Intestinal cholecalciferol absorption in the elderly and in younger adults

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(Received 12 January 1978; accepted 16 April 1978)

Summary

1. A method for assessing cholecalciferol absorption in man is described.
2. The intestinal absorption of \(^{3}\text{H}\)cholecalciferol was studied in 20 female geriatric patients, most of whom were vitamin D-depleted.
3. The plasma \(^{3}\text{H}\)cholecalciferol response after oral ingestion was significantly lower than that of a group of younger female subjects.
4. The plasma response of labelled polar metabolites of cholecalciferol was also lower in the geriatric than in the younger group, suggesting that increased removal of label by conversion into more polar metabolites could not account for the reduced plasma \(^{3}\text{H}\)cholecalciferol response.
5. There was no evidence that alteration in gastrointestinal motility could account for the different rate of appearance of the labelled vitamin in the plasma in the two groups.
6. It is suggested that there is a defect in intestinal absorption of cholecalciferol in the elderly.

Key words: age, cholecalciferol, intestinal absorption.

Abbreviation: 25(OH)D\(_3\), 25-hydroxycholecalciferol.

Introduction

It is probable that dietary vitamin D requirements are greater in the elderly than in young adults (Dent, 1970; Lester, Skinner & Wills, 1977). Low plasma concentrations of 25-hydroxycholecalciferol (25(OH)D\(_3\)) may be found in the elderly despite oral doses of vitamin D normally thought to be adequate (Corless, Beer, Boucher, Gupta & Cohen, 1975) and plasma 25(OH)D\(_3\) concentrations in long-stay geriatric patients on 2000 i.u. of vitamin D daily are only marginally higher than in subjects on 500 i.u. of vitamin D daily (MacLennan & Hamilton, 1977). Taken together these findings suggest that factors other than simple dietary vitamin D deficiency or lack of exposure to ultraviolet light might contribute to the low plasma 25(OH)D\(_3\) values and associated osteomalacia, which are commonly found in elderly subjects (Anderson, Campbell, Dunn & Runciman, 1966). Additional factors which must therefore be considered in this age-group include impaired absorption of dietary vitamin D and an age-related reduction in hepatic 25-hydroxylation.

Relatively few studies of the intestinal absorption of cholecalciferol have been made in man. Most approaches to the problem have either had to make assumptions about the contribution of differing removal rates of the vitamin from the plasma, or involved ingestion of unphysiological amounts of cholecalciferol or 25(OH)D\(_3\), or have not taken into account biliary excretion of metabolites of vitamin D (e.g. Thompson, Lewis & Booth, 1966; Stamp, 1974). We have developed an alternative approach to measuring cholecalciferol absorption and have applied it to a group of elderly females, many of whom were vitamin D-deficient.

Subjects

Approval for these studies was obtained from the Ethical Sub-committees of the hospitals involved,
and informed consent obtained from both control subjects and patients. Thirteen healthy male and nine female members of staff (age 30–58 years) were studied. All had a dietary vitamin D intake of at least 100 i.u. (6.25 nmol; 1 nmol = 16 i.u. of cholecalciferol) daily except for one female from the Indian sub-continent whose intake was low. The elderly subjects consisted of 20 female patients ranging in age from 68 to 94 years who had been resident in a long-stay hospital for at least 6 months. Their diagnoses ranged over a wide variety of illnesses, of which Parkinson’s disease, chronic respiratory disease, osteoarthritis and other locomotor diseases were the commonest. No patient had a previous history of renal, hepatic, biliary, pancreatic or gastrointestinal disease (other than diverticulosis), nor was any patient taking vitamin D supplements or any drug likely to interfere with vitamin D metabolism. The average vitamin D content of the ward diet was estimated to be 2.5 nmol (40 i.u.) daily. Two male and three female patients (age 31–66 years) with mal-absorption syndromes (faecal fat 7–26 g/24 h) were also studied. Diagnoses were: jejunal diverticulosis; short-gut syndrome (two patients); coeliac disease; Crohn’s disease of the small intestine. Two of these had previously been hypocalcaemic and three had elevated plasma alkaline phosphatase activities at the time of study. One subject was receiving calciferol supplements and one had recently been exposed to intense sunshine.

