Treatment of bone disease after jejunoileal bypass for obesity with oral 1α -hydroxyvitamin D_3

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SUMMARY The effects of oral 1α -hydroxyvitamin D_3 have been investigated in 12 patients with bone disease after jejunoileal bypass for obesity. Bone histology became normal or improved greatly after four to 12 months' treatment in eight patients but showed little change or worsened in four. There was a significant rise in plasma calcium and fall in plasma alkaline phosphatase concentration with 1α -hydroxyvitamin D_3 therapy in the patients with a good histological response. Administration of metronidazole and cotrimoxazole to two patients who had failed to respond to 1α -hydroxyvitamin D_3 resulted in clinical and biochemical improvement; in one of these patients histological improvement was also documented. It is concluded that oral 1α -hydroxyvitamin D_3 can be effective in healing post-bypass bone disease; the failure of some patients to respond may be related to bacterial contamination of the small intestine and in those patients antibiotics may also be indicated.

Metabolic bone disease is common after jejunoileal bypass for obesity and is characterised histologically by a combination of osteomalacia and hyperparathyroidism.¹ The pathogenesis of this bone disease is incompletely understood; its failure to correlate with either the length of time after bypass or the plasma 25-hydroxyvitamin D (25OHD) concentration suggests that malabsorption of vitamin D and 25OHD is not always the major cause of bone disease and raises the possibility of an abnormality in the metabolism of vitamin D beyond the stage of 25-hydroxylation.

In this study we report the clinical, biochemical, and histological effects of oral 1α -hydroxyvitamin D_3 (1α OHD₃), a synthetic analogue of 1,25 dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) in patients with post-bypass bone disease and provide some preliminary evidence to suggest that interference with the absorption or metabolism of vitamin D by intestinal bacteria may contribute to bone disease.

Methods

PATIENTS

Twelve patients, two male and 10 female, aged 41 to 59 years (mean 46.5) were studied. The length of time since bypass at the time of the initial biopsy ranged from 12 to 81 months (mean 55.0). All patients had undergone end-to-side anastomosis, in most cases of 10 cm of jejunum to 25 cm of ileum. The mean weight loss achieved after the operation was 46.3 kg (range 25-83 kg). All patients were treated with oral 1α OHD₃ 2 μ g daily. The duration of treatment ranged from four to 12 months (mean 7.2 months). No patient was taking any other vitamin supplements, or any drugs known to interfere with vitamin D metabolism or absorption. Renal function as judged by plasma creatinine levels was normal in all patients and none had evidence of severe liver dysfunction. Sunlight exposure was estimated to be within normal limits in all patients studied. Dietary intakes were not assessed.

BONE HISTOLOGY

8 μ m undecalcified sections of transiliac biopsy specimens were stained by haematoxylin and eosin,

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the von Kossa technique, or 1% toluidine blue and were quantified with a Zeiss 25 point eye-piece graticule. 10 μ m unstained sections were examined by fluorescence microscopy after the oral administration of demethylchlortetracycline. Before the first biopsy only a single label was given (900 mg 48 hours before the biopsy). Two labels were given before the second biopsy, for periods of two and four days respectively separated by two weeks, the last dose being taken three to five days before the biopsy. The mineral appositional rate was calculated from the distance between the two fluorescent bands divided by the number of days between the midpoint of the two labels.² Calcification fronts were assessed both on sections stained with 1% toluidine blue and by fluorescence microscopy of unstained sections. Hyperparathyroidism was qualitatively assessed by two independent observers. Control values for cancellous bone volume, osteoid volume, calcification fronts, and mineral appositional rate were obtained from transiliac biopsies taken from 20 normal subjects, eight male and 12 female, aged 24 to 73 years (mean 48.1 years).

