Effect of Late Night Calcium Supplements on Overnight Urinary Calcium Excretion in Premenopausal and Postmenopausal Women

P. E. BELCHETZ, MARGARET H. LLOYD, R. G. S. JOHNS, R. D. COHEN

British Medical Journal, 1973, 2, 510–512

Summary

The overnight urinary calcium/creatinine ratio is higher in the early years after the menopause than before it. However, the increment of urinary calcium/creatinine after a late evening calcium supplement is less in early postmenopausal than in premenopausal women. It is suggested that calcium therapy in postmenopausal osteoporosis may be best administered as a single late evening dose rather than in divided doses throughout the day.

Introduction

It has recently been suggested that loss of bone tissue in postmenopausal osteoporosis takes place mainly during the night (Nordin, 1971). The argument was that absorption of calcium from a meal continues for only three to five hours and thus by midnight any effect of the evening meal in raising the plasma calcium will have ceased. Parathormone secretion would thus be relatively unsuppressed for the remainder of the night. This would be of comparatively little importance in premenopausal women, in whom the relatively high levels of oestrogen desensitize the bone to the action of parathormone. In postmenopausal women, however, the relative oestrogen insufficiency would sensitize the bone to this action, and dissolution would occur. In support of this view was the observation that the fasting urine formed between 6 a.m. and 8 a.m. contains more calcium per 100 ml glomerular filtrate in postmenopausal than in premenopausal women.

A corollary of this hypothesis is that there should be some advantage in the treatment of postmenopausal osteoporosis from giving calcium supplements in one large dose at night rather than in divided doses throughout the day (Cohen, 1971). By this means the nocturnal period of relatively unsuppressed parathyroid secretion would be shortened. If other factors (such as absorption and renal excretion) did not differ before and after the menopause, a late night supplement of calcium should cause less rise in urinary calcium in postmenopausal than in premenopausal women, since there would be a greater suppression of nocturnal bone dissolution in the former than in the latter group. The object of the present study was to test this expectation.

Medical Unit and Department of Metabolism and Endocrinology, The London Hospital, Whitechapel, London E1 1BB

P. E. BELCHETZ, M.R.C.P., Lecturer
MARGARET H. LLOYD, M.R.C.P., Lecturer
R. G. S. JOHNS, M.A., Chief Biochemist
R. D. COHEN, M.D., F.R.C.P., Reader in Medicine

Subjects and Methods

Two groups of subjects were studied. Group A consisted of 59 premenopausal and 36 postmenopausal women in good health, mainly members of the medical, nursing, and lay staff of The London Hospital. A subject was regarded as postmenopausal if, after the age of 45, menstruation had not occurred for the last six months. The study took place on two successive nights. Fifty of the premenopausal and 32 of the postmenopausal women completed the programme on both nights, and the numbers of these women in each of the second to seventh decades of life were 3, 28, 15, 6, 19, and 11 respectively, the oldest subject being aged 66 years and the youngest 17 years. All subjects over the age of 50 were postmenopausal, and two in the fifth decade were also postmenopausal. Only four of the 50 premenopausal subjects were taking oral contraceptive drugs, and as their results were not clearly different from the other subjects they were included. Subjects with known renal or cardiovascular disease, disorders of calcium metabolism, and those taking diuretics were excluded.

Inquiry was made about the consumption of late night milk drinks, and subjects were asked to keep to their usual dietary habits during the study. The proportion of late night milk drinkers was about the same in both groups (26% premenopausal and 33% postmenopausal). Each subject was provided with two plastic bottles for urine collection and asked to make overnight collections on two successive nights. They were instructed to pass urine immediately before retiring to bed and to discard it. All urine passed during the night and immediately on rising the next morning was collected. The procedure on the second night differed from the first only in that each subject took two Sandocal tablets (800 mg calcium as the lactate-gluconate) in about 150 ml water immediately before retiring. Subjects were asked to record the times of starting and finishing the urine collections. The volumes of the collections were measured and the urine was analysed for calcium and creatinine.

In order to establish whether renal calcium clearance (urinary calcium excretion/total plasma calcium) and creatinine excretion vary systematically with age, these were measured in a different group of 49 women (group B), including members of the hospital staff and patients admitted for minor procedures (carried out after the studies). Their ages ranged from 17 to 78 years; 19 of them being older than 50 years. After an overnight fast, urine collections were made between 9 a.m. and noon, and a plasma sample was obtained at 10.30 a.m.

