

# Scanning electron microscopic examinations on retarded bone defect healing in spontaneously diabetic BB/O(ttawa)K(arlsburg) rats

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**Summary.** To date, no detailed knowledge from animal experiments is available on the kind and extent of osseous and mineral metabolic disorders in genetically determined, insulin-dependent Type I diabetes. The purpose of this study was to examine the influence of the diabetic metabolic state in spontaneously diabetic BB/O(ttawa)K(arlsburg) rats on bone defect healing.

Eighty spontaneously-diabetic BB/OK rats with a blood-glucose value of  $391 \pm 106$  mg% (mean  $\pm$  SD) at the time of manifestation were used in the study. Based on blood-glucose values at the time of surgery (mg%), postoperative blood-glucose course (mg%) and postoperative insulin requirements (IU/kg), the animals were divided into groups with well-compensated ( $n=40$ ,  $170 \pm 101$  mg%;  $221 \pm 120$  mg%;  $2.1 \pm 1$  IU/kg) or poorly compensated ( $n=40$ ;  $371 \pm 158$  mg%;  $357 \pm 83$  mg%;  $5.2 \pm 1.4$  IU/kg) metabolic state. Forty LEW.1A rats served as the normoglycemic controls ( $95 \pm 18$  mg%). Using a 1-mm-diameter Kirschner wire, a hole of femoral bone ca. 1 cm proximal to the knee joint space was centrally drilled. Ten animals from each group were killed on postoperative days 7, 14, 24, and 42, and specimens were taken for analysis. Using SEM to measure regions of new bone semiautomatically and quantitatively, also determining the number, area, and circumference of regions not yet filled with new bone.

Up to postoperative day 14, very significant differences ( $p < 0.0001$ ) for all investigated characteristics were found between the spontaneously-diabetic BB/OK rats and the control animals – in favor of the controls – and up to postoperative day 24 within the group of spontaneously-diabetic BB/OK rats, where the well-compensated animals had significantly better results in terms of number and area of regions of bone not yet filled with new bone formations. Forty-two days postoperatively, SEM observations showed no

differences between examination groups.

The process of bone defect healing in spontaneously-diabetic rats was disturbed only in the early phase and exhibited retardation in its progression. After 42 days, bone defect healing was complete, regardless of the diabetic metabolic state; no differences were detected with the SEM between examination groups at this time point.

**Key words:** Spontaneously-diabetic rats, New bone formation, Scanning electron microscopy, Remodelling, Bone defect healing

## Introduction

The coexistence of Diabetes mellitus and disturbed bone and mineral metabolism has been shown in studies both with diabetic patients and on animals with experimentally induced insulin-dependent Diabetes mellitus (Hough et al., 1981). Despite this, the pathogenesis of these alterations, their natural development, and their response to therapy have not been clarified in every detail. In this context, poor metabolic state, ketoacidosis, reduced level of circulating immunoreactive parathyroid hormone (PTH), and alterations in vitamin D metabolism have been discussed. In contrast, other investigations have not demonstrated any dependency of Diabetes mellitus on circulating PTH and 1,25 dihydroxycholecalciferol, and even question the possibility of alterations in bone and mineral metabolism in the presence of insulin-dependent Diabetes mellitus (Gertner et al., 1979; Hough et al., 1981).

Insulin-dependent type 1 diabetes (IDDM) in humans is frequently associated with osteoporosis, although an increased frequency of fractures has not been observed in a mixed group of IDDM and non-insulin-dependent diabetic subjects (Verhaeghe et al., 1990).

Scanning electron microscopy was chosen as the

method of observation. This method is ideally suited for directly examining the bone surface, and makes it simpler to understand the whole 2-dimensional structure. Several SEM studies have already emphasized the good assessability of trabecular bone structures, the remodelling process, or bone cell functions and their interactions (Draenert and Draenert, 1980; Matsuda et al., 1992; Braidotti et al., 1997). The differential representation of the development of vessel and cell proliferation during the individual phases of the remodelling process made scanning electron microscopy a viable alternative method of morphological examination and documentation (Mosekilde, 1990).

Most previous studies have dealt with short-term diabetes induced by a potentially nephrotoxic drug (streptozotocin). This study examined disturbances in bone defect healing in spontaneously diabetic BB/O(ttawa)K(arlsburg) rats instead of in animals with experimentally (streptozotocin) induced Diabetes mellitus. The spontaneously-diabetic BB/OK rat develops an autoimmune insulin-dependent type 1 diabetes (IDDM) closely resembling human disease including rapid onset, daily requirement of exogenous insulin, presence of autoantibodies and of T cells specific for insulin-producing  $\beta$  cells as well as T cell infiltrates within the islets of Langerhans. As in human beings, disease development in the BB/OK rat is complex and polygenic (Klötting and Voigt, 1991; Klötting et al., 2001) so that the BB/OK rat is most suitable as model of human type 1 diabetes and its complications.

## Materials and methods

### Animals

Spontaneously-diabetic BB/O(ttawa)K(arlsburg) (F60/61) (Klötting and Voigt, 1991) and non-diabetic LEW.1A rats (F72) used as normoglycemic controls (Klötting, 1987) were bred and kept in our own animal facility under strict hygienic conditions and were free of major pathogens as described previously. They were given a laboratory diet (Ssniff, Soest, Germany) and acidulated water ad libitum. The animals were kept with a rhythm of 12 h light (5 a.m. to 5 p.m.):12 h dark and housed 3 per cage (Macrolon type III, Ehret GmbH, Emmendingen, Germany) preoperatively but alone postoperatively.

Diabetes in BB/OK rats was diagnosed on the basis of glucosuria (Diabur-Test 5000, Boehringer, Mannheim, Germany) followed by measurement of blood glucose concentrations  $>300$  mg/dl on two consecutive days as described (Klötting and Voigt, 1991).

