Body weight was maintained within  $\pm 2\%$  of the control period weight and body composition was unchanged during this experiment (data not shown).

**Magnesium intake, excretion and balance.** Dietary magnesium increased significantly during the depletion and repletion periods (Table 2). The increase was attributed principally to an unanticipated change by the manufacturer in the anticaking agent of the beverage mix, which increased the magnesium content (30–40 mg/d) of the diet that was used in the depletion and repletion periods for all volunteers, as well as a modest increase in the food intake (0.85 MJ/d or 200 kcal/d) required to maintain body weights of three of the women.

Magnesium excretion paralleled dietary magnesium (Table 2). Urinary and fecal magnesium losses decreased significantly (30 and 40%, respectively) with dietary magnesium restriction (53%) relative to the control period. Despite the increased magnesium intake associated with the magnesium contamination of the beverage mix, magnesium balance in the magnesium depletion period was negative because the amount of magnesium in the anticaking agent was small, as shown by the tendency of fecal magnesium losses to increase ( $P = 0.10$ ) from 107 to 119 mg/d. This agent most likely did not influence magnesium bioavailability in general because urinary magnesium excretion did not decrease.

Magnesium balance decreased significantly (e.g., net loss of magnesium of  $-42$  mg/d) during magnesium depletion compared with a net retention ( $+32$  and  $+38$  mg/d) when dietary magnesium was adequate. Magnesium balances were different ( $P < 0.05$ ) than 0.

**Blood biochemical and cellular measures of magnesium status.** Serum magnesium concentrations were within the range of normal values and showed slight responses to changes in dietary magnesium (Fig. 1). Compared with the control period, serum magnesium tended to decrease ( $P = 0.07$ ) from  $0.85 \pm 0.02$  to  $0.81 \pm 0.02$  mmol/L with restricted magnesium intake and then increase ( $P = 0.06$ ) to  $0.86 \pm 0.02$  mmol/L with magnesium repletion. Erythrocyte magnesium concentra-

<sup>4</sup> Abbreviations used: AAS, atomic absorption spectrometry; EAR, estimated average requirement; Hb, hemoglobin; ICAP, inductively coupled plasma emission spectroscopy; RDA, recommended dietary allowance.

TABLE 2

Serial determinations of magnesium intake and excretion when magnesium intake was adequate (control and repletion) and restricted (depletion) in postmenopausal women<sup>1</sup>

Variable	Dietary magnesium treatment					
	Control	Depletion, wk				Repletion
	Last 18 d	2-5	6-9	10-13	2-13 <sup>2</sup>	Last 18 d
	mg/d					
Diet	122 ± 17 <sup>c</sup>	138 ± 27 <sup>b</sup>	144 ± 19 <sup>b</sup>	155 ± 35 <sup>a</sup>	146 ± 19	160 ± 34 <sup>a</sup>
Supplement	200	0	0	0	0	200
Intake	322 ± 17 <sup>b</sup>	138 ± 27 <sup>d</sup>	144 ± 19 <sup>d</sup>	155 ± 35 <sup>c</sup>	146 ± 19	360 ± 34 <sup>a</sup>
Feces	184 ± 6 <sup>b</sup>	107 ± 4 <sup>c</sup>	114 ± 5 <sup>c</sup>	119 ± 4 <sup>c</sup>	114 ± 4	212 ± 5 <sup>a</sup>
Urine	107 ± 5 <sup>a</sup>	73 ± 4 <sup>b</sup>	75 ± 5 <sup>b</sup>	77 ± 8 <sup>b</sup>	76 ± 2	110 ± 4 <sup>a</sup>
Balance	+32 ± 6 <sup>a</sup>	-47 ± 5 <sup>b</sup>	-45 ± 4 <sup>b</sup>	-34 ± 6 <sup>b</sup>	-43 ± 3	+38 ± 6 <sup>a</sup>

<sup>1</sup> Values are means ± SEM ( $n = 10$ ) for consecutive 3-d balance periods during the entire period indicated in the column heading. <sup>abcd</sup> Means in a row with different letters differ,  $P < 0.05$ .

<sup>2</sup> Data are inclusive of dietary magnesium restriction for the entire period indicated; these data were not included in the repeated-measures ANOVA.