Materials and methods

[1α,2α(n)-3H]Vitamin D₃, 12.3 Ci/mmol, in benzene/ethanol, was obtained from The Radiochemical Centre (Amersham, Bucks., U.K.). Silica gel (60–120 mesh) was supplied by B.D.H. Chemicals Ltd, Poole, Dorset, and Sephadex LH 20 was supplied by Pharmacia Fine Chemicals, Uppsala, Sweden. Elution solvents (diethyl ether, n-heptane, chloroform and methanol) were AnalaR reagents.

Preparation of radioactive vitamin D for use

The benzene vehicle of [3H]cholecalciferol was removed by evaporation under oxygen-free nitrogen and the residue was resuspended in absolute ethanol. An aliquot was subjected to benzene, pyrogen and sterility tests, and the remainder was stored under N₂ in heat-sealed glass ampoules at 4°C until use. The radiochemical purity of each batch of labelled vitamin was ascertained by Sephadex LH 20 column chromatography. With this system, which gives clear separation of cholecalciferol from 25(OH)D₃ and other more polar metabolites (Holick & De Luca, 1971), a single peak of radioactivity in the cholecalciferol position was eluted with diethyl ether/heptane (1:1, v/v) solvent. In every instance this peak accounted for at least 99% of the radioactivity applied to the column; after storage for 1 month the radiochemical purity was 97% and, after 3 months, 90%. In each study the labelled vitamin was used within 7 days of preparation.

Experimental procedures

Experiment 1 was a preliminary observation carried out to determine the protocol for the definitive study (experiment 2).

Experiment 1. The requirement for and timing of a lipid meal in association with the oral dose of [3H]cholecalciferol were studied as follows: 1–3.5 pCi of [3H]cholecalciferol in ethanol (containing approximately 15–52 i.u. of vitamin D) was administered orally with a meal of low lipid content (8 g) comprising 270 g of milk and a bowl of cornflakes, followed 3 h (subjects 1 and 2) and 8 h (subject 3) later by a lipid (50 g) meal (ham, butter, bread). Subjects 4 and 5 received 1 μCi of [3H]cholecalciferol orally with the 50 g lipid meal at zero hours.

Experiment 2. Barragry (1977) has shown that the use of a semi-solid 30 g lipid meal rather than a solid 50 g lipid meal permits the absorption of [3H]cholecalciferol to proceed at an enhanced rate. It was considered that this form of meal was appropriate for use in elderly subjects. On the basis of this finding and of the results of experiment 1 the following definitive protocol was adopted in the subjects described above. All subjects fasted for 12 h before the start of the experiment; after the administration of 6 pCi of [3H]cholecalciferol in 1 ml of ethanol, vigorously stirred in milk and consumed with a 30 g lipid meal (double cream and cornflakes), no further food was permitted for the duration of the test. The total amount of vitamin D thus administered was 95 i.u. Venous blood samples drawn through an indwelling cannula at hourly intervals for 6 h were collected in heparinized tubes. The total volume of blood removed was 70 ml.

Extraction of vitamin D metabolites and chromatography

Lipid extraction of plasma in chloroform/methanol was carried out by the method of Folch, Lees & Sloane-Stanley (1957). The chloroform phase was
evaporated to dryness, redissolved in 2.0 ml of diethyl ether/heptane (1:3, v/v) and applied to a silica-gel column.

