Prenatal Exposure to IL-1β Results in Disturbed Skeletal Growth in Adult Rat Offspring

DIANA SWOLIN-EIDE, CECILIA NILSSON, AGNETA HOLMÄNG, AND CLAES OHLSSON

Center of Bone Research at the Sahlgrenska Academy, RCEM, Department of Internal Medicine [D.S.-E., C.O.], Göteborg Pediatric Growth Research Center, Institute for the Health of Women and Children, Queen Silvia Children's Hospital [D.S.-E.], and Wallenberg Laboratory [C.N., A.H.], Sahlgrenska University Hospital, S-413 45 Göteborg, Sweden

ABSTRACT

Events occurring early in life or prenatally are able to play important roles in the pathogenesis of diseases in adult life. Different sorts of stress or hormonal influences, during particular periods of pregnancy, may result in persistent or transient changes in physiology. IL-1 is a multifunctional cytokine that is involved in bone metabolism. The aim of the present study was to investigate whether exposure to IL-1β during fetal life has any effect on skeletal growth or bone mineral density in adult rat offspring. Pregnant rats were given intraperitoneal injections of IL-1β, 1 μg/rat, or saline on days 8, 10, and 12 of gestation. Male IL-1–exposed offspring showed reduced height, areal bone mineral density, and bone mineral content at vertebra L5. Tibial length was reduced in both male and female offspring. Peripheral quantitative computed tomography analyses revealed reduced cortical bone mineral content caused by a decreased cortical cross-sectional area as a result of a decreased cortical thickness, whereas there was no reduction in the amount of trabecular bone in the tibia of male offspring. Our results demonstrate that prenatal exposure to IL-1 can induce specific programming of skeletal tissue. In conclusion, prenatal IL-1 exposure results in decreased skeletal growth and a reduced amount of cortical bone but unchanged trabecular bone mineral density in adult rat offspring. (*Pediatr Res* 55: 598–603, 2004)

Abbreviations
DXA, dual-energy x-ray absorptiometry
BMC, bone mineral content
BMD, bone mineral density
pQCT, peripheral quantitative computed tomography
HPA, hypothalamic-pituitary-adrenal
TRAP 5b, tartrate-resistant acid phosphatase 5b

It has been shown that events occurring early in life or prenatally are able to play important roles in the pathogenesis of adult diseases in both humans and animals (1, 2). The process whereby a stimulus or insult at a sensitive or critical period of development has long-term effects is termed programming (3). A low birth weight and leanness at birth have been shown to be associated with insulin resistance in children and adolescents (4, 5). Furthermore, a recent clinical study suggests that a low birth weight is associated with lower adult bone and muscle mass, indicating that the risk of osteoporosis in later life might be programmed by genetic or environmental influences during gestation (6).

IL-1 is a multifunctional cytokine that is involved in the regulation of bone metabolism, hematopoiesis, and inflammatory responses (7). Maternal infection during pregnancy leads to a systemic inflammatory response, with release of cytokines and glucocorticoids, representing a stressful event for the fetus. Early onset of neonatal sepsis is associated with increased IL-1 levels in umbilical plasma (8). Lipopolysaccharides from bacteria have been shown, via the release of IL-1, IL-6, and tumor necrosis factor-α, to regulate the neuroendocrine axis in adult rats and humans (9–11). Prenatal treatment with IL-1 results in long-lasting alterations in psychomotor development, behavior, and the neuroendocrine system (12, 13).

IL-1 is also regarded as a bone-regulating cytokine. It is involved in the regulation of bone remodeling (7, 14–16), fracture healing (17), inflammatory bone diseases (18), and malignant bone destruction (19). IL-1 is a potent stimulator of osteoclast formation, associated with increased bone resorption

(14, 19, 20). Both IL-1α and IL-1β are produced by osteoblasts (21, 22), and IL-1 stimulates the proliferation of cultured osteoblasts (23). Thus the major effect of IL-1 on bone metabolism is to regulate bone resorption, but an effect on bone formation cannot be excluded.

The aim of the present study was to investigate whether prenatal IL-1 exposure affects skeletal growth or mineralization of bones in rat offspring.

METHODS

Animals. Timed-pregnant nulliparous Wistar rats (B&K Universal, Sollentuna, Sweden) were housed, one animal to a cage, until parturition. Pups were raised with a lactating mother until 4 wk of age. Thereafter, they lived in cages, with three to four animals from the same group per cage. The study was approved by the Animal Ethic Committee of Göteborg University. Rats were housed under controlled conditions, at a temperature of 21°–22°C, humidity of 55 to 65%, and light on from 0500 to 1900 h. They received a commercial rat chow [containing 18.7% protein, 4.7% fat, and 63% carbohydrates with sufficient supply of vitamins and minerals (B&K Universal)]. Tap water was available *ad libitum*.