The urine collections were acidified by the addition of hydrochloric acid and well shaken. Urine calcium was measured by atomic absorption spectrophotometry (Willis, 1960), plasma calcium by an edetic acid complexiметric method, and urine and plasma creatinine by AutoAnalyzer method No. N 11a (Technicon Corporation, Tarrytown, New York).

The significance of differences between means was assessed by Student's t test, except where significant skewness or kurtosis was present in either of the groups or where the variance in the two groups were significantly different (as judged by the F test). When any of these eventualities was present the Mann-Whitney U test was used instead. The significance of mean differences between night 1 and night 2 of subjects in the same groups was assessed using the paired t test.

The object of the study was fully explained to the participants, and their consent obtained.

Results

Since it was important to study the effect of late evening calcium supplements in normal women carrying on their normal life, it was impracticable to prescribe strict timings for the overnight collection of urine. Though an attempt was made in group A to obtain a record of the times of starting and finishing the urine collections this did not prove consistently successful. Because of the obvious likelihood of substantial variations in the duration of the collections, the overnight calcium excretion was expressed as the ratio of calcium to creatinine excretion (Ca/Cr).

The overnight Ca/Cr was significantly larger in the postmenopausal group but failed to rise as much after the late evening calcium supplements, so that on the second night Ca/Cr was not significantly different in the two groups (see table).

The volume of urine in the overnight collection was greater on the second than on the first night in both groups of women by amounts somewhat less than the fluid taken with the calcium supplement. The increment in urine volumes did not, however, differ significantly between the two groups. There was no significant difference in creatinine excretion between the two nights in either group. The mean creatinine excretion was slightly less in the postmenopausal than in the premenopausal group. Though, for the reasons given above, the increment in absolute calcium excretion is difficult to interpret, it was very significantly less in the postmenopausal compared with the premenopausal group.

Creatinine excretion in the timed collections in group B showed no regression with age. The mean creatinine excretion in the age group 17 to 49 years was 0·69 ± S.E. of mean 0·032 mg/min and in the range 50 to 66 years it was 0·71 ± 0·045 mg/min; these are not significantly different. Mean calcium excretion in the younger group was 0·076 ± 0·009 mg/min and in the older group 0·098 ± 0·017 mg/min. The difference between these means is not significant ($0·1 < P < 0·2$), but if the dividing age is taken as 40 rather than 50 years the older women have a highly significantly greater fasting urinary calcium excretion (means 0·056 ± 0·008 and 0·110 ± 0·012 mg/min respectively; $P < 0·001$). There was a significant regression of calcium excretion per minute (CaB) on age (A) in this group ($CaB = 0·0011$ (± S.E. 0·0005) $A + 0·0366$; $P < 0·05$), but none of creatinine excretion on age ($P > 0·5$).

The subjects in group B showed no significant regression of creatinine clearance (C_{Cr}) with age (though two of the lowest values were found in subjects aged 76 and 78). Both calcium clearance (C_{Ca}), calculated in ml/min from the calcium output and the total plasma calcium, and the ratio C_{Ca}/C_{Cr} showed wide scatter but increased with age (A) according to the following regression equations:

$$C_{Ca} = 0·011 \ (\pm \text{ S.E. } 0·005)\ A + 0·409$$
$$C_{Ca}/C_{Cr} = 0·00014 \ (\pm \text{ S.E. } 0·00005)\ A + 0·0042$$

Both slopes were significantly different from zero ($P < 0·05$ and $< 0·02$ respectively; t test).

Discussion

The use of the calcium/creatinine ratio in the group A subjects is based on the assumption that differences in creatinine excretion between the premenopausal and postmenopausal women are related to differences in collection timings rather than in age. This assumption is supported by failure to find an effect of age in creatinine excretion in the timed collections of group B over an age range corresponding to that of group A. Furthermore, Bulusu *et al.* (1970) showed virtually no decrease in creatinine excretion with age until after the seventh decade in normal women in contrast to the earlier decline seen in men. Thus the increased overnight Ca/Cr is likely to be related to an alteration in calcium metabolism with age rather than in creatinine excretion.

