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Disorders of bone metabolism caused by small bowel resection in rats

Hiroshi UNO[1], Yoshihiro IZAWA[1], Tamotsu KOYAMA[1], Tokutaro MAKITA[1], Mitsugi SUGIYAMA[2], and Norimoto URAKAWA[3]
[1]*Second Bio-Medical Research Laboratories, Teijin Limited, Tokyo 191, Japan;*
[2]*Department of critical care and emergency medicine, Yokohama City University School of Medicine, Yokohama 232, Japan; and*
[3]*Department of Veterinary Pharmacology, Nippon Veterinary and Zootechnical College, Tokyo 180, Japan*

Summary: Disorders of bone metabolism caused by resection of three quarters of the small bowel in rats were investigated biochemically and histomorphologically. Metabolic bone disorders developing 90 days after in 75%-distal-small-bowel resected rats were characterized by reduction in ash content of the femur and by the disappearance of the trabecular bone in tibial metaphysis. Biochemical studies showed significant decrease in serum Ca and 1,25-dihydroxyvitamin D concentrations in 75% distal small bowel resected rats. These data suggest that 75% distal small bowel resection impairs intestinal absorption of calcium and results in a negative calcium balance, which may contribute to the development of bone metabolic disorder in rats. On the other hand, 75% proximal small bowel resection causes no obvious metabolic bone disorders in rats, possibly because of the adaptation by the remaining part of the intestine. *Gastroenterol Jpn 1990;25:693–699*

Key words: *bone metabolic disorder; small bowel resection*

Introduction

As is well known, the nutritional status is the essential factor to maintain bone metabolism. Recent advances in medical technology have enabled extensive resection of digestive organs. However, complications caused by various types of nutritional malabsorption are known to occur after extensive resections of digestive organs. For example, it is well known that gastrectomy results in metabolic bone diseases such as osteomalacia and/or osteoporosis[1-3]. On the other hand, the effects of small bowel resection on bone mineral metabolism are still obscure[4].

Therefore, in order to investigate the mechanism of development of disorders of bone metabolism dependency on the site of resection, we performed 75% small bowel resection in rats.

Materials and Methods

Forty-five 6-week-old male Wistar rats were used in the experiment. Thirty animals underwent resection of 75% of the small bowel. Under pentobarbital anesthesia the abdomen was opened with a midline incision, and the small bowel was spread out on a moist towel. According to Nygaard's report[5], the section of small bowel to be removed was defined by counting successive segments of equal length (4–5 cm). In every instance the measurement was carried out at least twice, once from above downwards, and once in the opposite direction. The desired length of small bowel was then removed, and countinuity was re-established by an end-to-end anastomosis. The mean length of resected intestine was 68.0 cm. In 75% proximal resections the anastomosis was made 1 cm below the ligament of Treitz (proximal resection group),

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Address for correspondence: Hiroshi Uno, 2ND Bio-Medical Research Laboratories, Teijin Limited, 4-3-2 Asahigaoka, Hino, Tokyo 191, Japan.

and in 75% distal resections 1 cm above the ileocaecal valve (distal resection group). The remaining 15 animals were subjected to laparotomy as a sham surgical procedure. The resected animals were reared without any treatment for 2 weeks after surgery. All animals were kept in individual stainless steal wire cages and fed a standard rat chow diet (CE-2, Crea Japan Inc., Tokyo, Japan) containing 1% calcium, 1% phosphorus and adequate amounts of vitamin D. Tap water was available *ad libitum*.

General symptoms were observed daily, and body weight and food intake were measured once a week throughout the duration of the experiment. An analysis of fat in the feces was performed at 3,6,9 and 12 weeks after the initiation of the observation. The fecal samples were obtained from animals in metabolic cages (Natsume Inc., Tokyo, Japan). The fat content in the feces and the diet were determined by using a modification of Saxon's method[6].

Blood samples were drawn from the carotid arteries of all animals under pentobarbital anesthesia after the end of 45 days and 90 days of observation. The total protein (T. Protein), albumin, creatinine (Cr), Blood urea nitrogen (BUN), Alp, Ca and inorganic phosphorus (Pi) were measured using an autoanalyzer (Hitachi 736-20, Hitachi Co. Ltd., Tokyo, Japan). The serum concentrations of 25-hydroxyvitamin D (25(OH)D), 24,25-dihydroxyvitamin D (24,25-$(OH)_2D$) and 1,25-dihydroxyvitamin D (1,25$(OH)_2D$) were also measured. 25(OH)D and 24,25$(OH)_2D$ were measured using competitive protein binding assay[7]. 1,25$(OH)_2D$ was measured using a radioreceptor assay[8].