Dams and litters. Dams were randomly divided and given i.p. injections of IL-1β (1 μg/rat; Boehringer Mannheim Biochemica, Mannheim, Germany) on days 8, 10, and 12 of gestation ($n = 7$). Control dams ($n = 7$) were injected i.p. with physiologic saline. The IL-1β dose (1 μg/rat) was chosen as it is known to stimulate the HPA axis in the adult rat (24). It is also known to result in no fetal anomalies and no abortion (unpublished observation, A. Holmäng). This dose had also been used by Götz *et al.* (12), who reported giving it i.p. from day 17 to 21 of pregnancy in rats, resulting in alterations in psychomotor development and behavior.

Gestation lasted for 22 d in all dams. Body weight and food intake of the dams were recorded throughout the pregnancy. All the dams were presented with the same amount of food, and food intake was measured the next day by subtracting the remaining uneaten food. This was calculated as food intake in grams per rat and day. Core body temperature of the dams was measured by a rectal probe 3 h after the IL-1 injection, as previously described (25). One IL-1 dam and one control dam did not deliver. Litters were weighed at birth and placed with their dams within each experimental group. Male and female offspring were brought up together, and each dam had equal numbers of pups (7, 8) to guarantee similar feeding conditions. Litters were adjusted so that they contained approximately the same ratio of male to female pups. Pups were left undisturbed until weaning at 4 wk of age, except for the occasion when they were weighed. Offspring were killed by an overdose of anesthesia at 10 (male) or 12 (female) wk of age.

Food intake. On two different occasions, when the rat offspring were 5 and 9 wk of age, their food consumption was recorded for the whole day. All were presented with the same amount of food, and their intake was measured the next day by subtracting the remaining uneaten food. This was done for 1 wk, and food intake was calculated in grams per rat and day.

Hormone analysis. Blood was collected in heparinized microtubes and centrifuged immediately in a microcentrifuge at 4°C. Testosterone was measured with a solid-phase RIA (Coat-A-Count Total Testosterone, Diagnostic Products, Los Angeles, CA, U.S.A.), and 17β-estradiol was determined with an ultrasensitive estradiol RIA (Diagnostic Systems Laboratories, Webster, TX, U.S.A.). Progesterone was assayed with a commercially available enzyme immunoassay (progesterone ELISA; Biomar Diagnostic Systems, Marburg, Germany). Corticosterone was determined with an RIA (RSL ^{125}I corticosterone RIA; ICN Biomedicals, Costa Mesa, CA, U.S.A.; detection level above 25 ng/mL). Leptin was determined by an RIA (Rat Leptin RIA kit; Linco Research, St. Louis, MO, U.S.A.). Serum IGF-I was measured by a double-antibody IGF binding protein–blocked RIA (IGF-I, IGFBP blocked, Mediagnost, Tubingen, Germany). Serum osteocalcin levels were measured by an ELISA (Rat-MID osteocalcin ELISA, Osteometer BioTech A/S, Herlev, Denmark). TRAP 5b was analyzed in serum with a solid-phase immunofixed enzyme activity assay (Rat TRAP; Suomen Bioanalytiikka Oy, SBA Sciences, Oulu, Finland). A C-terminal telopeptide fragments of type I collagen cross-links were analyzed with a RatLaps kit (Osteometer Bio TechA/S).

DXA

BMC and areal BMD (BMC per square centimeter) were measured with the pDEXA Sabre and Sabre Research software (both from Norland Medical Systems Inc., Fort Atkinson, WI, U.S.A.). *Ex vivo* measurements of tibia and vertebra L6 were performed on excised bones placed on a 1-cm-thick Plexiglas table. All bones compared were measured in the same scan as previously described (26).

PQCT

Computed tomography was performed with the Stratec pQCT XCT Research M (software version 5.4B; Norland Medical Systems Inc.) operating at a resolution of 70 μm (27).

Mid-diaphyseal pQCT scans of the tibia were performed to determine the cortical BMC, the cortical volumetric BMD, the cortical cross-sectional area, the cortical thickness, and the periosteal and endosteal circumference. The mid-diaphyseal region of the femur and tibia in rats contains mostly cortical bone (26).

Metaphyseal pQCT scans of the left tibia were performed to measure trabecular volumetric BMD. The scan was positioned in the metaphysis at a distance from the proximal growth plate corresponding to 5% of the total length of the tibia (an area containing a central portion of trabecular bone). The trabecular bone region was defined by setting an inner threshold of 45% of the total area. The interassay coefficients of variation for the pQCT measurements were less than 2%.

