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# Calcium Regulation and Bone Mass Loss After Total Gastrectomy in Pigs

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## Objective

Total gastrectomy often results in postgastrectomy bone disease with decreased bone mass and increased fracture risk. To further elucidate the mechanisms of postgastrectomy bone disease, the authors investigated calcium metabolism and bone mineral density after total gastrectomy in pigs.

## Summary Background Data

Postgastrectomy bone disease can present as osteomalacia, osteoporosis in excess of normal aging, or a combination of both. The underlying mechanisms are insufficiently understood and need further investigation.

## Methods

Growing minipigs were gastrectomized and compared with fed-matched, sham-operated control pigs for 1 year. Calcium absorption, serum calcium, parathyroid hormone, 25-(OH)-vitamin D, 1,25-$(OH)_2$-vitamin D, alkaline phosphatase, and computed tomography bone mineral density were measured in three monthly intervals.

## Results

Total gastrectomy resulted in impaired calcium absorption, reduced serum calcium and 25-(OH)-vitamin D, increased parathyroid hormone and 1,25-$(OH)_2$-vitamin, and reduced bone mineral density compared with fed-matched, sham-operated control pigs.

## Conclusions

The authors data indicate that a reduced serum calcium activates counter-regulatory mechanisms, resulting in calcium mobilization from the bone. Possibly, calcium and vitamin D supplementation after total gastrectomy might prevent postgastrectomy bone mass loss.

The term "postgastrectomy bone disease" was devised to describe bone disorders after gastrectomy operations. It may occur as osteomalacia, osteoporosis in excess of normal aging, or a combination of both.[1-3] Postgastrectomy bone disease results in a decreased cortical and trabecular bone mass and in a considerably increased fracture risk.[3-9] The cause of this entity still is a matter of experimental and clinical investigation. Decreased absorption of calcium or vitamin D as well as insufficient oral intake of calcium or vitamin D have been suggested as possible causes of postgastrectomy bone disease.[2,3,8,10-13]

Several problems arise when investigating postgastrectomy bone disease in patients. First, it is difficult to recruit sufficient patient numbers. This is because of a decreasing number of gastrectomies for benign gastric ulcer,[14] a decreasing incidence of gastric cancer,[15] thus reducing the number of gastrectomies performed, and a dismal prognosis of gastric cancer in western countries, leaving few patients as tumor-free survivors.[16] Second, the gastrectomy patient collective is often inhomogenous with respect to age and body constitution, which are both important parameters when investigating bone metabolism.[17-19] Third, food intake and food preferences vary widely in humans. Intake of nutrients like calcium or vitamin D, which are important for bone metabolism, will vary in patients, so that uncontrolled nutritional influences will interfere with study results. Finally, bone disease usually is not apparent until several years after gastrectomy,[7] making prospective studies difficult to perform.

To overcome these problems, experimental studies are necessary. Under controlled experimental conditions, parameters influencing bone metabolism and bone mineral density (BMD) can be evaluated prospectively in a homogenous animal population.

We investigated postgastrectomy bone disease in growing pigs because of several reasons. First, the pig is omnivorous just as humans are. Second, the pig has been recognized as suitable for food intake studies,[20] an important aspect when investigating postgastrectomy sequelae, because severe disturbances of food intake have been described after gastrectomy.[21] Third, it can be expected that changes in bone metabolism will delineate sooner in growing bone than in adult bone, so that postgastrectomy bone disease should present in a reasonable time span. Fourth, repeated blood draws to evaluate serum parameters are possible in pigs, which could be a problem when investigating small animals. Fifth and finally, vertebra size of pigs is suitable for computed tomographic (CT) scan, so that quantification of BMD by CT scan is feasible.

In our study, serum calcium, serum phosphate, parathyroid hormone (PTH), alkaline phosphatase, 25-(OH)-vitamin D, 1,25-$(OH)_2$-vitamin D, calcium absorption, and BMD were evaluated before and for 1 year after total gastrectomy in growing minipigs. Gastrectomized pigs were compared with free-fed pigs that were not operated on and with sham-operated, fed-matched pigs as control pigs. Under these conditions, uncontrolled nutritional influences on bone metabolism can be excluded, which has not been done previously.

## METHODS

### Animals

Seventeen female Göttingen miniature pigs (Institut für Tierzucht und Haustiergenetik, Göttingen, Germany) were purchased at the age of 3 months. Before surgery, pigs were accustomed to their home box while fed *ad libitum* with Altromin standard diet (Altromin International, Lage, Germany). Pigs were housed together, but were separated for feeding, in a surrounding of constant temperature (18 C) and illumination (lights on 6 A.M.–6 P.M.) with a 10% fraction of ultraviolet, a radiation for photobiosynthesis of vitamin D in the skin. The institutional guidelines for the care and use of laboratory animals were followed throughout the study. Three pigs remained untreated, six were sham operated, and eight underwent total gastrectomy.

### Surgery

Pigs having equal body weight were either sham operated (n = 6) or gastrectomized (n = 8) at the age of 4 months (11.9 ± 0.7 *vs.* 12.4 ± 0.7 kg, respectively). Premedication before surgery was flunitrazepam (Hoffmann-La Roche, Grenzach, Germany, 2 mg), ketamine (Parke–Davis, Berlin, Germany, 10 mg), and atropine (Köhler, Alsbach, Germany, 1 mg). After tracheal intubation, general anesthesia was maintained with halothane (Hoechst, Frankfurt, Germany) and a nitrous oxide–oxygen mixture (2:1 V:V). The sham operation consisted of a laparotomy with handling of the stomach and the small intestine for 90 minutes. Total gastrectomy, which lasted approximately 90 minutes, was done by removal of the stomach, closing of the transected duodenum, and an end-to-side esophagojejunostomy. The proximal jejunum was anastomosed to the descending limb of the jejunum side-to-side to prevent alkaline reflux into the esophagus (Schloffer reconstruction). There were no deaths in the sham-operated group, whereas two of the pigs that were

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gastrectomized died. One pig suffered an anastomotic breakdown and a subsequent peritonitis on the 2nd postoperative day. Another pig died of a prolonged postoperative ileus 4 weeks after surgery. Both pigs were excluded from analysis.

