Circadian variation in copper and zinc in man

M. D. LIFSCHITZ AND R. I. HENKIN
National Heart and Lung Institute, Bethesda, Maryland 20014

IT IS WELL KNOWN that there is in man a circadian variation in the urinary excretion of several electrolytes. This pattern has been observed for monovalent metals such as potassium and sodium, for divalent metals such as calcium and magnesium, and for some anionic groups such as chloride and phosphate (19, 25).

It is also well known that there is in man a circadian variation in the serum concentration or urinary excretion of several hormones. This pattern has been observed for steroid hormones such as cortisol (18), aldosterone (27), and 17-hydroxyprogesterone (24), and for polypeptide hormones such as parathyroid hormone (16), adrenocorticotropic hormone (21), and renin (9).

Some investigators have related the circadian variation in the urinary excretion of several electrolytes including sodium and potassium to changes in the secretion of adrenal cortical hormones (2, 6). Oral administration of carbohydrate-active steroids has been shown to block the circadian rhythm; steroid effects on metals in blood and urine; urinary excretion of copper and zinc; metal concentrations of copper and zinc; metal binding in relation to renal excretion of metal.
CIRCADIAN VARIATION IN COPPER AND ZINC IN MAN

Study was expressed as a percentage of his 24-hr mean for that day and then this percentage was combined with those of the other subjects for that given time period corresponding to that given day of their study. This resulted in a mean ± SEM for all subjects at each similar time point of the study. Tests of statistical significance were obtained by comparing the mean ± SEM of each time point with that of the 24-hr mean ± SEM. There were no systematic differences in the representation of the data either as absolute values or as percent change from the mean. Ceruloplasmin in serum was measured by an autoanalyzer modification of the oxidase method of Holmberg and Laurell (15) and expressed as milligrams per 100 ml of serum. Serum and urine were analyzed for creatinine by the method of Chasson et al. (5). Serum albumin and total serum protein were measured by the bromocresol (7) and biuret (10) methods, respectively. All blood and urine from each subject were analyzed at the same time.

All serum values of copper, zinc, ceruloplasmin, creatinine, albumin, and total protein were within normal limits as were similar values in urine, except for ceruloplasmin, albumin and protein, which were not measured. All creatinine clearances were normal also.

RESULTS

There is a circadian variation in serum copper concentration which is greater than the mean (as great as 6% above the mean) at 10:00 AM and 2:00 PM, is essentially equal to the mean at 6:00 PM and 10:00 PM, and falls to a level significantly below the mean (as great as 8% below the mean, P < 0.01) at 2:00 AM and 6:00 AM (Fig. 1). This pattern persisted for up to 56 hr which was the longest study conducted in this series. Serum concentration of ceruloplasmin showed a similar but less marked circadian pattern of variation (Fig. 2). This was as great as 4% above the mean at 10:00 AM to as great as 7% below the mean at 6:00 AM.

There is a circadian variation in serum zinc concentration which is also similar to that of serum copper concentration. It was greater than the mean from 10:00 AM to 10:00 PM (as great as 7% above the mean) and between 2:00 AM and 6:00 AM fell to a low point which was significantly below the mean (as great as 8% below the mean, P < 0.01) (Fig. 3). This pattern, similar to that of serum copper concentration, persisted for up to 56 hr.

Urinary copper excretion tended to be lower from 8:00 PM to 4:00 AM than at other times of the day (Fig. 4). This was true whether the data were expressed as micrograms excreted per 4 hr or micrograms excreted per milligram of creatinine per 4 hr. The fit of a 24-hr cosine curve to the urinary copper data according to Halberg (12) yielded an amplitude, C, of 1.53, the standard error (SE) of this amplitude being 0.127. The ratio SE/C was 0.083. This finding can be used to reject the assumption of a zero circadian amplitude. The detection of a circadian rhythm in urinary copper excretion by this approach is significant below the 1% level.
Urinary zinc excretion showed no obvious circadian pattern of variation over 24 hr, but did seem to be higher in the 4-hr collections after eating (Fig. 5). It is of interest that the urinary zinc excretion during the period from 8:00 PM to 12:00 midnight was significantly below most other periods of the study. It is of further interest that the serum concentration of zinc was at its highest level at the midpoint of this period, i.e., 10:00 PM.

