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Skeletal Overexpression of Noggin Results in Osteopenia and Reduced Bone Formation

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Skeletal cells synthesize bone morphogenetic proteins (BMPs) and BMP antagonists. Noggin is a glycoprotein that binds BMPs selectively and antagonizes BMP actions. Noggin expression in osteoblasts is induced by BMPs and noggin opposes the effects of BMPs on osteoblastic differentiation and function *in vitro*. However, its effects *in vivo* are not known. We investigated the direct *in vivo* effects of noggin on bone remodeling in transgenic mice overexpressing noggin under the control of the osteocalcin promoter. Noggin transgenics suffered long bone fractures in the first month of life. Total, vertebral, and femoral bone mineral densities were reduced by 23–29%. Static and dynamic histomorphometry of the femur revealed that noggin transgenic mice had decreased trabecular bone volume, number of trabeculae, and bone formation rate. Osteoblast surface and number of osteoblasts/trabecular area were not significantly decreased, indicating impaired osteoblastic function. Osteoclast surface and number were normal/decreased, there was no increase in bone resorption, and the tissue had the appearance of woven bone. Vertebral microcomputed tomography scanning confirmed decreased trabecular bone volume and trabecular number. In conclusion, transgenic mice overexpressing noggin in the bone microenvironment have decreased trabecular bone volume and impaired osteoblastic function, leading to osteopenia and fractures. (*Endocrinology* 144: 1972–1978, 2003)

SKELETAL CELLS SYNTHESIZE a number of growth factors including bone morphogenetic proteins (BMP)-2, -4, and -6 (1, 2). BMPs are unique because they induce the differentiation of mesenchymal cells toward cells of the osteoblastic lineage and enhance the function of the osteoblast, playing an autocrine role in osteoblast formation and function (3–7).

BMPs bind to specific receptors and signal either by activating the P38 MAPK pathway or by phosphorylating the cytoplasmic proteins mothers against decapentalegic (Smad) 1 and 5, which form heterodimers with Smad 4, and following nuclear translocation regulate transcription (8–10). BMP activity is modulated by intracellular and extracellular antagonists, including pseudoreceptors that compete with signaling receptors, inhibitory Smads that block signaling, intracellular binding proteins that bind Smad 1 and 5, and factors that induce ubiquitination and proteolysis of signaling Smads (11–15). In addition, a large number of extracellular proteins that bind BMPs and prevent their binding to signaling receptors have emerged. They include the components of the Spemann organizer, noggin, chordin, and follistatin, members of the Dan/Cerberus family, and twisted gastrulation (16–23). The antagonists tend to be specific for BMPs and have been used to block BMP action and study its role in selected tissues.

Noggin is one of the BMP antagonists that has been studied in greater detail. Noggin is a secreted glycoprotein with a molecular mass of 64 kDa, originally characterized as a component of the Spemann organizer and shown to induce dorsalization and the formation of neural tissue (16–18). Noggin binds with various degrees of affinity BMP-2, -4, -5, -6, and -7, and growth and differentiation factors 5 and 6, but not other members of the TGFβ family of peptides (17, 24). Homozygous null mutations of the noggin gene in mice result in serious developmental skeletal abnormalities and joint lesions, and heterozygous gene mutations in humans result in multiple joint lesions (25, 26). These findings indicate embryonic and adult detrimental consequences secondary to the lack of noggin, either directly or indirectly due to tissue overexposure to BMPs. The phenotypic lethality of noggin null mice has not allowed the definition of an adult skeletal phenotype in this model.

Osteoblasts express noggin following BMP treatment and the addition of noggin to osteoblasts and stromal cell cultures decreases the stimulatory effects of BMPs on osteoblastic function and impairs osteoblastogenesis and osteoclastogenesis (7, 27). However, the consequences of noggin overexpression in the bone microenvironment *in vivo* are not known. The intent of this study was to investigate the direct effects of noggin on bone remodeling. For this purpose, we created transgenic mice overexpressing noggin under the control of the osteoblastic specific osteocalcin promoter, and determined their skeletal phenotype.

Abbreviations: BMC, Bone mineral content; BMD, bone mineral density; BMP, bone morphogenetic protein; BV/TV, trabecular relative bone volume; μCT, microcomputed tomography; d, deoxy; L3, lumbar 3; Tb.Th., trabecular thickness.