## Feeding Protocol

After weaning, pigs were accustomed to a minipig pellet diet (Altromin standard diet fortified, Altromin International, Lage, Germany). The pellet diet consists of crude protein 13.5%, crude fat 2.5%, crude fiber 13%, ash 7.5%, nitrogen-free extract 50.5%, amino acids 5.5%, minerals 3.5%, trace elements 580 mg/kg, and vitamins. The metabolizable energy of the diet was 10.5 MJ/kg. Three pigs that were not operated on were fed *ad libitum* for 1 year, and body weight was measured monthly. The animals that were operated on were fed as follows: Starting the night before the operation until the 2nd postoperative day, pigs were fasted with free access to water. The next 2 weeks, the diet pellets were crushed and soaked in water before feeding. During the first 3 postoperative months, pigs were fed three times a day for 2 hours (total feeding time, 6 hours). From the 4th to the 12th postoperative month, pigs were fed twice daily for 2 hours (total feeding time, 4 hours). According to a study with a large number of miniature pigs (n = 204), these feeding periods are adequate for their age.[22] The amount of food given every day was determined as follows: On the 3rd postoperative day, all pigs were given free access to food. The amount of food eaten by the pig eating the least amount of food was recorded. During the next feeding period, this amount of food was given to all pigs. When all pigs had eaten the given amount of food, more food was added in 20 g amounts until one pig stopped eating. Food then was withdrawn from all pigs, and this amount of food was taken as the starting food portion for the next feeding period. Thereby, sham-operated and gastrectomized pigs received exactly the same quantity of food. Food intake was measured daily and body weight was measured monthly for 1 year. At the end of the experiment, esophagojejunal anastomoses were checked for stenosis. All anastomoses were patent with no evidence of stenosis.

## Serum Parameters

Calcium, phosphate, and alkaline phosphatase in serum were measured using a commercially available laboratory test automat (Serum Multiple Analyzer plus, Technikon, Bad Vilbel, Germany). The PTH in serum was measured by radioimmunoassay using a commercially available test kit with an antibody, which recognizes the midregion of PTH (parathyroid hormone$^{44\text{-}68}$; RIA-mat PTH-MM, Mallinckrodt Diagnostica, Dietzenbach, Germany). $^{125}$J-[Tyr$^{43}$]-hPTH$^{44\text{-}68}$ was used as tracer and human PTH to plot standard curves for radioimmunoassay. The lower detection limit of the test was 25 pmol/L. The intra-assay variance was 6.8%, and the interassay variance was 7.5%. The antibody does not cross-react with C-terminal or N-terminal fragments of PTH. The 25-(OH)-vitamin D in serum was measured by a commercially available radioimmunoassay after extraction with acetonitrile (25-(OH)-vitamin D RIA, Immuno Nuclear Corporation, Stillwater, Minnesota).

The $^{3}$H-25-(OH)-vitamin $D_3$ was used as tracer and synthetic 25-(OH)-vitamin $D_3$ to plot standard curves for radioimmunoassay. The lower detection limit of the test was 1.7 ng/mL. The intra-assay variance was 4%, and the interassay variance was 7.5%. The antibody does not cross-react with vitamin D and 1,25-$(OH)_2$-vitamin D, whereas vitamin $D_2$ and vitamin $D_3$ are not differentiated. The 1,25-$(OH)_2$-vitamin D was measured by a commercially available radioreceptor assay after extraction with acetonitrile and purification of vitamin D metabolites using a $C_{18}$-cartridge (1,25-$(OH)_2$-vitamin D radio receptor assay, Incstar Corp, Stillwater, Minnesota).[23,24] The $^{3}$H-1,25-$(OH)_2$-vitamin D was used as tracer and synthetic 1,25-$(OH)_2$-vitamin D to plot standard curves for radioreceptor assay. The lower detection limit of the test was 5 pg/mL. The intra-assay variance was 6.4%, and the interassay variance was 6.9%. Approximately 0.1% of 25-(OH)-vitamin $D_3$, 24,25-$(OH)_2$-vitamin $D_3$, and 25,26-$(OH)_2$-vitamin $D_3$ cross-react with the assay. The assay does not differentiate between vitamin $D_2$ and vitamin $D_3$.

## Calcium Absorption

Calcium absorption was estimated by measuring plasma radioactivity with a gamma counter (1282 Compu gamma, LKB Instruments, Gräfeling, Germany) 2 hours after a test meal containing $^{47}$Ca. The fraction of $^{47}$Ca absorbed was calculated according to the following equation[25]:

$$^{47}Ca_{abs} = \frac{^{47}Ca_{plasma} \times V}{^{47}Ca_{total}} \times 100\ \%,$$

where $^{47}Ca_{abs}$ is the absorbed calcium fraction, $^{47}Ca_{plasma}$ is the amount of $^{47}$Ca in plasma, V is extracellular volume, estimated as 0.15 x body weight, and $^{47}Ca_{total}$ is the amount of $^{47}$Ca contained in the test solution or in the test meal. The test solution to measure absorption of $CaCl_2$ consisted of 90 MBq $^{47}CaCl_2$ in 180 mg calcium gluconate in a total volume of 30 mLs. The test meal to measure absorption of $CaCO_3$ consisted of a crushed minipig pellet

diet, soaked with a solution of 90-MBq $^{47}CaCO_3$. Radiolabeled $^{47}CaCO_3$ solution was prepared by adding sodium carbonate to 90-MBq $^{47}CaCl_2$, thereby precipitating $^{47}CaCO_3$. The total amount of radioactivity of every test solution or test meal was 90 MBq. Absorption tests were done after an overnight fast with free access to water. The test solution to measure absorption of $CaCl_2$ was instilled into the anastomotic region endoscopically in a short anesthesia before surgery and at 3, 6, 9, and 12 months after surgery. The test meal to measure absorption of $CaCO_3$ was given before gastrectomy and at 3, 6, 9, and 12 months after gastrectomy. Before surgery and at 6, 9, and 12 months after surgery, the test meal consisted of 100 g of food containing 1000 mg of $CaCO_3$ with 90 MBq of $^{47}CaCO_3$. The feeding period was approximately 10 minutes in gastrectomized and sham-operated pigs. Three months after surgery, the test meal consisted of 50 g of food containing 500 mg of $CaCO_3$ with 90 MBq of $^{47}CaCO_3$. Gastrectomized pigs needed 30 minutes to eat up that amount of food, and sham-operated animals were given approximately one third of the 50 g every 10 minutes, because they ate much faster than did gastrectomized pigs. The $^{47}Ca$ in plasma was measured 2 hours after completion of the test meal, and the absorbed fraction was calculated according to the equation given above.

