Reduced plasma half-life of radio-labelled 25-hydroxyvitamin D_3 in subjects receiving a high-fibre diet

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1. The plasma disappearance of ^{3}H-labelled 25-hydroxyvitamin D_3 (25(OH)D_3) was studied in healthy volunteers on normal and high-fibre diets, using ^{3}H-labelled tracer doses given intravenously.

2. The mean (±SEM) plasma half-life in the high-fibre-diet group was 19·2±1·7 d, which was significantly shorter than in the group on normal diets (27·5±2·1 d, $P < 0.02$).

3. This finding suggests that a high-fibre diet leads to enhanced elimination of 25(OH)D_3 by an action within the intestinal lumen. This may involve interference with an enterohepatic circulation of the metabolite, perhaps by binding of 25(OH)D_3 to dietary fibre.

4. The reduced plasma half-life of ^{3}H-labelled 25(OH)D_3 associated with a high-fibre diet may explain the development of vitamin D deficiency in Asian immigrants with normal exposure to u.v. light.

The aetiology of rickets and osteomalacia among Asian immigrants to Britain has been the subject of debate and investigation over the past two decades. Several studies have failed to demonstrate any correlation between these diseases and dietary vitamin D intake or sunlight exposure (Dunnigan & Smith, 1965; Dunnigan *et al.* 1975), but a relationship with the consumption of high-extraction chappatti flour has been reported (Ford *et al.* 1976; Hunt *et al.* 1976; Robertson *et al.* 1977). Furthermore, Ford *et al.* (1972) demonstrated biochemical improvement in ten patients with rickets or osteomalacia following the substitution of white leavened bread for chappattis in the diet. It has also been suggested that components of dietary fibre could form complexes with bile salts and vitamin D in the gut (Reinhold, 1976) thus leading to increased faecal vitamin D excretion. In the presence of an enterohepatic circulation of vitamin D metabolites, such as 25-hydroxyvitamin D_3 (25(OH)D_3), (Arnaud *et al.* 1975) a similar mechanism could lead to loss of endogenous as well as exogenous vitamin D.

We have examined the possibility that a high-fibre diet may increase loss of endogenous 25(OH)D_3 by measuring the plasma disappearance of radio-labelled 25(OH)D_3 in healthy volunteers, taking either a normal or a high-fibre diet.

METHODS

Thirteen healthy Caucasian subjects were studied, twelve male and one female, with a mean age of 32·5 years (range 28–44 years). The project received the approval of the hospital ethical committee and all subjects gave their informed consent.

The subjects were allocated to either normal (*n* 6) or high-fibre (*n* 7) diets. The high-fibre group took 20 g fine bran (Allinson's Bran Plus) three times daily (equivalent to approximately 20 g dietary fibre/d) in addition to their normal diet, while the only restriction placed on the other group was that they should avoid high-extraction breakfast cereals, wholemeal bread and bran. The diets were started 24 h before the injection of radio-labelled 25(OH)D_3 and continued for 30 d.

^{3}H-labelled 25(OH)D_3 (Amersham International, Amersham, Bucks) in the 23,24(n)

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position (specific activity 120 Ci/mmol) or in the 26,27-methyl position (specific activity 9·6 Ci/mmol) was prepared for injection in aqueous ethanol (150 ml/l water). Each subject received between 2 and 5 μCi ^{3}H-labelled 25(OH)D_3 given intravenously over a period of 5 min, four subjects in each group receiving the high specific activity isotope and the remainder the low specific activity compound. Venous blood samples (5 ml) were collected into heparinized containers 10 min after completion of the injection, hourly for the following 4–8 h and thereafter at intervals of 1–3 d for up to 30 d. Portions (1 ml) of plasma were added to 10 ml of micellar scintillation fluid (Nuclear Enterprises 260) and the radioactivity of the samples measured in an LKB Wallac 1215 Rakbeta liquid-scintillation counter.

