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CONTRIBUTION OF ACTIVITY TO THE CIRCADIAN RHYTHM IN EXCRETION OF MAGNESIUM AND CALCIUM

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In man, the urinary excretion of the divalent cations, magnesium and calcium, appears to conform to a general circadian periodicity.⁹ However, there is little agreement among the reports available on the time of day during which excretion levels are maximal and minimal. Although the influence of diet on the excretion levels of calcium and magnesium seems to be well recognized, the effect of activity in generating or moderating the periodicity in excretion has been given less attention.

Of the studies concerned with the "diurnal variation" in excretion of magnesium and calcium, several do not permit an accurate evaluation of periodicity because of the use of too long a collection interval^{2,3} or the examination of only a single 24-hour cycle.^{5,6,14} It is the purpose of this report to examine the reproducibility of the circadian pattern in magnesium and calcium excretion and to consider the role of physical activity in this pattern.

Methods

The Circadian Pattern of Magnesium and Calcium Excretion: Eight men, 16-17 years of age, were given a standardized, balanced dietary regimen for 144 hours (Table 1). Each meal consisted of 8 ounces of Metrecal liquid, five Metrecal wafers and 8 ounces of distilled water.

TABLE 1. Physical Characteristics of Subjects Used in Circadian Excretion Studies.

Number	8
Height (cm)	177.5 (2.2)*
Initial weight (kg)	63.9 (2.4)
Final weight (kg)	63.7 (2.3)

*Values in parentheses are the S.E.

Meals were taken at 4-hour intervals over the course of the study. This regimen provided 2,100 kcal per day or a daily mean for the group of 32.9 kcal/kg body weight. Magnesium in the diet amounted to 52.1 MEQ/day (daily mean for the group, 0.82 MEQ/kg body weight); calcium amounted to 277.2 MEQ/day (daily mean for the group, 4.34 MEQ/kg body weight). Activity of the subjects was not controlled by design, but was uniform over the period of the study. School activities occupied most of the daytime hours; evenings were devoted to studies. Subjects slept between 2200 and 0600 hours.

During the first day of the study subjects followed the dietary routine, but collected no samples. Urine sample collections were started at 0600 on the second day of the study and continued at 4-hour intervals for 120 hours. Each 4-hour sample volume was recorded and a portion of urine reserved for analysis. Samples were analyzed for magnesium⁸, calcium⁷, and creatinine.¹¹ Chloride was measured by the method of Cotlove.⁴ Because the creatinine excretion did not vary with a regular pattern in this study, the excretion of magnesium, calcium and chloride was expressed per unit excretion of creatinine.

The Effect of Activity on the Excretion of Magnesium and Calcium: Twenty male subjects (20-29 years of age) were arbitrarily assigned to an experimental group or to a control group. Table 2 gives the physical characteristics of each group. One control and one experimental subject were studied simultaneously. All tests were conducted in the morning and all subjects were post-absorptive at the start of the tests. The format for experimental subjects was: 60 min-

TABLE 2. Physical Characteristics of Experimental and Control Subjects Used in Activity Study

	<i>Experimental</i>	<i>Control</i>
Number	10	10
Age (years)	23 (0.9)*	25 (1.0)
Height (cm)	177.1 (2.1)	175.1 (1.9)
Weight (kg)	75.0 (2.6)	76.6 (4.2)

*Values in parentheses are the S.E.

utes rest (recumbent), 60 minutes activity (treadmill walk at 3.56 ± 0.02 (SE) mph; 4° inclination), 120 minutes rest (recumbent). To insure an adequate urine flow over the course of the test, distilled water was given to experimental subjects in the following amounts: 3 ml/kg body weight at the start of the first rest period; 6 ml/kg immediately prior to the activity period; 1.5 ml/kg at the end of the activity period and every 30 minutes thereafter for 2 hours. One-hour urine samples were collected covering the initial 60-minute rest period and the 60-minute activity period; four 30-minute samples were collected over the 2-hour recovery period. Venous blood samples were taken at the end of the initial rest period, at the end of the activity period, and at the end of the 2-hour recovery period.

Control subjects followed the same schedule for urine and blood sampling outlined above, but rested in a recumbent position over the 4-hour test period. Distilled water was given to control subjects at a dose of 3 ml/kg/hour for the first 2 hours and 1.5 ml/kg/30 minutes over the remaining 2 hours. Urine volumes over the designated periods were recorded and a portion of each urine sample reserved for analysis. Magnesium and calcium levels in both urine and plasma were measured by techniques indicated earlier. Urine was also analyzed for creatinine. The excretion of magnesium and calcium was expressed per unit excretion of creatinine.

