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Low serum levels of 1.25-dihydroxyvitamin D and histomorphometric evidence of osteomalacia after jejunoileal bypass for obesity

L MOSEKILDE, F MELSEN, I HESSOV, M S CHRISTENSEN, BJ LUND, BI LUND, AND O H SØRENSEN

From Medical Department III and University Institute of Pathology, Aarhus County Hospital, Aarhus; Surgical Department of Gastroenterology L, Aarhus Municipal, Aarhus; Department of Clinical Chemistry, Copenhagen County Hospital, Glostrup; Department of Orthopaedic Surgery, Frederiksborg County Hospital, Hillerød; Department of Medicine E, Frederiksberg University Hospital and Department of Medicine F, Herlev University Hospital, Copenhagen, Denmark

SUMMARY Twenty-seven unselected patients were investigated three to eight years after jejunoileal bypass for morbid obesity. The serum levels of calcium, magnesium, and phosphorus, and the renal excretions of calcium and magnesium were reduced. The serum alkaline phosphatase levels were increased. The serum levels of the two vitamin D metabolites 25-hydroxyvitamin D (25-OHD) and 1·25-dihydroxyvitamin D (1·25-$(OH)_2$D) were reduced and inversely related to the increased serum levels of immunoreactive parathyroid hormone (iPTH). Serum 1·25-$(OH)_2$D correlated positively and serum iPTH inversely with serum concentrations and renal excretion rates of calcium. Iliac crest bone biopsies after *in vivo* tetracycline double-labelling showed a reduced bone turnover with an increased amount of osteoid due to an increase in both surface extent and mean width of osteoid seams. The increased volume of osteoid was caused by a decreased osteoblastic function with a longer life-span of bone-forming sites and a prolongation of the mineralisation lag time. The amount of trabecular bone was normal. The results indicate an impaired vitamin D metabolism with osteomalacia and secondary hyperparathyroidism.

In the last 15 to 20 years jejunoileal bypass surgery has been used for the treatment of severe and otherwise intractable morbid obesity. However, a great number of serious complications to this surgical procedure have been reported,[1 2] including a disturbed calcium-phosphorus homeostasis with malabsorption of calcium,[3] vitamin D, and 25-hydroxyvitamin D (25-OHD).[4 5] Low serum levels of 25-OHD[6–9] and 1·25-$(OH)_2$D[10] have been reported. Reduced bone mineral content[3 8 9] and histological evidence of an increased amount of unmineralised bone (osteoid) has also been found.[6 8]

Twenty-seven patients were studied three to eight years after jenunoileal bypass for severe obesity. The vitamin D metabolism was evaluated by measurement of the major circulating form 25-OHD, which is produced in the liver, and 1-25-$(OH)_2$D, the ultimate metabolically active metabolite, which is produced in the kidney.[11] The relationships between these metabolites and the serum levels of calcium, phosphorus, alkaline phosphatase, and iPTH were studied. Furthermore, bone morphology and dynamics were described in detail using tetracycline double-labelling and point counting on sections of undecalcified iliac crest bone.

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Methods

PATIENTS

The study comprised 27 outpatients (26 females and one male) aged 29–64 years (mean 40·0 years), who three to eight years (mean 5·4 years) previously had undergone jejunoileal bypass for morbid obesity. The patients were not selected for their symptoms. All patients gave informed consent to the investigation and none of the patients refused bone biopsy. There is no overlap between the present population and the patients studied by Hay *et al.*[9] and Lund

et al.[10] The mean preoperative weight was 123 kg (range 97–173 kg). During operation 35 cm of jejunum was anastomosed end-to-side to 15 cm of the distal ileum in 25 of the patients. In the last two patients 15 cm of jejunum was anastomosed end-to-side to 35 cm of the distal ileum. The average weight loss at the time of study was 38 kg (range 16–77 kg) or 31% (range 8–56%) of the initial weight. No patients had more than three stools per day. Eleven of the patients received a daily oral dose of 400–1200 IU vitamin D_2 without calcium supplementation. None of the patients had bone pains or bone tenderness. All had a normal serum creatinine concentration and none received anticonvulsants or were immobilised.

