Effects of Exercise and Disuse on Bone Remodeling, Bone Mass, and Biomechanical Competence in Spontaneously Diabetic Female Rats

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Diabetes is associated with low bone formation. In this study we investigate the effect of additional or reduced mechanical loading on indices of bone formation and resorption, bone mass, and biomechanical properties in spontaneously diabetic BB rats. Female diabetic (mean age 13 weeks) and age-matched control rats were each allocated to three experimental groups: no-intervention; supervised running exercise program (Ex); and unloading induced by unilateral sciatic neurectomy (USN). The study period was 8 weeks. We measured biochemical parameters of bone formation (plasma osteocalcin) and resorption (urinary deoxypyridinoline [Dpd]); bone mineral density (BMD) by dual-energy X-ray absorptiometry (DXA) at middiaphyseal and metaphyseal regions of the femur; histomorphometry of the proximal tibial metaphysis (PTM); and biomechanical properties of the femur (neck, diaphysis, and metaphysis) and lumbar vertebra (L-5). In nondiabetic rats, Ex did not affect parameters of bone formation/resorption and BMD, and had little effect on biomechanical properties. USN increased Dpd excretion, whereas there was a decreased trabecular bone formation rate (BFR) on morphometry of PTM in both paralyzed and intact limbs. Compared with intact limbs, paralyzed limbs of USN rats showed decreased trabecular bone volume at the PTM, and decreased BMD and biomechanical properties at the distal femoral metaphysis (DFM) and, to a lesser extent, femoral neck. Diabetic rats of the three experimental groups had low plasma osteocalcin levels and Dpd excretion, as well as low BFR on morphometry. The BMD and biomechanical properties of both femur and L-5 were unchanged in diabetic rats. Diabetic Ex rats, however, showed a lower maximum load and stress at DFM than control Ex rats. Diabetic USN rats showed no increase in Dpd excretion; their paralyzed limbs showed decreased maximum load at DFM, but there was no significant decrease in trabecular bone volume at PTM or BMD at DFM. Thus, the running exercise does not affect low bone formation in diabetic rats; however, trabecular bone loss caused by disuse is less pronounced in diabetic rats, probably as a result of low

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Key Words: Diabetes; Exercise; Unloading; Biomechanical properties.

Introduction

There is extensive evidence that untreated or poorly treated diabetes mellitus—from the very onset—is associated with low bone formation, in both humans and experimental animals. Experimental models for type 1 diabetes include the streptozotocin (SZ)-diabetic rat and the spontaneously diabetic BB rat. In bone sections from SZ-diabetic and BB rats, cuboidal osteoblasts as well as osteoid tissue are scarce, and the bone surface is covered by bone-lining cells; plasma osteocalcin levels are <30% of controls after 4–5 weeks of diabetes (for review, see refs. 24 and 28). Thus, severe diabetes appears to revert active osteoblasts into inactive bone-lining cells; in addition, there is also suggestive evidence of decreased proliferation of preosteoblastic cells.³¹ These changes are prevented or reversed by insulin treatment.^{24,28,31} Because there is no trabecular or cortical bone loss (as indicated by chemical analysis, dual-energy X-ray absorptiometry [DXA] measurements, and histomorphometry) even after long-term diabetes in BB rats,^{24,26} bone resorption must be dampened accordingly. Indeed, a decrease in osteoclast number has been shown by histomorphometry^{24,26}; on electron microscopy, osteoclasts in diabetic rats show signs of decreased activity (no ruffled border, lower acid-phosphatase activity).¹⁴ However, Epstein et al.⁷ found a significant increase in the eroded perimeter at trabecular bone surfaces. Biochemical bone resorption indices have not been reported in diabetic rats to date.

The effect of diabetes on bone strength is uncertain. Several, but not all, studies found an increased fracture risk in type 1 diabetic subjects.^{4,8} However, it is unknown to what extent this would be due to intrinsic factors (reduced bone strength) vs. extrinsic factors (greater propensity to fall). In diabetic rats, torsion loading of the femoral diaphysis has shown decreased maximum load.^{6,25,26} In addition, the maximum load at the femoral neck has been shown to be lower in untreated diabetic rats.¹⁰

Mechanical stimulation has distinct effects on bone formation and remodeling, as well as on bone strength. There is evidence

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from rat studies that mechanical stimulation produces both an immediate and a delayed stimulation to bone formation. The immediate response is mediated by activation of bone-lining cells into osteoblasts,⁵ whereas the delayed response is the result of preosteoblast proliferation and differentiation into osteoblasts.²² In vivo, mechanical stimulation in the form of exercise improves biomechanical competence.^{18,21} In contrast, unloading (disuse) produces a drop in preosteoblast proliferation,³ as well as decreased bone formation on histomorphometry^{1,30,33} and decreased bone strength.¹⁵ Because bone resorption is stimulated by unloading, bone loss ensues.^{1,30,33} Thus, the resorption-formation sequence is “uncoupled” in unloaded bones.