Administration of carbohydrate-active steroids did not suppress the circadian variation for serum concentration of either copper or zinc, but the normal pattern for serum zinc was less apparent (Figs. 6 and 7). The absolute levels of serum copper were consistently higher during prednisolone administration (Fig. 8) and this also occurred for serum zinc in four of six comparisons (Fig. 9). The pattern of serum ceruloplasmin was unchanged by this administration as were the absolute levels. The pattern of urinary copper and zinc excretion did not change significantly during carbohydrate-active steroid administration except that the possible dietary influence on urinary zinc was no longer present. Although there were no definite changes in the patterns of serum or urine copper or zinc, the absolute levels in the serum were generally higher and the amount excreted in the urine was uniformly higher during prednisolone administration than in control periods (Figs. 10 and 11).

**DISCUSSION**

These data demonstrate that a circadian pattern of variation is present for serum concentration of copper and zinc in man. This pattern was apparent during a time when the subjects' life pattern under study was rigidly controlled; i.e., dietary intake was regulated as to content and time of intake, blood and urine samples were collected at fixed intervals over several days, activity was carefully monitored, and each subject was confined to a metabolic ward in which spontaneous idiosyncratic activity was limited. Under these conditions a circadian pattern of variation of these transition metals in serum was apparent. This pattern was
independent of age and was apparent in one man as well as in nine women. There was no obvious circadian pattern of variation noted for serum albumin or for total protein although such a pattern for total serum protein has been previously observed (11). Serum ceruloplasmin, however, which is the major copper-carrying protein in serum, followed a pattern of variation similar to that of total serum copper. Previous workers have shown a similar pattern for serum copper (20), but have failed to show a similar pattern for serum zinc (13).

The changes observed in total serum copper concentration may be due to changes in serum ceruloplasmin. This is suggested by the similarities observed in their circadian patterns of variation. This question will not be definitively answered until free copper can be measured consistently in serum. Though there may well be specific zinc-carrying proteins in serum they are not well characterized presently and were not measured in this study.

The presence of a circadian rhythm in urinary copper excretion has been previously observed (2). The demonstration of a circadian pattern of variation in both total serum copper and copper bound to ceruloplasmin, and a circadian pattern of excretion of urinary copper suggests that there is also a circadian pattern of variation of "free" or nonceruloplasmin-bound copper. Since ceruloplasmin-bound copper is presumably too large to pass the glomerular membrane under normal conditions then the cyclic fluctuation observed in urinary copper excretion may be due primarily to the cyclic variations in free or nonceruloplasmin-bound copper.

The lack of a discernible circadian rhythm in urinary zinc excretion in this study is in contrast to a previous report of the existence of such a pattern for this ion (8) and previous demonstrations of circadian rhythms for excretion of most urinary constituents. Indeed, the lack of a circadian pattern of urinary excretion of zinc is surprising since both serum concentrations of copper and zinc and urinary copper and creatinine excretion (23) have nadirs during the early morning hours. At this time the low serum levels of copper and zinc and the low creatinine clearances should lead to a lower filtered level of these metals. The inability to demonstrate a circadian rhythm for this metal in these studies is therefore difficult to explain. Perhaps the amount of free (and thus filterable) zinc changes during the day or the processes by which tubular reabsorption of zinc take place change. In addition, dietary intake may affect one ion more than another and hence obscure the circadian variation; e.g., urinary creatinine has been said to be more affected by dietary intake than serum creatinine (1) and the same affect may influence zinc more than copper. Some investigators also report no significant relationships between circadian variations in serum concentration or urinary excretion of potassium, phosphate, creatinine or sodium (3).

What role ACTH played in these changes is not known since it is not clear whether or not administration of doses of prednisolone of this size suppresses ACTH secretion. Higher doses of prednisolone than those given here could affect many functions including any of those mentioned above.

The Zeitgeber for the circadian rhythm of serum copper and zinc has not been determined. These results suggest that diet and fluid intake as well as carbohydrate-active steroids are not significant factors. It may well be other factors such as the sleep-wake cycle, or the regulation of serum proteins such as evidenced by the circadian rhythm of serum ceruloplasmin which are important in this respect.

The authors gratefully acknowledge the technical assistance of Miss Silja Meret and also of the staff of the Clinical Chemistry Service, National Institutes of Health.

Received for publication 22 June 1970.

REFERENCES


7. Dow, D., and P. V. C. Pinto. Determination of serum albumin...