The controls for bone histomorphometry consisted of 25 normal volunteer females and one male, all double-labelled with tetracycline[12] to be, approximately, sex- and age-matched to the patient group. The controls for serum 25-OHD comprised 60 normal individuals selected from a larger material[13] to be matched to the patient group according to age, vitamin D supplementation, and months of investigation (November-December). The controls for serum 1·25-$(OH)_2$D comprised 65 normal individuals, approximately sex- and age-matched to the patients and selected from a larger material, which, in part, has been described previously,[14] The controls for serum iPTH comprised 28 normal individuals, 26 females and two males, aged 25 to 59 years.

BIOCHEMISTRY

Blood samples were taken in the fasting state. Serum calcium (mmol/l) was corrected for individual variations in serum albumin (mmol/l) according to the formula: s-calcium, corrected=1·2 (0·700–s-albumin)+s-calcium. This formula was derived from the regression equation of s-calcium on s-albumin in 160 normal individuals. Urinary excretions of calcium, phosphorus, and magnesium were determined on an unrestricted diet and expressed in mmol/mol excreted creatinine.

Serum 25-OHD was measured by a competitive protein binding assay.[13] The intra- and interassay coefficients of variation were, respectively, 0·09 at the level of 20 ng/ml and 0·14 at the level of 15 ng/ml. The lower detection limit was 0·8 ng/ml. Serum 1·25-$(OH)_2$D was determined by a radio assay based on competitive binding to an intestinal cytosol protection from rachitic chicks.[14] Both intra- and interassay coefficients of variation were 0·11 at the level of 30 pg/ml. The lower detection limit was 6 pg/ml. Serum immunoreactive parathyroid hormone (iPTH) was measured by a sensitive radioimmunoassay on extracts of serum.[15,16] The extraction procedure induced a five-fold higher iPTH concentration in extracts than in serum. The antiserum used was AS211/41, which reacts with both the N-terminal and C-terminal part of PTH. Chromatography of serum showed that the dominant part of the iPTH in primary hyperparathyroid sera coeluted with PTH (1–84). The intra- and interassay coefficients of variation were, respectively, 0·08 and 0·15 at the level of 80 pg/ml. The sensitivity of the assay was 15 pg bovine PTH present in the incubation mixture.

BONE HISTOMORPHOMETRY

Bone biopsies were performed by transfixing the right iliac crest[17] after *in vivo* double-labelling with tetracycline with an interval of 10 days.[12] The following parameters were determined on undecalcified bone sections using point counting and simple measurements: trabecular bone volume in decimal fraction of the total bone volume; trabecular osteoid volume in decimal fraction of the trabecular bone volume; osteoid covered surfaces, labelled surfaces and resorption surfaces in decimal fractions of the total trabecular bone surface; mean width of osteoid seams in μm, and appositional rate in μm/day. The appositional rate, which reflects the function of the active osteoblasts, was determined as the mean distance between the tetracycline lines in all double-labelled zones divided by the interval in days between the labellings. The following parameters were calculated from the values above[18]: (1) Bone formation rate in $\mu m^3/\mu m^2$/day, which gives the average amount of mineralised bone being formed per day per unit osteoid covered surface and reflects the average activity of active and inactive osteoblasts; at steady state situations with regard to osteoid thickness this parameter equals the linear appositional rate of osteoid; (2) bone formation rate, tissue level, in $\mu m^3/\mu m^2$/day, which gives the amount of the new bone mineralised in unit time per unit trabecular bone surface and reflects the bone turnover or the number of new remodelling units initiated in unit time; (3) mineralisation lag time in days, which gives the average period of time between the apposition and subsequent mineralisation of osteoid.

STATISTICS

The statistical significance of differences in group means was determined by the Wilcoxon test for two samples and correlation coefficients by Spearman's rank correlation. Normal values were expressed as mean ± 2 SD for normal or lognormal distributions.