In this study, we investigate the effects of extra mechanical stimulation (exercise) and unloading on bone formation and bone strength in spontaneously diabetic BB rats. The research questions were as follows: (1) Because mechanical stimulation reactivates bone-lining cells into osteoblasts and stimulates the proliferation of osteoblastic precursors (both processes appear to be deficient in diabetic rats), can exercise by itself activate bone formation and improve bone strength in diabetic rats? (2) Because bone resorption appears to be depressed in diabetic rats, are the effects of unloading on bone loss and biomechanical competence less pronounced than in nondiabetic rats? Several experimental models for unloading (apart from space flight) have been described: tail or back suspension; hindlimb casting or taping; sciatic nerve resection or hemicordotomy; and resection of knee or calcaneal tendons. Because we aimed to investigate the effects of an 8 week period of unloading in diabetic rats that show marked polyphagia, polydipsia, and polyuria, unilateral sciatic neurectomy (USN) appeared to be the best and most practical method after preliminary experiments.

Materials and Methods

Animals

The experiments described were approved by the ethics committee for animal research at Leuven University. Female (virgin) diabetic rats were obtained from the Leuven/pfd Wistar rat colony, inbred since 1983. Between 30% and 40% of rats in this colony (38% in 1997) develop spontaneous type 1-like diabetes at a mean age of 13 weeks. Diabetes was diagnosed by checking three times per week for glycosuria, which invariably corresponds to a capillary (tail) blood glucose level of >300 mg/dL. After onset of diabetes, the rats were untreated unless ketonuria was detected (checked five times per week with KetoDiabur sticks, Boehringer Mannheim, Mannheim, Germany), in which case 1 U of Ultralente MC (Novo Nordisk, Bagsværd, Denmark) was administered subcutaneously (s.c.); in our experience, this regime leaves the diabetic rats untreated from a biologic viewpoint, while preventing deaths due to ketosis. The rats were given tap water and a rat chow containing 1.03% calcium, 0.76% phosphate, and 200 IU/kg vitamin D₃ (Trouw, Ghent, Belgium), all available ad libitum. Diabetic rats were included in the study on a twice-weekly basis, and the duration of diabetes (glycosuria) was 0–4 days at inclusion. For each of the diabetic rats, an age-matched control (nondiabetic) rat was obtained, preferably from the same litter. The diabetic and control rats were placed into three study groups: no-intervention (8 diabetic and control rats); exercise (Ex; 8 diabetic and control rats); or unloading by USN (18 diabetic and control rats). Rats were allocated to the different groups to have an average starting age of 13 weeks (range 10–17) in each group. Ex animals ran on a treadmill (Scholz, Overijse, Belgium), 5 days/week, with increasing velocity (20 or 60 m/min) for longer periods of time (gradually increasing from 5 to 60 min/day); that is, the projected distance

was 100 m during the first 2 days, 200 m during the subsequent 3 days of week 1, 400 m/day during week 2, 1200 m/day during week 3, 2400 m/day during week 4, and 3600 m/day during weeks 5–8 (total projected distance 92,800 m). The Ex group was supervised so that if the animal stopped running the treadmill was gently put in motion to encourage the animal. The actual running distance was measured in each animal; during the 8 week period, diabetic Ex rats ran $96,292 \pm 1252$ m (mean $\pm$ SEM), and control Ex rats $98,929 \pm 1373$ m, that is, 104% and 107% of the projected distance, respectively ($p = 0.18$ between diabetic and control rats). Unloading consisted of USN of the right hindlimb through a small incision in the gluteal area, under anesthesia (atropine 0.25 mg/kg, xylazine hydrochloride 10 mg/kg, and ketamine hydrochloride 50 mg/kg intraperitoneally [i.p.]). All rats were kept in plastic cages, one rat per cage. The study period was 8 weeks. During week 4 and week 8, 24 h urine collections were performed in a metabolic cage. Calcein labeling (16 mg/kg i.p.) was given 11 days and 24 h prior to the end of the study. All Ex experiments were ended on a Friday, after the animals had exercised. The rats were anesthetized and weighed, and a blood sample was taken from the abdominal aorta. The left tibia was removed for histomorphometry, immersed in Burckhardt's fixative for 24 h at 4°C, and subsequently kept in 70% ethanol; the left femur was removed to obtain wet and dry weights, and for DXA analysis. The right femur and the fifth lumbar vertebra (L-5) were removed for biomechanical measurements. In the diabetic and control USN groups, two subgroups were necessary: in subgroup A, the tibiae of both paralyzed and intact limbs were removed for histomorphometry, and the femora for DXA analysis; in subgroup B, both femora and L-5 were removed for biomechanical analysis. The USN groups were therefore twice as large as the intact and Ex groups; plasma and urine were available from both subgroups.