Results

Figure 1 presents the pattern of excretion of magnesium and calcium, chloride, and creatinine over the 120-hour test period. The variation in urine flow over this time is shown in the lower panel. A clear circadian periodicity in excretion of both magnesium and calcium was observed. Peak excretion levels for magnesium were observed between the hours 0200-0600 and were reproducible from day to day. Trough levels

for magnesium excretion occurred between the hours 1000 and 1400 and were also consistent. Peak excretion levels for calcium were only slightly less regular than those for magnesium. During days 1, 3, and 4, calcium peaks coincided with magnesium peaks (0200-0600); on days 2 and 5, calcium peaks occurred between 2200 and 0200. Trough levels for calcium excretion always occurred between 1000 and 1400.

The pattern of chloride excretion was also circadian, but out of phase with the magnesium/calcium cycles. Peak levels for chloride excretion occurred each day between 0600 and 1000. Minimum excretion levels of chloride were observed between 2200 and 0600. A secondary peak in chloride excretion was noted on days 2-5 occurring between the hours 1800-2200. No regular pattern was observed in either creatinine excretion or in urine flow.

The influence of activity on the urinary excretion of magnesium and calcium is shown in Figure 2. Exercise induced a marked decrease in the excretion of both ions to about 45% of beginning excretion levels. During the period of rest following exercise the excretion of magnesium and calcium tended to return to the original level; however, after two hours of rest, the excretion of both ions was still only 60% of the original level. By comparison, only small changes were detected in the calcium and magnesium excretion of control (resting) subjects over the 4-hour period.

Creatinine excretion fluctuated slightly over the 4-hour test in both groups. The changes observed, however, were similar for both groups and were not statistically significant. Urine flow gradually increased in the control group as a result of water loading. In the exercise group urine flow decreased, as was expected, during the exercise period and during the first 30-minute post-exercise rest period. Beyond that time urine flow increased rapidly to the same levels occurring in control subjects.

Plasma levels of magnesium and calcium for both experimental and control groups are given in Table 3. No significant difference between groups was detected in either ion at the times sampled.

Discussion

Under the conditions described in this paper we have observed (1) a reproducible circadian