Table 1 *Biochemical values in serum and urine in 27 patients three to eight years after jejunoileal bypass for obesity and in normal control*

	Normal controls			*Intestinal bypass*			
	N	$\bar{X}$	*SE*	*N*	$\bar{X}$	*SE*	P
S-calcium (mmol/l)	160	2·501	0·007	27	2·351	0·020	<0·001
S-albumin (mmol/l)	160	0·695	0·004	27	0·683	0·007	NS
S-calcium (corr., mmol/l)	160	2·508	0·006	27	2·374	0·019	<0·001
S-phosphorus (mmol/l)	60	1·18	0·02	27	1·05	0·03	<0·001
S-magnesium (mmol/l)	60	0·98	0·01	27	0·85	0·02	<0·001
S-alkaline phosphatase (U/l)	60	147	4	27	190	18	<0·001
S-1·25-$(OH)_2$D (pg/ml)	65	33	2	27	23	3	<0·001
S-25-OHD (ng/ml)	60	28	2	27	9	1	<0·001
S-iPTH (pg/ml)	28	37	2	22	62	16	<0·05
U-calcium*	60	430	13	27	240	26	<0·001
U-phosphorus*	60	1965	45	27	2153	138	NS
U-magnesium*	60	356	15	27	201	19	<0·001

*Expressed in mmol per mol excreted creatinine.

Results

Table 1 gives the biochemical values measured in serum and urine from the 27 intestinal bypass patients and from various groups of normal controls. The mean serum levels of total calcium, albumin adjusted calcium, magnesium, and phosphorus were reduced in the patient group, whereas the mean serum level of alkaline phosphatase was increased. Serum albumin levels were normal. The renal excretion rate of calcium and magnesium was markedly decreased. The renal excretion of phosphorus was normal. Table 2 shows the prevalence of abnormal serum values in the patient group within 95% confidence limits.

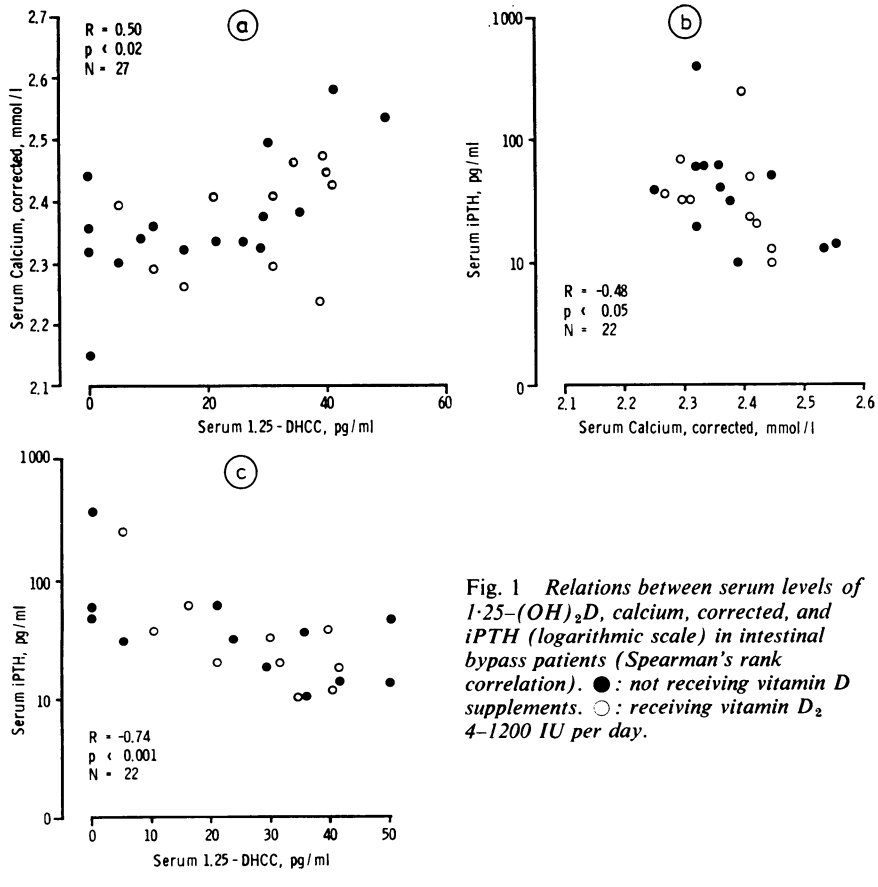

Fig. 1 *Relations between serum levels of 1·25-$(OH)_2$D, calcium, corrected, and iPTH (logarithmic scale) in intestinal bypass patients (Spearman's rank correlation).* ● *: not receiving vitamin D supplements.* ○ *: receiving vitamin* D_2 *4–1200 IU per day.*