The results also show that overnight urinary calcium excretion, expressed as Ca/Cr, shows a smaller increase after a late evening calcium supplement in early postmenopausal compared with premenopausal women. This difference is unlikely to be due to less absorption of the calcium supplement in the postmenopausal women, since analysis of individual data taken from the figure in Bullamore *et al.* (1970) shows that only in more advanced old age is there a clear fall in calcium absorption. The mean hourly fractional calcium absorption in women between the ages of 20 and 49 was, however, not significantly different ($P > 0·5$) from that in women between the ages of 50 and 66. These age ranges are nearly identical to those of groups A and B in the present study though Bullamore *et al.* studied fewer subjects below the age of 40. However, the measurements of calcium absorption of Bullamore *et al.* were made using radioactive calcium in a small amount of carrier, in contrast to the present study where the calcium load was much greater. The possibility that absorption from a calcium load is less in early postmenopausal women than in premenopausal women cannot be entirely excluded.

Relatively impaired renal excretion of calcium in the elderly could also account for these results. In the separate group of 49 women (group B), however, calcium clearance and the ratio of calcium/creatinine clearance increased rather than fell with age. This makes it unlikely that impaired renal excretion is responsible for the findings. In order strictly to exclude this possibility, however, it would be necessary to have information on the variation with age of the rate of increase of urinary calcium per unit increase in plasma calcium at the prevailing level of plasma calcium. Such information is obviously difficult to obtain. However, if the shape of the curve of urinary calcium versus plasma calcium (Nordin and Peacock, 1969) does not in fact vary markedly with age, then an explanation in terms of poorer renal excretion of calcium in the postmenopausal women would not be compatible with the increase in calcium clearance

Calcium and Creatinine Excretion and Ratios (Ca/Cr) and Urine Volumes in Premenopausal and Postmenopausal Women, before and after Late Evening Calcium Supplements. Results expressed as Mean ± S.E. of Mean

	Premenopausal	Postmenopausal	P (Two-tailed)
Ca/Cr (night 1)	0·162 ± 0·010 (n = 59)	0·215 ± 0·019 (n = 36)	0·02 < P < 0·03*
Ca/Cr (night 2)	0·277 ± 0·015 (n = 50)	0·293 ± 0·026 (n = 32)	P > 0·4*
Increment of Ca/Cr (night 2-night 1)	0·117 ± 0·012 (n = 50)	0·077 ± 0·012 (n = 32)	0·02 < P < 0·05† ($t = 2·23$)
Urine calcium (mg) (night 1)	54·44 ± 3·33 (n = 59)	60·86 ± 6·51 (n = 36)	0·3 < P < 0·4*
Urine creatinine (mg) (night 1)	338·5 ± 12·00 (n = 50)	289·1 ± 13·75 (n = 32)	P < 0·01*
Increment of total urine calcium (mg)	43·3 ± 4·09 (n = 50)	26·50 ± 6·56 (n = 32)	P < 0·01*
Increment of urinary creatinine (mg)	22·36 ± 11·69 (n = 50)	32·16 ± 19·9 (n = 32)	P > 0·6†
Increment of volume (ml)	102·1 ± 17·57 (n = 50)	82·56 ± 41·7 (n = 32)	0·3 < P < 0·4

*P derived by Mann-Whitney U test.
†P derived by Student's t test.

with age and the slightly higher levels of plasma calcium often found after the menopause (Young and Nordin, 1967).

We therefore conclude that the failure of urinary Ca/Cr and calcium to rise after late evening calcium supplements as much in the postmenopausal as in the premenopausal women may well be due to a greater effect in the former group of suppression of nocturnal bone dissolution, as outlined above.

Not consistent with this hypothesis is the recent finding of Jubiz *et al.* (1972) that the normal rise of plasma parathormone which occurs in the early hours of the morning cannot be suppressed by an overnight intravenous infusion of calcium sufficient to raise the plasma calcium to about 11·0 mg/100 ml. This observation was confined to two young subjects and requires further confirmation, and its significance is in any case rendered uncertain by current difficulties in the understanding of the relation of parathormone immunoassay measurements to biological activity (Parsons and Potts, 1972). Thus, Reiss (1970) could detect little fall of serum immunoreactive parathormone after calcium infusion in patients with parathyroid adenomas, whereas Potts *et al.* (1971) using an antiserum to the biologically active N-terminal region of the parathormone molecule found a brisk fall in similar studies.

If the present arguments are valid it follows that calcium supplements in the treatment of osteoporosis should be given as a single large dose immediately before retiring rather than in divided doses throughout the day, when ample dietary calcium is being absorbed. Late evening calcium supplements would have advantages over more elaborate treatments that may be used for osteoporosis, both in terms of economics and liability to unwanted effects.

Requests for reprints should be addressed to: Dr. R. D. Cohen, Medical Unit, The London Hospital, Whitechapel, London E1 1BB.