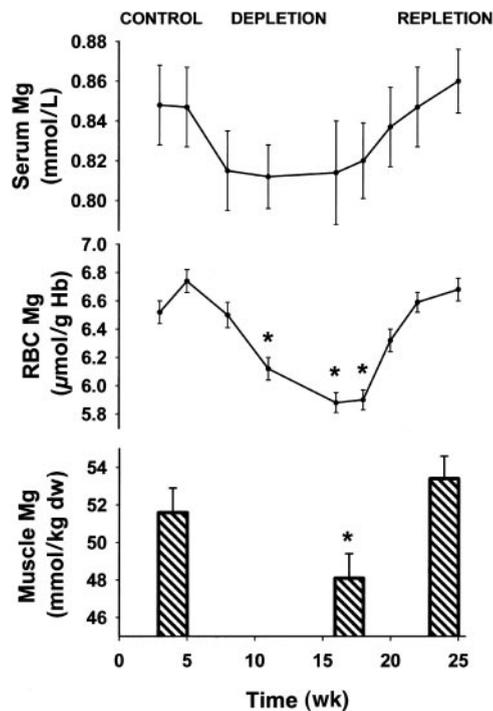
tion decreased ( $P < 0.05$ ) when dietary magnesium was low [ $5.91 \pm 0.07 \mu\text{mol/g}$  hemoglobin (Hb)] and increased when magnesium intake was greater during the control and repletion periods ( $6.74 \pm 0.08$  and  $6.68 \pm 0.08 \mu\text{mol/g}$  Hb).

Magnesium intake significantly affected skeletal muscle magnesium concentration. Compared with the repletion and

control periods ( $53.4 \pm 1.2$  and  $51.6 \pm 1.3 \text{ mmol/kg}$  dry weight, respectively), dietary magnesium restriction was associated with a decrease ( $P < 0.05$ ) in muscle magnesium concentration ( $48.1 \pm 1.3 \text{ mmol/kg}$  dry weight).

**Functional responses during submaximal exercise.** The duration of submaximal ergocycle exercise and hence total work was maintained despite dietary magnesium restriction. Magnesium intake, however, affected metabolic and cardiovascular responses during exercise (Table 3). Peak oxygen uptake, total and cumulative net oxygen utilization and heart rate increased significantly during magnesium restriction compared with the periods when magnesium intake was adequate.

Increased oxygen use during submaximal exercise was related to cumulative magnesium loss during dietary magnesium restriction. During magnesium depletion, the increase in total oxygen uptake during exercise for each woman, expressed as a percentage of total oxygen use during the control or adequate magnesium intake period, was correlated significantly ( $r = 0.89$ ,  $P < 0.05$ ) with the cumulative or total magnesium



**FIGURE 1** Serial determinations of serum magnesium (top), red blood cell magnesium (middle) and skeletal muscle magnesium (bottom) concentrations in 10 postmenopausal women fed diets containing adequate magnesium (control, 0-5 wk), restricted in magnesium (depletion, 6-18 wk) and supplemented with magnesium (repletion, 19-25 wk); dw, dry weight. Values are means ± SEM. ANOVA with a repeated-measures design with Tukey-Kramer contrasts and adjustments for multiple comparisons was used to identify significant differences. \*Diet effect,  $P < 0.05$ .

TABLE 3

Functional and metabolic responses during submaximal exercise of postmenopausal women consuming diets adequate (control and repletion) and restricted (depletion) in magnesium<sup>1</sup>

	Diet		
	Control	Depletion	Repletion
Duration, min	11.8 ± 0.7	11.2 ± 0.8	11.9 ± 0.6
Resting $\text{VO}_2$ , mL/min	245 ± 11	256 ± 13	248 ± 12
$\text{VO}_2$ peak, mL/min	1118 ± 31 <sup>b</sup>	1293 ± 43 <sup>a</sup>	1128 ± 31 <sup>b</sup>
Total $\text{VO}_2$ , <sup>2</sup> mL	8139 ± 521 <sup>b</sup>	8981 ± 712 <sup>a</sup>	8207 ± 549 <sup>b</sup>
$\text{CNVO}_2$ , <sup>3</sup> mL	5238 ± 227 <sup>b</sup>	6111 ± 232 <sup>a</sup>	5319 ± 216 <sup>b</sup>
Heart rate, bpm	129 ± 1 <sup>b</sup>	136 ± 2 <sup>a</sup>	128 ± 1 <sup>b</sup>

<sup>1</sup> Values are means ± SEM ( $n = 10$ ). <sup>ab</sup> Values in a row with different letters differ,  $P < 0.05$ .