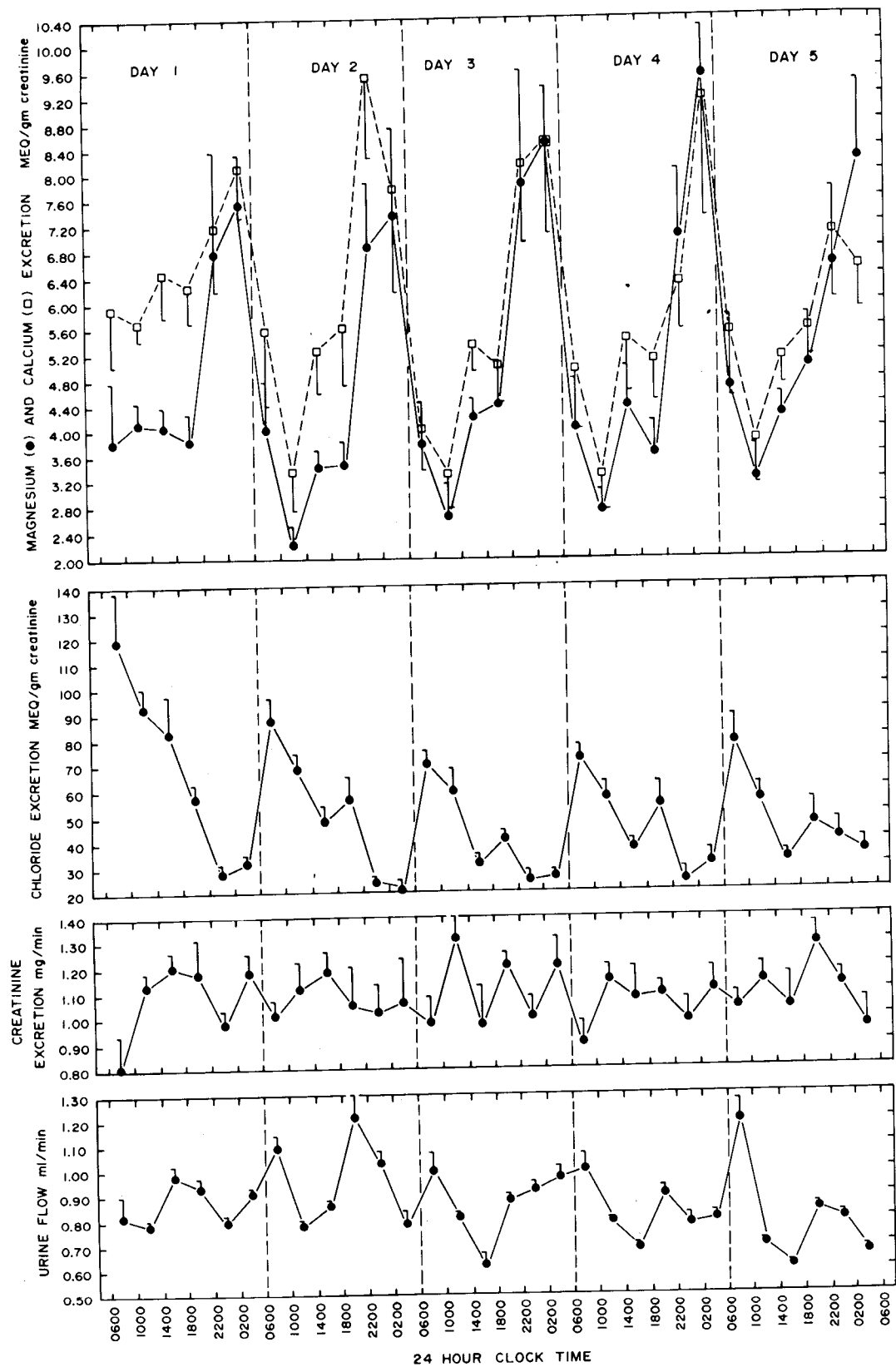


FIGURE 1. Circadian excretion cycles of magnesium and calcium (upper panel) and chloride (middle panel). Creatinine excretion and urine flow (lower two panels) show no regular rhythm.

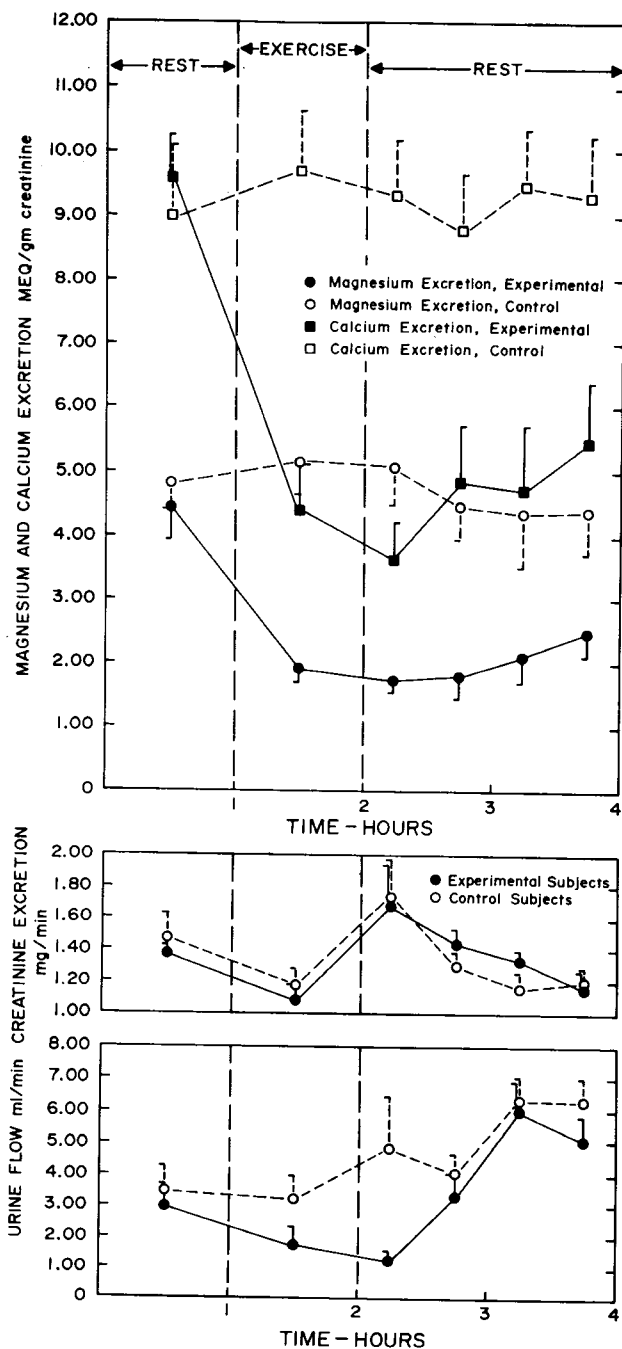


FIGURE 2. Reduced excretion of magnesium and calcium during and following moderate exercise (upper panel). Creatinine excretion and urine flow are shown in the lower panels.