BIOCHEMISTRY

Blood and urine for all measurements were obtained in the fasting state. Plasma levels of calcium, phosphate, alkaline phosphatase, albumin, bilirubin, and aspartate aminotransferase were measured on a Vickers M 300 analyser. Plasma calcium levels were corrected for plasma albumin concentration.³ Serum magnesium was measured by atomic absorption spectroscopy. Plasma 25OHD concentrations were measured by a competitive protein-binding assay⁴ using normal human serum as binding protein and plasma 1,25(OH)₂D₃ levels in patient

11 were measured by radioimmunoassay.⁵ Plasma parathyroid hormone (PTH) levels were measured by an immunoradiometric assay using an antiserum supplied by the Medical Research Council (Code BW 211/41).⁶ Plasma and urine creatinine were measured by the alkaline picrate method on a Mark 1 autoanalyser. Urinary phosphate was measured with acid molybdate and aminonaphthol-sulphonic acid as reducing agent on a Mark 1 autoanalyser. The maximum tubular reabsorption capacity for phosphate relative to the glomerular filtration rate ((Tm_{PO₄}/GFR) was calculated from the phosphate/creatinine clearance ratio.⁷

STATISTICS

Statistical analyses were performed using Student's *t* test for paired and unpaired data.

Results

BONE HISTOLOGY

The details of quantitative bone histology before and after 1 α OHD₃ therapy are shown in Table 1. In six patients (1-6) osteoid volume and calcification fronts were normal after treatment and evidence of hyperparathyroidism, initially present in all but patient 1, had resolved except in patient 5. In patients 7 and 8 the osteoid volume had decreased but was still slightly greater than normal, calcification fronts had returned to normal, and evidence of hyperparathyroidism had disappeared. The mineral appositional rate was normal after treatment in patients 3-7; patients 1 and 8 had received only a single label of demethylchlortetracycline and in patient 2 no double labelling could be detected.

In the four remaining patients (9-12), despite an

Table 1 Quantitative bone histology in 12 patients before and after 1 α OHD₃ therapy

Patient no.	Cancellous bone volume % total bone area		Osteoid volume % total cancellous volume		Calcification fronts % total osteoid surface		Mineral appositional rate μ m/day	Hyperparathyroidism	
	Before	After	Before	After	Before	After	After	Before	After
1	23.4	26.4	6.2	2.1	23.9	67.5	*	0	0
2	42.9	31.8	12.1	2.7	74.8	88.3	ND	++	0
3	52.0	19.5	14.0	2.0	67.1	94.8	0.68	±	0
4	27.1	28.3	8.0	1.7	6.4	84.4	0.71	±	0
5	33.5	29.5	6.2	1.6	66.0	86.6	0.53	±	±
6	21.4	28.3	10.3	3.7	45.4	78.9	0.41	±	0
7	37.2	32.0	13.6	6.1	2.7	73.4	0.47	±	0
8	30.0	25.0	9.8	6.2	64.0	88.9	*	±	0
9	30.5	34.5	14.7	13.2	19.0	84.8	0.26	±	++
10	30.4	43.5	20.7	13.0	8.6	42.3	0.24	++	++
11	32.5	55.4	32.7	35.4	2.2	24.7	ND	++	++
12	32.9	35.3	26.0	29.0	4.7	36.6	ND	±	±
Normal mean $\pm$ SD	24.4 $\pm$ 5.4		1.74 $\pm$ 1.1		73.9 $\pm$ 7.4		0.55 $\pm$ 0.13		

*No double label given. ND: no double labelling detected. Hyperparathyroidism: 0 = none, $\pm$ = mild, ++ = moderate.

increase in calcification fronts, especially in patient 9, the osteoid volume remained grossly abnormal, increasing in patients 11 and 12 and showing little change in patients 9 and 10 when allowance was made for the large increase in cancellous bone volume in the second biopsy of patient 10. Histological evidence of hyperparathyroidism persisted in these four patients, increasing in patient 9, and a reduced mineral appositional rate was found after treatment in patients 9 and 10; in patients 11 and 12 no double label could be detected.