## Measurement of Bone Mineral Density and Trabecular Bone Mineral Density

Measurements were done by CT scan (Somatom DR 3 scanner, Siemens AG, Erlangen, Germany). Dual-energy CT with rapid kilovolt switching (85 and 125 kV), minimizing errors due to varying fat content of bone marrow, was used.[26–28] Data were evaluated on an Evaluoskop (Siemens AG, Erlangen, Germany) with the help of dedicated software (Dialog program EVA, Version D, Siemens AG, Erlangen, Germany). For each CT scan, the density of a calcium hydroxyapatite body was measured as reference value. The standard deviation of the reference value was below 1% throughout the study. The BMD and trabecular BMD (TBMD) were measured before surgery and in monthly intervals thereafter. For CT scans, animals were anesthetized for approximately 15 minutes by intramuscular injection of flunitrazepam (Hoffmann-La Roche, Grenzach, Germany, 2 mg) and ketamine (ketalar, Parke–Davis, Berlin, Germany, 10 mg) and brought into a supine position. Midvertebral slices of 4-mm thickness were selected from a lateral digital tomogram of the fourth lumbar vertebra. The borders of the vertebral body were defined automatically by the program, whereas the borders toward the epiphysis of the vertebral arches were drawn manually on the monitor. The selected region to determine BMD included cortical and trabecular vertebral bone, thus giving an average density of the whole area. In contrast, the area to measure TBMD was selected manually from the middle of the vertebral body, thus measuring density of trabecular vertebral bone only. To measure BMD and TBMD, an algorithm was used that registered signals between 200 and 2000 Hounsfield units (HU) only.

**Table 1. POSTOPERATIVE BODY WEIGHT (kg)***

| Age (mos) | Free Fed: Unoperated (n = 3) | Controlled Food Intake: Sham Operated (n = 6) | Controlled Food Intake: Total Gastrectomy (n = 6) |
|---|---|---|---|
| 4 | 12.2 ± 0.17 | 11.6 ± 0.72 | 11.8 ± 0.71 (NS) |
| 7 | 15.2 ± 0.17 | 12.5 ± 0.78† | 11.1 ± 0.7† |
| 10 | 21.5 ± 0.4 | 16.5 ± 1.02† | 12.4 ± 0.7†‡ |
| 13 | 28.6 ± 0.59 | 21.6 ± 1.34† | 13.7 ± 0.87†§ |
| 16 | 33.8 ± 0.64 | 27.0 ± 1.94† | 16.0 ± 0.87†§ |

NS = not significant.
* Sham-operated and gastrectomized animals underwent surgery at 4 months of age. Values are mean ± standard error of the mean.
† $p < 0.001$ *vs.* unoperated.
‡ $p < 0.005$ *vs.* sham operated.
§ $p < 0.001$ *vs.* sham operated.

## Statistical Analysis

Data of serum parameters are presented as mean ± standard error of the mean. Differences between groups were determined by analysis of variance followed by Fisher's least significant differences test. Data of BMD measurements are presented as mean ± standard deviation. Differences between groups were determined by two-sided Mann–Whitney test. Differences within groups were determined by two-sided Wilcoxon matched pairs signed-rank test. A probability of $p < 0.05$ was taken as significant.

# RESULTS

## Body Weight and Food Intake

Free-fed animals that were not operated on increased their body weight as expected from large breeding studies.[22,29] They weighed significantly more than did fed-matched sham-operated, or gastrectomized pigs throughout the study. However, the body weight increase over time was the same for pigs that were not operated on and for sham-operated pigs, the only difference being that weight increase was delayed by 3 months in sham-operated pigs (Table 1). There was a significant body weight

**Table 2. FOOD INTAKE AND CALCIUM INTAKE OF FED-MATCHED, SHAM-OPERATED, AND GASTRECTOMIZED PIGS**

| Postoperative Month | Food Intake [MJ/mo] | Calcium Intake [g/die]* |
|---|---|---|
| 1 | 54.5 | 0.86 |
| 2 | 61.1 | 0.97 |
| 3 | 63.0 | 1.0 |
| 4 | 65.2 | 1.18 |
| 5 | 68.3 | 1.52 |
| 6 | 71.5 | 2.03 |
| 7 | 76.7 | 2.59 |
| 8 | 92.3 | 3.24 |
| 9 | 101.1 | 3.55 |
| 10 | 117.5 | 4.06 |
| 11 | 132.0 | 4.47 |
| 12 | 132.6 | 4.96 |

* Mean of month.

difference between sham-operated and gastrectomized pigs from the 5th postoperative month onward (14.9 ± 0.9 kg *vs.* 11.8 ± 0.7 kg, $p < 0.002$). The daily food intake, which was designed equal for sham-operated and gastrectomized pigs, increased steadily over the course of the experiment. Accordingly, daily calcium intake also increased (Table 2).

## Serum Parameters

Serum calcium, serum phosphate, PTH, alkaline phosphatase, 25-(OH)-vitamin D, and 1,25-$(OH)_2$-vitamin D were not significantly different between groups at the beginning of the study. All parameters were not significantly different between free-fed and fed-matched sham-operated control pigs throughout the study.