It should be emphasized that the DXA technique gives the areal BMD, whereas the pQCT gives the real, volumetric BMD. Thus, DXA gives the mineral content per area, not per volume. Therefore, a factor regulating the outer dimensions of a bone will affect the areal BMD (DXA) but not the volumetric BMD (pQCT).

HISTOMORPHOMETRIC ANALYSIS

Histomorphometric analysis was performed on the rat distal femur of 10-wk-old male rats (Pharmatest Services Ltd., Turku, Finland). The variables were calculated from one section of each bone sample. Six fields (two rows, total area 4.6 mm^2) were measured 0.9 mm above the growth plate in each section. The following variables were determined: trabecular bone volume relative to tissue volume, trabecular thickness, osteoclast surface relative to bone surface, and number of osteoclasts relative to bone surface.

STATISTICAL METHODS

Distribution of variables is given as mean $\pm$ SEM. A Mann-Whitney nonparametric U test was used for comparison between the treatment groups and the control groups. A two-tailed p value of <0.05 was considered statistically significant.

RESULTS

Dams and litters. The body weight of the dams increased throughout pregnancy, and there was no significant difference in body weight between the two groups at any time (data not shown). There was an insignificant tendency to an increase in core body temperature in the IL-1–exposed animals (37.7° $\pm$ 0.8°C) compared with control dams (37.0° $\pm$ 0.3°C). A significant reduction in food intake was seen in IL-1–exposed dams, on days 9, 11, 13, and 14 (Fig. 1). However, no significant difference was found in litter size for the IL-1–exposed group compared with the control group [IL-1, 6.0 $\pm$ 1.1; controls, 7.2 $\pm$ 0.9 ($n = 10$ in each group)].

Body weights of offspring. There was no difference in body weight between the newborn pups in the IL-1–injected group and the controls [male IL-1, 6.80 $\pm$ 0.16 g ($n = 15$); controls, 6.68 $\pm$ 0.1 g ($n = 22$), female IL-1, 6.49 $\pm$ 0.12 g ($n = 20$); controls, 6.41 $\pm$ 0.12 ($n = 21$)]. Body weight was recorded

from week 1 to week 10 for male offspring and from week 1 to week 11 for female offspring. No significant effect on body weight was seen at any time (data not shown). Food intake was measured in control offspring and IL-1–treated offspring at 5 wk of age and between 9 and 10 wk of age. There was no difference in food intake between IL-1–treated and control offspring (data not shown).

Organ weights. The weights of different organs were measured at 12 wk of age for female rats and at 10 wk of age for male rats. No difference were found in the weights of muscle, adipose tissue, gonads, thymus, spleen, or heart, but the adrenals of male IL-1–treated offspring were slightly lighter than the adrenals of control offspring [IL-1, 0.20 $\pm$ 0.01 g; controls, 0.23 $\pm$ 0.01 g; ($n = 9$–12), $p < 0.05$].

Skeletal growth. The height of vertebrae L5 and L6 and the length of the tibia were measured at 10 wk of age for male rats and at 12 wk of age for female rats (Table 1). Male IL-1–treated offspring showed clearly reduced vertebral heights (Table 1). Tibial lengths were reduced in both male and female IL-1–treated offspring (Table 1).

Bone mineral status as determined by DXA. Areal BMD of the spine and tibia were examined by DXA. IL-1–treated male offspring had a reduced areal BMD in the spine but not in the tibia compared with control rats (Table 2).

Trabecular bone as determined by pQCT and histomorphometric analyses. The pQCT analysis was performed to investigate whether there were any specific effects on cortical or trabecular bone, which could not be separated by DXA analysis. The trabecular volumetric BMD in the proximal metaphysis of the tibia was unchanged in the IL-1–treated offspring [male IL-1, 0.269 $\pm$ 0.009 mg/mm^3; controls, 0.249 $\pm$ 0.012 mg/mm^3, female IL-1, 0.516 $\pm$ 0.032 mg/mm^3; controls 0.457 $\pm$ 0.027 mg/mm^3 ($n = 10$ in each group)]. Histomorphometric analyses verified that the trabecular bone variables were unchanged in the IL-1–treated male offspring (Table 3).

Cortical bone as determined by pQCT. Cortical bone variables were analyzed in the mid-diaphyseal region of the tibia. Male IL-1–treated offspring showed a clear reduction in cortical BMC caused by a decreased cortical cross-sectional area although a slight increase in cortical volumetric BMD was seen. The reduced cortical cross-sectional area was the result of decreased cortical thickness (Fig. 2). Female IL-1–treated offspring showed a nonsignificant tendency for reduced amount of cortical bone (data not shown).