Assays

Plasma glucose was measured by the glucose-oxidase method with a YSI 2300 Stat Plus glucometer (Yellow Springs Instruments, Yellow Springs, OH). Insulin was measured by radioimmunoassay (RIA) with a rat insulin standard.²⁸ Insulin-like growth factor-I (IGF-I) was determined by RIA after acid ethanol extraction,²⁶ and osteocalcin by RIA using a rat standard.²⁹ Calcium (Ca) in plasma and urine (Sigma Diagnostics, St. Louis, MO) and phosphate in plasma were measured by colorimetry, and total protein by the Biuret method; “diffusible” Ca levels were calculated as $6.25[\text{Ca}] - 0.375[\text{TP}]/6.25 + [\text{TP}]$.²⁶ Plasma carboxyterminal crosslinked telopeptide of type I collagen (ICTP) was measured by an RIA double-antibody kit specific for rat ICTP (Orion Diagnostica, Espoo, Finland). Parathyroid hormone (PTH) was measured with an IRMA kit specific for rat PTH(1-34) (Immutopics, San Clemente, CA). Urinary free deoxypyridinoline crosslinks (Dpd) were measured by enzyme immunoassay (Pyrilinks-D, Metra Biosystems, Mountain View, CA). Dpd excretion was calculated both as the 24 h urinary excretion, and as Dpd/creatinine excretion; the average of the two 24 h collections was calculated.

Bone Studies

Histomorphometry. Histomorphometry was performed as described previously.²⁵ Briefly, the undecalcified proximal tibia was embedded in methylmethacrylate, and 5- μm -thick longitudinal sections were cut. Three unstained sections per bone, 100 μm apart, were evaluated for dynamic morphometry. We measured the trabecular bone surface (thus excluding the endocortical surface) covered by a double or single calcein label (dLS/BS

Table 1. Body weight, plasma glucose, insulin, and IGF-I concentrations

	No intervention		Exercise		USN		One-way ANOVA
	Control (n = 8)	Diabetic (n = 8)	Control (n = 8)	Diabetic (n = 8)	Control (n = 18)	Diabetic (n = 18)	
End body weight (g)	290 (12)	176 (9) ^a	292 (11)	231 (10) ^{a,b}	249 (9) ^{b,c}	201 (8) ^{a,c}	$p < 0.001$
Plasma glucose (mmol/L)	11 (0.2)	46 (3) ^a	11 (0.3)	33 (4) ^{a,b}	11 (0.3)	41 (2) ^a	$p < 0.001$
Plasma insulin (pmol/L)	1012 (170)	36 (20) ^a	592 (88) ^b	37 (35) ^a	685 (90)	27 (11) ^a	$p < 0.001$
Plasma IGF-I (nmol/L)	107 (6)	34 (7) ^a	117 (7)	61 (10) ^{a,b}	102 (7)	45 (6) ^a	$p < 0.001$

KEY: ANOVA, analysis of variance; IGF-I, insulin-like growth factor-I; USN, unilateral sciatic neurectomy.

Number of animals in each group shown in parentheses. Data expressed as means (SEM).

^aSignificant difference between diabetic groups and respective control groups (Fisher's LSD post hoc test).

^bSignificant difference vs. respective (control/diabetic) no-intervention groups.

^cSignificant difference vs. respective (control/diabetic) exercise groups.

and sLS/BS, respectively) in the metaphysis under fluorescence microscopy, in as many fields as possible, starting 1 mm from the distal growth plate. The mineralizing surface (MS) was calculated as dLS/BS + 1/2sLS/BS. The mineral apposition rate (MAR) was measured as the mean distance between all double calcein labels, each double label measured at three equidistant intervals. The bone formation rate, surface referent (BFR/BS), was calculated as MS/MAR $\times \pi/4$. If dLS/BS was 0 and MAR measurements could not be performed, then the BFR was defined as 0 as well. Trabecular bone volume (BV/TV) was measured in three von Kossa-stained sections, 100 μm apart, in the central metaphysis, starting 1 mm from the distal growth plate.