Materials and Methods

Osteocalcin/noggin construct

Noggin was expressed under the control of the osteocalcin promoter so that transcription would occur specifically in cells of the osteoblastic

lineage (28). For this purpose, a 1.7-kb fragment of the rat osteocalcin promoter (Dr. R. Derynck, San Francisco, CA) was used to direct transcription of a 0.7-kb fragment coding for murine noggin (16, 29). The second intron of the rabbit β-globin gene (0.6 kb) was included between the osteocalcin promoter and noggin coding sequences, which were followed by a 0.2-kb fragment containing polyadenylation coding sequences from the bovine GH gene. Before the generation of transgenic mice, the *in vitro* activity of the osteocalcin promoter construct was demonstrated by documenting its ability to direct green fluorescent protein gene expression in transiently transfected MC3T3 cells by fluorescence microscopy (30, 31). The ability of the construct to drive noggin *in vitro* was confirmed by transiently transfecting the transgene construct into ROS17/2 osteosarcoma cells and identification of noggin transgene mRNA (by Northern blot analysis) and protein (by Western blot analysis; data not shown).

Generation of transgenic mice

Microinjection of linearized DNA into pronuclei of fertilized oocytes from CD-1 outbred albino mice (Charles River Laboratories, Inc., Cambridge, MA), and transfer of microinjected embryos into pseudopregnant mice were carried out at the transgenic facility of The University of Connecticut Health Center (Farmington, CT). Positive founders for osteocalcin driven noggin transgene were identified by Southern blot analysis of tail DNA (32). Founder mice were bred to wild-type CD-1 outbred albino mice. Heterozygous and nontransgenic littermates from the F1 and subsequent generations were selected by Southern blotting of genomic DNA. Heterozygous mice were intercrossed but failed to generate a homozygous offspring, possibly due to embryonic lethality. Transgenic mice overexpressing noggin were compared with wild-type littermates. All animal experiments were approved by the Animal Care and Use Committee of Saint Francis Hospital and Medical Center.

Northern blot analysis and RT-PCR

Calvarial or femoral RNA was isolated by the acid guanidium thiocyanate-phenol-chloroform extraction method followed by purification with an RNeasy column (QIAGEN, Valencia, CA; Ref. 33). RNA was quantitated by spectrometry. For Northern blot analysis, equal amounts of RNA were loaded on a formaldehyde agarose gel following denaturation. The gel was stained with ethidium bromide to visualize ribosomal RNA and confirm equal RNA loading of the samples. The RNA was blotted onto GeneScreen Plus charged nylon (Perkin-Elmer, Norwalk, CT) and uniformity of transfer confirmed by revisualization of ethidium bromide-stained ribosomal RNA. A 1.0-kb murine noggin cDNA (Regeneron Pharmaceuticals, Inc.), and a 752-bp murine 18S ribosomal RNA (American Type Culture Collection, Manassas, VA) were purified by agarose gel electrophoresis. cDNAs were labeled with [α-^{32}P]-deoxy (d)-CTP (50 μCi at a specific activity of 3000 Ci/mmol; Perkin-Elmer) using Ready-To-Go DNA-labeling beads (dCTP) kit (Amersham Pharmacia Biotech, Piscataway, NJ), in accordance with manufacturer's instructions. Hybridizations were carried out at 42 C for 16–72 h, followed by two post-hybridization washes at room temperature for 15 min in 1× saline sodium citrate and a wash at 65 C for 20 to 30 min in 0.5× or 1× saline sodium citrate. The bound radioactive material was visualized by autoradiography on X-AR5 film (Eastman Kodak Co., Rochester, NY), employing Cronex Lightning Plus (Perkin-Elmer) or Biomax MS (Eastman Kodak Co.) intensifying screens.

For RT-PCR, 1 μg of deoxyribonuclease I-treated total RNA from bone extracts was reverse transcribed with murine Moloney leukemia virus reverse transcriptase (Invitrogen, Rockville, MD) in the presence of 20 μM reverse primer corresponding to an 18-bp fragment of bovine GH polyadenylation sequence present in the noggin vector. One microliter of reverse transcription reaction was amplified by 30 cycles of PCR at 64 C annealing temperature in the presence of 20 μM 5′ to 3′ primer spanning bp +355 to +375 of noggin coding sequence, [α-^{32}P]-dCTP and 2.5 U of *Taq* polymerase to yield a product of a 388-bp predicted size. The products of the PCR were resolved by electrophoresis on a 6% polyacrylamide gel and visualized by autoradiography. Northern analysis and RT-PCR shown are representative of three samples.