Table 2 *Prevalence of abnormal biochemical values in 27 patients after intestinal bypass for morbid obesity*

	%	*95% confidence limits*	*Normal ranges*
Low S-calcium, corrected	52	32–71	2·37–2·65 mmol/l
Low S-phosphorus	15	4–34	0·9–1·5 mmol/l
Low S-magnesium	15	4–34	0·75–1·20 mmol/l
Raised S-alkaline phosphatase	22	9–42	85–210 U/l
Low S-1·25-$(OH)_2D$	41	22–61	19–52 pg/ml
Low S-25-OHD	56	35–75	9–71 ng/ml
Raised S-iPTH	18	5–40	13–60 pg/ml

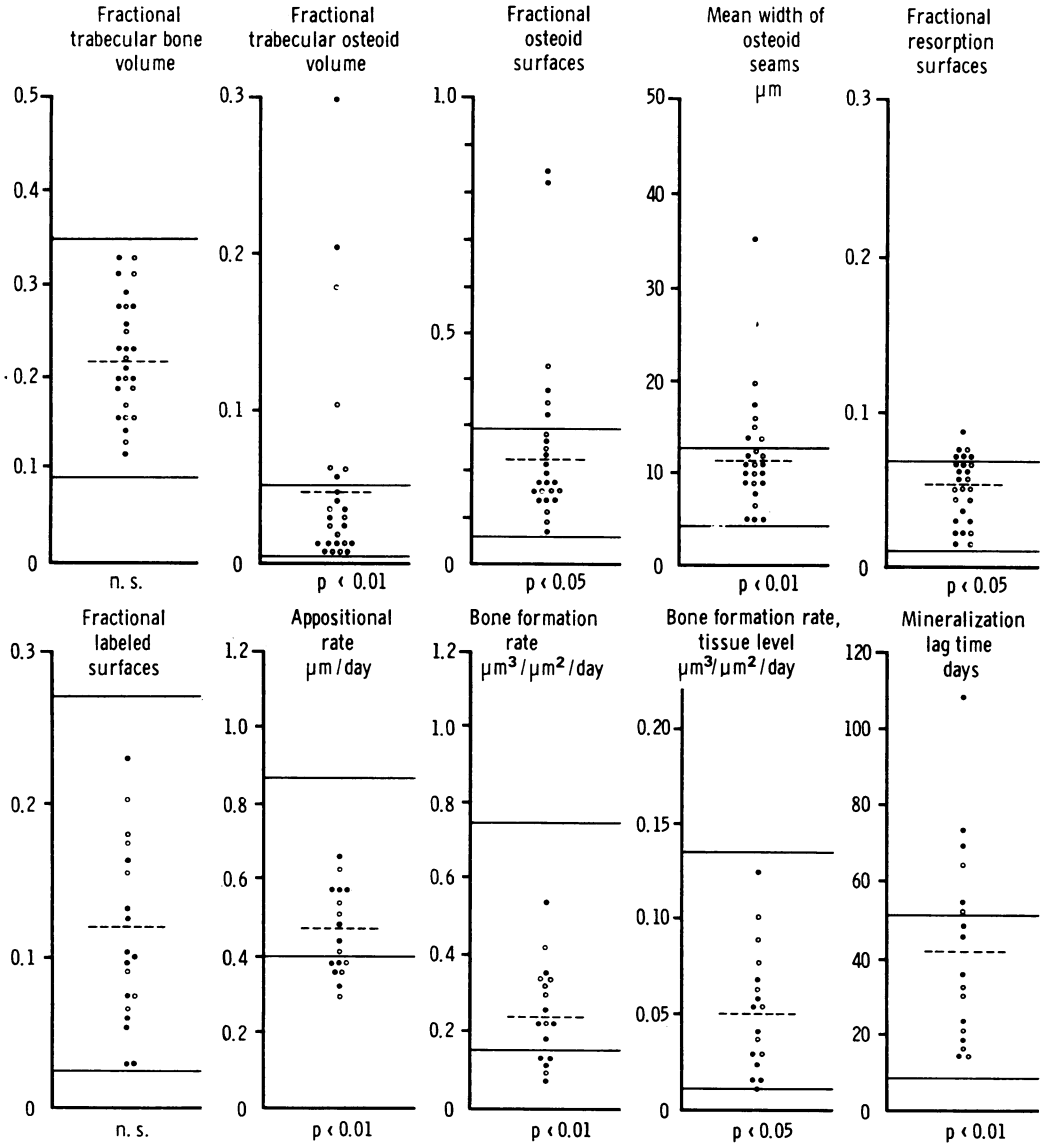

Fig. 2 *Static and dynamic histomorphometric values from iliac crest biopsies in intestinal bypass patients and normal controls. - - - mean values. ——: normal ranges. ● : not receiving vitamin D supplements. ○ : receiving vitamin D_2 4–1200 IU per day.*

VITAMIN D METABOLITES

The mean serum level of 1·25-$(OH)_2D$ was moderately reduced. Subnormal concentrations were found in 11 patients. Serum 1·25-$(OH)_2D$ correlated positively with albumin corrected serum calcium ($r=0·60$, $P<0·02$) (Fig. 1) with the renal excretion rate of calcium ($r=0·49$, $P<0·02$). A highly significant inverse correlation was found between serum 1·25-$(OH)_2D$ and serum iPTH ($r=-0·74$, $P<0·001$) (Fig. 1). The mean serum level 25-OHD was markedly reduced and subnormal values were found in 15 of the patients. Serum 25-OHD was positively related to the renal excretion rate of calcium ($r=0·45$, $P<0·05$) and inversely related to serum iPTH ($r=-0·58$, $P<0·01$).

SERUM iPTH