Comparison of the responders (patients 1-8) and the non-responders (9-12) revealed no significant difference in age, stool frequency, length of time since bypass, duration of $1\alpha\text{OHD}_3$ therapy, or post-bypass weight loss. The non-responders had more severe bone disease initially and a significantly higher mean pre-treatment plasma PTH concentration (1.38 ± 0.56 vs 0.75 ± 0.32 $\mu\text{g/l}$) (mean $\pm$ SD; $P < 0.025$). There were no significant differences in pre-treatment plasma calcium, phosphate, magnesium, alkaline phosphatase, or 25OHD concentrations.

BIOCHEMISTRY

In the group of 12 patients the mean plasma calcium before treatment (2.27 ± 0.14 mmol/l) was significantly lower than after treatment (2.38 ± 0.14 mmol/l; $P < 0.005$) and there was a significant fall in plasma alkaline phosphatase activity during treatment (17.2 ± 9.6 vs 15.2 ± 11.2 King Armstrong Units/dl; $P < 0.025$). The mean plasma creatinine after treatment (68.8 ± 15.8 $\mu\text{mol/l}$) was significantly higher than before treatment (56.8 ± 10.8 $\mu\text{mol/l}$; $P < 0.0025$). There was no significant change in plasma phosphate, 25OHD , PTH, serum magnesium, or TmPO_4/GFR with $1\alpha\text{OHD}_3$ therapy.

When the patients were divided into responders and non-responders on the basis of their histological

Fig. 1 Corrected plasma calcium before and after $1\alpha\text{OHD}_3$ therapy in the responders and non-responders. The normal range is shown between the two continuous horizontal lines.

response to treatment, significant changes in plasma calcium and alkaline phosphatase levels occurred in the responders but not in the non-responders (Table 2, Figs. 1 and 2). There was no significant change in plasma phosphate, 25OHD , PTH, serum magnesium or TmPO_4/GFR in either group during $1\alpha\text{OHD}_3$ therapy. No consistent pattern was seen in the plasma PTH response to $1\alpha\text{OHD}_3$ and post-treatment plasma PTH levels correlated poorly with histological evidence of hyperparathyroidism in the responders, being raised (1.85 and 2.4 $\mu\text{g/l}$) in patients 1 and 7. However, plasma PTH levels remained raised in the four non-responders after $1\alpha\text{OHD}_3$ therapy.

Table 2 Biochemistry before and after $1\alpha\text{OHD}_3$ therapy in responders (patients 1-8) and non-responders (9-12)

	Responders (n=8)			Non-responders (n=4)			Normal range
	Before	After	P	Before	After	P	
Corrected plasma calcium (mmol/l)	2.31 ± 0.12	2.44 ± 0.06	<0.01	2.21 ± 0.18	2.26 ± 0.18	NS	2.25-2.55
Plasma phosphate (mmol/l)	0.94 ± 0.21	1.01 ± 0.15	NS	0.85 ± 0.31	0.83 ± 0.19	NS	0.8-1.4
Serum magnesium (mmol/l)	0.80 ± 0.08	0.76 ± 0.09	NS	0.80 ± 0.08	0.80 ± 0.07	NS	0.7-0.9
Plasma alkaline phosphatase (kau/dl)	14.9 ± 6.7	12.0 ± 8.4	<0.01	21.8 ± 13.7	21.5 ± 14.6	NS	4-13
Plasma 25OHD (nmol/l)	12.5 ± 6.6	11.8 ± 8.5	NS	7.4 ± 8.5	8.4 ± 6.1	NS	20-100
Plasma PTH ($\mu\text{g/l}$)	0.75 ± 0.32	0.91 ± 0.79	NS	1.38 ± 0.56	1.90 ± 0.26	NS	0-0.9
TmPO_4/GFR (mmol/l)	0.86 ± 0.30	0.87 ± 0.15	NS	0.67 ± 0.06	0.79 ± 0.24	NS	0.8-1.35

Results are expressed as mean $\pm$ SD.