DXA analysis. DXA analysis of the femur was performed as described previously.^{25,26} We measured the BMC of the total femur, of a middiaphyseal area (consisting of cortical bone), and of a metaphyseal area at 20% from the distal end of the femur (consisting predominantly of trabecular bone). BMD was calculated as BMC/area. At the middiaphyseal level, we also calculated the bone mineral apparent density (BMAD) as BMC/area²,¹⁶ to correct for the smaller bone size in diabetic animals.

Biomechanical analysis. We performed biomechanical testing of the fifth lumbar vertebrae (L-5) and of the femora, at the neck, diaphysis, and distal metaphysis. The methods have been described in detail previously.¹⁹ Briefly, the biomechanical properties of L-5 were tested by a compression test of a vertebral body cylinder along the proximodistal axis. The femoral necks were tested with a vertical load applied to the top of the femoral head. The femoral middiaphyses were tested by a three-point bending test. At the femoral metaphysis, a compression test was performed in a 4-mm-thick section along the proximodistal axis, similar to that with the lumbar vertebrae. The following parameters were obtained: load, F_{max} (N, the maximal load applied at fracture of the specimen; ash density, ρ (mg/mm³), the ash weight divided by total tissue volume (bone + marrow); load/ash density; maximum stress, σ_{max} (MPa), the maximum stress on the stress-strain curve; Young's modulus, E (GPa), the maximum slope of the stress-strain curve.

Data Analysis

A statistical software program was used (NCSS, Kaysville, UT). Data are presented as means $\pm$ SEM. Statistical analysis included two-factor analysis of variance (ANOVA) to assess: (1) the effects of exercise vs. diabetes on bone parameters; and (2) the effects of paralysis on bone parameters in diabetic vs. control rats. One-way ANOVA was used to detect overall differences between the study groups regarding general parameters, plasma and urinary measurements, and bone parameters. If two-factor

ANOVA analysis detected an interactive effect ($p < 0.15$) between both variables, we proceeded to one-way ANOVA to detect overall differences between groups. If one-way ANOVA indicated an overall significant effect ($p < 0.05$), Fisher's least-significant-difference (LSD) post hoc test was used to detect significant differences ($p < 0.05$) between individual groups.

Results

The average age at the start of the study was 13 weeks in all groups; there was no difference in body weight among the six groups (ANOVA $p = 0.36$). At the end of the 8 week study period, body weight was 20% lower in the diabetic groups than in the respective control groups (**Table 1**); body weight of Ex diabetic rats was higher than that of no-intervention and USN diabetic rats. All diabetic groups showed severe hyperglycemia and hypoinsulinemia, and markedly lower IGF-I concentrations. Glucose levels were lower and IGF-I levels were higher in Ex than in no-intervention diabetic rats, despite similarly low insulin levels, reflecting increased tissue insulin sensitivity induced by Ex. Insulin levels were lower in Ex than in no-intervention control rats, perhaps also reflecting increased insulin sensitivity.

Plasma total Ca levels, as well as "diffusible" Ca levels (data not shown), were lower in Ex control than in no-intervention control rats (**Table 2**). Urine volume (75–93 mL in diabetic groups vs. 9–13 mL in control groups; ANOVA $p < 0.001$), and urinary Ca excretion were many times higher in diabetic than in control rats of the three experimental groups. Despite hypercalciuria, PTH concentrations were lower in the diabetic groups. Ex diabetic rats had lower urinary Ca excretion, but not urine volume, than no-intervention or USN diabetic rats. Diabetic rats had markedly lower plasma osteocalcin levels and urinary Dpd excretion, irrespective of whether the urinary Dpd was calculated per creatinine excretion or as pyridinoline excretion per 24 h (**Figure 1**). However, plasma ICTP levels were not significantly different among groups (ANOVA $p = 0.12$); mean values varied between 14.2 and 20.5 $\mu\text{g/L}$. Dpd excretion was increased in USN compared with no-intervention control rats.

The effects of diabetes, Ex, and disuse (paralyzed and intact limbs) on bone parameters were assessed for statistical purposes in three ways: (1) two-factor ANOVA assessing the effects of Ex (vs. no-intervention) and diabetes (vs. control rats) (**Table 3**); (2) two-factor ANOVA assessing the effects of paralysis (vs. intact limbs) and diabetic (vs. control) rats (**Table 4**); and (3) one-way ANOVA comparing the data of all groups. Table 3 shows that Ex had no significant effect on bone size, ash density, or BMD of the femur, nor was there a significant effect on the biomechanical properties at the femoral neck and diaphysis, and L-5. There was a trend for a decreased BV/TV in the Ex groups. Diabetes was

Table 2. Plasma calcium, phosphate, and parathyroid hormone (PTH) concentrations, and urinary calcium excretion