X-ray analysis and bone mineral density (BMD)

Radiography was performed on mice anesthetized with tribromoethanol (Sigma, St. Louis, MO) on a Faxitron x-ray system (model MX 20, Faxitron X-Ray Corp., Wheeling, IL). Total, vertebral and femoral bone mineral content (BMC; grams), skeletal area (square centimeters), and BMD (grams per square centimeter) were measured on anesthetized mice using the PIXImus small animal DEXA system (GE Medical Systems/Lunar Corp., Madison, WI; Ref. 34). Calibrations were performed with a phantom of a defined value, and quality assurance measurements were performed before each use. The coefficient of variation for total BMD is less than 1% (n = 9).

Microcomputed tomography (μCT) scanning

μCT scanning of trabecular vertebral bone of transgenic and wild-type mice was determined using a μCT-20 system (μCT-20; Scanco Medical, Zurich, Switzerland; Ref. 35). Tridimensional analysis was performed to calculate morphometric indices of the lumbar spine (L3), including bone volume density [bone volume (BV)/tissue volume (TV), %], bone surface (BS) to volume ratio (BS/TV, $mm^2/mm^3 = 1/mm$) trabecular thickness (Tb.Th., μm), trabecular number (1/mm), and trabecular separation (mm).

Bone histomorphometric analysis

Static and dynamic histomorphometry was carried out in mice following calcein injections, 20 mg/kg, 7 d, and 2 d before the mice were killed by CO_2 asphyxiation. Femora were dissected and fixed in 70% ethanol. After dehydration, bone samples were embedded in methyl methacrylate and 5-μm-thick longitudinal sections were cut on a Jung polycut (Reichert-Jung, Heidelberg, Germany) or a Microm microtome (Microm, Richards-Allan Scientific, Kalamazoo, MI) and stained with Masson, Masson-Goldner trichrome or toluidine blue stains. Static parameters of bone formation and resorption were measured at a standardized site using a BioQuant image analysis system (Nashville, OH), and an OsteoMeasure morphometry system (Osteometrics, Atlanta, GA). Trabecular relative bone volume (BV/TV, %), Tb.Th. (micrometers) and trabecular number (per millimeter), and trabecular osteoblast and osteoclast surfaces (percentage) were measured. To determine the number of osteoblasts and osteoclasts/bone or trabecular area (square millimeters), the number of cells present in 20 fields of 180 μm^2 each of bone area were counted. For dynamic histomorphometry, mineral apposition rate (micrometers per day) was measured on unstained sections under UV light, using a B-2A set long pass filter consisting of an excitation filter ranging from 450–490 nm, a barrier filter at 515 nm, and a dichroic mirror at 500 nm. Bone formation rate (cubed micrometers per square micrometers per day) was calculated. The terminology and units used are those recommended by the Histomorphometry Nomenclature Committee of the American Society for Bone and Mineral Research (36).

Statistical analysis

Results are expressed as means ± SEM. Statistical significance was determined by Student's *t* test.

Results

Generation of transgenic mice and examination of transgene expression

Two transgenic lines with analogous skeletal phenotype were generated, and one was studied in detail and is described. For this purpose, a founder expressing four to six copies of the transgene was used to generate heterozygous mice, which were compared with wild-type littermates. Attempts to generate a homozygous offspring were unsuccessful, suggesting that high levels of noggin expression may compromise viability. However, there was no evidence of heterozygous lethality following heterozygote-wild-type matings, which generated the expected 50% heterozygous/50% wild-type offspring. RT-PCR of RNA extracted from

calvariae (Fig. 1) and femora (not shown) demonstrated the presence of noggin transgene in bone from transgenic mice and not in wild-type littermate controls. Noggin mRNA was not detected by Northern blot analysis of calvariae from 6-, 10-, or 28-wk-old wild-type mice. In contrast, noggin transcripts were clearly detectable in calvariae from noggin transgenic mice, although their levels declined as the animals aged (Fig. 1). This is probably a function of decreased activity of the osteocalcin promoter in older mice (28).