Fig. 2 Plasma alkaline phosphatase activity before and after $1\alpha\text{OHD}_3$ therapy in the responders and non-responders. The normal range is shown between the two continuous horizontal lines.

CLINICAL SYMPTOMS

Two of the three responders who complained of bone pains before treatment with $1\alpha\text{OHD}_3$ noticed a definite improvement during therapy. Bone pains persisted despite treatment in all four non-responders. No patient had clinical evidence of proximal myopathy before or after treatment.

EFFECT OF ANTIBIOTIC THERAPY IN PATIENTS 10 AND 11

Patient 10 (Fig. 3)

Plasma calcium remained low and plasma alkaline phosphatase and PTH levels high in this patient despite seven months' treatment with $1\alpha\text{OHD}_3$. A post-treatment bone biopsy showed little improvement of his bone disease and metronidazole 400 mg three times daily and cotrimoxazole (trimethoprim 80 mg + sulphamethoxazole 400 mg/tablet) two tablets twice daily were added to the treatment regime. Over the following five months there was a progressive rise in plasma calcium concentration and plasma alkaline phosphatase and PTH levels fell to within the normal range. A third bone biopsy after five months of combined therapy showed a reduction in osteoid volume to 9% and an increase in calcification fronts and mineral appositional rate to normal. There was also an improvement in bone pain during this time.

Patient 11

Investigations carried out in October 1977 revealed hypocalcaemia (corrected plasma calcium 1.94 mmol/l) and severe histological bone disease. In December 1977 treatment was started with oral $1\alpha\text{OHD}_3$, 2 μg daily, but because of persistent hypocalcaemia the dose was increased to 3 μg daily in April 1978 and 4 μg daily in May 1978. In September 1978 the plasma calcium was still low (2.04 mmol/l) and a second bone biopsy showed that the bone disease had become more severe. Plasma $1,25(\text{OH})_2\text{D}_3$ was undetectable (<8 ng/l; normal range 20–60). Treatment was started with metronidazole 400 mg three times daily and cotrimoxazole two tablets twice daily; $1\alpha\text{OHD}_3$ was continued at a dose of 4 μg daily. One month later the plasma calcium had risen to 2.42 mmol/l. After three months of combined treatment with antibiotics and $1\alpha\text{OHD}_3$ the plasma alkaline phosphatase activity had fallen from 42 to 23 KA Units/dl and the plasma $1,25(\text{OH})_2\text{D}_3$ levels measured on two occasions were 20 and 23 ng/l respectively.

Fig. 3 Corrected plasma calcium and PTH concentrations in patient 10 during treatment with $1\alpha\text{OHD}_3$ alone and with combined $1\alpha\text{OHD}_3$ and antibiotic therapy. The continuous horizontal lines show the normal range for plasma calcium and plasma PTH. Bone biopsies were carried out at 0, 7, and 12 months.

Discussion

This study has shown that, although oral $1\alpha\text{OHD}_3$ therapy was effective in healing post-bypass bone disease in some patients, one-third failed to respond. Although the non-responders had more severe bone disease before treatment than the responders, there appeared to be a genuine difference in the histological response shown by the two groups. In the responders histological parameters became normal or improved greatly and post-treatment mineral appositional rates were normal, whereas in the non-responders the osteoid volume either showed little change or increased, histological evidence of hyperparathyroidism persisted or worsened and the mineral appositional rate was low. The increase in calcification fronts in the non-responders suggests an increase in the extent of mineralisation of osteoid; however, the lack of change in osteoid amount and the low mineral appositional rate in these patients indicate that the overall mineralisation rate did not increase significantly in response to $1\alpha\text{OHD}_3$.