Serum calcium and serum phosphate levels were decreased significantly 3, 6, and 9 months after surgery in gastrectomized pigs. However, serum calcium and serum phosphate started to increase in gastrectomized pigs from the 9th postoperative month onward and were not significantly different from those of control pigs by the end of the study (Table 3). The PTH was increased significantly in gastrectomized pigs throughout the study (Fig. 1). The 25-(OH)-vitamin D increased significantly in control pigs throughout the study, whereas it decreased slowly in gastrectomized animals. Accordingly, 25-(OH)-vitamin D was decreased significantly in gastrectomized pigs throughout the study (Table 4). The 1,25-$(OH)_2$-vitamin D decreased in control pigs during the study, being decreased further in gastrectomized pigs. However, from the 9th postoperative month onward, 1,25-$(OH)_2$-vitamin D increased in gastrectomized pigs and was increased significantly by the end of the study compared with that of control pigs (Table 4). The ratio of 1,25-$(OH)_2$-vitamin D25-(OH)-vitamin D decreased continuously in control pigs, whereas it was increased significantly in gastrectomized pigs (Fig. 2).

Alkaline phosphatase decreased throughout the study in free-fed and fed-matched sham-operated control pigs. It was decreased further in gastrectomized pigs, but increased toward the end of the study, not being significantly different from that of control pigs by the end of the study (Table 4).

## Calcium Absorption

Absorption of calcium carbonate and calcium chloride decreased significantly over the course of the study in

**Table 3. SERUM CALCIUM (MVAL/L) AND PHOSPHATE (MG/DL) LEVELS***

| | Free Fed | | Controlled Food Intake | | | |
|---|---|---|---|---|---|---|
| | Unoperated (n = 3) | | Sham Operated (n = 6) | | Total Gastrectomy (n = 6) | |
| Month | Calcium | Phosphate | Calcium | Phosphate | Calcium | Phosphate |
| Preoperative | 4.84 ± 0.01 | 7.53 ± 0.11 | 4.85 ± 0.04 | 7.48 ± 0.18 | 4.85 ± 0.02 | 7.50 ± 0.14 |
| 1 | 4.88 ± 0.01 | 7.73 ± 0.08 | 4.81 ± 0.04 | 7.63 ± 0.17 | 4.65 ± 0.02† | 6.57 ± 0.09† |
| 3 | 4.85 ± 0.01 | 7.50 ± 0.07 | 4.83 ± 0.02 | 7.00 ± 0.14 | 4.48 ± 0.05† | 5.82 ± 0.08† |
| 6 | 4.85 ± 0.02 | 6.70 ± 0.07 | 4.86 ± 0.02 | 6.75 ± 0.13 | 4.26 ± 0.05† | 5.33 ± 0.15† |
| 9 | 4.78 ± 0.02 | 5.87 ± 0.13 | 4.79 ± 0.05 | 5.87 ± 0.13 | 4.45 ± 0.05† | 5.37 ± 0.10† |
| 12 | 4.64 ± 0.02 | 5.73 ± 0.04 | 4.66 ± 0.02 | 5.72 ± 0.13 | 4.62 ± 0.04 | 5.70 ± 0.07 |

* Values are mean ± standard error of the mean.
† $p < 0.01$ *vs.* unoperated or *vs.* sham operated.

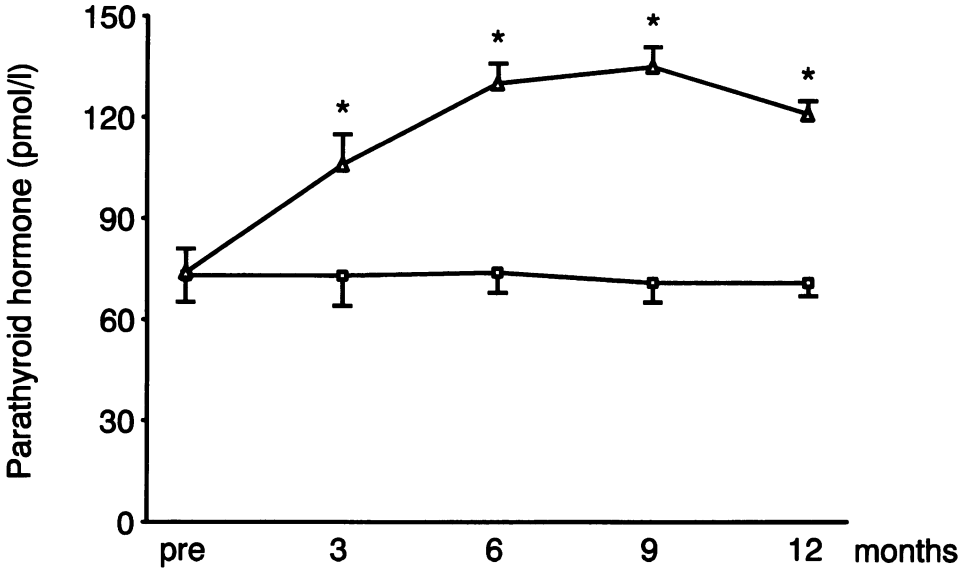


**Figure 1.** Parathyroid hormone (pmol/L) in gastrectomized pigs (triangles) and sham-operated control pigs (squares) before surgery, and at 3, 6, 9, and 12 months after surgery, respectively. Values are given as mean ± standard error of the mean. * $p < 0.01$ gastrectomized *vs.* sham-operated control pigs.

free-fed and fed-matched, sham-operated pigs, with no significant difference between calcium carbonate or calcium chloride absorption. Calcium absorption was decreased significantly in gastrectomized pigs 3 and 6 months after surgery (Table 5). Calcium chloride was absorbed significantly better than was calcium carbonate 3 months after surgery, but the difference was only minor (absorbed fraction; calcium chloride: 0.13 ± 0.016; calcium carbonate: 0.08 ± 0.008; $p < 0.05$). Thereafter, calcium absorption increased in gastrectomized pigs, being significantly increased compared with that of control pigs by the end of the study, with no difference between