<sup>2</sup> Sum of minute oxygen uptake during exercise uncorrected for resting energy utilization.

<sup>3</sup>  $\text{CNVO}_2$ , cumulative net oxygen uptake =  $\Sigma$ [total oxygen uptake - resting oxygen uptake].

losses assessed by using magnesium balance measurements determined during the last 12 wk of consumption of the low magnesium diet.

## DISCUSSION

The biochemical consequences of restricted magnesium intake depend on the physiologic state of the individual, the amount of magnesium intake and the duration of consumption of the low magnesium diet (1,32–36). The findings of significantly increased magnesium loss (e.g., negative magnesium balance) in parallel with decreased RBC and skeletal muscle magnesium when the postmenopausal women consumed the low magnesium diet indicate magnesium depletion. They reveal that 155 mg magnesium/d is not adequate, and they are consistent with current recommendations of 320 mg/d for women (7).

Functional deficits associated with magnesium deprivation are well characterized. Severe magnesium deficiency in humans results in neuromuscular dysfunction (34). Functional deficits with moderate magnesium depletion are not well characterized. The results of the present study show that low dietary magnesium, in amounts consumed by some women in the United States, is associated with an overall loss of magnesium from the body, decreased cellular magnesium, serum magnesium in the low end of the range of normal values and decreased work economy during submaximal work. The finding of impaired work economy (increased oxygen utilization to accomplish the same work output) is consistent with some previous results (11,12) in physically active adults and elite athletes and, therefore, provides evidence for a role of magnesium in physical activity.

Reports of decreased magnesium intake and reduced serum magnesium concentrations within the range of normal values in some groups of physically active persons heighten interest in the role of magnesium in physical training and performance (14,37,38). Evidence of impaired physiological function during physical activity or exercise performance and documented magnesium depletion or deficiency, however, is sparse (18,39).

Magnesium supplementation of humans has yielded inconsistent reports of improved exercise metabolism and performance. In a double-blind, crossover trial, magnesium supplementation (390 mg/d for 25 d with a 3-wk washout) of male athletes resulted in increased peak oxygen uptake and total work output during work capacity tests (3). During submaximal work, supplemental magnesium elicited reductions in heart rate, ventilation, oxygen uptake and carbon dioxide production (40). Physically active collegians experienced significant increases in endurance performance and decreased oxygen utilization during standardized, submaximal exercise after magnesium supplementation at 8 mg/(kg · d) (17). Similarly, men supplemented with magnesium (420 mg/d) had significant strength gains during resistance training compared with their unsupplemented counterparts consuming 250 mg magnesium daily (15). In contrast, other studies have found no functional or performance improvements resulting from magnesium supplementation of physically active persons with serum magnesium and muscle magnesium concentrations in the range of normal values (18,39). The contradictory response in function and performance to magnesium supplementation apparently reflects differences in the magnesium nutritional status of the participants.

Supplementation of magnesium-depleted individuals consistently results in significant improvements in physiologic function and some measures of performance. After magnesium supplementation of 360 mg/d for 3 wk, female competitive

athletes with low plasma magnesium concentrations within the range of normal values responded with significant decreases in circulating total creatine kinase and the creatine kinase isozyme from skeletal muscle after training, compared with other low magnesium status athletes receiving a placebo (11). Similarly, elite male rowers supplemented with 360 mg of magnesium daily for 4 wk used less oxygen during a controlled rowing test compared with other rowers receiving a placebo (41).

The findings of the present study confirm the impaired cardiorespiratory and cardiovascular function during submaximal work when dietary magnesium was limited and demonstrate for the first time that the impairments were associated with magnesium depletion. Thus, this report provides the first evidence that low dietary magnesium, in amounts consumed by some groups of physically active individuals, impairs function during conditions of increased energy expenditure and that functional limitations occur despite the level of physical fitness (e.g., trained athletes vs. relatively sedentary postmenopausal women).