Soft tissue was removed from the left femur. After measuring the bone volume using a plethysmometer (TK-101, Unicom Co., Chiba, Japan), the left femur was dried for 5 days at 110°C in an oven and the dry weight was recorded. After weighing the dried bones, they were reduced to ashes at 650°C for 5 hours using porcelain crucibles to obtain the ash weight. The ash was dissolved in 6N-HCl solution to determine Ca and Pi contents.

The tibial bones were fixed in 70% ethanol and embedded undecalcified in methylmethacrylate. Frontal sections of proximal tibia were sawed at 200 μm, ground to 80 μm thickness. Then, the sections were examined histologically using contact microradiography (CMR).

The data were expressed as means±SD, and the statistical analysis was performed by Student's

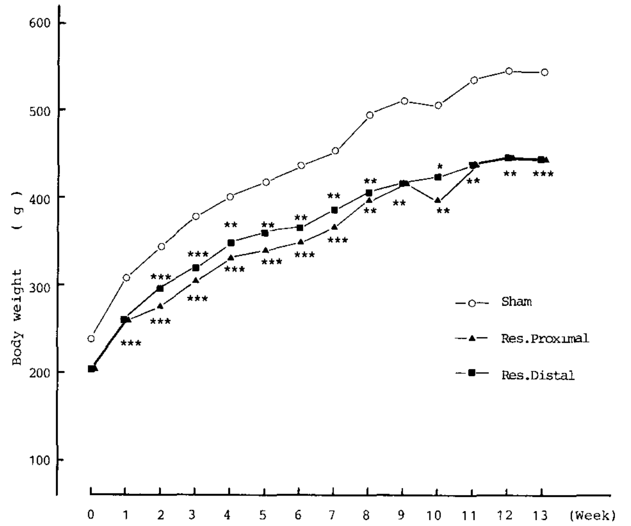

Fig. 1 Body weight changes in small-bowel-resected rats.
Res: Resection
Significantly different from sham group
(*: P<0.05, **: P<0.01, ***: P<0.001)

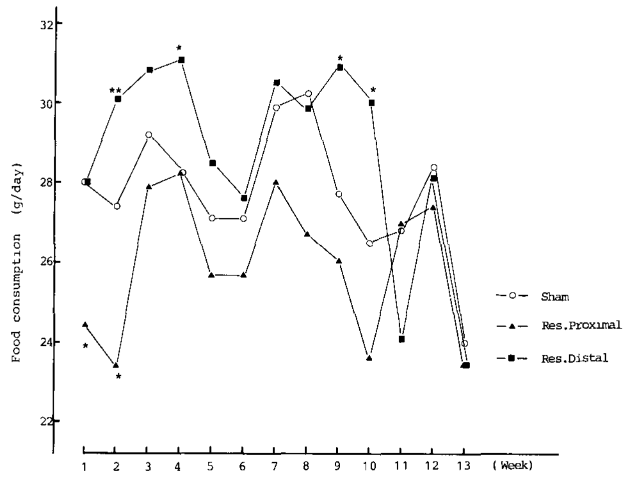

Fig. 2 Food consumption changes in small-bowel-resected rats.
Res: Resection
Significantly different from sham group
(*: P<0.05, **: P<0.01)

t-test (two tailed) at a significance level of more than 95%.

Results

Changes in body weight during the experiment are shown in **Figure 1**. The body weights of the proximal and the distal resection rats were significantly lower than those of the sham rats throughout the experiment. No obvious differences were observed in the proximal and distal resection groups.

As shown in **Figure 2**, food consumption data revealed a significant decrease in the proximal resection group compared to the sham group sporadically during the experiment. On the other hand, the food consumptions of the distal resection group were sporadically significantly higher than those of the sham group. **Table 1** shows the results of the fecal analysis. The fecal fat and the percent excretion of administered fat into feces were significantly higher or tended to be higher in the distal resection group, as compared with the sham group. On the other hand, no obvious changes were observed in the proximal resection group.