A 10 cm × 1 cm column was packed to a height of 6 cm with silica gel. [3H]Cholecalciferol applied to this column could be eluted almost entirely (92%) with a diethyl ether/heptane solvent but could be eluted in 14 min by chloroform/methanol solvent at a flow rate of 5 ml/min. Thus although contamination of labelled cholecalciferol by 25(OH)D₃ could not occur with this chromatographic system, a minor amount of labelled cholecalciferol would fail to be eluted by diethyl ether-heptane solvent and its subsequent removal by chloroform/methanol solvent would cause a small but spurious increase in the polar radioactivity obtained. A correction for this 'carry-over' was effected by adding an aliquot of radiochemically pure labelled cholecalciferol to plasma drawn in the fasting state. This 'standard sample' was extracted and chromatographed in the same way as each of the samples drawn after the oral administration of labelled cholecalciferol; the radioactivity counts in these latter samples were corrected for 'carry-over' by employing the relative amounts of radioactivity of the radiochemically-pure cholecalciferol eluted with these solvent systems in the standard sample. The mean carry-over of labelled cholecalciferol into the chloroform/methanol-eluted phase in subsequent experiments was 8.2 ± SEM 1.4%. The mean recovery of [3H]cholecalciferol added to the plasma and subjected to the entire extraction procedure was 89.0 ± SEM 2.2% (n = 12). The diethyl ether eluate from plasma extracts after ingestion of [3H]cholecalciferol presumably could contain, to an unknown extent, esters of [3H]cholecalciferol as well as the free vitamin. The chloroform/methanol eluate, referred to hereafter as the 'polar fraction', contains mainly 25-hydroxycholecalciferol but presumably also other polar metabolites. These considerations are unlikely to affect appreciably the interpretation of the results.

Measurement of plasma radioactivity

The two fractions from the column were evaporated to dryness under N₂ at 80°C and the residue was transferred by washing with toluene/POPOP/PPO scintillation fluid into counting vials. Radioactivity counting (to 2000 counts above background) was performed on a Nuclear Enterprises NE 8312 Liquid Scintillation Spectrometer, the counting efficiency being 40%. A quench correction factor for each sample was derived by recounting after the addition of a known [3H]hexadecane internal standard. The plasma content of radioactivity was calculated, assuming plasma volume to be 5% of body weight, and expressed as a percentage of the dose of label administered. The conversion of [3H]cholecalciferol into its more-polar metabolites was calculated by expressing at each sampling time after 1 h the radioactivity in the chloroform/methanol eluate (the 'polar fraction') as a percentage of the total plasma radioactivity at that time (polar/total; P/T).

Other investigations

Plasma creatinine, calcium, phosphorus and alkaline phosphatase were estimated by using standard auto-analyser methods. Plasma 25(OH)D₃ assays were carried out by the competitive protein-binding method of Edelstein, Charman, Lawson & Kodicek (1974) with the rat-kidney binding protein of Haddad & Chyu (1971). The assay was performed without preparative chromatography (Belsey, De Luca & Potts, 1974), β-lipoprotein being used as solubilizing agent. Plasma triglyceride concentrations were measured both in the fasting state and hourly after the ingestion of the lipid meal in healthy and elderly subjects by the method of Eggstein & Kreutz (1966).

The statistical significance of differences between the mean values in the groups studied was evaluated with Student's t-test and, where variances were non-homogeneous or if there was gross non-normality, by the use of the Mann/Whitney U test (Siegel, 1956). Since all elderly subjects studied were female, statistical comparisons were made with the younger female subjects.

Results

Experiment 1: requirement for and timing of lipid meal in relation to dose of [3H]cholecalciferol

After the administration of [3H]cholecalciferol with a meal of low lipid content little radioactivity was detected in the plasma until a lipid-containing meal was ingested at 3 h (subjects 1 and 2) or 8 h (subject 3). When a 50 g lipid meal was administered at the outset (subjects 4 and 5) there was a gradual and sustained rise in plasma ³H radioactivity starting at 1 h (Fig. 1).
Experiment 1: effect of timing of lipid-containing meal on the appearance of $[^{3}H] $cholecalciferol in plasma after an oral dose. $[^{3}H] $Cholecalciferol was administered at zero hours; a lipid (50 g) meal was administered at 3 h in subjects 1 and 2, at 8 h in subject 3 and at zero hours in subjects 4 and 5.

Experiment 2: $[^{3}H] $cholecalciferol absorption in healthy and elderly subjects and in the malabsorption syndrome

Table 1 shows the mean plasma calcium, phosphorus, alkaline phosphatase and 25(OH)D$_3$ values in the healthy young adults, in elderly subjects and in the patients with malabsorption syndromes. Only five of the elderly subjects had plasma 25(OH)D$_3$ values within the normal range and eight had undetectable concentrations; the remainder had subnormal values. Plasma creatinine concentrations in the elderly group were within normal limits.