Magnesium depletion may elicit impaired work economy by diverse mechanisms. In rats, decreased skeletal muscle magnesium and serum magnesium caused a partial uncoupling of the respiratory chain in liver mitochondria with a reduced ADP to oxygen ratio (42). Similar effects of magnesium deficiency on mitochondrial respiratory chain have been described in heart muscle cells (43). Thus, magnesium depletion increases the oxygen requirement to maintain ATP production.

Magnesium depletion may increase the energy cost of muscle contraction. Ergocycle work is a combination of muscle contraction and relaxation. Relaxation accounts for an important fraction of total energy needs during the short, repeated muscle contractions associated with pedaling a cycle ergometer (44). We hypothesize that the hyperexcitability of magnesium deficiency (1) boosts the energy cost, and hence oxygen use, of cycling by increasing the cocontraction activity of agonist and antagonist muscles and decreasing muscle relaxation during submaximal work. Therefore, the combination of augmented mitochondrial respiratory activity and enhanced neuromuscular excitability may explain the increased oxygen requirement during controlled exercise observed in the present study.

The chemical balance data from the present study are consistent with the recent dietary recommendations for magnesium intake in women aged 51–70 y (7). The estimated average requirement (EAR) for magnesium for older women is based on an extrapolation of the balance data of 10 women, aged 25–53 y, who consumed self-selected diets ranging from 164 to 301 mg/d in magnesium intake with an average intake of  $231 \pm 80$  mg/d (35). The EAR for magnesium was estimated to be 265 mg/d on the basis of the anticipated decline in renal function with aging (7). The estimate for a recommended dietary allowance (RDA) of magnesium is 320 mg/d (7), which is similar to the value of 322 mg/d determined in the present study. Importantly, the findings of altered cardiorespiratory function at low dietary magnesium intake provide independent confirmation of the current RDA for magnesium in older women.

In conclusion, the present study demonstrates that magnesium depletion may be induced by dietary magnesium restriction in otherwise healthy postmenopausal women. It provides independent data that support the current RDA for magnesium for women aged 51–70 y on the basis of various experimental approaches including chemical balance, biochemical and functional markers. The decreased work economy as a functional impairment of restricted dietary magnesium is

unique and extends preliminary observations in young elite athletes, in whom magnesium status was not examined, to older women not participating in intense physical activity.

### ACKNOWLEDGMENTS

The authors gratefully recognize the valuable contributions of other members of our human studies research team: B. S. Hoverson supervised diet development and preparations; D. B. Milne and S. K. Gallagher supervised clinical and mineral analysis laboratories; J.E. Burrell performed the muscle biopsies; W. A. Siders and C. B. Hall administered the work capacity tests. We are especially grateful for the dedication and conscientious participation of the women without whom this study would not have been successful.