**Table 4. SERUM PARATHYROID HORMONE [PTH], 25-(OH)-VITAMIN D, 1,25-$(OH)_2$-VITAMIN D AND ALKALINE PHOSPHATASE LEVELS IN UNOPERATED (N = 3), SHAM-OPERATED (N = 6) AND GASTRECTOMIZED (N = 6) PIGS PREOPERATIVELY, AND 3, 6, 9, AND 12 MONTHS POSTOPERATIVELY***

| | | Months Postoperative | | | |
|---|---|---|---|---|---|
| | Preoperative | 3 | 6 | 9 | 12 |
| PTH [pmol/L] | | | | | |
| Unoperated | 73 ± 3 | 72 ± 1 | 71 ± 2 | 71 ± 1 | 71 ± 1 |
| Sham operation | 74 ± 6 | 73 ± 7 | 74 ± 4 | 71 ± 4 | 71 ± 2 |
| Gastrectomy | 74 ± 5 | 106 ± 7† | 130 ± 4† | 135 ± 4† | 121 ± 2† |
| 25-(OH)-vitamin D [ng/mL] | | | | | |
| Unoperated | 15 ± 1.7 | 20 ± 0.6 | 26 ± 0.2 | 30 ± 0.4 | 43 ± 1.3 |
| Sham operation | 15 ± 1.8 | 20 ± 1.3 | 21 ± 2.7 | 27 ± 3.2 | 40 ± 6.1 |
| Gastrectomy | 14 ± 1.5 | 12 ± 1.4† | 12 ± 1.3† | 12 ± 2.2† | 12 ± 0.3† |
| 1,25-$(OH)_2$-vitamin D (pg/L) | | | | | |
| Unoperated | 108 ± 2 | 126 ± 2 | 116 ± 2 | 79 ± 2 | 42 ± 5 |
| Sham operation | 109 ± 3 | 117 ± 3 | 116 ± 2 | 85 ± 2 | 46 ± 5 |
| Gastrectomy | 108 ± 6 | 84 ± 5† | 68 ± 1† | 71 ± 1† | 80 ± 1† |
| Alkaline phosphatase (units/L) | | | | | |
| Unoperated | 192 ± 4 | 199 ± 2 | 169 ± 1 | 100 ± 2 | 95 ± 1 |
| Sham operation | 190 ± 3 | 197 ± 2 | 174 ± 2 | 99 ± 1 | 95 ± 2 |
| Gastrectomy | 193 ± 4 | 83 ± 3† | 57 ± 2† | 51 ± 1† | 91 ± 1 |

* Values are mean ± standard error of the mean.
† $p < 0.01$ *vs.* unoperated or *vs.* sham operated.

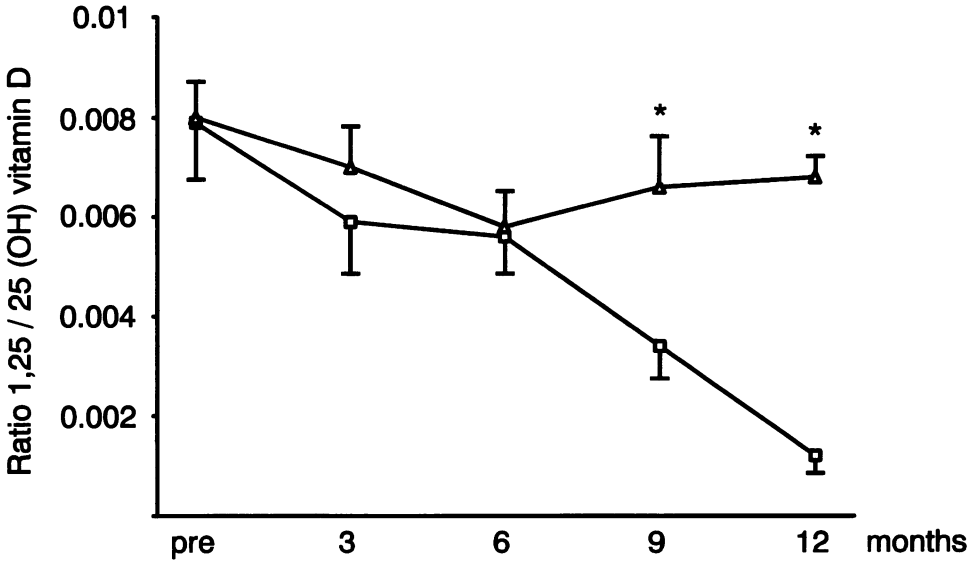


**Figure 2.** Ratio of 1,25-$(OH)_2$-vitamin D to 25-(OH)-vitamin D in gastrectomized pigs (triangles) and sham-operated control pigs (squares) before surgery, and at 3, 6, 9, and 12 months after surgery, respectively. * $p < 0.01$ gastrectomized *vs.* sham-operated control pigs.

calcium chloride and calcium carbonate absorption (Table 5).

## Bone Mineral Density

The BMD and TBMD were not significantly different between groups at the beginning of the study (BMD: 358.0 ± 2.2, 357.0 ± 6.3, 357.0 ± 2.6 mg calcium hydroxyapatite/mL, not significant). In free-fed pigs that were not operated on and in fed-matched sham-operated pigs, BMD and TBMD increased slightly, reaching a plateau of approximately 400-mg calcium hydroxyapatite/mL by the end of the study. In contrast, BMD and TBMD of gastrectomized pigs were decreased throughout the study compared with that of control pigs. The difference was significant from the 1st postoperative month onward (BMD: free-fed pigs that were not operated on: 369.0 ± 1.7; fed-matched pigs that were not operated on: 360.0 ± 6.5; gastrectomized pigs: 334.0 ± 2.7; $p < 0.01$). The BMD of gastrectomized pigs increased slightly toward the end of the study, whereas TBMD stayed low (Table 6, Fig. 3).

## DISCUSSION

In the present study, we investigated bone metabolism in free-fed pigs that were not operated on, fed-matched,

**Table 5. CALCIUMCARBONATE AND CALCIUMCHLORIDE ABSORPTION OF UNOPERATED (N = 3), SHAM OPERATED (N = 6), AND GASTRECTOMIZED (N = 6) PIGS PREOPERATIVELY, AND 3, 6, 9, AND 12 MONTHS POSTOPERATIVELY***

| | | Months Postoperative | | | |
|---|---|---|---|---|---|
| | Preoperative | 3 | 6 | 9 | 12 |
| Calciumcarbonate | | | | | |
| Unoperated | 65 ± 0.7 | 53 ± 0.8 | 40 ± 1.1 | 32 ± 1.1 | 18 ± 1.8 |
| Sham operation | 63 ± 0.8 | 50 ± 1.1 | 41 ± 0.8 | 31 ± 0.9 | 20 ± 1.2 |
| Gastrectomy | 63 ± 1.5 | 8 ± 0.8† | 14 ± 1.2† | 27 ± 1.8 | 31 ± 1.0† |
| Calciumchloride | | | | | |
| Unoperated | 64 ± 3.6 | 52 ± 2.5 | 39 ± 1.0 | 27 ± 1.1 | 15 ± 1.1 |
| Sham operation | 62 ± 1.8 | 50 ± 1.4 | 40 ± 1.0 | 28 ± 0.8 | 16 ± 0.9 |
| Gastrectomy | 63 ± 1.4 | 13 ± 1.6†‡ | 17 ± 1.6† | 30 ± 1.1 | 32 ± 1.4† |

* Values are % of applied radioactivity in serum 2 hours after oral application and expressed as mean ± standard error of the mean.
† $p < 0.01$ *vs.* unoperated or *vs.* sham operated.
‡ $p < 0.05$ *vs.* calciumcarbonateabsorption of gastrectomized pigs 3 months postoperatively.