Prediction of the histological response from plasma biochemistry was often difficult, as, even though in the responders as a group there were significant changes in plasma calcium and alkaline phosphatase levels, in individual patients these changes were usually small and often occurred within the normal range. The response of the plasma PTH to $1\alpha\text{OHD}_3$ was variable; its rise in some patients with a good histological response was unexpected and contrasts with the fall in plasma PTH levels which usually occurs during treatment of renal bone disease with $1\alpha\text{OHD}_3$.

The aetiology of post-bypass bone disease is not yet fully understood. Low plasma 25OHD levels are common after bypass^{1 9 10} and possible contributory factors include a low dietary vitamin D intake, reduced endogenous vitamin D synthesis, malabsorption of dietary vitamin D and 25OHD, and malabsorption of 25OHD undergoing enterohepatic circulation.¹¹ Malabsorption of vitamin D may occur as a result of the reduced intraluminal bile acid concentration in the upper small intestine after bypass¹² and some evidence that this occurs is provided by the failure of post-bypass patients to achieve normal plasma 25OHD concentrations during oral vitamin D supplementation.^{10 13} Indirect evidence for malabsorption of 25OHD has also been reported,⁹ although bile acids may not be required for the absorption of this metabolite;¹⁴ thus increased faecal losses of dietary and endogenous 25OHD may occur. Calcium malabsorption has also been reported after bypass¹⁵ and may contribute to bone disease.

The reason for the failure of four patients to respond to $1\alpha\text{OHD}_3$ is not clear. Non-compliance with therapy is unlikely, as all four patients were co-operative and attended regularly for blood tests. None had evidence of severe hepatic dysfunction, which sometimes occurs after bypass¹⁶ and could impair the 25-hydroxylation of $1\alpha\text{OHD}_3$, and sunlight exposure, age, weight loss, time since bypass, and duration of therapy were comparable in responders and non-responders. Our earlier finding that plasma 25OHD levels were often similar in patients with and without bone disease¹ suggested either that factors unrelated to vitamin D deficiency were involved in the bone disease or that there might be an abnormality of vitamin D metabolism beyond the stage of 25-hydroxylation. The effect of antibiotics in two patients raises the possibility that bacteria present in the small intestine after bypass might affect vitamin D metabolism. After bypass, increased numbers of the bacteria normally found in the lower intestine have been demonstrated in the jejunum,¹² some of which can metabolise bile acids and steroid hormones by a variety of reactions including dehydroxylation.^{17 18} If vitamin D metabolites undergo enterohepatic circulation they could be inactivated by bacteria present within the intestinal lumen. Alternatively, the beneficial effect of antibiotics might be due to a reduction of bile acid deconjugation, thus increasing the intraluminal bile acid concentration and improving vitamin D absorption. Variability in the degree of bacterial colonisation of the small intestine after bypass could explain the rapid development of bone disease in some patients and its complete absence after many years in others, and might also explain 'resistance' to vitamin D therapy in some patients. However, additional studies are required before definite conclusions can be drawn about the role of bacterial contamination in post-bypass bone disease.

Effective prophylaxis or treatment of post-bypass bone disease is important as it is a common and sometimes severe complication of jejunoileal bypass. Our results indicate that oral $1\alpha\text{OHD}_3$ can be effective and is safe. There is no evidence that $1\alpha\text{OHD}_3$ is more effective than other forms of vitamin D, but the practical advantages associated with its narrow therapeutic dose range and short plasma half-life may make it preferable to treatment with the parent vitamin.¹⁹ No serious side-effects were encountered and, in particular, hypercalcaemia never occurred during fortnightly or monthly monitoring, even when $1\alpha\text{OHD}_3$ was continued for up to six months after the bone disease had healed. Some patients, however, respond poorly to $1\alpha\text{OHD}_3$; this may be related to bacterial colonisa-

tion of the small intestine and antibiotics may be indicated in these patients in addition to vitamin D therapy.