### LITERATURE CITED

- Shils, M. E. (1998) Magnesium. In: Handbook of Nutritionally Essential Mineral Elements (O'Dell, B. L. & Sunde, R. A., eds.), pp. 117-152. Marcel Dekker, New York, NY.
- Nadler, J. L., Buchanan, T., Natarajan, R., Antonipillai, I., Bergman, R. & Rude, R. K. (1993) Magnesium deficiency produces insulin resistance and increased thromboxane synthesis. *Hypertension* 21: 1024-1029.
- Rude, R. K. (1993) Magnesium metabolism and deficiency. *Endocrinol. Metab. Clin. N. Am.* 22: 377-395.
- Paolisso, G., Scheen, A., D'Onofrio, F. D. & Lefebvre, P. (1990) Magnesium and glucose homeostasis. *Diabetologia* 33: 511-514.
- Joffres, M. R., Reed, D. M. & Yano, K. (1987) Relationship of magnesium intake and other dietary factors to blood pressure: the Honolulu heart study. *Am. J. Clin. Nutr.* 45: 469-475.
- Ma, J., Folsom, A. R., Melnick, S. L., Eckfeldt, J. H., Sharrett, A. R., Nabulsi, A. A., Hutchinson R. G. & Metacalf, P.A. (1995) Associations of serum and dietary magnesium with cardiovascular disease, hypertension, diabetes, insulin, and carotid arterial wall thickness: The ARIC study. *Atherosclerosis Risk in Community Study. J. Clin. Epidemiol.* 48: 927-940.
- Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, Food and Nutrition Board, Institute of Medicine (1997) Magnesium. In: Dietary Reference Intakes for Calcium, Phosphorus, Magnesium, Vitamin D, and Fluoride, pp. 190-249. National Academy Press, Washington, DC.
- Pennington, J.A.T. (2000) Current dietary intakes of trace elements and minerals. In: Clinical Nutrition of the Essential Trace Elements and Minerals (Bogden, J. D. & Klevay, L. M., eds.), pp. 49-67. Humana Press, Totowa, NJ.
- Anderson, R. A., Bryden, N. A. & Polansky, M. M. (1993) Dietary intake of calcium, chromium, copper, iron, magnesium, manganese, and zinc: duplicate plate values corrected using derived nutrient intake. *J. Am. Diet. Assoc.* 93: 462-464.
- Rayssiguier, Y., Guezennec, C. Y. & Durlach, J. (1990) New experimental and clinical data on the relationship between magnesium and sport. *Magnes. Res.* 3: 93-102.
- Golf, S. W., Böhmer, D. & Nowacki, P. E. (1994) Is magnesium a limiting factor in competitive exercise? A summary of relevant scientific data. In: Magnesium 1993 (Golf, S., Dralle, D. & Vecchiet, L., eds.), pp. 209-220. John Libbey & Company, London, UK.
- Brilla, L. R. & Pateson Lombardi, V. (1999) Magnesium in exercise and sport. In: Macroelements, Water and Electrolytes (Driskell, J. & Wolinsky, L., eds.), pp. 48-90. CRC Press, Boca Raton, FL.
- Lukaski, H. C. (1999) Vitamin and mineral metabolism and exercise performance. In: Metabolic Basis of Performance in Exercise and Sport. Perspectives in Exercise Science and Sports Medicine, vol. 12 (Lamb, D.R. & Murray, R., eds.), pp. 261-314. Cooper Publishing Group, Carmel, IN.
- Lukaski, H. C. (2000) Magnesium, zinc and chromium nutrition and physical activity. *Am. J. Clin. Nutr.* 72 (suppl.): 585S-593S.
- Brilla, L. R. & Haley, T. F. (1992) Effect of magnesium supplementation on strength training in humans. *J. Am. Coll. Nutr.* 11: 326-329.
- Ripari, P., Peralisi, G., Giamberardino, M. A. & Vecchiet, L. (1989) Effects of magnesium pidolate on some cardiorespiratory submaximal effort parameters. *Magnes. Res.* 2: 70-74.
- Brilla, L. R. & Gunther, K. B. (1995) Effect of magnesium supplementation on exercise time to exhaustion. *Med. Exerc. Nutr. Health* 4: 230-233.
- Manore, M., Merkel, J., Helleksen, J. M., Skinner, J. S. & Carroll, S. S. (1995) Longitudinal changes in magnesium status in untrained males: effect of two different 12-week exercise training programs and magnesium supplementation. In: Sports Nutrition: Minerals and Electrolytes (Kies, C.V. & Driskell, J.A., eds.), pp. 179-187. CRC Press, Boca Raton, FL.
- U.S. Department of Agriculture, Agricultural Research Service (1997) Data tables: results from USDA's 1994-96 Continuing Survey of Food Intakes by Individuals and 1994-96 Diet and Health Knowledge Survey. CD-ROM, NTIS Accession Number PB98-500457.
- U.S. Department of Agriculture, Human Nutrition Information Service (1992) USDA Nutrient Database for Standard Reference. Release 10, (computer tape). National Technical Information Service, Springfield, VA.
