SHORT COMMUNICATION

Metabolism in vitro of 25-hydroxycholecalciferol in chicks fed on phosphorus-deficient diets

R. SWAMINATHAN*, BARBARA A. SOMMERVILLE AND A. D. CARE

Department of Animal Physiology and Nutrition, University of Leeds, Leeds, U.K.

(Received 17 June 1977; accepted 24 October 1977)

Summary

1. Three groups of 10-days-old chicks were fed on one of three diets having phosphorus contents of 0·08 mol/kg, 0·14 mol/kg or 0·21 mol/kg. Ten days later duodenal calcium absorption by the ligated loop technique in vivo, and plasma calcium and phosphorus concentrations, were measured. In addition the metabolism in vitro of 25-hydroxycholecalciferol [25-(OH)D₃] by kidney homogenates was studied.

2. In the low phosphorus group (0·08 mol/kg) calcium absorption and the activity of 25-(OH)D₃-1-hydroxylase were significantly higher than those of the high phosphorus group (0·21 mol/kg). However, in the medium phosphorus group (0·14 mol/kg), calcium absorption was significantly higher although the activity of 25-(OH)D₃-1-hydroxylase was not significantly higher when compared with the high phosphorus group (0·21 mol/kg).

3. It is concluded that in phosphorus deprivation, unlike in calcium deprivation, a diet very low in phosphorus is required to stimulate the renal 25-(OH)D₃-1-hydroxylase activity.

Key words: 1,25-dihydroxycholecalciferol, phosphorus deficiency.

Abbreviations: 25-(OH)D₃, 25-hydroxycholecalciferol; 1,25-(OH)₂D₃, 1,25-dihydroxycholecalciferol.

Introduction

Vitamin D₃ is metabolized to the active metabolite 1,25-dihydroxycholecalciferol [1,25-(OH)₂D₃] in the kidney after preliminary hydroxylation in the liver (Lawson, Fraser, Kodicek, Morris & Williams, 1971). The formation of this metabolite is influenced by the vitamin D and calcium status of the animal (Henry, Midgett & Norman, 1974; Swaminathan, Sommerville & Care, 1977b). Stimulation of the renal 25-hydroxycholecalciferol-1-hydroxylase [25-(OH)D₃-1-hydroxylase] has been shown to be the mechanism by which animals increase their efficiency of intestinal calcium absorption in response to calcium deprivation (Henry et al., 1974; Hughes, Brumbaugh, Haussler, Wergedal & Baylink, 1975; Swaminathan et al. 1977b). Animals fed on a diet low in phosphorus increase their efficiency of intestinal calcium absorption (Carlsson, 1953; Morrissey & Wasserman, 1971; Fox, Swaminathan, Murray & Care, 1977), possibly by an increase in the production of 1,25-(OH)₂D₃ (Tanaka & DeLuca, 1973). However, Henry et al. (1974) failed to find an increase in the activity of 25-(OH)D₃-1-hydroxylase in chicks fed on a phosphorus-deficient diet. Furthermore, the increase in calcium absorption in response to phosphorus deprivation was not abolished when the 1-hydroxylation step was by-passed by feeding dihydrotachysterol (Bar & Wasserman, 1973), 1α-hydroxycholecalciferol (Bar, Hurwitz & Edelstein, 1975) or 1,25-(OH)₂D₃ (Ribovich & DeLuca, 1975). We therefore examined the metabolism in vitro of 25-hydroxycholecalciferol [25-(OH)D₃] by kidney homogenates in vitamin D-replete chicks fed on different phosphorus diets.
Materials and methods