Plasma taken at weekly intervals from all subjects was extracted with chloroform/methanol (2:1 v/v) and chromatographed on silicic acid columns to assess the proportion of plasma radioactivity eluting as ^{3}H-labelled 25(OH)D_3. Plasma 25(OH)D levels at the start of the experiment were measured by a competitive protein-binding assay using normal human serum as binding protein (Edelstein *et al.* 1974).

By plotting log plasma concentration of radioactivity *v.* period after administration for each subject, two major exponential functions were demonstrated. An initial relatively rapid distribution phase (slope α) was followed by a slow elimination phase (slope β), with a zone of overlap lasting from approximately 4 h to 4 d. Least squares regression analysis of the values from the 4th day onwards was used to define the gradient of the β slope, from which the elimination half-time ($T_{\frac{1}{2}\beta}$) could be simply derived ($T_{\frac{1}{2}\beta}$ = ln 2/gradient). The apparent total volume of distribution of the injected isotope was calculated by dividing the dose given by the plasma concentration at time zero obtained from extrapolation of the β slope back to its intercept with the vertical axis.

Differences between the plasma half-lives of ^{3}H-labelled 25(OH)D_3 in the two groups were assessed by means of Student's *t* test.

RESULTS

The plasma half-life of ^{3}H-labelled 25(OH)D_3 during the initial distribution phase ($T_{\frac{1}{2}\alpha}$) ranged between 4·3 and 7·8 h, with a mean ($\pm$SEM) of 5·9$\pm$0·5 in the high-fibre group, and 5·5$\pm$0·5 in the group on a normal diet. The mean elimination half-life ($T_{\frac{1}{2}\beta}$) of the subjects on a normal diet was 27·5$\pm$2·1 d and this was significantly longer than the value for the high-fibre group, 19·5$\pm$1·7 d ($P < 0·02$) (Fig. 1). There was no significant difference between the mean values for total volume of distribution of the injected isotope in the high-fibre group (8·7$\pm$1·2 l) and in the group on a normal diet (8·4$\pm$1·7 l).

Extraction and chromatography of plasma samples after administration of either isotope demonstrated that at 7 d over 90% and at 28 d 80% of the radioactivity eluted as ^{3}H-labelled 25(OH)D_3.

The plasma 25(OH)D level in all subjects was normal, ranging from 34 to 142 nmol/l. The mean value ($\pm$SEM) in the normal diet group (65$\pm$8·1) was not significantly different from that in the high-fibre group (80$\pm$17·0). There was no significant correlation between the plasma 25(OH)D concentration and plasma half-life of ^{3}H-labelled 25(OH)D_3 for each subject in the high-fibre or normal group (*r* 0·04 and 0·08 respectively).

DISCUSSION

Our results indicate that a high-fibre diet is associated with more rapid elimination of 25(OH)D_3 resulting in a reduction of its mean plasma half-life from 27·5 to 19·2 d. Bolus administration of even tracer doses of ^{3}H-labelled 25(OH)D_3 is non-physiological but, considering the plasma disappearance only, after allowing 4 d for distribution equilibrium to be attained probably provides values representative of the endogenous metabolite.

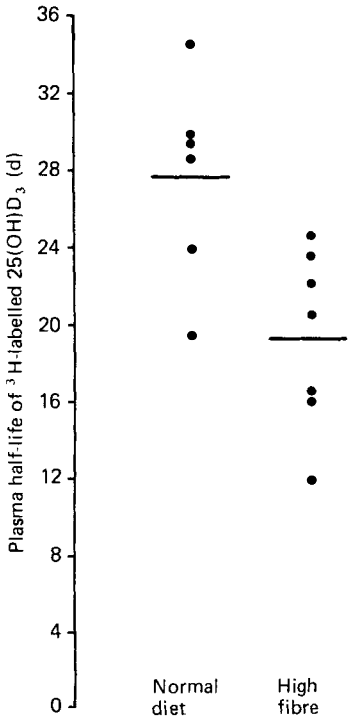

Fig. 1. Plasma half-life of ^{3}H-labelled 25-hydroxyvitamin D_3 (25(OH)D_3) in subjects on a normal and high-fibre diet. (——), Mean values for each of the two groups; the values were significantly different ($P < 0{\cdot}02$).