Table 1 Fecal analysis in small-bowel-resected rats

Fecal fat (g/100g feces)

Group	Observation period (Week)			
	3	6	9	12
Sham	2.52±0.59	4.20±0.27	2.77±0.44	2.83±0.41
Res. Proximal	2.63±0.89	4.43±0.67	3.25±0.90	3.39±1.02
Res. Distal	4.31±1.17*	6.71±1.39*	5.67±2.05	3.67±1.02

Excreted fat ratio to food intake (%)

Group	Observation period (Week)			
	3	6	9	12
Sham	41.1±10.7	33.1± 2.3	17.0± 4.9	15.7± 4.2
Res. Proximal	35.6±15.7	34.6± 9.5	21.1± 8.1	19.8± 6.3
Res. Distal	55.1± 7.3	55.2±22.0	48.9±24.1	30.4±10.4**

Res: Resection
Significantly different from sham group (*: P<0.05, **: P<0.01)
Mean±SD (n= 4-7)

Table 2 Serum biochemical findings in small-bowel-resected rats

(after 45-day observation period)

Group	T.P. (g/dl)	Alb. (g/dl)	Cr. (mg/dl)	BUN (mg/dl)	Alp (u/l)	Ca (mg/dl)	Pi (mg/dl)
Sham	5.6±0.3	2.3±0.1	0.52±0.04	19± 3	174±29	10.04±0.19	6.4±0.5
Res. Proximal	5.1±0.2**	2.0±0.4*	0.55±0.09	22± 6	161±39	10.21±0.43	7.3±0.9*
Res. Distal	4.9±0.2**	1.9±0.1**	0.54±0.11	24±11	182±48	10.00±0.40	8.6±1.9*

(after 90-day observation period)

Group	T.P. (g/dl)	Alb. (g/dl)	Cr. (mg/dl)	BUN (mg/dl)	Alp (u/l)	Ca (mg/dl)	Pi (mg/dl)
Sham	5.1±0.2	1.9±0.1	0.58±0.09	15.7±3.5	117±35	10.03±0.32	6.1±1.2
Res. Proximal	5.3±0.3	2.0±0.1	0.63±0.08	18.1±1.7	100±31	9.89±0.38	5.9±0.7
Res. Distal	4.6±0.6*	1.7±0.2	0.60±0.12	18.8±4.0	138±47	9.56±0.46*	6.8±1.6

Res: Resection
Significantly different from sham group (*: P<0.05, **: P<0.01)
Mean±SD (n= 7, 8)

Table 3 Serum concentrations of vitamin D metabolites

(after 45-day observation period)

Group	25(OH)D (ng/ml)	1,25(OH)$_2$D (pg/ml)	24, 25(OH)$_2$D (ng/ml)
Sham	8.8±2.2	75.0±30.0	8.7±0.4
Res. Proximal	6.4±1.1	46.8± 9.1	9.3±2.5
Res. Distal	6.2±2.8	36.2±21.5*	6.7±3.8

(after 90-day observation period)

Group	25(OH)D (ng/ml)	1,25(OH)$_2$D (pg/ml)	24, 25(OH)$_2$D (ng/ml)
Sham	9.7±4.0	50.2±15.6	6.3±1.0
Res. Proximal	8.2±1.7	35.4± 8.3	6.4±3.5
Res. Distal	6.6±1.3	30.5±10.0*	4.1±0.4**

Res: Resection
Significantly different from sham group (*: $P<0.05$, **: $P<0.01$)
Mean±SD (n= 4-6)

The results of serum biochemical tests are shown in **Table 2**. At the end of 45 days of observation, the total protein and albumin were significantly lower in the proximal and distal resection groups, Pi was significantly higher in the proximal and distal resection groups than in the sham group. At the end of 90 days of observation, the total protein and serum Ca were significantly lower in the distal resection group than in the sham group.

Table 3 shows the serum concentrations of vitamin D metabolites. At the end of 45 days of observation, the 1,25(OH)$_2$D concentration was significantly lower in the distal resection group than in the sham group. On the other hand, the 1,25(OH)$_2$D and 24,25(OH)$_2$D concentrations were significantly decreased in the distal resection group compared to the sham group at the end of 90 days of observation.

The results of analysis of the chemical composition of the femur are given in **Table 4**. At the end of 45 days of observation, no significant differences were observed between the proximal and distal resection groups. On the other hand, the ash content per unit volume was significantly lower in the proximal and distal resection groups than in the sham group at the end of 90 days of observation. The ash content per unit dry weight was also significantly lower in the distal resection group than in the sham-operated group. Furthermore, Ca and Pi contents per unit dry weight tended to be lower in the distal resection group than in the sham group.