**Plasma $[^{3}H] $cholecalciferol response** (Fig. 2). After the oral administration of $[^{3}H] $cholecalciferol the plasma $[^{3}H] $cholecalciferol values in the healthy female subjects were slightly greater than in the male subjects with mean 6 h values of 13.2 ± SEM 1.55% and 10.8 ± 1.08% of the dose administered respectively. However, this difference was not significant ($P > 0.05$). The pooled 6 h mean was 12.2 ± 1.0%. The plasma $[^{3}H] $cholecalciferol response in the elderly subjects was consistently lower than in the younger female group, reaching a value at 6 h of 7.64 ± 0.87%. The differences between the mean values of the groups were significant at 3, 4, 5 and 6 h ($P < 0.01$). In the subjects with the malabsorption syndrome the plasma $[^{3}H] $cholecalciferol response (3.8 ± 0.3% at 6 h) was substantially lower than in the combined healthy group. The differences between the mean values of these two groups were significant at 3 h ($P < 0.05$), 4, 5 and 6 h ($P < 0.01$).

**Table 1. Biochemical data from subjects studied**

<table>
<thead>
<tr>
<th>Group</th>
<th>Plasma Calcium (mmol/l)</th>
<th>Plasma Phosphorus (mmol/l)</th>
<th>Plasma Alkaline phosphatase (i.u./l)</th>
<th>Plasma 25(OH)D$_3$ (nmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy subjects</td>
<td>2.39 ± 0.03</td>
<td>0.97 ± 0.05</td>
<td>61.7 ± 2.9</td>
<td>102 ± 16.7†</td>
</tr>
<tr>
<td>Elderly subjects</td>
<td>2.33 ± 0.03</td>
<td>1.04 ± 0.04</td>
<td>89.3 ± 3.8*</td>
<td>10.88 ± 2.95*</td>
</tr>
<tr>
<td>Malabsorption syndrome</td>
<td>2.26 ± 0.08</td>
<td>0.91 ± 0.03</td>
<td>89.0 ± 4.0*</td>
<td>48.35 ± 22.5</td>
</tr>
</tbody>
</table>

* $P < 0.01$ compared with healthy subject values.
† Lower limit of normal range = 20 nmol/l.
Cholecalciferol absorption in the elderly

Plasma 3H-labelled polar metabolite response (Fig. 3). In the group of healthy young subjects the 3H-labelled polar metabolite response (reflecting mainly [3H]25(OH)D$_3$) in female members was slightly greater than in male members, with mean 6 h values of 21.7 ± 3.4% and 10.0 ± 1.9% respectively. However, only the 6 h values differed significantly (P < 0.05). In the elderly subjects the response was lower throughout than in the younger female group, and the 6 h value was 7.8 ± 0.9%. The differences between the mean values of the groups were significant at 3, 4, 5 and 6 h (P < 0.01). The response in subjects with the malabsorption syndrome (6.2 ± 1.1% at 6 h) was lower than in the combined group of healthy subjects and the difference between the mean values of these two groups was significant at 5 and 6 h (P < 0.05).

Total plasma 3H radioactivity. The total plasma 3H radioactivity, i.e. the sum of plasma [3H]cholecalciferol and 3H-labelled polar metabolites, reflected the above patterns, with significantly greater values in females than males at 5 h (28.2 ± 3.2% and 17.8 ± 2.5% respectively) (P < 0.05) and at 6 h (34.9 ± 3.8% and 20.8 ± 2.3% respectively) (P < 0.05). The response in the elderly subjects was consistently lower than in the younger healthy group of females, reaching 6 h values of 15.6 ± 1.5% and 34.9 ± 3.8% respectively. The differences between the mean values were significant at 3, 4, 5 and 6 h (P < 0.01). In subjects with malabsorption the total plasma 3H radioactivity was lower than that of the healthy group, reaching a mean value of 10.1 ± 0.85% at 6 h. The differences between the mean values in each group were significant (P < 0.01) at 3, 4, 5 and 6 h.

Polar/total plasma 3H radioactivity. The polar/total (P/T) values in female members of the healthy group were not significantly greater than those of the male members. The P/T values in the elderly subjects were somewhat lower than those of the younger group, although the differences were not significant (P > 0.05) (Fig. 4). The P/T values in the malabsorption group were greater than those of the healthy group although the differences were not significant (P > 0.05).