Moreover, all the subjects studied were vitamin D replete and plasma 25(OH)D levels were similar in the two groups, excluding possible effects of differences in vitamin D status on the plasma disappearance. Furthermore, our results cannot be explained on the basis of differing volumes of distribution in the two groups for the metabolite, as we were unable to demonstrate any significant differences in this respect.

25(OH)D_3 is the major circulating form of vitamin D in the body and appears to act as a pool for the production of further metabolites. Studies of the disappearance of radio-labelled 25(OH)D_3 given intravenously (Bec *et al.* 1972; Gray *et al.* 1974) have previously suggested a long plasma half-life for this compound (13·3–23 d), as have studies following the plasma disappearance after labelled (Mawer *et al.* 1971; Smith & Goodman, 1971) or unlabelled (Haddad & Rojanasathit, 1976) vitamin D_3 administration (19·6–43·6 d). The large variation is likely to be at least partly due to the differences in vitamin D status of the subjects studied (Mawer *et al.* 1971) and the number of normal subjects investigated has been small.

Biliary excretion of vitamin D and its metabolites is well documented (Avioli *et al.* 1967; Bell & Kodicek, 1969; Mawer *et al.* 1972), although quantitative estimates of 25(OH)D_3 excretion in man vary widely (Arnaud *et al.* 1975; Gray *et al.* 1974). The presence of an enterohepatic circulation has been postulated (Arnaud *et al.* 1975) but not conclusively demonstrated; others have suggested that biliary excretion serves primarily to eliminate the metabolite from the body (Mawer, 1979). If a conservative enterohepatic circulation does exist, its interruption would lead to increased faecal loss of both exogenous and endogenous 25(OH)D_3. The demonstration of a reduced plasma half-life of 25(OH)D_3 in subjects

receiving a high-fibre diet provides some indirect evidence for a conservative enterohepatic circulation, although increased faecal losses of $25(OH)D_3$ would have to be demonstrated to prove that loss from the intestine was responsible for the reduction. Since dietary fibre is not absorbed in the small intestine, it seems likely that its effect on the elimination of $25(OH)D_3$ from the body results from an effect within the intestinal lumen; for example, binding of $25(OH)D_3$ thus preventing its reabsorption. However, the effects of dietary fibre are complex and other mechanisms to explain our results cannot be excluded, such as changes mediated via an influence of fibre on the circulating bile acid pool. Another possible explanation for our findings is that changes in calcium balance induced by the high-fibre diet indirectly affected vitamin D metabolism; this explanation would not require the presence of a conservative enterohepatic circulation of $25(OH)D_3$.

In our study the subjects in the high-fibre group consumed approximately 20 g fibre/d in addition to that provided by their normal diets. The fibre content of the Asian diet varies widely, but in one study rachitic Asian children were consuming over 200 g chappatti flour/d (Ford *et al.* 1976), which would provide approximately 20 g cereal fibre in addition to that derived from other sources. Previous theories to explain the possible link between high-extraction flour consumption and Asian rickets and osteomalacia have included incrimination of the high phosphate content (Ford *et al.* 1976), and the high levels of phytate leading to interference with calcium absorption (Wills *et al.* 1972). The possibility that dietary fibre could be involved by interrupting the enterohepatic circulation of vitamin D metabolites has received little attention. Such a process would increase the requirements for vitamin D, the major source of which is from endogenous synthesis in the skin (Haddad & Hahn, 1973). Thus rickets and osteomalacia among Asians in Britain may be the product of a high-fibre diet, together with a traditional way of dress and social customs which limit sunlight exposure, in a latitude where the large amounts of u.v. radiation found in their native lands are no longer available.

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