Weight, x-rays, and BMD

Transgenic heterozygous mice overexpressing noggin were smaller than wild-type littermates and the decrease in weight was sustained for a 6-month period (Table 1). The phenotype appeared limited to the skeleton because noggin expression was under the control of the osteocalcin promoter, and no obvious abnormalities were noted in extraskeletal tissues. Contact radiography of noggin transgenics revealed the presence of osteopenia and fractures of long bones at 4–5 wk of age (Fig. 2). Fractures occurred in tibiae, humeri, and occasionally in femora, but they were not detected in other bones. Of the fractures detected, tibial fractures were the most common and noted in virtually all transgenic mice overexpressing noggin. The osteopenia was sustained and fractures of long bones of upper and lower extremities occurred for 8–12 wk, and at 24 wk lucent areas of what appeared poorly healed fractures were noted. Accordingly, transgenic mice overexpressing noggin had significant and sustained decreases in BMC when compared with wild-type controls (Table 1). At 5–24 wk of age, total BMC was 28–37% lower in noggin transgenics than in wild-type control mice. Although noggin transgenics had reduced skeletal area, total BMD in 5- to 24-wk-old noggin transgenics was 15–24% ($P < 0.05$) lower than in wild-type controls, indicating a sustained decrease in total BMD (Table 1). This decrease was generalized and observed in total, vertebral, and femoral bones (Table 2). At 5 wk of age, the decrease in BMD in transgenic

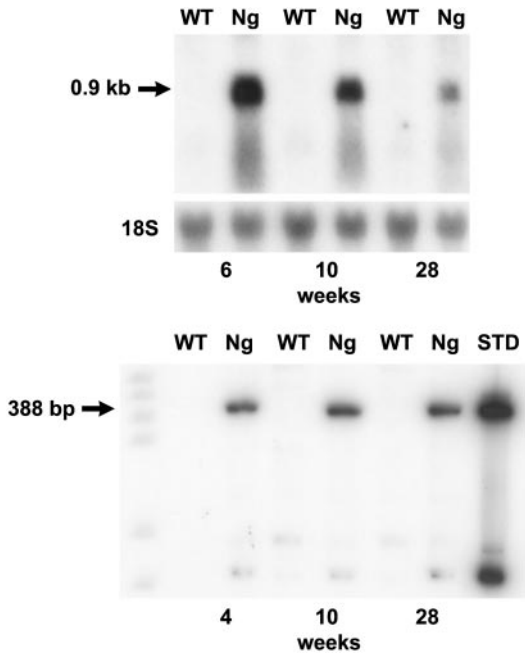

FIG. 1. Expression of noggin mRNA by Northern blot analysis (*upper panel*) and noggin transgene mRNA by RT-PCR (*lower panel*) in extracted calvariae from noggin transgenic (Ng) and wild-type (WT) control mice. Total RNA was extracted from calvariae of noggin transgenics and wild-type controls of the indicated ages. In the *upper panel*, RNA was resolved by gel electrophoresis, transferred to a nylon membrane, hybridized with [α-^{32}P]-labeled mouse noggin and 18S cDNAs, and visualized by autoradiography. In the *lower panel*, RNA was reverse transcribed, amplified by PCR in the presence of [α-^{32}P]-dCTP, resolved by gel electrophoresis, and visualized by autoradiography. *Right lane* represents osteocalcin noggin plasmid standard (STD).

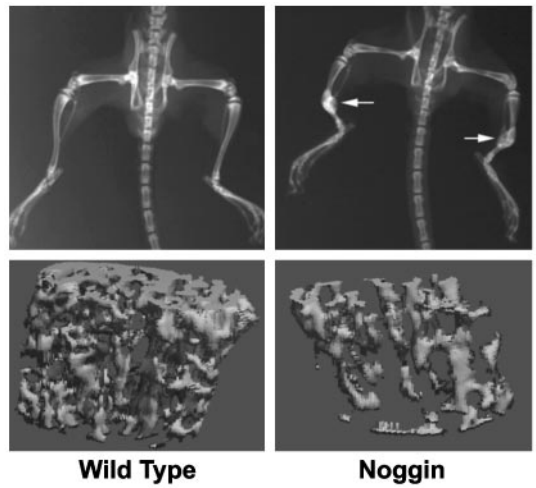

FIG. 2. The *upper panel* shows a representative skeletal radiograph of a 4-wk-old heterozygous transgenic overexpressing noggin and a wild-type littermate control mouse. *Arrows* indicate fractures and calluses. The *lower panel* shows a representative μCT scan of trabecular bone of an L3 vertebral body from a 4.5-wk-old heterozygous transgenic overexpressing noggin, with a BV/TV of 12.1%, and a wild-type littermate control, with a BV/TV of 26.7%. Images were obtained at a resolution of 18 μm, and the cortical shell was artificially removed for a better display of trabecular bone.