Ten-days-old male 909 chicks (Thornber, Hebden Bridge, Yorkshire, U.K.) were given one of three diets (phosphorus content 0.08 mol/kg, 0.14 mol/kg or 0.21 mol/kg; calcium content 0.25 mol/kg; vitamin D$_3$ content 65 nmol/kg). The composition of the diet was similar to that described earlier (Swaminathan et al., 1977b). When the chicks were 20 days old, they were starved for 4 h and duodenal calcium absorption was measured by the ligated loop in vivo technique as described previously (Swaminathan & Care, 1975). A solution containing tracer calcium, $^{47}$Ca, was injected into a loop of the duodenum and after 30 min the animal was killed. A blood sample was taken by cardiac puncture before death for plasma calcium and phosphate determinations. Immediately after death the kidneys were removed and a 10% kidney homogenate was prepared in Tris/acetate buffer (15 mmol/l, pH 7.4) containing MgCl$_2$ (1.9 mmol/l), sodium succinate (5 mmol/l) and sucrose (200 mmol/l). A portion (1.5 ml) of this homogenate was incubated at 39°C with 160 pmol of 25-[26,27-3H](OH)D$_3$ (16 Ci/mol; The Radiochemical Centre, Amersham, Bucks., U.K.). Lipid extracts of this incubation mixture were prepared and chromatographed on Sephadex LH20 (Pharmacia Ltd, Uppsala, Sweden) (glass column 0.55 m x 0.14 m, flow rate 0.7 ml/min) with chloroform/hexane (65:35, v/v) as the eluent as described previously (Swaminathan et al., 1977). The results were expressed as pmol of 1,25-(OH)$_2$D$_3$ formed 15 min$^{-1}$ g$^{-1}$ of tissue. Plasma calcium concentration was measured by the method of Gitelman (1967) and plasma phosphate by an automated method (Technicon Method N-4B).

Results

Duodenal calcium absorption was highest in chicks fed on the low phosphorus diet (0.08 mol/kg) and lowest in the group fed on the 0.21 mol/kg diet (Table 1). Calcium absorption in the low (0.08 mol/kg) and medium (0.14 mol/kg) phosphorus groups was significantly different from that of the high phosphorus (0.21 mol/kg) group (P < 0.01). Plasma calcium concentration was highest and plasma phosphate concentration was lowest in the low phosphorus group (0.08 mol/kg). The increase in plasma calcium concentration was previously shown to be accompanied by an increase in ionized calcium concentration (Swaminathan, Care & Wasserman, 1977a). There was no significant difference in the plasma calcium and phosphate concentrations between the medium phosphorus (0.14 mol/kg) group and the high phosphorus (0.21 mol/kg) group (Table 1). In chicks fed on a low phosphorus diet (0.08 mol/kg) there was a significantly greater proportion of 25-(OH)D$_3$ converted into 1,25-(OH)$_2$D$_3$ by the kidneys (P < 0.01). However, there was no significant difference in the production in vitro of 1,25-(OH)$_2$D$_3$ between medium (0.14 mol/kg) and high (0.21 mol/kg) phosphorus groups.

Discussion

The increase in calcium absorption in response to phosphorus-deficient diets agrees with the observations reported earlier (Carlsson, 1953; Morrissey & Wasserman, 1971; Swaminathan et al., 1977a). We have previously shown that in vitamin D-replete chicks, given calcium-deficient diets, the absorption of calcium is directly related to the renal

### Table 1. Metabolism in vitro of 25-[26,27-3H]hydroxycholecalciferol by kidney homogenates and duodenal calcium absorption in chicks fed on three different phosphorus diets

<table>
<thead>
<tr>
<th>Dietary phosphorus (mol/kg)</th>
<th>Duodenal calcium absorption (%)</th>
<th>Concentration of plasma calcium (mmol/l)</th>
<th>Concentration of plasma phosphate (mmol/l)</th>
<th>Metabolism in vitro of 25-(OH)$_2$D$_3$†</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.08 (5)</td>
<td>90.7 ± 3.6**</td>
<td>3.90 ± 0.12*</td>
<td>1.08 ± 0.17*</td>
<td>25.6 ± 0.82*</td>
</tr>
<tr>
<td>0.14 (7)</td>
<td>56.4 ± 5.0*</td>
<td>2.78 ± 0.08</td>
<td>2.45 ± 0.20</td>
<td>15.4 ± 0.73</td>
</tr>
<tr>
<td>0.21 (7)</td>
<td>31.9 ± 2.4</td>
<td>2.85 ± 0.08</td>
<td>2.77 ± 0.16</td>
<td>11.7 ± 1.31</td>
</tr>
</tbody>
</table>