**Table 6. BONE MINERAL DENSITY (BMD) AND TRABECULAR BONE MINERAL DENSITY (TBMD) OF UNOPERATED (N = 3), SHAM OPERATED (N = 6) AND GASTRECTOMIZED (N = 6) PIGS, PREOPERATIVELY AND 3, 6, 9 AND 12 MONTHS POSTOPERATIVELY**

| | | Months Postoperative | | | |
|---|---|---|---|---|---|
| | Preoperative | 3 | 6 | 9 | 12 |
| Bone mineral density | | | | | |
| Unoperated | 358 ± 2.2 | 390 ± 1.4 | 398 ± 0.9 | 401 ± 0.2 | 402 ± 0.4 |
| Sham operation | 357 ± 6.3 | 380 ± 7.2 | 393 ± 9.9 | 400 ± 10.3 | 402 ± 13.5 |
| Gastrectomy | 356 ± 2.6 | 299 ± 3.3† | 262 ± 3.2† | 259 ± 3.0† | 274 ± 3.0†‡ |
| Trabecular bone mineral density | | | | | |
| Unoperated | 388 ± 2.40 | 382 ± 1.5 | 388 ± 1.2 | 393 ± 0.5 | 397 ± 0.2 |
| Sham operation | 387 ± 2.5 | 388 ± 2.1 | 384 ± 2.6 | 391 ± 2.3 | 396 ± 2.8 |
| Gastrectomy | 388 ± 10.0 | 286 ± 7.3† | 247 ± 9.5† | 243 ± 8.9† | 245 ± 9.6† |

* Values are given as mg calcium hydroxylapatite/mL bone volume of the 4th lumbar vertebral body and expressed as mean ± standard deviation of mean.
† $p < 0.01$ *vs.* unoperated or *vs.* sham operated.
‡ $p < 0.01$ *vs.* bone mineral density of gastrectomized pigs 9 months postoperatively.

sham-operated, and gastrectomized pigs over a period of 12 months. Free-fed pigs that were not operated on and fed-matched, sham-operated pigs did not differ significantly in any parameter investigated, except for body weight gain, which was delayed by 3 months in sham-operated pigs, thus probably reflecting the operative trauma. To ease understanding, we only will refer to sham-operated and gastrectomized animals in our discussion.

Gastrectomy in pigs resulted in a serum calcium decrease and reduced calcium absorption, which was decreased by 85% 3 months after gastrectomy. A reduced calcium absorption after gastrectomy is well documented.[30,31] This probably is due to exclusion of the duodenum and the upper jejunum from the food passage by surgical reconstruction, due to both are the major sites of calcium absorption under physiological conditions.[32] Additionally, rapid food transit allowing less time for absorption and steatorrhea leading to the formation of insoluble calcium soaps might contribute to calcium malabsorption.[3,33,34] Calcium absorption improved in gastrectomized pigs by the end of the study. This correlates with an increase in 1,25-$(OH)_2$-vitamin D in gastrectomized pigs toward the end of the study, since 1,25-$(OH)_2$-vitamin D stimulates the transport of calcium from the small intestinal lumen into circulation.[35] Different calcium prep-

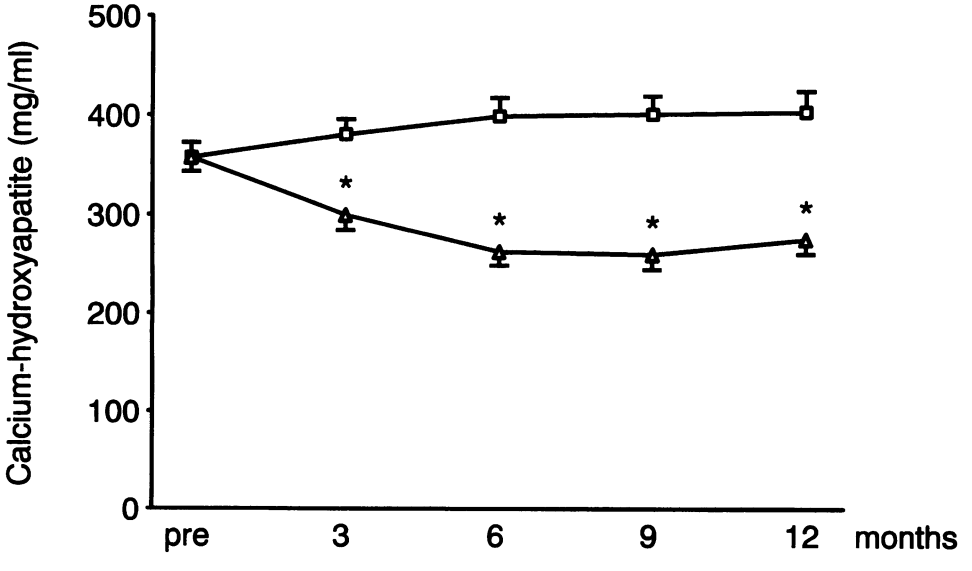


**Figure 3.** Bone mineral density (calcium hydroxyapatite, mg/mL) in gastrectomized (triangles) and sham-operated control pigs (squares) before surgery, and at 3, 6, 9, and 12 months after surgery, respectively. Values are given as mean ± standard deviation. * $p < 0.01$ gastrectomized *vs.* sham-operated control pigs.