	No intervention		Exercise		USN		One-way ANOVA
	Control	Diabetic	Control	Diabetic	Control	Diabetic	
Calcium (mmol/L)	2.57 (0.11)	2.57 (0.13)	2.27 (0.05) ^b	2.35 (0.06)	2.34 (0.03)	2.54 (0.05) ^a	<i>p</i> = 0.005
Phosphate (mmol/L)	1.80 (0.04)	1.85 (0.05)	1.78 (0.03)	1.76 (0.03)	1.71 (0.02)	1.75 (0.04)	<i>p</i> = 0.08
PTH(1-34) (pmol/L)	6.5 (1.0)	3.5 (1.3) ^a	4.0 (0.3) ^b	2.5 (0.2) ^a	5.4 (0.6)	2.3 (0.3) ^a	<i>p</i> < 0.001
Urine calcium (nmol/mmol creatinine)	0.48 (0.04)	3.87 (0.45) ^a	0.50 (0.04)	1.51 (0.20) ^{a,b}	0.53 (0.07)	3.79 (0.35) ^{a,c}	<i>p</i> < 0.001

See Table 1 for number of animals in each group and abbreviations. Data expressed as means (SEM).

^aSignificant difference between diabetic groups and respective control groups (Fisher's LSD post hoc test).

^bSignificant difference vs. respective (control/diabetic) no-intervention groups.

^cSignificant difference vs. exercise diabetic group.

associated with smaller femoral size (lower weight and area), and markedly lower bone formation parameters on dynamic histomorphometry. The biomechanical properties at the femur and L-5 were unaltered in intact diabetic rats, except for load/density at the femoral metaphysis. The biomechanical properties at the femoral metaphysis were influenced differently by Ex in diabetic versus control rats: some biomechanical properties (load/density, stress) improved by Ex in control but not in diabetic Ex rats. Consequently, several biomechanical properties (load, load/density, stress) at the distal metaphysis were higher in Ex control than in Ex diabetic rats (Table 3 and **Figure 2**). Table 4 shows ANOVA analysis of the effects of paralysis in control and diabetic rats. Diabetes, but not paralysis, had a significant effect on bone formation parameters at the proximal tibia. Paralyzed limbs had lower biomechanical properties at the femoral metaphysis, and at the femoral neck to a lesser extent (Young's modu-

lus), but not at the diaphysis (increased Young's modulus). At the metaphysis, two-way ANOVA indicated a significant effect of paralysis in both control and diabetic rats for several biomechanical properties (load, load/density, stress, Young's modulus). However, there was an interactive effect between paralysis and diabetes for BMD and ash density at the femoral metaphysis, as well as for the trabecular bone volume at the proximal tibial metaphysis. Subsequent analysis by one-way ANOVA followed by post hoc testing showed that BMD, ash density, and BV/TV were decreased only in paralyzed limbs of control but not diabetic rats (**Figure 3**). **Table 5** shows the dynamic histomorphometry data. Diabetes was associated with decreased bone formation parameters in all groups. Confirming the analysis of Table 4, there was no difference in the bone formation rate between paralyzed and intact tibiae in either USN control or diabetic rats, and the BFR of both tibiae was lower than in intact rats.

Figure 1. Plasma osteocalcin levels and urinary deoxypyridinoline excretion in control and diabetic rats. Statistical analysis: ^asignificant difference between diabetic rats and the respective controls (Fisher's post hoc test); ^bsignificant difference in USN control rats compared with no-intervention and exercise control rats.

Discussion

This study examined the effects of exercise and USN on bone formation/resorption parameters, bone density, and biomechanical competence in control and diabetic rats. The results confirm and extend previous conclusions on the effects of diabetes on bone in rats. First, diabetes is characterized by a marked drop in bone formation, as shown by histomorphometric analysis and plasma osteocalcin levels. This study demonstrates that urinary Dpd excretion, a measure of bone resorption, is greatly decreased as well. Surprisingly, plasma ICTP levels were unaltered by diabetes, but urinary Dpd is generally considered to be superior to plasma ICTP as a biochemical marker of bone resorption. Because bone resorption and formation appear to be dampened to a comparable degree in diabetic animals (i.e., there is no uncoupling of the resorption-formation sequence), no bone loss ensues.