Up to surgery diabetic animals were treated with subcutaneous applications of a sustained release insulin implant using a trocar/stylet (LINPLANT™, ©LINSHIN Canada, INC., Scarborough, Ontario, Canada). The sustained release insulin implant contains bovine insulin in an erodible palmitic acid matrix and is characterised

by an insulin release rate of about 2 IU/day for more than 4 weeks. Following the application of the insulin implant the diabetics were monitored weekly for body weight and blood glucose concentration. When the blood glucose values exceeded  $>200$  mg% a new insulin implant was applied. Before surgery diabetics with blood glucose values  $<200$  mg% did not obtain a new implant to avoid hypoglycemia during surgery. They were daily treated with insulin (Lente™, Novo Nordisk, Denmark). The insulin dose per animal changed between 1 IU/kg and 6 IU/kg depending on stable body weight gain and blood glucose concentrations of animals as described (Klötting and Voigt, 1991).

### Surgery

The animals were anesthetized with an intraperitoneal injection of a Rompun® (Xylazin, Bayer, Leverkusen, Germany) (0.2 ml/kg)/Ketanest® (Ketamin, Sanofi, Berlin, Germany) (0.4 ml/kg) mixture. The right distal femur was shaved and then disinfected with 70% alcohol. Subsequently, an incision was made parallel to the long axis of the femur. After dividing the fascia and M. biceps femoris, the bone was grasped with 2 small Hohmann's retractors and exposed. Using a 1-mm-diameter Kirschner wire, a hole ca. 1 cm proximal to the knee joint space was centrally drilled, also penetrating the corticalis of the opposite side. The wound was closed with resorbable sutures.

### Experimental protocol

Eighty spontaneously-diabetic BB/OK rats with a blood-glucose value of  $391 \pm 106$  mg% (mean  $\pm$  SD) at the time of manifestation and at an age of  $95 \pm 18$  days were used in the study after a diabetes duration of  $115 \pm 15$  days. Based on blood-glucose values at the time of surgery (mg%), postoperative blood-glucose course (mg%) and postoperative insulin requirements (IU/kg), the animals were divided into groups with well-compensated ( $n=40$ ,  $170 \pm 101$  mg%;  $221 \pm 120$  mg%;  $2.1 \pm 1$  IU/kg) or poorly compensated metabolic state ( $n=40$ ;  $371 \pm 158$  mg%;  $357 \pm 83$  mg%;  $5.2 \pm 1.4$  IU/kg). Forty LEW.1A rats aged  $182 \pm 25$  d served as the normoglycemic controls ( $95 \pm 18$  mg%). Ten animals from each group were killed on postoperative days 7, 14, 24, and 42, and specimens were taken for analysis. The statistical differences in terms of the characteristics chosen for grouping are shown in Table 1 by sampling day.

### Tissue preparation

After thoroughly washing the specimens, they were treated for 2 hours at room temperature with 1.25% glutaraldehyde and 4% paraformaldehyde in 0.1 mol/l phosphate buffer (pH 7.4). Organic substances were subsequently dissolved by placing specimens in 5% sodium hypochloride for 20 min at room temperature.

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The specimens were then rinsed 3 times with 0.1 mol/l phosphate buffer (pH 7.4). They were fixed in 1% osmium tetroxide solution in 0.1 mol/l phosphate buffer (pH 7.4) for 90 min, prepared fresh each time from 4% osmium tetroxide. Subsequently, specimens were dehydrated in an ascending series of 30, 50, 70, 90, and 96% ethanol, followed by 3 15-minute treatments with 100% ethanol. Further treatment was conducted at an ethanol:hexamethyldisilazane (HMDS) ratio of 1:1 (Perdigao et al., 1995); two 10-min exposures to pure HMDS followed. Specimens were air dried, using filter paper on the surfaces and constant suction.

### Measuring the regions of new bone

After gold-sputtering the specimens, they were examined with a scanning electron microscope (DSM 940 A, Zeiss, Jena, Germany). In addition to the surface examination, the total area of each hole were measured and used as reference and the regions of new bone were measured semiautomatically and quantitatively (analySIS, SIS, Münster, Germany), also determining the number, area, and circumference of regions not yet filled with new bone.

### Data analysis

The significance of differences between groups was calculated with the unpaired Student's t test. The level of significance was pre-set at  $p < 0.05$ . All statistical analyses were carried out according to Steel and Torrie (1981) using a computer program (SPSS/PC+TM 4.0, Base Manual for the IBM PC/XT/AT and PS/2V, Release 4.0, SPSS Inc., Chicago, USA, 1990).

## Results

### Postoperative day 7

Between animals with well- and those with poorly

compensated metabolic states, the blood-glucose values at the time of surgery ( $p=0.047$ ) and the postoperative blood-glucose course ( $p=0.009$ ) were statistically significantly different, and the postoperative insulin requirement also differed highly significantly ( $p < 0.0001$ ) (Table 1).

In animals with a well-compensated metabolic state (Fig. 1a) and in the controls (Fig. 3), SEM observations showed newly formed bone with a spongy-like appearance. In the majority of cases, there was still a considerable amount of space between the newly formed bone and the margin of the hole. The newly grown bone consisted of thin trabeculae, and these, in turn, were made of an aggregation of many small spherical mineral clusters. The trabeculae enclosed numerous vessel canals. Howship's lacunae, with sharp edges upon which fibrous structures were obvious, were representative of resorptive processes in the vicinity of the incipient vessel spaces. On the margin of the hole, Howship's lacunae were found next to spongy-like newly formed bone. Between the lamellae of the corticalis of the drilled hole's margin and the newly formed spongy-like bone inside the hole, fine interlamellar fibers were distinguishable which partially fused into fiber bundles (Fig. 1b).