Figure 3 shows the CMR of each group. At the end of 45 days of observation, no obvious changes were observed in the proximal and distal resection groups. On the other hand, remarkable reduction in trabecular bone of the metaphysis was observed in the distal resection group compared to the sham group at the end of 90 days of observation.

Discussion

In this study, we found bone metabolic disorders

Table 4 Composition of the femur in small-bowel-resected rats

(after 45-day observation period)

Group	Ash/Vol (mg/ml)	Ash/Dry W. (%)	Ca/Dry W. (%)	Pi/Dry W. (%)
Sham	900.1±33.0	68.5±0.7	21.6±3.6	11.0±1.7
Res. Proximal	870.2±71.3	68.3±0.8	21.3±3.8	10.7±1.8
Res. Distal	878.2±46.1	69.0±0.4	20.6±3.3	10.6±1.6

(after 90-day observation period)

Group	Ash/Vol (mg/ml)	Ash/Dry W. (%)	Ca/Dry W. (%)	Pi/Dry W. (%)
Sham	805.2±31.5	70.6±0.6	25.0±0.6	12.1±0.3
Res. Proximal	774.9±16.9*	70.1±0.5	24.9±0.6	12.2±0.5
Res. Distal	719.5±49.1**	68.9±1.4*	21.8±4.8	10.8±2.2

Res: Resection
Significantly different from sham group (*: $P<0.05$, **: $P<0.01$)
Mean±SD (n= 7, 8)