No correlation was observed between the plasma 25(OH)D$_3$ concentrations and plasma [3H]cholecalciferol response, 3H-labelled polar metabolite response or P/T values.

Plasma triglyceride response (Fig. 5). The mean fasting plasma triglyceride concentrations in the control group (0.93 ± 0.11 mmol/l) and in the geriatric group (1.23 ± 0.19 mmol/l) were similar (P > 0.05). To determine the change in plasma triglyceride concentration after the lipid meal the fasting value was subtracted from each hourly value. The peak response in plasma triglyceride concentration occurred at 3 h in both groups, at which time the mean increment in plasma triglyceride was 0.33 ± 0.07 mmol/l in the younger group and 0.34 ± 0.07 mmol/l in the geriatric group.
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mainly with chylomicrons. Barragry (1977) has shown that the relative contributions of $[^3]$H]cholecalciferol and $[^3]$H-labelled polar metabolites to total plasma $[^3]$H radioactivity are altered by the simultaneous administration of large doses of unlabelled cholecalciferol (300–1500 i.u.), so that rather more radioactivity is present as $[^3]$H]cholecalciferol and less as $[^3]$H-labelled polar metabolites than when a 1 $\mu$Ci dose of $[^3]$H]cholecalciferol (containing 15 i.u.) is given alone. These findings demonstrate the importance of the type and timing of the meal administered and of its content of vitamin D when assessing vitamin D absorption under physiological conditions. The interpretation of absorption tests in which large quantities of vitamin D or its metabolites are administered without fat (Thompson et al., 1966; Stamp, 1974) is therefore uncertain.

Theoretically the most satisfactory method of allowing for simultaneous removal from the plasma of an absorbed substance is to observe the disappearance of a dose of the substance injected intravenously at the same time as the oral dose, and to deconvolute the apparent absorption curve by using the intravenous curve. Because of the complexity and variability of the disappearance of intravenously injected cholecalciferol (Avioli, Lee, McDonald, Lund & De Luca, 1967; Barragry, 1977) this is not applicable for cholecalciferol. An alternative means of defining the true absorption of cholecalciferol is to measure the apparent absorption of orally administered labelled cholecalciferol and the subsequent appearance of labelled polar metabolites in the plasma. A correction for removal of absorbed label by hydroxylation to these polar metabolites may be made by expressing the amount of polar metabolite-associated radioactivity as a percentage of the total plasma radioactivity. It is recognized that the use of this procedure is not necessarily equivalent to correcting for overall removal of absorbed label. Possible alternative routes of removal of $[^3]$H]cholecalciferol are the conversion in the liver into other metabolites, which are then secreted into the circulation or into bile, and the uptake of label into tissue stores. The rate of disappearance of $[^3]$H]cholecalciferol from the plasma might also depend on the pool size of circulating unlabelled cholecalciferol if the removal processes are zero order rather than first order.

It is evident that this technique of assessing cholecalciferol absorption is capable of discriminating between subjects with normal intestinal absorption and those with malabsorption. Studies on elderly subjects revealed a substantially reduced plasma $[^3]$H]cholecalciferol response compared with
younger subjects. Possible interpretations of this finding include a prolongation of intestinal transit time in the elderly, an enhanced rate of conversion of the label into its more polar tritiated metabolites in the vitamin D-deficient subject or an impairment of intestinal absorption of vitamin D.

By measuring the hourly increase in plasma triglyceride after the ingestion of the lipid meal and by comparing the timing of the peak triglyceride response in both the control and geriatric groups it was expected that any substantial slowing of gastric emptying or of transit to the site of intestinal absorption would manifest itself as a later peak. In fact the plasma triglyceride response was similar in both groups, reaching a peak value at 3 h, suggesting that differences in gastric emptying and transit time were not responsible for the present findings.