arations have been used to supplement the diet, including calcium chloride and calcium carbonate, but there was little difference in intestinal utilization of calcium from these salts.[32] In our study, calcium chloride and calcium carbonate were absorbed equally. In contrast to our experimental data, a decrease in serum calcium often is not observed after gastrectomy in humans.[3,7–9,11] However, when patients who were gastrectomized are compared with matched healthy subjects, a significant decrease in serum calcium has been reported, although serum calcium remains in the normal range.[13,36] Possibly, a slight decrease in serum calcium after gastrectomy is followed by immediate counter-regulation of the body, because maintenance of circulating calcium within a narrow range is critical for body homeostasis and survival.[32] Any tendency toward hypocalcemia is counteracted by an increased rate of PTH release.[35] Therefore, an increase in PTH secretion could be expected after gastrectomy. In our study, serum levels of PTH increased by 90% after gastrectomy compared with that of fed-matched, sham-operated control pigs. In accordance with our data, increased serum PTH levels and increased urinary excretion of nephrogenic cyclic adenosine monophosphate (cAMP), an extremely sensitive parameter of parathyroid function, have been observed in gastrectomized patients.[11,37,38]

Intestinal calcium absorption, vitamin D, and bone metabolism are closely related.[35,39] Vitamin D, which is either formed from cholesterol by photobiogenesis in the skin or contained in food and absorbed from the small intestine, is hydroxylated consecutively to 25-(OH)-vitamin D in the liver and to 1,25-$(OH)_2$-vitamin D, its active form, in the kidney.[35,39] In our study, 25-(OH)-vitamin D in sham-operated pigs increased significantly during the study, possibly reflecting an increased need for vitamin D in the growing phase. In gastrectomized pigs, 25-(OH)-vitamin D was reduced by 70% compared with that of sham-operated pigs by the end of the study. In gastrectomized patients, most studies report a decrease of 25-(OH)-vitamin D in serum.[6,11,13,36,38,40] Impaired absorption, insufficient intake, or both have been suggested as the cause of low 25-(OH)-vitamin D after gastrectomy.[12,41–43] Since vitamin D intake was identical in sham-operated pigs and gastrectomized pigs in our study, vitamin D malabsorption may be the major cause of low 25-(OH)-vitamin D in serum after gastrectomy. The 25-(OH)-vitamin D is hydroxylated to 1,25-$(OH)_2$-vitamin D by 1$\alpha$-hydroxylase in the kidney.[35] The 1$\alpha$-hydroxylase is stimulated by PTH as a counter-regulatory mechanism to hypocalcemia.[35] Since we measured increased PTH levels in serum after gastrectomy, increased 1,25-$(OH)_2$-vitamin D in serum would be expected. In fact, we observed increased 1,25-$(OH)_2$-vitamin D levels after gastrectomy in pigs by the end of the study. Additionally, the 1,25-$(OH)_2$-vitamin D25-(OH)-vitamin D ratio was increased threefold. These data are consistent with increased 1,25-$(OH)_2$-vitamin D levels in patients after gastrectomy.[2,8,38]

The BMD and TBMD were significantly decreased after gastrectomy in pigs. Quantitative CT scan osteodensitometry is a reliable method to measure BMD and TBMD with a coefficient of variation below 5%.[26–28,44,45] Using this method, we could show that BMD and TBMD were reduced by 32% and 38%, respectively, 1 year after gastrectomy in pigs. This is in accordance with a variety of studies, which report a reduced BMD after gastrectomy in patients.[2,3,6–9,40,45–48] The incidence of postgastrectomy bone disease varies widely, and incidence rates up to 69% in females who were gastrectomized have been described.[6]

Gastrectomized pigs were underweight significantly compared with sham-operated pigs. Malabsorption and malnutrition *per se* could have disturbed normal bone growth and bone calcification. Calcium absorption was decreased significantly in gastrectomized pigs after surgery, and a mild degree of fat or protein malabsorption after gastrectomy has been reported repeatedly.[49,50] Recently, we reported a reduced food intake and a decrease in serum albumin and total protein 6 months after gastrectomy in pigs,[51] indicating a certain degree of malnutrition. Because protein intake and serum albumin have been shown to be correlated positively with bone mass or bone mineral content in humans,[52,53] postgastrectomy malabsorption and malnutrition might have interfered with normal bone growth and bone calcification, at least temporarily.

When bone metabolism is investigated, bone can be divided histologically into areas of quiescence, resorption, or formation. Each bone remodeling unit continuously runs through the cycle of quiescence, bone resorption, and bone formation, a process called "bone turnover."[54,55] In metabolic bone disease, low, regular, and high bone turnover states exist and describe disturbances in the dynamic process of bone remodeling.[55] Depending on whether the resorptive or the formative phase dominates, a negative or a positive bone mass balance results, with bone mass loss or bone mass gain, respectively. Accordingly, bone mass loss can be present with low, regular, or high bone turnover.

Bone turnover can be evaluated by biochemical markers in serum or by histomorphometric evaluation of the bone.[2,55] We measured alkaline phosphatase, which is a well-known parameter of bone turnover.[55] In our study, we were investigating pigs in the growing phase, where an increased bone formation is expected and probably reflected by increased serum levels of alkaline phosphatase, as seen in sham-operated pigs. In growing gastrectomized pigs, alkaline phosphatase was reduced, possibly