TABLE 1. Weight, total BMC, bone area, and BMD of 5- to 24-wk-old transgenics overexpressing noggin and wild-type littermate mice

Weeks	Weight		BMC		Skeletal area		BMD	
	Wild-type	Noggin	Wild-type	Noggin	Wild-type	Noggin	Wild-type	Noggin
5	20.6 ± 0.6	13.3 ± 0.7[a]	0.324 ± 0.01	0.206 ± 0.01[a]	8.2 ± 0.2	6.3 ± 0.2[a]	0.040 ± 0.001	0.033 ± 0.001[a]
8	28.0 ± 2.2	19.5 ± 1.3[a]	0.456 ± 0.04	0.316 ± 0.02[a]	9.1 ± 0.5	7.5 ± 0.3[a]	0.050 ± 0.001	0.042 ± 0.001[a]
12	37.8 ± 1.6	25.6 ± 1.1[a]	0.611 ± 0.01	0.383 ± 0.02[a]	10.6 ± 0.2	8.7 ± 0.3[a]	0.058 ± 0.003	0.044 ± 0.001[a]
24	31.4 ± 1.8	26.0 ± 0.3[a]	0.620 ± 0.05	0.444 ± 0.01[a]	10.6 ± 0.4	9.2 ± 0.1[a]	0.058 ± 0.002	0.049 ± 0.002[a]

Weight (g), BMC (g), skeletal area (cm^2), and BMD (g/cm^2) were obtained from 5-, 8-, 12-, and 24-wk-old noggin heterozygous transgenic mice and wild-type littermates. Values are means ± SEM (n = 3–14).

[a] Significantly different from wild-type controls, $P < 0.05$.

TABLE 2. BMD of 5- to 24-wk-old transgenic overexpressing noggin and wild-type littermate mice

Weeks	Total		Femoral		Vertebral	
	Wild-type	Noggin	Wild-type	Noggin	Wild-type	Noggin
5	0.040 ± 0.001	0.033 ± 0.001[a]	0.053 ± 0.002	0.038 ± 0.003[a]	0.042 ± 0.001	0.030 ± 0.002[a]
8	0.050 ± 0.001	0.042 ± 0.001[a]	0.067 ± 0.003	0.049 ± 0.002[a]	0.058 ± 0.002	0.042 ± 0.002[a]
12	0.058 ± 0.003	0.044 ± 0.001[a]	0.081 ± 0.002	0.058 ± 0.002[a]	0.066 ± 0.002	0.039 ± 0.002[a]
24	0.058 ± 0.002	0.049 ± 0.002[a]	0.079 ± 0.003	0.061 ± 0.002[a]	0.065 ± 0.006	0.045 ± 0.001[a]

BMD was obtained from 5, 8, 12, and 24-wk-old noggin heterozygous transgenic mice and wild-type littermates. BMD is expressed as g/cm^2 and values are means ± SEM (n = 3–14) of total, vertebral, and femoral BMD.

[a] Significantly different from wild-type controls, $P < 0.05$.

TABLE 3. Three-dimensional microstructural parameters of vertebral bones from 4.5-wk-old mice overexpressing noggin and wild-type littermate mice

	Wild-type	Noggin
BV/TV (%)	22.6 ± 2.2	12.0 ± 1.4[a]
Trabecular thickness (mm)	0.052 ± 0.002	0.046 ± 0.009
Trabecular number (1/mm)	5.7 ± 0.2	4.3 ± 0.3[a]
Trabecular separation (mm)	0.17 ± 0.01	0.24 ± 0.02[a]
Bone surface to volume ratio (1/mm)	37.7 ± 2.9	45.0 ± 1.8[a]

Morphometric indices were obtained by μCT scanning of vertebral bones from 4.5-wk-old noggin heterozygous transgenic mice and wild-type littermates. Values are means ± SEM (n = 6–7).