In animals with poorly compensated metabolic states, the SEM merely revealed isolated islands of newly formed bone in the center of the drilled hole (Fig. 2a). Neither did the margin of the hole exhibit any tendency toward the formation of delicate collagenous fibrils (Fig. 2b).

It was only possible to obtain descriptive results seven days postoperatively, since at this time point, it was not possible to adequately mark the still very delicate structures of spongy-like appearance for the quantitative semiautomatic computer analysis.

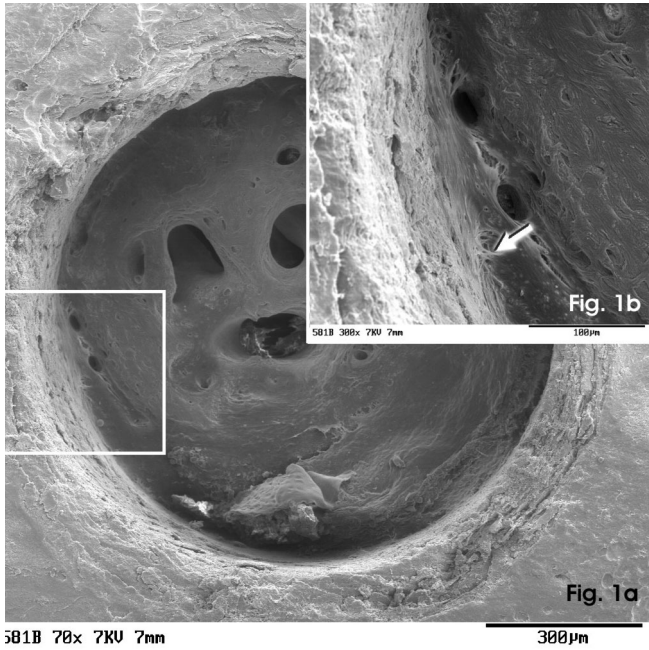
### Postoperative day 14

Between animals with well- and those with poorly

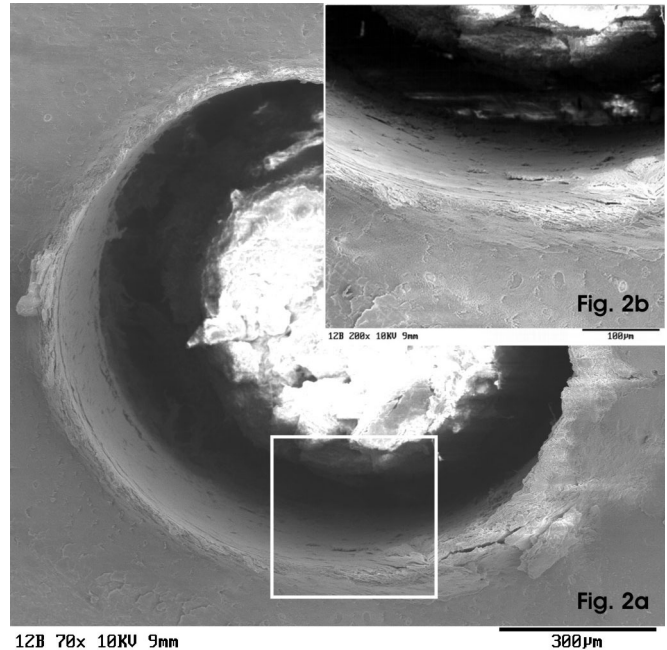
**Table 1.** Grouping of spontaneously-diabetic BB/OK rats.

| TIME OF PREPARATION | METABOLIC COMPENSATION (mean $\pm$ SD) | BLOOD GLUCOSE AT TIME OF OP IN MG% (mean $\pm$ SD) | POSTOPERATIVE BLOOD GLUCOSE COURSE IN MG% (mean $\pm$ SD) | POSTOPERATIVE INSULIN REQUIREMENT IN IU/KG BODY MASS (mean $\pm$ SD) |
|---------------------|--|--|---|--|
| 7 Days              | Well                                   | 245.15 $\pm$ 108.80                                | 223.29 $\pm$ 96.71  | 1.36 $\pm$ 1.27  |
|                     | Poor                                   | 351.25 $\pm$ 109.35                                | 331.01 $\pm$ 66.52  | 4.85 $\pm$ 0.98  |
|                     | Significance                           | $p=0.047$  | $p=0.009$   | $p < 0.0001$   |
| 14 Days             | Well                                   | 170.60 $\pm$ 101.47                                | 220.60 $\pm$ 120.17                                       | 2.10 $\pm$ 0.99  |
|                     | Poor                                   | 371.30 $\pm$ 158.40                                | 356.90 $\pm$ 82.66  | 5.22 $\pm$ 1.34  |
|                     | Significance                           | $p=0.003$  | $p=0.008$   | $p < 0.0001$   |
| 24 Days             | Well                                   | 247.60 $\pm$ 118.62                                | 264.30 $\pm$ 63.74  | 2.33 $\pm$ 1.04  |
|                     | Poor                                   | 362.30 $\pm$ 52.12                                 | 405.80 $\pm$ 38.81  | 6.08 $\pm$ 0.96  |
|                     | Significance                           | $p=0.012$  | $p < 0.0001$  | $p < 0.0001$   |
| 42 Days             | Well                                   | 223.45 $\pm$ 12.34                                 | 265.25 $\pm$ 87.22  | 2.33 $\pm$ 1.14  |
|                     | Poor                                   | 315.30 $\pm$ 79.64                                 | 374.40 $\pm$ 30.61  | 5.85 $\pm$ 1.38  |
|                     | Significance                           | $p=0.067$ n.s.                                     | $p=0.002$   | $p < 0.0001$   |