The mean serum level of iPTH was increased and raised levels were found in four of 22 patients. Serum iPTH was inversely related to serum calcium, corrected ($r=-0·48$, $P<0·05$) (Fig. 1) and to the renal excretion rate of calcium ($r=-0·70$, $P<0·01$). Serum iPTH was inversely related to the serum levels of vitamin D metabolites as mentioned above.

BONE HISTOMORPHOMETRY

The histomorphometric values for trabecular bone remodelling in the patients are shown in Fig. 2. The trabecular bone volume was normal. The mean trabecular osteoid volume was markedly increased and raised values were found in seven patients. The increase in osteoid volume was caused by an increase in both fractional osteoid surfaces and mean width of osteoid seams. Increased osteoid surfaces were found in six patients and eight had an increased mean width of osteoid seams. The mean extent of resorption surfaces was slightly increased. The surface extent of labelled zones was normal. The mean appositional rate and the mean bone formation rate were reduced, indicating reduced activity of the osteoblasts. Subnormal values were found in seven and five patients, respectively. The bone formation rate at tissue level was slightly reduced, indicating a low bone turnover. The mineralisation lag time was prolonged and raised values were found in six patients. The difference in osteoid volume and osteoid seam width between a normal individual and an intestinal bypass patient and normal tetracycline double-lines are demonstrated in Fig. 3.