cycle in the urinary excretion of magnesium and calcium, and (2) a marked decrease in the urinary excretion of magnesium and calcium during and following a period of moderate activity.

Although other (shorter term) studies have suggested a circadian rhythm in the excretion of calcium and magnesium^{5,6,10} the time at which

excretion is maximal has varied widely among the several reports. This variability has precluded any statement linking the excretion of these ions with other exogenous rhythms. The reproducibility of our own data leads us to suggest that the excretion of both ions is greatest during the sleeping hours and decreases during the waking hours. Since the sleep-wake cycle also represents an activity cycle it is a reasonable assumption that the circadian periodicity in magnesium and calcium excretion simply reflects the exogenous activity pattern. The demonstration of a marked decrease in magnesium and calcium excretion after exercise supports this view.

The mechanism behind the change in excretion with activity remains obscure. In our experiments the excretion of both ions was not simply related to urine flow. During the post-exercise recovery period excretion of calcium and magnesium was only 60% of control at the same time that urine flow was nearly 200% of control. Nor did changes in blood levels of the ions account for the decreased excretion after exercise. The decrease therefore, must have been the result of a decrease in renal blood flow, or an increase in tubular reabsorption of magnesium and calcium, or both.

In contrast to the excretion pattern of the divalent ions, chloride ion excretion increases and peaks during waking (active) hours and decreases to its lowest levels during the sleeping (inactive) hours (Figure 1). The circadian pattern of chloride excretion observed in the present study is consistent, with respect to peaking times, with several other studies of monovalent ion excretion.^{9,13} The fact that chloride (along with sodium and potassium) excretion is lowest during the sleeping (inactive) hours at the same time that magnesium and calcium excretion is highest suggests several possibilities for the role of the kidney. Assuming no active changes in the renal handling of the ions in question, the above findings might be the reflection of a circadian cycle in the filtered load of those ions due to rhythmically fluctuating plasma concentrations. This idea has not been generally substantiated^{3,14} although some workers have reported day-night differences in plasma magnesium levels.^{1,12} Two other possibilities are (1) the rhythmic response of the kidney to other regularly fluctuating blood levels of "renal regu-

TABLE 3. Plasma Concentrations of Magnesium and Calcium in Resting and Active Subjects.

Experimental Subjects (10)			
	<i>After 60 min. rest</i>	<i>After 60 min. exercise</i>	<i>After 120 min. rest following exercise</i>
Magnesium MEQ/l	1.72 (0.04)*	1.67 (0.05)	1.67 (0.20)
Calcium MEQ/l	6.36 (0.09)	6.57 (0.07)	6.48 (0.10)
Control Subjects (10)			
	<i>After 60 min. rest</i>	<i>After 120 min. rest</i>	<i>After 240 min. rest</i>
Magnesium MEQ/l	1.72 (0.03)	1.72 (0.03)	1.71 (0.03)
Calcium MEQ/l	6.47 (0.09)	6.57 (0.11)	6.53 (0.09)

*Values in parentheses are the S.E.

Neither magnesium nor calcium plasma levels were significantly different between experimental and control group at times sampled.

lators" [aldosterone, "glomerulokin¹⁴"], and (2) an excretion/reabsorption regulating system operating under the influence of an intrinsic "renal clock." The involvement of the above "mechanisms" as factors responsible for the circadian excretion cycles is largely speculative, and reflects the lack of data appropriate to the question.

The results of this study suggest the general conclusion that the circadian periodicity observed in the excretion of magnesium and calcium is primarily a reflection of the circadian

activity cycle. Data are not yet available, either from the present study or from other studies concerned with the problem, to answer the question whether the excretion of magnesium and calcium follows an endogenous circadian cycle.

Appropriate experiments to test this point are necessarily difficult to accomplish⁸, and, in addition to controlling diet and activity factors, must exclude all exogenous time cues. Further attempts to distinguish an endogenous excretory pattern for magnesium and calcium are presently under consideration.

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