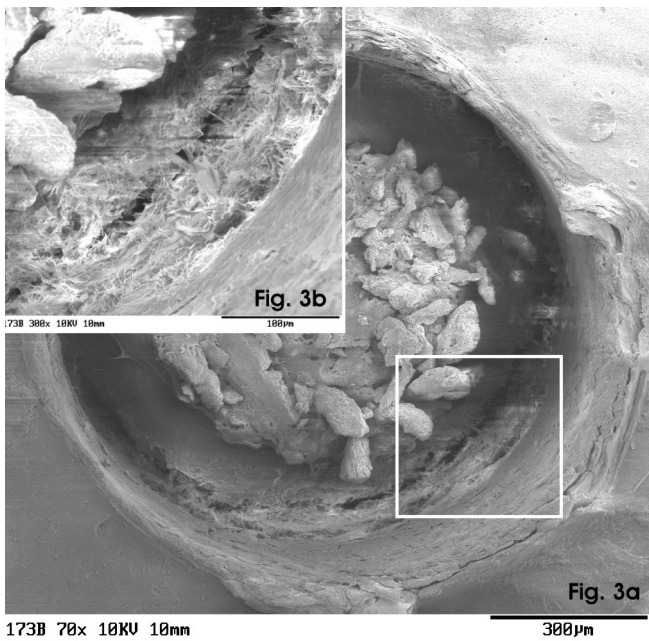
*Retarded bone defect healing*



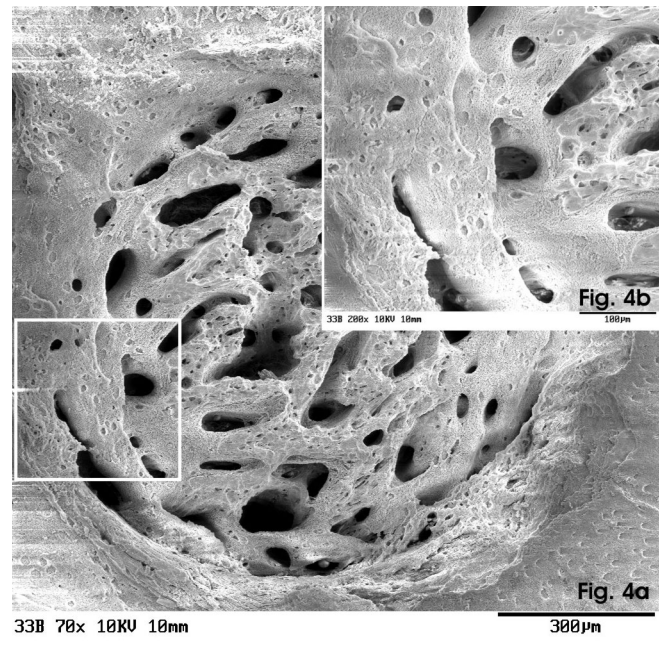
**Fig. 1.** Postoperative day 7, well-compensated metabolic state: Formation of fine interlamellar fibers (arrow) between lamellae of corticalis of drill hole margin (1b, x 300) and the spongy-like newly formed bone within the hole (1a, x 70), partially fused into fiber bundles.



**Fig. 2.** Postoperative day 7, poorly compensated metabolic state: In the center of the hole are isolated islands of new spongy-like bone (2a, x 70). No tendency toward formation of delicate collagenous fibrils from the hole margin is seen (2b, x 200).



**Fig. 3.** Postoperative day 7, nondiabetic control animals: Fine fibers between lamellae of corticalis of drill hole margin (3b, x 300) and the spongy-like newly formed bone within the hole (3a, x 70).



**Fig. 4.** Postoperative day 14, well-compensated metabolic state. Most of the newly formed bone at the margins of the former hole (3b, x 200) is completely remodelled and still shows many vascular spaces in the center (3a, x 70).

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compensated metabolic states, the blood-glucose values at the time of surgery ( $p=0.003$ ) and the postoperative blood-glucose course ( $p=0.008$ ) were statistically significantly different, and the postoperative insulin requirement also differed very significantly ( $p<0.0001$ ) (Table 1).

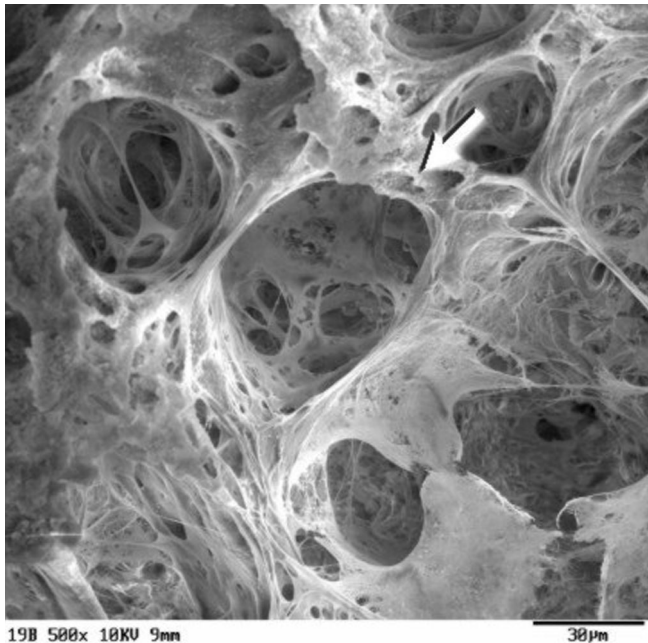
In animals with well-compensated metabolic states, SEM observation showed that the drilled hole was completely filled with new bone (Fig. 4a). In the transition zone, the newly formed bone corresponded to normal cortical bone structure outside of the hole (Fig. 4b). The new spongy bone in the center of the hole still exhibited an abundance of vessel spaces. These were constructed of collagenous fiber bundles with a regular arrangement. The latter, in turn, were composed of thin

fibers containing spherical mineral aggregations (Fig. 5). In addition to the already completely remodelled bone areas, regions of similar extent still in the process of remodelling were found in what was formerly the margin of the drilled hole.