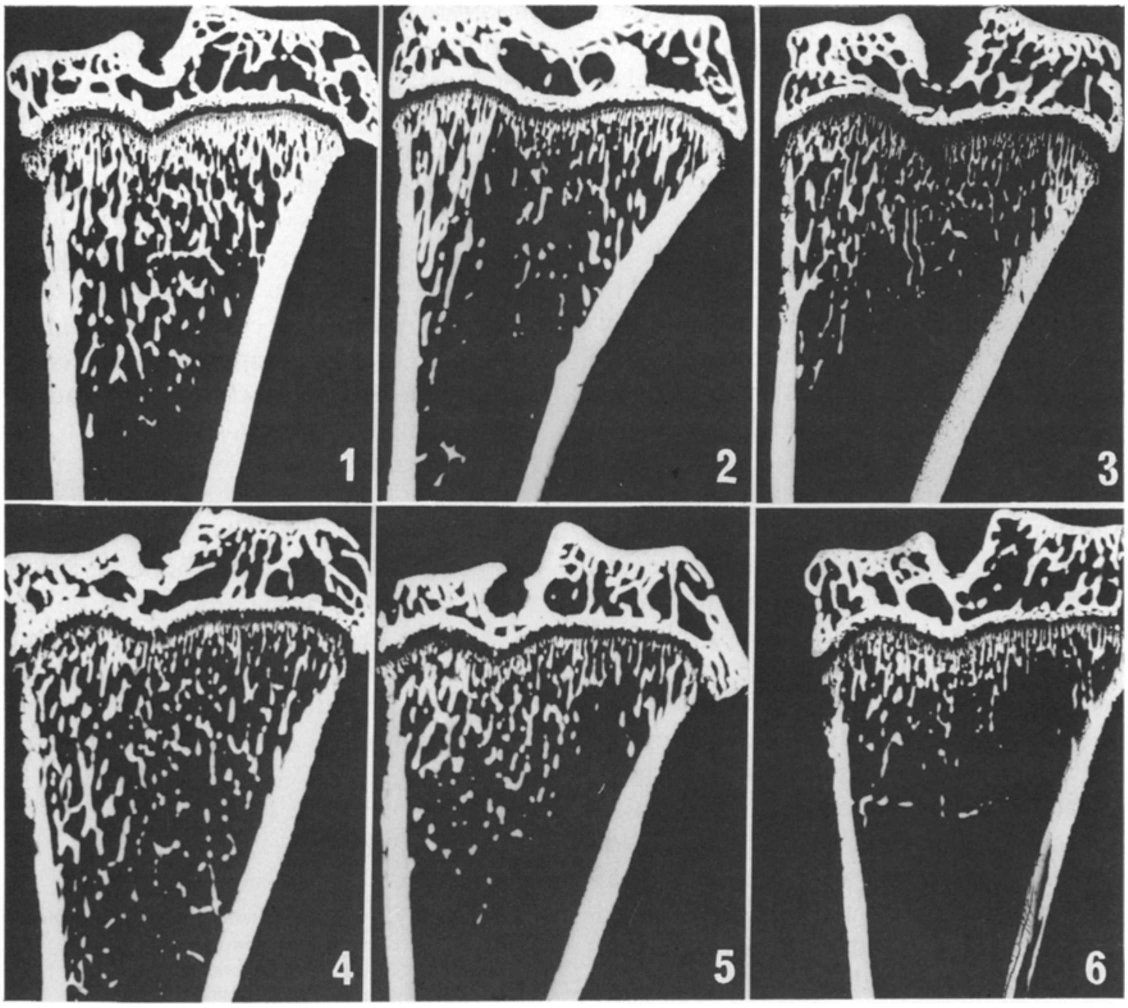

Fig. 3 Contact microradiogram of the longitudinal ground section from the proximal end of tibia. (×8)
1: Sham at the end of 45-day observation
2: Proximal resection at the end of 45-day observation
3: Distal resection at the end of 45-day observation
4: Sham at the end of 90-day observation
5: Proximal resection at the end of 90-day observation
6: Distal resection at the end of 90-day observation

at 90 days following removal of 75% of the small bowel of 6-week-old male rats. Specifically, as shown in the photo, there was a marked decrease in the tibial metaphyseal trabecular bone in the 75% distal resection group compared with the sham-operated group. In serum biochemical tests, serum Ca concentration levels were significantly lower in the distal resection rats than in sham-operated animals. This data suggests that suppressed Ca absorption in the intestines is an important cause of disorders of bone metabolism found in 75%-distal-small-bowel-resected rats.

The causes of decreased Ca absorption in the intestine following small bowel resection include not only a decreased volume of Ca absorption sites, but also steatorrhea[9]. Specifically, insoluble Ca soap may be produced from the fatty acids and Ca that remain in the intestines as a results of fat malabsorption. Steatorrhea is a well-recognized complication of ileal resection[10]. In this study, the significant increase in the fecal fat and the percent excretion of administered fat into feces in the distal resection group, indicating that fat malabsorption was caused by 75% distal small bowel resection. There are few reports about the concentrations of vitamin D metabolites after small bowel resection. According to Compston et al., 25(OH)D plasma levels were significantly reduced in a group of patients with small bowel resections when compared with normal controls[11]. In contrast, Colette et al. reported that plasma $1,25(OH)_2D$ concentration was markedly reduced in a group of patients with extensive small bowel resections compared with controls[12]. Thus, much remains unclear about the correlation between the causes of small bowel resection bone pathology and blood vitamin D metabolite levels.

In this study, we found no significant difference in blood 25(OH)D levels between the distal resection and sham operation groups. The reason why bowel resection in our experiment did not cause a reduction in blood 25(OH)D levels is not yet clear, but we speculate that the fat absorption disorder caused by the resection was not severe enough to induce a vitamin D deficiency. Additionally, food consumption was not decreased after the operation; on the contrary, food consumption increased sporadically during the experimental period. We assume it is difficult to induce vitamin D deficiency under standard diet feeding conditions. On the other hand, blood $1,25(OH)_2D$ levels were lower in the distal resection group than in the sham group. In this study, we found no significant difference in serum creatinine and BUN levels between the distal resection and sham operation groups. These observations suggest that the distal bowel resection does not have a deteriorative effect on renal function. Consequently, there is the possibility that the decrease in serum $1,25(OH)_2D$ concentrations in the distal resection group was due to enhanced degradation rather than suppressed $25(OH)D_3$-1α-hydroxylase activity in the kidneys. Many reports have confirmed the marked acceleration of intestinal Ca absorption[13] and bone resorption[14] as major pharmacological effects of $1,25(OH)_2D_3$. A decrease of serum $1,25(OH)_2D$ concentrations is therefore believed to suppress intestinal Ca absorption. The above findings suggest that 75% distal small bowel resection impairs intestinal absorption of Ca and causes the decreased bone mass due to the negative calcium balance.

In 75% proximal small bowel resection, no obvious bone changes were observed at the end of 90 days of observation. According to Nygaard's report[5], the adaptation of proximal resection was followed by an almost complete adaptation within 3 months, whereas adaptation was incomplete or lacking after distal resections. Adaptation following intestinal resection may play an important role in the development of disorders of bone metabolism.

In conclusion, 75% distal small bowel resection can develop the metabolic bone disorders which were characterized a reduction of bone volume in rats. On the other hand, 75% proximal small bowel resection can develop no obvious metabolic bone disorders in rats, possibly because of the adaptation by the remaining part of the intestine.

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