Both biochemical and histomorphometric values

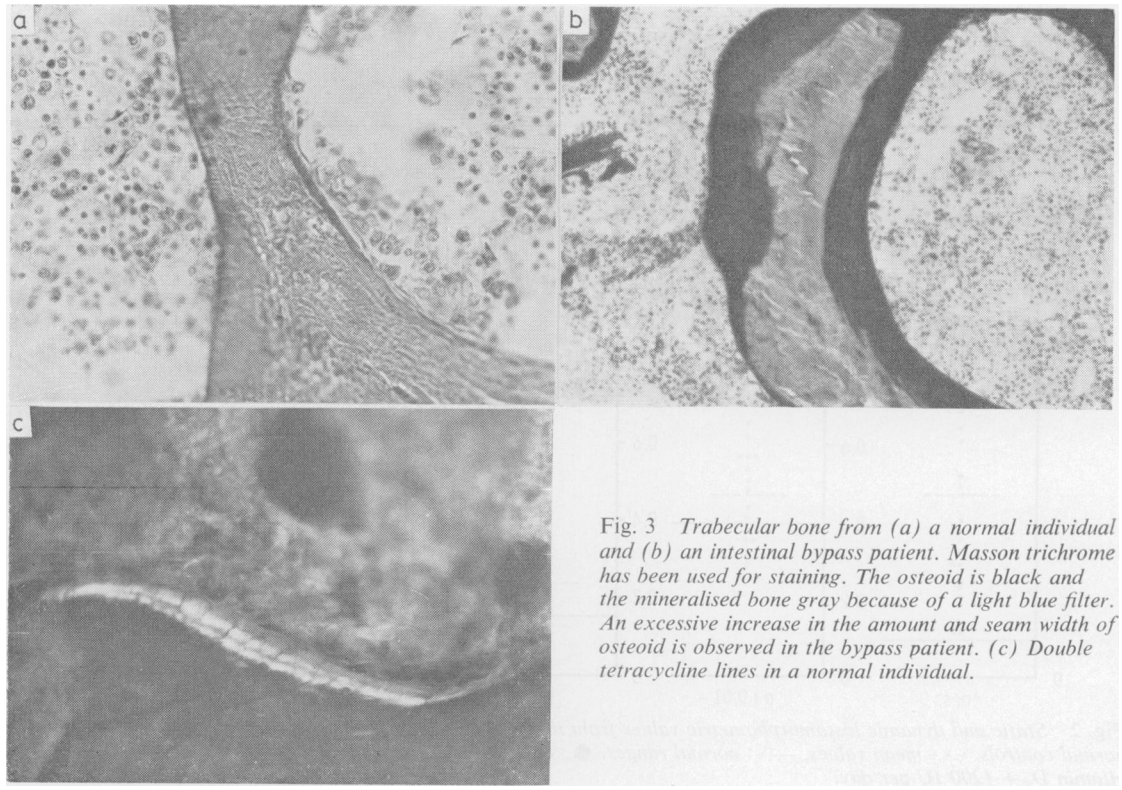

Fig. 3 *Trabecular bone from (a) a normal individual and (b) an intestinal bypass patient. Masson trichrome has been used for staining. The osteoid is black and the mineralised bone gray because of a light blue filter. An excessive increase in the amount and seam width of osteoid is observed in the bypass patient. (c) Double tetracycline lines in a normal individual.*

were unrelated to the absolute or relative weight loss, to vitamin D supplementation in the doses as given here, and to the time since operation. No significant correlations were found between biochemical and histomorphometric values.

Discussion

The present study demonstrates impaired vitamin D metabolism and severely disturbed osteoid mineralisation and trabecular bone remodelling several years after jejunoileal bypass for obesity. The low serum levels of 25-OHD found in the present study support previous reports.[6–9] In accordance with most other studies, we could not confirm the increase in serum 25-OHD with time since operation found by Teitelbaum *et al.*[16] The observed reduction in serum 25-OHD may be caused by a reduced exposure to sunshine for cosmetic reasons, malabsorption of vitamin D[4] and 25-OHD,[5] loss of 25-OHD from the enterohepatic circulation,[19] and/or reduced hepatic hydroxylation of vitamin D due to complicating liver affection.[1 2]

The serum levels of 1·25-$(OH)_2$D were also reduced in the intestinal shunt patients. The mean serum concentration of 1·25-$(OH)_2$D was, however, less reduced (70% of normal mean) than the mean serum level of its parent metabolite, 25-OHD (32% of normal mean) and no significant relation was found between the two metabolites. These findings may be explained by an increased activity of the renal 1α-hydroxylase at low serum levels of vitamin D metabolites mediated in a complex way by the increased serum levels of PTH[20] and the reduced serum levels of calcium and phosphorus.[21 22] The relation between PTH and vitamin D metabolism is supported by the correlation found in the present study between serum iPTH levels and the serum concentrations of 25-OHD and 1·25-$(OH)_2$D.