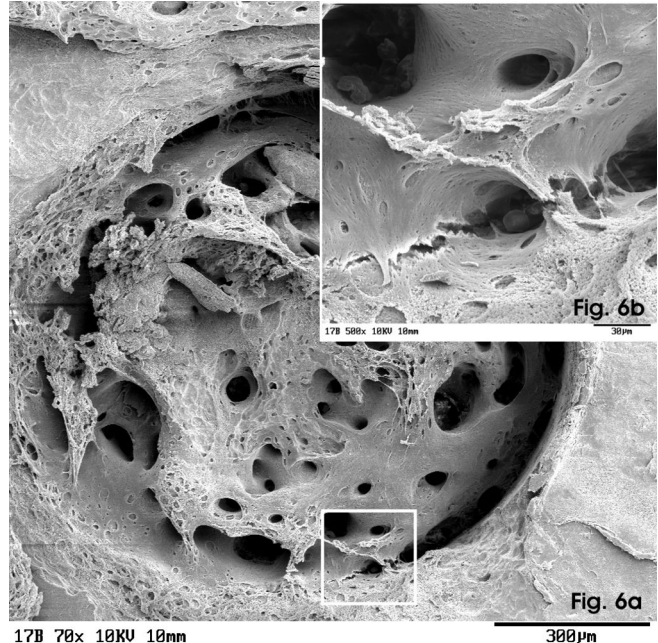
In animals with poorly compensated metabolic states, SEM examination revealed newly formed spongy-like bone which also filled the entire hole; however, the remodelling process was not complete at any location along the former hole margin (Fig. 6a). In the entire area of transition to cortical femoral bone, processes actively forming the new surface were found to coincide with resorptive processes of new bone formation, i.e., Howship's lacunae filled with a network of delicate collagenous fibrils (Fig. 6b). Compared to the

**Table 2.** Statistical comparison of quantitatively-measured examination characteristics.

| TIME OF PREPARATION | METABOLIC COMPENSATION | AREAS OF BONE NOT YET FILLED WITH NEW BONE FORMATIONS |                                   |  |
|---------------------|------------------------|---|-----------------------------------|--|
|                     |                        | Number (mean±SD)                                      | Area in $\mu\text{m}^2$ (mean±SD) | Circumference in $\mu\text{m}$ (mean±SD) |
| 14 days             | Well                   | 88.50±5.48  | 79 441.86±69857.10                | 12 210.84±1182.24                        |
|                     | Poor                   | 53.30±7.20  | 165 178.08±10862.54               | 16 393.08±1288.00                        |
|                     | Significance           | $p<0.0001$  | $p=0.001$                         | $p<0.0001$                               |
|                     | Control animals        | 30.80±6.34  | 29 460.61±6664.37                 | 10 655.12±5615.42                        |
|                     | Significance           | $p<0.0001$  | $p<0.0001$                        | $p<0.0001$                               |
| 24 days             | Well                   | 59.00±7.66  | 41 809.84±3923.80                 | 5399.99±1268.12                          |
|                     | Poor                   | 48.00±6.06  | 48 796.09±5204.47                 | 5555.74±1102.31                          |
|                     | Significance           | $p=0.004$   | $p=0.003$                         | $p=0.773$ n.s.                           |



**Fig. 5.** Postoperative day 14, well-compensated metabolic state: Vessel spaces form from collagenous fiber bundles with regular arrangement, which in turn are composed of thin fibers containing spherical mineral aggregations (arrow) (x 500).



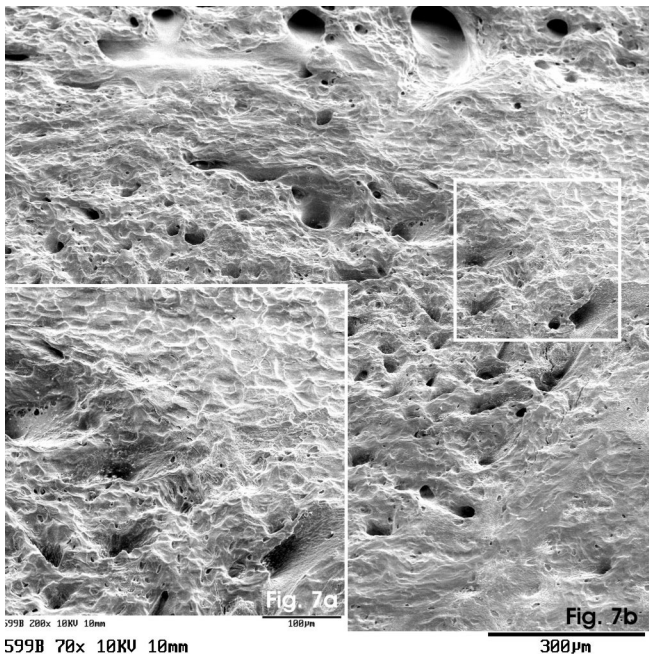
**Fig. 6.** Postoperative day 14, poorly compensated metabolic state: The remodelling process is not complete at any site (5a, x 70). In the transitional zone to cortical femoral bone, a network of collagenous fibrils is visible (5b, x 500).

architecture of newly formed spongy bone in animals with well-compensated metabolic states, animals in this group exhibited much less new bone. This was primarily due to markedly larger but fewer vascular spaces. Between animals with well- and those with poorly compensated metabolic states, the number (well-compensated:  $88.50 \pm 5.48$ ; poorly compensated:  $53.30 \pm 7.20$ ) and circumference (well-compensated:  $12210.84 \pm 1182.24 \mu\text{m}$ ; poorly compensated:  $16393.08 \pm 1288.00 \mu\text{m}$ ) of the still incompletely ossified new bone regions were very statistically significantly different ( $p < 0.0001$ ), and the total area (well-compensated:  $79441.86 \pm 69857.10 \mu\text{m}^2$ ; poorly compensated:  $165178.08 \pm 10862.54 \mu\text{m}^2$ ) occupied by such regions also differed significantly ( $p = 0.001$ ) (Table 2).