An enhanced rate of removal of absorbed \(^{3}H\)cholecalciferol by conversion into more active polar metabolites, especially in vitamin D-depleted subjects (Mawer, Lumb, Schaefer & Stanbury, 1971), might provide an explanation for the reduced concentrations of \(^{3}H\)cholecalciferol noted in the elderly subjects. Such enhanced conversion could be due to stimulation of 25-hydroxylation in the vitamin D-depleted elderly subjects or to a low circulating pool of stable cholecalciferol in these subjects. Though cholecalciferol concentrations have not been measured we have assumed in the following arguments that they were directly related to the measured plasma 25(OH)D\(_3\) concentration (Krawitt, Grundman & Mawer, 1977) and therefore were relatively low in the elderly subjects, the majority of whom were vitamin D-deficient (Corless et al., 1975; Preece, Tomlinson, Ribot, Pietrek, Korn, Davies, Ford, Dunnigan & O’Riordan, 1975). If either or both of these reasons for enhanced conversion obtain, a comparable level of total \(^{3}H\) plasma radioactivity and increased concentrations of \(^{3}H\)-labelled polar metabolites and P/T values would be expected in the geriatric group. In fact a different pattern is seen, with lower overall values of \(^{3}H\) radioactivity in the plasma, lower concentrations of \(^{3}H\)-labelled polar metabolites and mean P/T values indistinguishable from normal. Enhanced conversion of labelled cholecalciferol into its more polar labelled metabolites is therefore unlikely to account for the reduced \(^{3}H\)cholecalciferol response seen in the elderly.

The absorption test procedure described does not detect removal of absorbed labelled cholecalciferol by means other than conversion into more polar metabolites. Specifically, no measurement is made of the uptake of label into adipose stores and muscle. It is thus conceivable that enhanced removal of absorbed label by means other than conversion into more polar metabolites could account for the reduced plasma \(^{3}H\)cholecalciferol response observed in the overall relatively vitamin D-depleted geriatric subjects. If this were the case the peripheral removal of label would be lower among those five geriatric subjects whose vitamin D status as assessed by plasma 25(OH)D\(_3\) concentration was normal and this would be reflected in a relatively greater plasma \(^{3}H\)cholecalciferol response than in the vitamin D-depleted elderly subjects. However, no such difference was noted. Moreover, in the vitamin D-depleted subject it is likely that conversion of cholecalciferol into 25(OH)D\(_3\) constitutes the major metabolic path for the disposal of cholecalciferol (Mawer et al., 1971). In contrast, in vitamin D-replete subjects the initial disappearance of administered cholecalciferol has been shown to be accounted for not by metabolic transformation but by distribution to the tissues (Mawer, Backhouse, Holman, Lumb & Stanbury, 1972). Because of these considerations the P/T value is likely to be a closer representation of total removal of ingested cholecalciferol in vitamin D-deficient subjects than in vitamin D-replete ones. Hence the observed ‘normal’ P/T values in the vitamin D-deficient geriatric subjects are inappropriately low when compared with the vitamin D-replete group of younger subjects whose P/T values represent only one facet of cholecalciferol removal from the plasma. It is possible that an age-related impairment of hepatic 25-hydroxylation of cholecalciferol might account for the low ratio of polar metabolites in these elderly subjects despite a reduced body pool of the vitamin.

The studies of disappearance of polar metabolites (presumably mainly \(^{3}H\)25(OH)D\(_3\)) showed that the half-time was not significantly different in the younger and geriatric subjects. This contrasts with the faster disappearance of labelled 25(OH)D\(_3\) seen in younger depleted subjects (Mawer et al., 1971; Krawitt et al., 1977). However, the lower intercept on the ordinate in the geriatric subjects suggests that less labelled 25(OH)D\(_3\) was formed in these subjects. Since 25-hydroxylation is the principal route of disposal of \(^{3}H\)cholecalciferol in vitamin D-depleted subjects, this lower intercept may be interpreted as further evidence of either impaired absorption or impaired 25-hydroxylation (or both) in the elderly.

Thus neither a reduction in intestinal motility nor an increase in removal of absorbed \(^{3}H\)cholecalciferol can readily account for the reduced plasma
[3H]cholecalciferol concentrations seen in the geriatric group. We suggest that elderly subjects malabsorb cholecalciferol, thus compounding a vitamin D-deficient state induced by insufficient exposure to ultraviolet light and an inadequate diet.

Acknowledgments

This work was supported by a grant from the Department of Health and Social Security. We express our thanks to the nursing staff of wards A3, 4, 5 and 6 of the Eastern Hospital and to the Dietetic Staff of the London Hospital for their interest and help in this study.

References