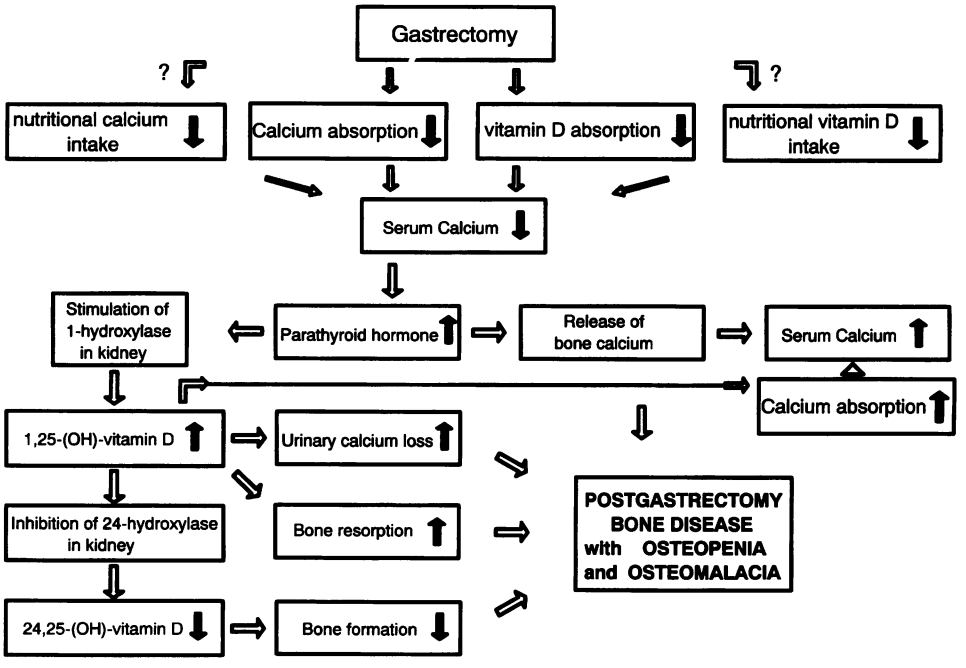


**Figure 4.** Proposed mechanism for postgastrectomy bone disease, adapted from the literature and our study (refer to Discussion section).

reflecting a reduced bone formation compared with that of sham-operated pigs, This contrasts with that of gastrectomy patients, where an increased alkaline phosphatase often is observed[2,6,8,38,40] and probably reflects an increased bone turnover with a net bone mass loss.[2,9,55] Reduced bone formation, as assumed in growing gastrectomized pigs, or bone mass loss as observed in gastrectomized patients,[2,3,6–9,40,45–48] would both result in calcium gain for body homeostasis, suggesting that a decrease in alkaline phosphatase in growing gastrectomized pigs could be consistent with increased levels of alkaline phosphatase in gastrectomized patients.

Additional parameters to evaluate bone turnover-like osteocalcin, propeptide of type I procollagen, urinary hydroxyproline, or histomorphometric bone analysis exist.[2,40,55] In patients who were gastrectomized, osteocalcin, a biochemical marker of osteoblast activity, propeptide of type I procollagen, a marker of collagen formation, and urinary excretion of hydroxyproline, a parameter of bone resorption, all have been found to be increased.[40] Histomorphometric analysis of bone turnover after gastrectomy indicated an increased osteoid volume, but mineralization lag time and tetracycline label takeup results were normal.[2] Taken together, biochemical markers and histomorphometric data indicate an increased bone turnover and an increased bone resorption with a net bone mass loss after gastrectomy. However, the critical defect of postgastrectomy bone disease still is unknown.

The underlying mechanism of postgastrectomy bone disease might be a calcium deficit, which in turn increases PTH release. The PTH stimulates osteoclast activity and 1$\alpha$-hydroxylase in the kidney, resulting in increased 1,25-$(OH)_2$-vitamin D.[35] The 1,25-$(OH)_2$-vitamin D increases calcium absorption from the small intestine, but also increases bone resorption and urinary calcium loss, resulting in a net calcium loss.[56] Further, 1,25-$(OH)_2$-vitamin D inhibits 24-hydroxylation of 25-(OH)-vitamin D to 24,25-$(OH)_2$-vitamin D,[56,57] which is thought to be essential for bone formation.[58] We did not measure 24,25-$(OH)_2$-vitamin D, but studies have shown that 24,25-$(OH)_2$-vitamin D is decreased after gastrectomy in patients.[2,8,38] Thus, the endocrinologic data from our study showing an increase in PTH and 1,25-$(OH)_2$-vitamin D would suggest increased bone resorption and reduced bone formation, which are consistent with reduced BMD in gastrectomized pigs.

A variety of factors possibly contributing to postgastrectomy bone disease have been suggested: calcium or vitamin D malabsorption, reduced food intake, reduced calcium or vitamin D intake, changed food preferences, milk intolerance, reduced sun exposure, or reduced body exercise. It is impossible to control all these factors in patients, and it is thereby difficult to decide which factors are decisive. In our study, food intake, including calcium and vitamin D intake, was designed to be equal in sham-operated and gastrectomized pigs. Further, exposure to ultraviolet, a radiation for photobiosynthesis of vitamin D in the skin, was equal for all pigs, and body exercise was not obviously different between sham-operated and gastrectomized pigs. Therefore, from our experimental

data, we would suggest that reduced calcium and vitamin D absorption are major contributors to reduced BMD after gastrectomy.

From the literature and our data, we would propose the following mechanism for postgastrectomy bone disease (Fig. 4): Calcium and vitamin D absorption are reduced after gastrectomy, resulting in decreased serum calcium. Any decline in serum calcium is counter-regulated by PTH release to ensure calcium and body homeostasis. The PTH stimulates osteoclast activity in the bone and 1$\alpha$-hydroxylase activity in the kidney, resulting in the release of bone calcium and the increase of 1,25-$(OH)_2$-vitamin D in serum. The 1,25-$(OH)_2$-vitamin D increases calcium absorption from the small intestine, bone resorption, and urinary calcium loss, resulting in a net calcium loss. Further, 1,25-$(OH)_2$-vitamin D inhibits 24-hydroxylase in the kidney, resulting in reduced 24,25-$(OH)_2$-vitamin D levels in the serum, which is necessary for bone formation. Increased bone resorption and decreased bone formation will result in bone mass loss after gastrectomy.

In summary, this is the first study comparing bone metabolism of gastrectomized pigs with that of fed-matched sham-operated control pigs. Obviously, such a study is impossible to perform in patients. From our experimental data, we propose that patients who are gastrectomized require calcium and vitamin D supplementation, as it has been suggested previously.[10,59] However, convincing data proving that calcium and vitamin D supplementation is successful in preventing postgastrectomy bone disease still are lacking, and there currently is no generally acknowledged supplementation regimen. Further studies are needed to investigate the benefit of calcium and vitamin D supplementation for gastrectomy patients to prevent the detrimental consequences of postgastrectomy bone disease.

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