- Nielsen, F. H. & Milne, D. B. (1999) Calcium balance in postmenopausal women with dietary calcium intakes between 650 and 850 mg/day. *FASEB J.* 13: A869 (abs.).
- Harris, J. A. & Benedict, F. G. (1919) A Biometric Study of Basal Metabolism in Men, Carnegie Publication no. 279. J. P. Lippincott, Philadelphia, PA.
- Wills, M. R., Sunderman, F. W. & Savory, J. (1986) Methods for the estimation of serum magnesium in clinical laboratories. *Magnesium* 5: 317-327.
- Agarwal, R. P., Chencharick, J. D. & Henkin, R. I. (1985) A simple method for simultaneous estimation of zinc and copper in erythrocytes: comparison of a new methodology with a standard technique. *Biol. Trace Elem. Res.* 7: 199-208.
- Sims, R. L., Mullen, L. M. & Milne, D. B. (1990) Application of inductively coupled plasma emission spectroscopy to multielement analysis of food-stuffs used in metabolic studies. *J. Food Comp. Anal.* 3: 27-37.
- Analytical Methods Committee (1960) Methods of destruction of organic matter. *Analyst* 85: 643-656.
- Bergstrom, J. (1962) Muscle electrolytes in man determined by neutron activation analysis on needle biopsy specimens. A study of normal subjects, kidney patients and patients with chronic diarrhea. *Scand. J. Clin. Investig. (suppl.)* 68: 1-110.
- Ferrannini, E. (1988) The theoretical bases of indirect calorimetry: a review. *Metabolism* 37: 287-301.
- Lukaski, H. C. & Bolonchuk, W. W. (1989) Maintenance of aerobic capacity and body composition of volunteers residing on a metabolic research unit. *J. Sports Med. Phys. Fitness* 29: 273-278.
- Lukaski, H. C., Hall, C. B. & Siders, W. A. (1991) Altered metabolic response of iron-deficient women during graded, maximal exercise. *Eur. J. Appl. Physiol.* 63: 140-145.
- SAS Institute Inc. (1999) SAS/STAT User's Guide, version 6, vols. 1-3. SAS Institute, Cary, NC.
- Seelig, M. S. (1964) The requirement of magnesium by the normal adult. *Am. J. Clin. Nutr.* 14: 342-390.
- Dunn, M. J. & Walser, M. (1966) Magnesium depletion in normal man. *Metabolism* 15: 884-895.
- Shils, M. E. (1969) Experimental human magnesium depletion. *Medicine* 48: 61-85.
- Lakshmanan, L. F., Rao, R. B., Kim, W. W. & Kelsay, J. L. (1984) Magnesium intakes, balances, and blood levels of adults consuming self-selected diets. *Am. J. Clin. Nutr.* 40: 1380-1389.
- Klevay, L. M. & Milne, D. B. (2002) Low dietary magnesium increases supraventricular ectopy. *Am. J. Clin. Nutr.* 75: 550-554.
- Gatteschi, L., Galvani, P., Orsi, F., Rubenni, M. G. & Resina, A. (1994) Relationships between blood magnesium changes and physical performance. In: Magnesium 1993 (Golf, S., Dralle, D. & Vecchiet, L., eds.), pp. 247-251. John Libbey & Company, London, UK.
- Dragani, L., Resina, A., Giamberardino, M. A. & Vecchiet, L. (1994) Magnesium and physical activity. In: Magnesium 1993 (Golf, S., Dralle, D., & Vecchiet, L., eds.), pp. 265-272. John Libbey & Company, London, UK.
- Terblanche, S., Noakes, T. D., Dennis, S. C., Marais, D. W. & Eckert, M. (1992) Failure of magnesium supplementation to influence marathon performance or recovery in magnesium-replete subjects. *Int. J. Sports Nutr.* 2: 154-164.
- Vecchiet, L., Peralisi, G., D'Ovidio, M., Dragani, L., Felzani, G., Mincarini, A., Giamberardino, M. A., Borella, P., Bargellini, A. & Piovanelli, P. (1995) Effects of magnesium supplementation on maximal and submaximal effort. In: Magnesium and Physical Activity (Vecchiet, L., ed.), pp. 227-237. Parthenon Publishing Group, London, UK.
- Münch, J. (1992) Einfluß einer Langzeitmagnesiumsupplementierung auf Physiologische und Biochemische Parameter unter Wettkampfspezifischen Belastungen bei Ruderern. Doctoral thesis, Justus-Liebig-University, Giessen, Germany.
- Heaton, F. W. & Elie, J. P. (1984) Metabolic activity of liver mitochondria from magnesium-deficient rats. *Magnesium* 3: 21-28.
- Vitale, J. J., Nakamura, M. & Hegsted, D. M. (1957) Effect of magnesium deficiency on oxidative phosphorylation. *J. Biol. Chem.* 228: 573-576.
- Bergström, M. & Hultman, E. (1988) Energy cost and fatigue during intermittent electrical stimulation of human skeletal muscle. *J. Appl. Physiol.* 65: 1500-1505.