Bone formation is decreased not only at trabecular bone surfaces, but also at periosteal bone surfaces^{2,7}; in addition, endochondral bone formation is inhibited in diabetic rats (reviewed in ref. 24). Lower bone growth and modeling explain the second consistent feature of "diabetic bone disease": decreased bone size. The effect on bone size is correlated with the severity of diabetes in SZ-diabetic rats,¹⁷ and also with lower age at induction of SZ diabetes and longer duration of diabetes.⁶ After 8 weeks of diabetes, we found that some, but not all, parameters of femoral size were decreased (Tables 3 and 4); indeed, BB rats develop diabetes at a mean age of 13 weeks, when the growth rate is past its peak level.¹⁷ The reduction in femoral BMC in diabetic rats (data not shown) was commensurate with that of the area of measurement, and the calculated BMD or BMAD remained normal. Thus, all density measurements in diabetic animals (or humans, for that matter) should be corrected for

Table 3. Two- and one-way analysis of variance (ANOVA) (*p* values) of the effects of exercise and diabetes on bone parameters in no-intervention and exercise rats (*n* = 32)

	Two-factor ANOVA			One-way ANOVA	Fisher's LSD post hoc test
	Exercise	Diabetes	Interaction		
A. Femur:					
1. Weight and length					
Dry weight	n.s.	0.01[d]	n.s.		
Length	n.s.	n.s.	n.s.		
2. DXA analysis					
Total area	n.s.	0.002[d]	n.s.		
Diaphyseal BMD	n.s.	0.01[d]	n.s.		
Total BMD; metaphyseal BMD; diaphyseal BMAD	n.s.	n.s.	n.s.		
3. Biomechanical analysis					
(a) Femoral neck					
$F_{\max}$; $\sigma_{\max}$; E	n.s.	n.s.	n.s.		
(b) Diaphysis					
Area	n.s.	0.001	0.12	0.006	noI-D < noI-C
$F_{\max}$; E	n.s.	n.s.			
(c) Distal metaphysis					
$F_{\max}^a$	n.s.	<0.001	0.09	<0.001	Ex-D < Ex-C
$F_{\max}/\rho$	n.s.	<0.001	0.05	<0.001	noI-D < noI-C; Ex-D < Ex-C; Ex-C > noI-C
$\sigma_{\max}^a$	n.s.	0.008	0.02	0.006	Ex-D < Ex-C; Ex-C > noI-C
ρ ; E	n.s.	n.s.	n.s.		
B. Tibia					
Histomorphometry of proximal tibial metaphysis					
BV/TV	n.s. (0.07[d])	n.s.	n.s.		
dLS/BS	n.s.	<0.001[d]	n.s.		
MS/BS	n.s.	<0.001	0.07	<0.001	noI-D < noI-C; Ex-D < Ex-C
BFR/BS	n.s.	<0.001	0.05	<0.001	noI-D < noI-C; Ex-D < Ex-C
C. L-5					
1. Size					
Length; area	n.s.	n.s.	n.s.		
2. Biomechanical analysis					
$\sigma_{\max}$	n.s. (0.10[i])	n.s.	n.s.		
$F_{\max}$; ρ ; $F_{\max}/\rho$; E	n.s.	n.s.	n.s.		

KEY: BFR/BS, bone formation rate (surface referent); BMAD, bone mineral apparent density; BMD, bone mineral density; BV/TV, trabecular bone volume; C, control; D, diabetic; [d] and <, decreased; dLS/BS, double-labeled surface; DXA, dual-energy X-ray absorptiometry; E , Young's modulus; Ex, exercise; $F_{\max}$, load; i, intact limb; [i] and >, increased; MS/BS, mineralizing surface; noI, no intervention; n.s., nonsignificant; ρ , ash density; $\sigma_{\max}$, stress.

For statistical analysis see Materials and Methods.

^aDepicted in Figure 2.

changes in bone size, and be presented as BMD or, if appropriate, as BMAD results.

This study produced the largest analysis of biomechanical properties in diabetic animals to date, which were measured at the femoral neck, diaphysis, and distal metaphysis and at L-5. The statistical analyses did not show any consistent differences in these parameters at the femoral neck and diaphysis and at L-5 (Tables 3 and 4). The results of this study are not in complete agreement with previous biomechanical analyses in chronic diabetic rats. These included torsion loading tests of the femoral diaphysis^{6,25,26} and biomechanical analysis of the femoral neck.¹⁰ However, in some of these studies, the differences in femoral size and geometry between diabetic and control rats were much more pronounced than in this study, because SZ diabetes was induced at a young age (8 weeks¹⁰) or because the interval between diabetes induction and bone analysis was much longer (12 months⁶). This would confirm our previous finding that there is a correlation between maximum load and parameters of size in femora of diabetic rats.²⁵ The decrease in biomechanical competence in diabetic rats would thus depend on the severity, age at onset, and duration of diabetes.