The bone mineral content is reduced in intestinal bypass patients.[3 8 9] In a longitudinal study starting one and a half to two years after operation, however, no additional decrease was observed in bone mineral content for the following one and a half years.[8] Where bone mineral content remains unchanged, the renal excretion rate of calcium and magnesium will mainly reflect the net intestinal absorption of these minerals. The reduced excretions of calcium and magnesium found in the present study support the reduced fractional intestinal calcium absorption previously reported in intestinal shunt patients.[3] The malabsorption of calcium and magnesium may be caused by a variety of factors. Calcium is absorbed throughout the length of the small intestine. The duodenum exhibits the greatest transport capacity per unit length but the ileum and the jejunum are responsible for the absorption of most dietary calcium because of the length of the segments.[23] The reduction in intestinal absorptive surface after intestinal bypass operation will, therefore, directly induce malabsorption of calcium. Furthermore, steatorrhoea, besides reducing the intestinal absorption of vitamin D and 25-OHD, may decrease calcium absorption due to precipitation of insoluble calcium soaps.[24] The positive correlations found in the present study between the serum levels of 1·25-$(OH)_2$D and the serum concentrations and renal excretions of calcium suggest, however, that a lack of 1·25-$(OH)_2$D is the main reason for the malabsorption of calcium. The significance of the vitamin D metabolism for magnesium absorption is far less known.

Increased serum levels of iPTH have previously been reported in intestinal bypass patients.[7–9] We have confirmed the inverse relation found by Compston *et al.*[7] between serum 25-OHD and serum iPTH. Furthermore, the present study has demonstrated significant inverse relations between serum iPTH and serum levels of calcium and 1·25-$(OH)_2$D, indicating that the hyperparathyroidism is a part of an adaptive response to hypocalcaemia and vitamin D deficiency. The hypophosphataemia may be explained by a reduction in the maximal tubular reabsorption capacity for phosphorus[7] caused by the secondary hyperparathyroidism.

The bone changes in the intestinal bypass patients were characterised by a reduced function of the osteoblasts with low linear rates of formation and mineralisation of bone matrix. As a result of the decreased osteoblastic function the time taken to complete bone formation at a single site is prolonged.[25] The surface extent of osteoid depends on the life-span of bone-forming sites and the birth-rate of new remodelling units.[25] The increase in osteoid covered surfaces may be explained by the longer life-span of bone-forming sites, because the birth-rate of remodelling units is decreased as demonstrated by the low bone formation rate at tissue level. The observed increase in the mean width of osteoid seams may be explained by the longer mineralisation lag time, which is inversely related to the osteoblastic function.[18] The low Ca × P product may also contribute to the longer mineralisation lag time by inducing mineralisation in a later stage in the process of osteoid maturation. The trabecular osteoclastic resorption surfaces were slightly increased. The reduced bone formation and the normal amount of trabecular bone indicate, however, that the resorption was inactive and the increased surface extent may be explained by a longer lifespan of bone

resorption sites in accordance with the findings in most patients with primary hyperparathyroidism.[26]

The bone changes are characteristic for osteomalacia secondary to vitamin D deficiency.[27] No sign of osteopenia was found. The lack of correlation between the serum levels of 1·25-$(OH)_2$D and the bone changes may be explained by the number of aetiological factors involved in the development of the bone disease, including vitamin D metabolites, PTH, calcium, inorganic phosphate, and the acid base balance, as well as by the great intra- and interindividual variation in the measured parameters.[28]

We conclude that jejunoileal bypass operation for morbid obesity involves a high risk of development of a vitamin D deficient state with osteomalacia and secondary hyperparathyroidism. It seems reasonable to recommend that all patients routinely take a vitamin D supplement. The treatment should be guided by regular determinations of vitamin D metabolites and iPTH in order to identify those patients who should be treated with larger doses of vitamin D or with 1α-hydroxycholecalciferol or 1·25-$(OH)_2$D. The lack of significant correlations between histomorphometric and biochemical values in the present study and in the study of Compston *et al.*,[7] however, illustrates the importance of bone biopsy in the diagnosis of ostermalacia. Repeated bone biopsies may be necessary as a guide during treatment.

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