In the control group, SEM images depicted normal, completely remodelled bone structure (Fig. 7a) with numerous canaliculi over the major portion of the hole and in almost the entire marginal zone to the laminar cortical femur bone (Fig. 7b). The results for all investigated characteristics (number:  $30.80 \pm 6.34$ ; area:  $29460.61 \pm 6664.37 \mu\text{m}^2$ ; circumference:  $10655.12 \pm 5615.42 \mu\text{m}$ ) were very significantly different ( $p < 0.0001$ ), with the normoglycemic control animals showing better results than the spontaneously-diabetic BB/OK rats (Table 2).

#### Postoperative day 24

Between animals with well- and those with poorly

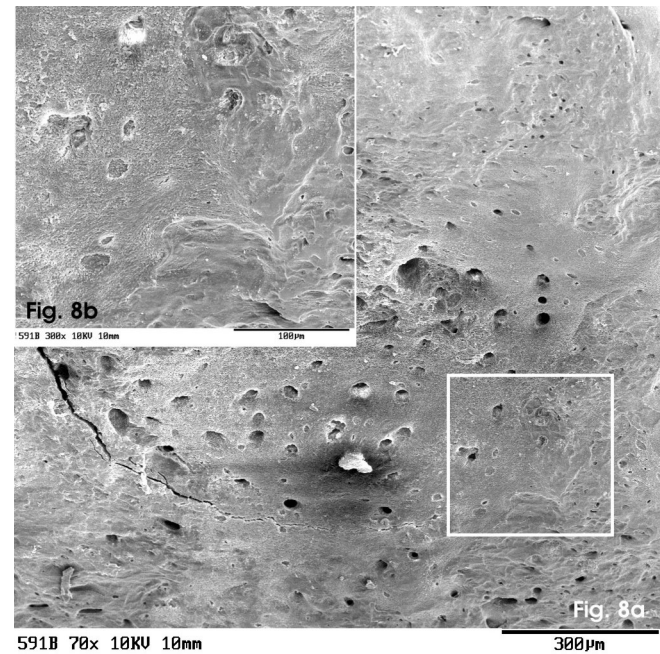


**Fig. 7.** Postoperative day 14, nondiabetic control animals: Most of the hole is re-ossified (6a, x 70), and in almost all of the border area (6b, x 200) to laminar cortical femoral bone, normal, completely remodelled bone structure is observed.

compensated metabolic states, the postoperative blood-glucose course and the postoperative insulin requirement were very statistically significantly different ( $p < 0.0001$ ), and the blood-glucose values at the time of surgery also differed significantly ( $p = 0.012$ ) (Table 1).

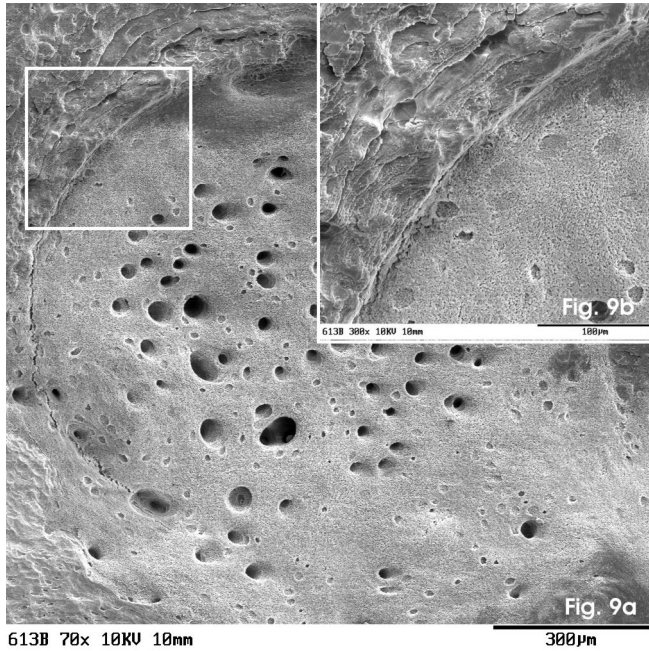
As shown by the SEM, animals with well-compensated metabolic states differed from those with poorly compensated metabolic states in terms of number and size of newly formed bone regions that were still incompletely ossified. Although there was a significantly ( $p = 0.004$ ) higher number (well-compensated:  $59.00 \pm 7.66$ ; poorly compensated:  $48.00 \pm 6.06$ ) of such incompletely reossified areas in animals with well-compensated metabolic states, they comprised a smaller area (well-compensated:  $41809.84 \pm 3923.80 \mu\text{m}^2$ ; poorly compensated:  $48796.09 \pm 5204.47 \mu\text{m}^2$ ) than those observed in the metabolically poorly compensated animals ( $p = 0.003$ ) (Fig. 9a). In contrast, a statistically significant difference in the circumferences of the areas (well-compensated:  $5399.99 \pm 1268.12 \mu\text{m}$ ; poorly compensated:  $5555.74 \pm 1102.31 \mu\text{m}$ ) examined was no longer present ( $p = 0.773$ ) (Table 2). The remodelling process was complete, with the exception of a few areas in the metabolically well-compensated animals; the boundaries between the newly formed bone and the surrounding cortical bone could no longer be distinguished (Fig. 8). The remodelling process in the marginal zone of animals with poorly compensated metabolic states was not yet complete (Fig. 9b).