[a] Significantly different from wild-type controls, $P < 0.05$.

mice was 17% for total BMD, and 28 and 29% for femoral and vertebral BMD.

μCT scanning, static and dynamic histomorphometry

μCT scanning of the L3 vertebral body revealed decreased bone volume due to decreased trabecular number (Table 3 and Fig. 2). Static and dynamic parameters of bone formation and parameters of bone resorption were determined by histomorphometric analysis of the femur. Cancellous bone volume was reduced 70% in noggin transgenic mice, and this was due to a reduction in the number of trabeculae, rather than a reduction in trabecular thickness (Table 4 and Fig. 3). Histomorphometric analysis revealed normal osteoblast and osteoclast surfaces in noggin transgenic mice. In accordance, the number of osteoblasts per trabecular area was not significantly altered in noggin transgenics at either 4 wk (Table 4) or 8 wk of age, when osteoblast number/trabecular area was (mean ± SE; n = 7–8) 86 ± 8/mm^2 in wild-type and 114 ± 18/mm^2 in transgenics. The number of osteoclasts/trabecular area was decreased in noggin transgenics at 4 wk (Table 4), but not at 8 wk, when osteoclast number was 4.1 ± 1.6 in wild-type and 6.3 ± 1.5 (n = 7–8, $P > 0.05$) in transgenics. The decrease in osteoclasts/bone area observed at 4 wk of age in noggin transgenics was related to the reduction in trabecular bone resulting in areas of bone tissue without trabeculae, and therefore without cells. Mineral apposition and bone formation rates were reduced by 85% in transgenic mice, indicative of a decrease in osteoblastic function (Table 4 and Fig. 3).

In noggin transgenic mice, dual labeling with calcein resulted in diffuse broad labels, at times disorganized, characteristic of woven bone, suggesting abnormal or incomplete mineralization (Fig. 3). In some areas, mineralization fronts were visible, and were used to calculate mineral apposition rate (Table 4). In addition to the effects on trabecular bone, noggin transgenics displayed decreased bone formation in cortical bone. Cortical bone formation rate in 4-wk-old wild-type was (mean ± SEM; n = 7–9) 2.6 ± 0.3 and in transgenic mice was 1.1 ± 0.3 μm/μm^2·d, $P < 0.05$. Growth plate architecture appeared normal, although in accordance with the generalized decrease in bone size, growth plate height and width were decreased in noggin transgenics by 20%, $P < 0.05$. Consistent with the activity of the osteocalcin promoter, the phenotypic impact of noggin overexpression, as determined by bone histomorphometry, occurred in the first 4–8 wk of life and then declined (Fig. 3 and Table 5). Dual calcein labeling of 12 wk and 6-month-old transgenics revealed normal and organized mineralization fronts and absence of woven bone (not shown).

Discussion

Our findings demonstrate that transgenic mice overexpressing noggin under the control of the osteocalcin promoter develop decreased trabecular bone volume and osteopenia. The skeletal phenotype, as determined by histomorphometry, was evident at 4 and 8 wk of age, a time of marked expression of the osteocalcin promoter, but it was not observed in older animals, presumably due to a decrease in the activity of the osteocalcin promoter (28). However, the decrease in bone mineral content, the osteopenia and poor healing of fractures persisted for 6 months. The osteopenia could be due to more persistent changes in cortical bone, but it is more likely due to early changes in bone structure with a consequent decrease in bone mineral content. Abnormal mineralization would be consistent with the marked initial disruption of the bone structure, and poor healing of fractures could be due to a suppression of BMP activity by noggin because BMPs play a role in fracture healing (37). However, fractures caused misalignment of bone structures that were not corrected, and this also could affect their healing. The decrease in trabecular bone volume observed in noggin transgenic mice was secondary to a decrease in trabecular number. This appeared to be due to decreased osteoblastic activity because mineral apposition rate was decreased and the number of osteoblasts per trabecular area was not decreased significantly. Furthermore, osteoclast number was not increased and bone resorption was not visibly affected. The substantial decrease in trabecular bone resulted in areas of tissue without trabeculae or cells in noggin transgenics. However, the number of osteoblasts/bone area was not significantly decreased, although the number of

TABLE 4. Femoral trabecular bone histomorphometry in 4-wk-old transgenic overexpressing noggin and wild-type littermate mice