References

Bullamore, J. R., Gallagher, J. C., Wilkinson, R., and Nordin, B. E. C. (1970). *Lancet*, **3**, 535.
Bulusu, L., Hodgkinson, A., Nordin, B. E. C., and Peacock, M. (1970). *Clinical Science*, **38**, 601.
Cohen, R. D. (1971). *Rheumatology and Physical Medicine*, **11**, 162.
Jubiz, W., Canterbury, J. M., Reiss, E., and Tyler, F. H. (1972). *Journal of Clinical Investigation*, **51**, 2040.
Nordin, B. E. C. (1971). *British Medical Journal*, **1**, 571.
Nordin, B. E. C., and Peacock, M. (1969). *Lancet*, **4**, 1280.
Parsons, J. A., and Potts, J. T. (1972). In *Clinics in Endocrinology and Metabolism*, ed. I. McIntyre, vol. 1, p. 62. London, Saunders.
Potts, J. T., jun., *et al.* (1971). *American Journal of Medicine*, **50**, 639.
Reiss, E. (1970). *Medical Clinics of North America*, **54**, 131.
Willis, J. B. (1960). *Spectrochimica Acta*, **16**, 259.
Young, M. M., and Nordin, B. E. C. (1967). *Lancet*, **2**, 118.

Metabolic Effects of Oestrogen Treatment in Patients with Carcinoma of Prostate: A Comparison of Stilboestrol and Conjugated Equine Oestrogens

M. SHAHMANESH, C. H. BOLTON, R. C. L. FENELEY, M. HARTOG

British Medical Journal, 1973, 2, 512–514

Summary

Serum cholesterol and triglyceride levels were estimated and oral glucose tolerance tests performed on 16 patients with carcinoma of the prostate before treatment and while receiving stilboestrol in doses of 1 mg, 7·5 mg, and 15 mg daily and conjugated equine oestrogens (Premarin) 15 mg daily. Serum triglyceride levels were greater than 170 mg/100 ml in nearly all the patients while receiving Premarin or stilboestrol 7·5 mg and 15 mg daily. In six out of 10 patients who were given stilboestrol 1 mg daily the serum triglycerides remained within the normal range. No significant effects on serum cholesterol levels or glucose tolerance tests were observed with the various oestrogen regimens. The results support previous suggestions that a daily dose of 1 mg of stilboestrol should be regularly used in the treatment of carcinoma of the prostate.

Introduction

The Veterans' Administration Urological Co-operative Research Group's (V.A.U.C.R.G.) report of an increased incidence of cardiovascular deaths in patients with carcinoma of the prostate treated with diethylstilboestrol 5 mg daily (V.A.U.C.R.G., 1967) has led to a search for alternative forms of oestrogen therapy. Kaplan (1968) suggested that conjugated equine oestrogens (Premarin) might prove more satisfactory than stilboestrol. More recently Byar (1972) reported the findings of the second V.A.U.C.R.G. that 1 mg diethylstilboestrol daily was equally effective against the cancer while apparently free from the cardiovascular complications of the larger dose.

Patients with carcinoma of the prostate are usually elderly and might be expected to show abnormalities of blood lipids and carbohydrate tolerance (Boyns *et al.*, 1969). Furthermore, synthetic oestrogens have been shown to raise plasma triglyceride levels (Hazzard *et al.*, 1969) and produce carbohydrate intolerance (Wynn and Doar, 1966). These abnormalities have been shown to be associated with an increased risk of atherosclerosis (Wahlberg and Thomasson, 1968). The present study was undertaken to compare the effects of different doses of stilboestrol and of Premarin on blood lipids and glucose tolerance in patients with carcinoma of the prostate.

Patients and Methods

Sixteen patients with histologically-proved carcinoma of the prostate were studied whose ages ranged from 49-83 (mean 71) years. All the patients had had the nature of the study explained to them and had agreed to take part. Oestrogen treatment was with stilboestrol in doses of 1, 7·5, or 15 mg daily or Premarin 15 mg daily for periods of 6-30 (mean

University Departments of Medicine and Surgery, Bristol Royal Infirmary, Bristol BS2 8HW
M. SHAHMANESH, M.R.C.P., Medical Registrar (Present appointment: Research Registrar)
C. H. BOLTON, B.SC., PH.D., Research Fellow
R. C. L. FENELEY, M.CHIR., F.R.C.S., Consultant Surgeon
M. HARTOG, D.M., F.R.C.P., Senior Lecturer