In the control group, it was no longer possible to

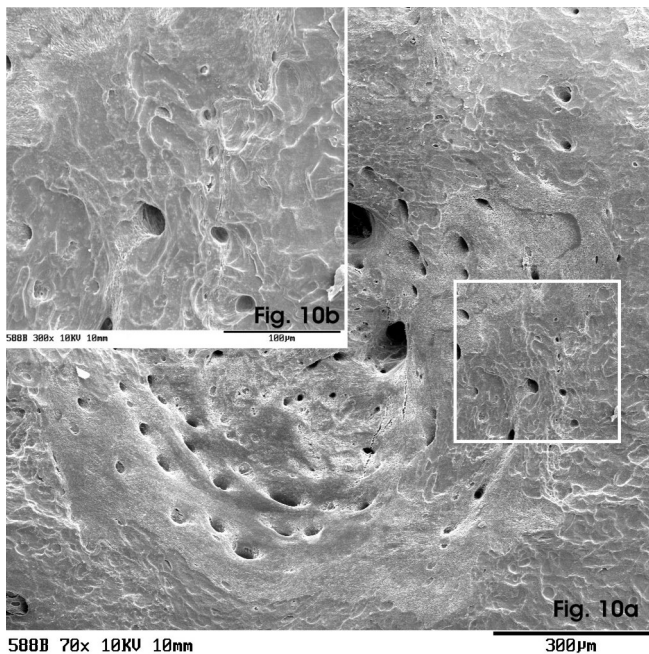


**Fig. 8.** Postoperative day 24, well-compensated metabolic state: With the exception of a few areas, the remodelling process is complete (7a, x 70). The borders between the newly formed bone and the cortical surroundings are no longer detectable (7b, x 300).

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**Fig. 9.** Postoperative day 24, poorly compensated metabolic state: The remodelling process in the border region is not yet complete. The hole margins are still distinct (8b, x 300). In the center, large, non-remodelled vascular spaces are evident (8a, x 70).



**Fig. 10.** Postoperative day 24, nondiabetic control animals: Remodelling is complete (9a, x 70). The newly formed bone cannot be distinguished from the cortical surroundings (9b, x 300).

distinguish the newly formed bone from its surroundings (Fig. 10).

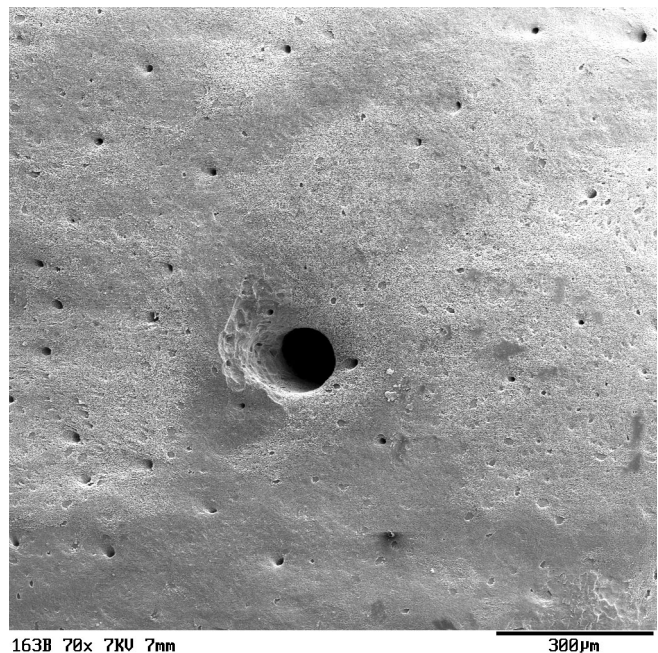
### Postoperative day 42

Between animals with well- and those with poorly compensated metabolic states, the postoperative insulin requirement was very statistically significantly different ( $p < 0.0001$ ), and postoperative blood-glucose course also differed significantly ( $p = 0.002$ ). A statistically significant difference in the blood-glucose values at the time of surgery was not present ( $p = 0.067$ ) (Table 1).

The SEM revealed no differences between the three groups examined. The remodelling process was entirely complete, and the borders between new bone and the cortical surroundings were no longer perceptible (Fig. 11).

### Discussion

The scanning electron microscopic results of our animal experiments show a retarded bone defect healing in the spontaneously diabetic BB/OK rats. Up to postoperative day 14, very significant differences for all investigated quantitatively-measured characteristics were found between the spontaneously-diabetic BB/OK rats and the control animals – in favor of the controls – and up to postoperative day 24 within the group of spontaneously-diabetic BB/OK rats, where the well-compensated animals had significantly better results in



**Fig. 11.** Postoperative day 42, poorly compensated metabolic state: Remodelling process complete. Single vessel spaces can be seen in the center (x 70).

terms of number and area of regions of bone not yet filled with new bone formations. This study demonstrates that the formation of new osseous tissue is fundamentally influenced by the diabetic metabolic state, since those spontaneously-diabetic animals with a poorly compensated metabolic state exhibited a much more pronounced disturbance of the process of bone defect healing. Scanning electron microscopic results have not been reported in spontaneously-diabetic rats to date. These results from animals with genetically-determined spontaneous diabetes agree only partially with those of comparable studies on the retarded bone defect healing in animals with Streptozotocin-induced diabetes. Shyng et al. (2001) found that cancellous bone volume and bone formation in the femur were greatly reduced in the Streptozotocin-induced diabetic model. These observations are also consistent with published reports of impaired osteoid formation (Shires et al., 1981), and decreased synthesis of both collagen (Schneir et al., 1979, 1990; Spanheimer, 1989) and proteoglycan (Weiss et al., 1981), in Streptozotocin-induced diabetes. Since osteoclast numbers are also reduced in diabetic rats (Hough et al., 1981; Shires et al., 1981) an overall reduction in bone turnover, rather than excessive bone resorption, is implicated in the pathogenesis of diabetic osteopenia (Bain et al., 1997).