	Wild-type	Noggin
Trabecular relative bone volume (%)	8.3 ± 2.4	2.1 ± 0.5[a]
Trabecular osteoblast surface (%)	42.5 ± 4.4	42.4 ± 6.3
Number of osteoblasts/trabecular area (mm^2)	254 ± 24	204 ± 19
Trabecular osteoclast surface (%)	24.3 ± 1.4	25.2 ± 8.0
Number of osteoclasts/trabecular area (mm^2)	13.4 ± 2.3	5.6 ± 1.2[a]
Trabecular thickness (μm)	22.1 ± 2.4	18.8 ± 3.1
Trabecular number (per mm)	3.4 ± 0.7	1.0 ± 0.2[a]
Trabecular spacing (μm)	688 ± 2	3573 ± 1896
Mineral apposition rate (μm/d)	1.27 ± 0.15	0.22 ± 0.16[a]
Bone formation rate ($\mu m^3/\mu m^2$/d)	0.28 ± 0.03	0.04 ± 0.04[a]

Bone histomorphometry was performed on femora from 4-wk-old noggin heterozygous transgenic mice and wild-type littermates. Values are means ± SEM (n = 7–8).

[a] Significantly different from wild-type controls, $P < 0.05$.

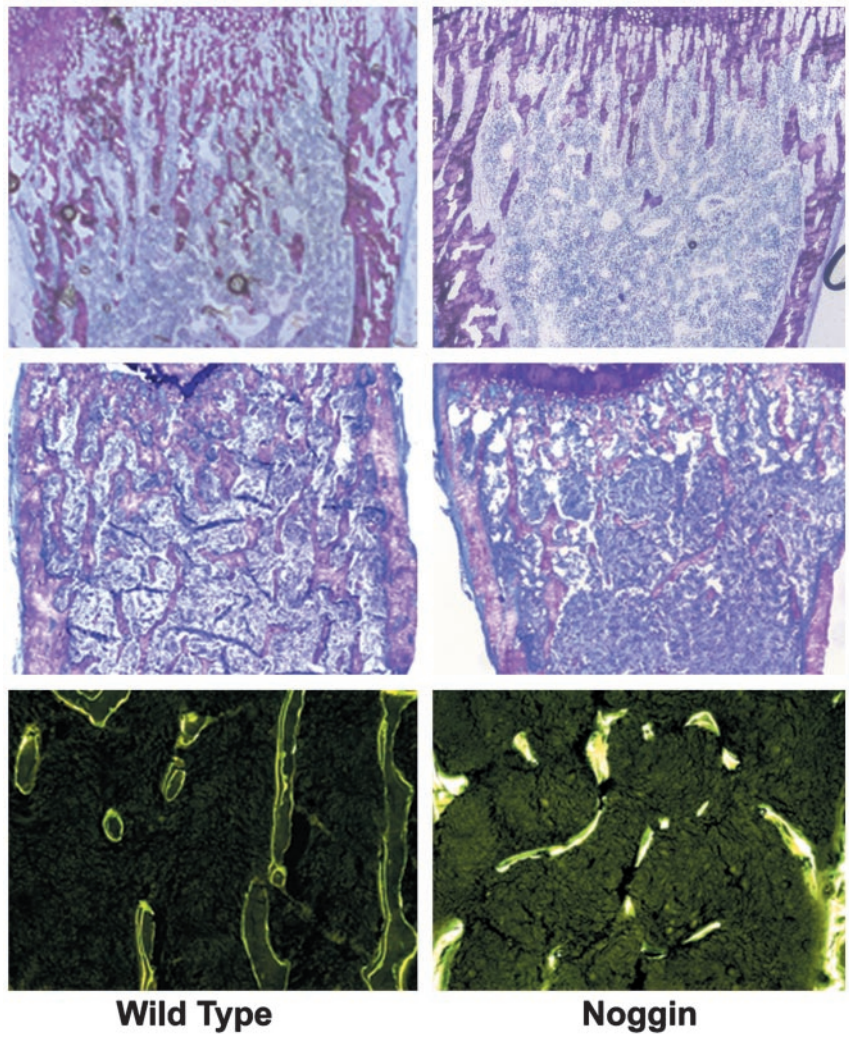

FIG. 3. Representative undecalcified sections of femora from heterozygous transgenics overexpressing noggin and wild-type littermate controls. In the *upper panel*, toluidine blue-stained sections were obtained from 4-wk-old mice (final magnification, ×100). In the *middle* and *lower panels*, toluidine blue-stained and unstained sections were obtained from the same area of 8-wk-old mice given sequential doses of calcein. Stained sections were visualized at a final magnification of ×40 and unstained sections were visualized by UV light microscopy at a final magnification of ×100.

osteoclasts present in a predefined area of bone were decreased.