† Kidneys from three chicks in the low (0.08 mol/kg), four chicks in the medium (0.14 mol/kg) and three chicks in the high (0.21 mol/kg) phosphorus groups were used to study the metabolism in vitro of 25-hydroxycholecalciferol [25-(OH)$_2$D$_3$]. Kidney homogenates were incubated with labelled 25-(OH)$_2$D$_3$. The lipids were extracted and chromatographed to determine the amount of 1,25-dihydroxycholecalciferol [11,25-(OH)$_2$D$_3$] formed. Results are expressed as pmol of 1,25-(OH)$_2$D$_3$ formed 15 min$^{-1}$ g$^{-1}$ of tissue.
Metabolism of 25-(OH)D₃ in phosphorus deficiency

25-(OH)D₃-1-hydroxylase activity (Swaminathan et al. 1977b). However, in phosphorus deficiency, although calcium absorption decreased with increasing dietary phosphorus, the activity of 25-(OH)D₃-1-hydroxylase was high only in the low phosphorus (0·08 mol/kg) group. In rats fed on a low phosphorus diet (0·03 mol/kg) more of the administered 25-(OH)D₃ was shown to be converted into 1,25-(OH)₂D₃ than in rats fed on a high phosphorus diet (Tanaka, Frank & DeLuca, 1973). Hughes et al. (1975) used a radioreceptor assay to measure circulating amounts of 1,25-(OH)₂D₃ in rats fed on low phosphorus (0·03 mol/kg) or normal phosphorus (0·18 mol/kg) diets (adequate vitamin D), and found that the concentrations of 1,25-(OH)₂D₃ in the low phosphorus group were higher than in the normal group. However, Henry et al. (1974) and Montecuccoli, Bar, Risenfeld & Hurwitz (1977) failed to detect any change in the activity of 25-(OH)D₃-1-hydroxylase in chicks fed on a low phosphorus diet. These contradictory results could be explained by the difference in the dietary content of phosphorus. Our results and those of Baxter & DeLuca (1976) show that only a diet containing 0·08 mol of phosphorus/kg or less stimulated the activity of 25-(OH)D₃-1-hydroxylase. The phosphorus content of the diet used by Montecuccoli et al. (1977) (0·12 mol/kg) was close to the medium phosphorus diet (0·14 mol/kg) in our experiment, which we found to have no significant effect on the 25-(OH)D₃-1-hydroxylase activity. Although Henry et al. (1974) claimed to have used a diet containing no phosphorus, it has been suggested that this diet could have contained as much as 0·1 mol of phosphorus/kg of diet (Baxter & DeLuca, 1976).

The lack of stimulation of 25-(OH)D₃-1-hydroxylase by a medium phosphorus diet indicates that the increase in calcium absorption in phosphorus deficiency cannot be directly dependent upon the stimulation of 25-(OH)D₃-1-hydroxylase. This is further confirmed by the studies in which the increase in calcium absorption in response to phosphorus deprivation was not abolished when the 1-hydroxylation step was bypassed (Bar & Wasserman, 1973; Bar et al., 1975; Ribovich & DeLuca, 1975). Furthermore, Baxter & DeLuca (1976) and Friedlander, Henry & Norman (1977) have found that the stimulation of renal 25-(OH)D₃-1-hydroxylase in phosphorus deprivation was not as great as that seen in calcium deprivation. Increased accumulation of 1,25-(OH)₂D₃ by the intestinal mucosa has been suggested as one possible mechanism by which calcium absorption is increased in phosphorus deficiency (Baxter & DeLuca, 1976; Montecucoli et al., 1977). The finding that the amount of 1,25-(OH)₂D₃ accumulated in phosphorus deprivation was as great as that in calcium deprivation (Edelstein, Harell, Bar & Hurwitz, 1975) supports this hypothesis.

It is concluded that in phosphorus deficiency a diet with very low phosphorus content (0·08 mol/kg or less) is required to stimulate the renal 25-(OH)D₃-1-hydroxylase activity.

Acknowledgments

This study was partly supported by a grant from the Medical Research Council to A.D.C. We are grateful to Mr J. Dowson for technical assistance.

References