Skeletal changes in diabetic rats have also been attributed to metabolic abnormalities which accompany the malnutrition of insulin deficiency (Shires et al., 1981). However, recent studies have shown that undernutrition can account for only 30% of the net deficit in collagen production in the Streptozotocin-induced diabetic rats (Umpierrez et al., 1989). Spanheimer et al. (1988) has shown that decreases in collagen production in the streptozotocin-induced diabetic rats are a consequence of the chronic diabetic state and not due to Streptozotocin toxicity in the days immediately after injection of this diabetogenic drug. Furthermore, exposing bone and cartilage in culture to Streptozotocin for short time had no direct effect on connective tissue metabolism (Spanheimer, 1989).

Compared to animals with Streptozotocin-induced diabetes, spontaneously-diabetic rats provide an excellent model for human insulin-dependent Type 1 Diabetes mellitus, since these model animals both clinically and etiopathogenetically exhibit a great number of analogies to human Type 1 diabetes (Yoon and Yun, 2001). Similarly to human beings, the class II genes of the major histocompatibility complex (MHC) of the haplotype RT1<sup>u</sup> (*Iddm1*) are associated with diabetes development. In contrast to human diabetes, the BB rat is characterised by an immune deficiency which finds expression in a profound T-cell lymphopenia. This lymphopenia is inherited as an autosomal recessive trait. By several crossing studies it has been demonstrated that the MHC class-II genes of the RT1<sup>u</sup> haplotype, *Iddm1*, and the lymphopenia, *Iddm2*, are essential but not sufficient for diabetes development in the BB rat. As in human beings, there are additional non-MHC genes

contributing to type 1 diabetes in the BB rat as recently described (Klötting et al., 1995, 1998). Nevertheless, the main genetic component in rat type 1 diabetes are the MHC class-II genes of the RT1<sup>u</sup> haplotype. A fact which is supported by two additional rat models of type 1 diabetes, the Komeda Diabetes-Prone and the LEW,1AR1/Ztm-iddm rat. Both rat strains are also characterised by the MHC class-II genes of the RT1<sup>u</sup> haplotype (Yokoi et al, 1997; Lenzen et al. (2001). Therefore, normoglycemic LEW.1A rats with diabetes-resistant MHC class II genes of the RT1a haplotype were used as controls representing a diabetes-resistant rat strain.

In the literature, only Verhaeghe et al. (2000) has been reported about low bone formation rate and low biochemical markers of bone formation in female spontaneously diabetic BB rats. Verhaeghe's (1989) experiments have also shown impaired epiphyseal cartilage proliferation and enchondral bone formations in young spontaneously diabetic BB rats.

On postoperative day 7, SEM observations revealed an obvious retardation of new bone formation in the diabetic BB/OK rats which, despite insulin therapy, exhibited an overall poorly compensated metabolic state in terms of postoperative blood-sugar courses and insulin requirements. The most dramatic differences were observed with SEM after 14 days. The chief reason for this was that in the rats with poorly compensated metabolic states, the remodelling process after 14 days was not complete at any location, and resorptive processes and new bone formation were still occurring simultaneously in the entire area of transition to cortical bone. Twenty-four days postoperatively, the remodelling process was complete in the control animals, and no difference between the former hole and surrounding corticalis could be seen. Up to this point in new bone formation, not only are the highly significant differences between diabetic BB/OK rats and normoglycemic control animals noteworthy, but also the scanning electron microscopic proof of statistically very significant differences within the diabetic BB/OK rats that depend upon the efficacy of insulin therapy on metabolic state. Despite the attempt to provide adequate insulin therapy where the overall metabolic state was poorly compensated, we found a statistically significant retardation of the remodelling process in rats with insulin-dependent Type 1 diabetes, because the bone and mineral metabolism could not be adequately corrected. Forty-two days postoperatively, the SEM images no longer showed any differences in surface morphology – as had existed in the early phase of the remodelling process.

Gene therapy utilizing recombinant viral constructs containing IGFs I and II may be of benefit during bone healing in an effort to augment clinical scenarios of poor or retarded bone repair. Steinbrech et al. (1999) have shown the temporal pattern of IGF I and IGF II gene expression during mandibular osteotomy healing using a rat model. The upregulation of IGF and IGF II during



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mandibular bone healing underscores the importance of these growth factors in bone repair. Thaller et al. (1995) reported that IGF-1 exerts a potentiating effect on the repair of critical-size calvarial defects in adult, male diabetes-induced rats. Furthermore, el-Hakim (1999) demonstrated that fibrin stabilizing factor (F. XIII) may enhance early stages of bone healing in uncontrolled diabetic rats. On the other hand, F. XIII did not significantly affect healing in non-diabetic rats.

Bone defect healing in the spontaneously-diabetic BB/OK rats was thus disturbed only in the early phase and proceeded with delay; however, it was independent of a diabetic metabolic state and complete after 42 days, showing no scanning electron microscopically detectable differences between the test groups.

### *Conclusions*

The process of bone defect healing in spontaneously-diabetic BB/OK rats is retarded due to disturbances in the early phase. If insulin therapy successfully achieves a compensated metabolic state, the extent of the disturbance is reduced, yet the difference remains very statistically significant compared to nondiabetic control animals. Even among the diabetic BB/OK rats, bone and mineral metabolism were not adequately correctable with insulin therapy where the existing diabetic state was poorly compensated, so that the differences recorded were statistically significant to highly significant.

After 42 days, bone defect healing was complete in all experimental animals, regardless of the presence (or absence) of a diabetic state. By that time, SEM examination could not distinguish between newly formed bone and the adjacent cortical bone.

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