The decreased osteoblastic function observed in noggin transgenics confirms previous *in vitro* observations, demonstrating that the addition of noggin to cultured calvariae results in decreased bone collagen synthesis and alkaline phosphatase activity (7). The mechanism of the impaired osteoblastic function in noggin transgenics could involve the binding and sequestering of BMPs by noggin in the bone microenvironment. There was no evidence of systemic effects of noggin, outside the skeleton, and immunoreactive noggin, as determined by ELISA, was undetectable in serum (not shown). The phenotypic changes observed are in agreement with what would be predicted from blocking the known effects of BMPs on osteoblastic function. BMPs have modest mitogenic activity in bone and have a more prevalent

TABLE 5. Selected histomorphometric parameters in 4- to 24-wk-old transgenic overexpressing noggin and wild-type littermate mice

Weeks	Trabecular bone volume (%)		Trabecular number (per mm)		Mineral apposition rate (μm/d)	
	Wild-type	Noggin	Wild-type	Noggin	Wild-type	Noggin
4	8.3 ± 2.4	2.1 ± 0.5[a]	3.4 ± 0.7	1.0 ± 0.2[a]	1.27 ± 0.15	0.22 ± 0.16[a]
8	11.9 ± 1.7	5.0 ± 0.7[a]	4.1 ± 0.5	2.1 ± 0.3[a]	1.00 ± 0.07	0.24 ± 0.07[a]
12	11.2 ± 2.2	9.8 ± 2.8	3.3 ± 0.5	3.0 ± 0.6	0.90 ± 0.16	0.81 ± 0.21
24	6.8 ± 2.5	5.7 ± 3.6	2.3 ± 0.5	1.8 ± 0.8	Not determined	Not determined

Bone histomorphometry was performed on femora from 4- to 24-wk-old noggin heterozygous transgenic mice and wild-type littermates. Values are means ± SEM (n = 3–8).

[a] Significantly different from wild-type controls, $P < 0.05$.

impact on the differentiated function of the osteoblast (4–7). However, noggin effects independent of BMPs are possible and were not excluded. Previous work has shown that BMPs induce the differentiation of mesenchymal cells toward cells of the osteoblastic lineage (38). However, our results suggest a more significant effect on osteoblastic function than in number in noggin transgenics. This is possibly due to the model used in the present study because the osteocalcin promoter becomes active only following osteoblastic cell maturation. Consequently, noggin is not overexpressed before the formation of mature osteoblasts, and in this model it might not affect the differentiation of immature cells. Recent work from our laboratory has demonstrated that stromal cells overexpressing noggin under the control of the cytomegalovirus promoter and primary cultures of stromal cells from noggin transgenic mice fail to differentiate into mature osteoblasts (39). This would confirm a dual role for BMPs in osteoblastic differentiation and function.

Our studies also demonstrate that a decrease in trabecular bone volume and osteoblastic function by noggin overexpression can be translated into significant and generalized osteopenia and fractures in the mouse. This would suggest a role for BMPs in the maintenance of a normal bone structure. The validity of the transgenic model used has been substantiated by a number of studies including the demonstration of an increase in bone formation by overexpressing IGF-I under the control of the osteocalcin promoter, and confirmation of the known anabolic actions of IGF I *in vitro* (40). The present studies suggest that noggin may act by trapping BMPs in a manner analogous to that described for IGF I and its binding proteins. Although we have postulated a need to temper the activity of locally produced skeletal growth factors, it is important to note that overexpression of proteins binding skeletal growth factors can have seriously detrimental consequences, such as osteopenia and fractures. For instance, transgenic mice overexpressing IGF binding protein 5 under the control of the osteocalcin promoter display decreased bone formation and osteopenia (31).

In conclusion, our studies demonstrate that *in vivo* skeletal overexpression of noggin, under the control of the osteocalcin promoter, causes decreased trabecular volume and severe osteopenia. This appears secondary to impaired osteoblastic function.

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