Exercise did not have a significant effect on bone formation indices, BMD, and most biomechanical properties in control or diabetic rats. The only significant difference was an increase in some biomechanical properties (load/density, stress) at the femoral metaphysis in control but not diabetic rats, so that several biomechanical properties (load, load/density, stress) at that site were lower in Ex diabetic than Ex control rats (Figure 2). In addition, there was a trend for an increase in maximum stress at L-5 ($p = 0.096$ by ANOVA). Treadmill exercise for 8 weeks in younger female rats (4 weeks at the start) has been reported to increase bone formation rate in the proximal tibial metaphysis.¹¹ One of us (Li.M.) previously found that long-term (4–12 months) treadmill exercise in 2-month-old female rats increased biomechanical competence (load) at L-5 and the femoral neck but not the femoral diaphysis.^{18,21} Recent studies have produced evidence that impact exercise (up-and-down movement of the treadmill, jumps) clearly raises maximum load of the femur and tibia in both young²³ and adult¹² rats. Future experiments should examine whether there is any effect of impact loading on bone formation and biomechanical properties in diabetic rats.

Diabetes has been associated with marked hypercalciuria.²⁶

Table 4. Two- and one-way analysis of variance (ANOVA) (*p* values) of the effects of paralysis and diabetes on bone parameters in rats after unilateral sciatic neurectomy (USN) (*n* = 36)

	Two-factor ANOVA			One-way ANOVA	Fisher's LSD post hoc test
	Paralysis	Diabetes	Interaction		
A. Femur					
1. Dry weight and length	n.s.	n.s.	n.s.		
2. DXA analysis					
Area	n.s.	n.s.	n.s.		
Total BMD	n.s. (0.08[d])	n.s.	n.s.		
Diaphyseal BMD	n.s.	n.s.	0.10	n.s.	
Metaphyseal BMD ^a	0.002[d]	n.s.	0.11	0.009	p-C < i-C; p-C < p-D
3. Biomechanical analysis					
(a) femoral neck					
<i>E</i>	0.01[d]	n.s.	n.s.		
$\sigma_{\max}$	n.s. (0.10[d])	n.s.	n.s.		
<i>F</i> _{max}	n.s.	n.s.	n.s.		
(b) diaphysis					
Area	n.s.	0.009[d]	n.s.		
<i>F</i> _{max}	n.s. (0.09[i])	n.s.	n.s.		
<i>E</i>	0.02[i]	n.s.	n.s.		
(c) distal metaphysis					
<i>F</i> _{max} ^a	<0.001[d]	n.s.	n.s.		
ρ^a	0.007[d]	<0.001[i]	0.13	<0.001	p-C < i-C; p-C < p-D
<i>F</i> _{max} / ρ	0.002[d]	n.s.	n.s.		
$\sigma_{\max}$	0.003[d]	n.s. (0.05[i])	n.s.		
<i>E</i>	<0.001[d]	n.s. (0.05[i])	n.s.		
B. Tibia					
Histomorphometry					
BV/TV ^a	0.01	n.s.	0.07	0.02	p-C < i-C; p-C < p-D
dLS/BS	n.s.	0.008[d]	n.s.		
MS/BS	n.s.	<0.001[d]	n.s.		
BFR/BS	n.s.	<0.001[d]	n.s.		

KEY: BFR/BS, bone formation rate (surface referent); BMD, bone mineral density; BV/TV, trabecular bone volume; C, control; D, diabetic; [d] and <, decreased; dLS/BS, double-labeled surface; DXA, dual-energy X-ray absorptiometry; *E*, Young's modulus; *F*_{max}, load; i, intact limb; [i] and >, increased; MS/BS, mineralizing surface; n.s., nonsignificant; p, paralyzed limb; ρ , ash density; $\sigma_{\max}$, stress.

For statistical analysis see Materials and Methods.

^aDepicted in Figure 3.

Despite the hypercalciuria, the PTH concentrations have been shown to be lower, which is presumably the result of high (passive) intestinal Ca absorption in diabetic rats induced by hyperphagia ("hyperabsorption hypercalciuria"), with downregulation of PTH secretion to prevent hypercalcemia.^{25,27} Exercise decreased the hypercalciuria, although urine volume was slightly increased (93 ± 8 vs. 75 ± 9 mL in the no-intervention group,

Figure 2. Biomechanical properties at the distal femoral metaphysis (DFM) in no-intervention and exercise control and diabetic groups. Statistical analysis (see also Table 3): ^asignificant difference between the exercise diabetic group compared with the exercise control group (Fisher's post hoc test); ^bsignificant difference between the exercise and no-intervention control groups.