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References

- ¹Compston JE, Horton LWL, Laker MF, *et al.* Bone disease after jejuno-ileal bypass for obesity. *Lancet* 1978; **2**: 1-4.
- ²Frost HM. Tetracycline-based histological analysis of bone remodeling. *Calcif Tissue Res* 1969; **3**: 211-37.
- ³Payne RB, Little AJ, Williams RB, Milner JR. Interpretation of serum calcium in patients with abnormal serum proteins. *Br Med J* 1973; **4**: 643-6.
- ⁴Edelstein S, Charman M, Lawson DEM, Kodicek E. Competitive protein-binding assay for 25-hydroxycholecalciferol. *Clin Sci Mol Med* 1974; **46**: 231-40.
- ⁵Clemens TL, Hendy GN, Graham RF, Baggiolini EG, Uskokovic MR, O'Riordan JLH. A radioimmunoassay for 1,25-dihydroxycholecalciferol. *Clin Sci Mol Med* 1978; **54**: 329-32.
- ⁶Addison GM, Hales CN, Woodhead JS, O'Riordan JLH. Immunoradiometric assay of parathyroid hormone. *J Endocrinol* 1971; **49**: 521-30.
- ⁷Walton RJ, Bijvoet OLM. Nomogram for derivation of renal threshold phosphate concentration. *Lancet* 1975; **2**: 309-10.
- ⁸Brownjohn KM, Goodwin FJ, Hatley W, Marsh FP, O'Riordan JLH. 1 α -hydroxycholecalciferol for renal osteodystrophy. *Br Med J* 1977; **2**: 721-3.
- ⁹Teitelbaum SL, Halverson JD, Bates M, Wise L, Haddad JG. Abnormalities of circulating 25-OH vitamin D after jejuno-ileal bypass for obesity. Evidence of an adaptive response. *Ann Intern Med* 1977; **86**: 289-93.
- ¹⁰Parfitt AM, Miller MJ, Frame B, *et al.* Metabolic bone disease after intestinal bypass for treatment of obesity. *Ann Intern Med* 1978; **89**: 193-99.
- ¹¹Arnaud SB, Goldsmith RS, Lambert PW, Go VLW. 25-hydroxyvitamin D₃: evidence of an enterohepatic circulation in man. *Proc Soc Exp Biol Med* 1975; **149**: 570-2.
- ¹²Danö P, Lenz K, Justesen T. Bile acid metabolism and intestinal bacterial flora after three types of intestinal shunt operation for obesity. *Scand J Gastroenterol* 1974; **9**: 767-74.
- ¹³Schoen MS, Lindenbaum J, Roginsky MS, Holt PR. Significance of serum levels of 25-hydroxycholecalciferol in gastrointestinal disease. *Amer J Dig Dis* 1978; **23**: 137-42.
- ¹⁴Nechama H, Hoff D, Harell A, Edelstein S. The intestinal absorption of vitamin D and its metabolites. *J Molec Med* 1977; **2**: 413-22.
- ¹⁵Danö P, Christiansen C. Calcium absorption and bone mineral content following intestinal shunt operation for obesity. A comparison of three types of operation. *Scand J Gastroenterol* 1974; **9**: 775-9.
- ¹⁶Drenick EJ, Simmons F, Murphy JF. Effect on hepatic morphology of treatment of obesity by fasting, reducing diets, and small bowel bypass. *N Engl J Med* 1970; **282**: 829-34.
- ¹⁷Hill MJ, Drasar BS. Degradation of bile salts by human intestinal bacteria. *Gut* 1968; **9**: 22-7.
- ¹⁸Eriksson H, Gustafsson JA, Sjovall J. Steroids in germfree and conventional rats. 21-dehydroxylation by intestinal microorganisms. *Eur J Biochem* 1969; **9**: 550-4.
- ¹⁹Kanis JA, Russell RGG. Rate of reversal of hypercalcaemia and hypercalciuria induced by vitamin D and its 1 α -hydroxylated derivatives. *Br Med J* 1977; **1**: 78-81.