significantly different according to Fisher's post hoc test). We speculate that this may be related to the increased insulin sensitivity induced by exercise, because insulin has been shown to have direct effects on tubular Ca reabsorption.⁹

In control rats, USN resulted in trabecular bone loss (proximal tibia metaphysis, distal femoral metaphysis), and unequivocally decreased biomechanical competence at the femoral metaphysis, and at the femoral neck to a lesser extent. It is well known from previous histomorphometric studies in rats that unloading results in trabecular bone loss through increased bone resorption, whereas bone formation is inhibited.^{30,33} The present study has also shown that trabecular bone formation was dampened in both paralyzed and intact limbs of USN rats; however, there was no effect of USN on plasma osteocalcin levels, possibly because the inhibition of bone formation was restricted to the hindlimb bones. Our histomorphometric results are at variance with previous data by Jee and Li,¹³ who found a small increase in the bone formation rate at the contralateral proximal tibia of rats in which the ipsilateral hindlimb had been immobilized by taping. In USN rats, the loading of the contralateral hindlimb may be decreased in response to a reduced activity level. Alternatively, a systemic factor could be involved. Indeed, the stimulation of bone formation as a consequence of ovariectomy has been attributed to one or more systemic factors, which may include IGF-I.³² However, plasma IGF-I levels were not decreased in USN rats in this study.

The effect of USN on trabecular bone loss was less pronounced in diabetic rats than in controls. ANOVA analysis

Figure 3. Selected histomorphometric, DXA, and biomechanical measurements in paralyzed and intact hindlimbs of control and diabetic rats after USN. Abbreviations: PTM, proximal tibial metaphysis; DFM, distal femoral metaphysis; BV/TV, trabecular bone volume; BMD, bone mineral density. Statistical analysis (see also Table 4): ^asignificant difference between paralyzed limbs of control rats compared with paralyzed and intact limbs of diabetic rats and intact limbs of control rats (Fisher's post hoc test); ^bsignificant effect of paralysis detected by two-way ANOVA in both control and diabetic rats.

indicated that USN had neither a significant effect on trabecular bone volume in the proximal tibia nor on the BMD and ash density at the distal femoral metaphysis; however, there was an effect of USN on some biomechanical properties (load, load/density) of the femoral metaphysis in both diabetic and control rats. This smaller effect of USN in diabetic rats was probably the result of decreased bone resorption. Indeed, Dpd excretion increased after USN in control but not diabetic rats. This should be confirmed by histomorphometric data in future studies. Our finding of reduced trabecular bone loss in the low-remodeling state of diabetes extends previous data showing that trabecular

bone loss induced by unloading in rats is aggravated in high-remodeling states such as ovariectomy or Ca deficiency.²⁰

In conclusion, this study produced the following answers to our primary research questions: (1) Running exercise does not affect low bone formation in diabetic rats. (2) Trabecular bone loss is smaller in diabetic than control rats after USN, presumably due to lower bone resorption.

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Table 5. Dynamic histomorphometry of the proximal tibia metaphysis: Bone formation parameters

	No intervention		Exercise		USN		ANOVA intact limbs
	Control	Diabetic	Control	Diabetic	Control	Diabetic	
Double-labeled surface (dLS/BS, %)	3.75 (1.15) [8]	0.36 (0.24) ^a [8]	3.97 (1.15) [8]	0.52 (0.22) ^a [8]	<i>i</i> : 1.18 (0.57) ^{b,c} [9]; <i>p</i> : 2.08 (1.03) [9]	0.10 (0.03) [9]; 0.14 (0.04) [9]	<i>p</i> < 0.001
Mineralizing surface (MS/BS, %)	17.4 (2.6)	3.5 (1.3) ^a	13.2 (1.2)	5.5 (0.9) ^a	<i>i</i> : 10.6 (1.3) ^b ; <i>p</i> : 11.5 (2.2)	2.2 (0.7) ^a ; 2.4 (0.7)	<i>p</i> < 0.001
Bone formation rate (BFR/BS) (μm ² /μm/day × 10 ⁻²)	11.6 (1.5)	2.4 (1.1) ^a	8.8 (1.1)	4.5 (0.9) ^a	<i>i</i> : 7.0 (0.5) ^b ; <i>p</i> : 7.2 (1.1)	1.7 (0.6) ^a ; 2.0 (0.6)	<i>p</i> < 0.001

KEY: *i*: intact limb; *p*: paralyzed limb. See Table 3 for other abbreviations. Data are means (SEM). Number of data given in brackets.

^aSignificant difference between diabetic and respective control groups (Fisher's LSD post hoc test).

^bSignificant difference between USN and no-intervention control groups.

^cSignificant difference between USN and exercise control groups.

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