Serum variables. Serum levels of TRAP 5b were increased in male offspring compared with females in the untreated group
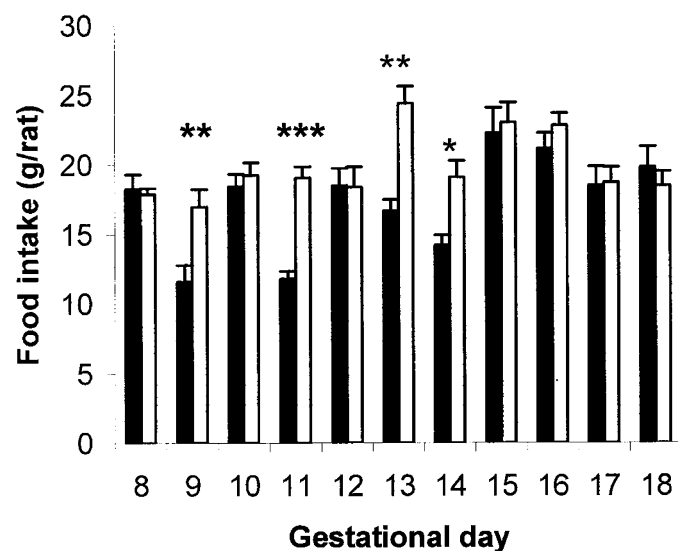

Figure 1. Food intake (g/rat and day) in IL-1–exposed dams (*solid bars*, $n = 6$) and control dams (*open bars*, $n = 6$) from gestational day 8 until day 18. Values are mean $\pm$ SEM. Data were analyzed with Mann-Whitney nonparametric U test. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$; IL-1 *vs* control.

Table 1. *Length of tibia and height of vertebrae*

	Male		Female	
	Control ($n = 10$)	IL-1 ($n = 10$)	Control ($n = 10$)	IL-1 ($n = 10$)
Vertebra L5 (mm)	7.37 $\pm$ 0.15	6.90 $\pm$ 0.09*	6.92 $\pm$ 0.11	6.51 $\pm$ 0.16
Vertebra L6 (mm)	7.40 $\pm$ 0.07	7.03 $\pm$ 0.11*	6.93 $\pm$ 0.11	6.99 $\pm$ 0.09
Tibia (mm)	37.90 $\pm$ 0.20	37.10 $\pm$ 0.20**	35.20 $\pm$ 0.20	34.60 $\pm$ 0.20*

Bone measurements were made at 10 wk of age (male) or 12 wk of age (female). Values are given as mean $\pm$ SEM. Data were analyzed with Mann-Whitney nonparametric U test. *$p < 0.05$, **$p < 0.01$; IL-1 *vs* control.

Table 2. *DXA measurements*

	Male rats		Female rats	
	Control ($n = 10$)	IL-1 ($n = 10$)	Control ($n = 10$)	IL-1 ($n = 10$)
Spine areal BMD (g/cm^2)	0.175 ± 0.003	0.164 ± 0.002**	0.166 ± 0.003	0.164 ± 0.003
Tibia areal BMD (g/cm^2)	0.155 ± 0.001	0.155 ± 0.002	0.156 ± 0.002	0.155 ± 0.001

DXA was performed at 10 wk of age for male rats and at 12 wk of age for female rats. Values are given as mean ± SEM. Data were analyzed with Mann-Whitney nonparametric *U* test. **$p < 0.01$, IL-1 *vs* control.

Table 3. *Histomorphometric analysis*

	BV/TV (%)	Tb Th (μm)	OcS/BS (%)	No. Oc/BS (/mm)
Control ($n = 5$)	19.9 ± 1.2	48.2 ± 4.5	13.8 ± 1.4	3.9 ± 0.3
IL-1 ($n = 6$)	18.9 ± 2.0	45.2 ± 2.8	10.6 ± 1.4	3.2 ± 0.4

Values are given as mean ± SEM. Data were analyzed with Mann-Whitney nonparametric *U* test.

trabecular bone volume/tissue volume (BV/TV), trabecular thickness (TB.Th), osteoclast surface/bone surface (Oc.S./B.S) and number of osteoclasts/bone surface (N.Oc/B.S.).

Abbreviations used: BV/TV, trabecular bone volume relative to tissue volume; TB Th, trabecular thickness; OcS/BS, osteoclast surface relative to bone surface; Oc/BS, osteoclasts relative to bone surface.

($p < 0.01$). After prenatal exposure to IL-1 this sex difference in TRAP 5b levels disappeared (Table 4). C-terminal type I collagen levels were reduced, whereas testosterone levels were increased in female IL-1–treated rats compared with controls. Serum levels of IGF-I, corticosterone, leptin, progesterone, estradiol, and osteocalcin were unchanged in IL-1–treated offspring compared with controls in both males and females (Table 4).

DISCUSSION

The programming hypothesis, whereby events early in life or during specific fetal periods have long-lasting effects later in
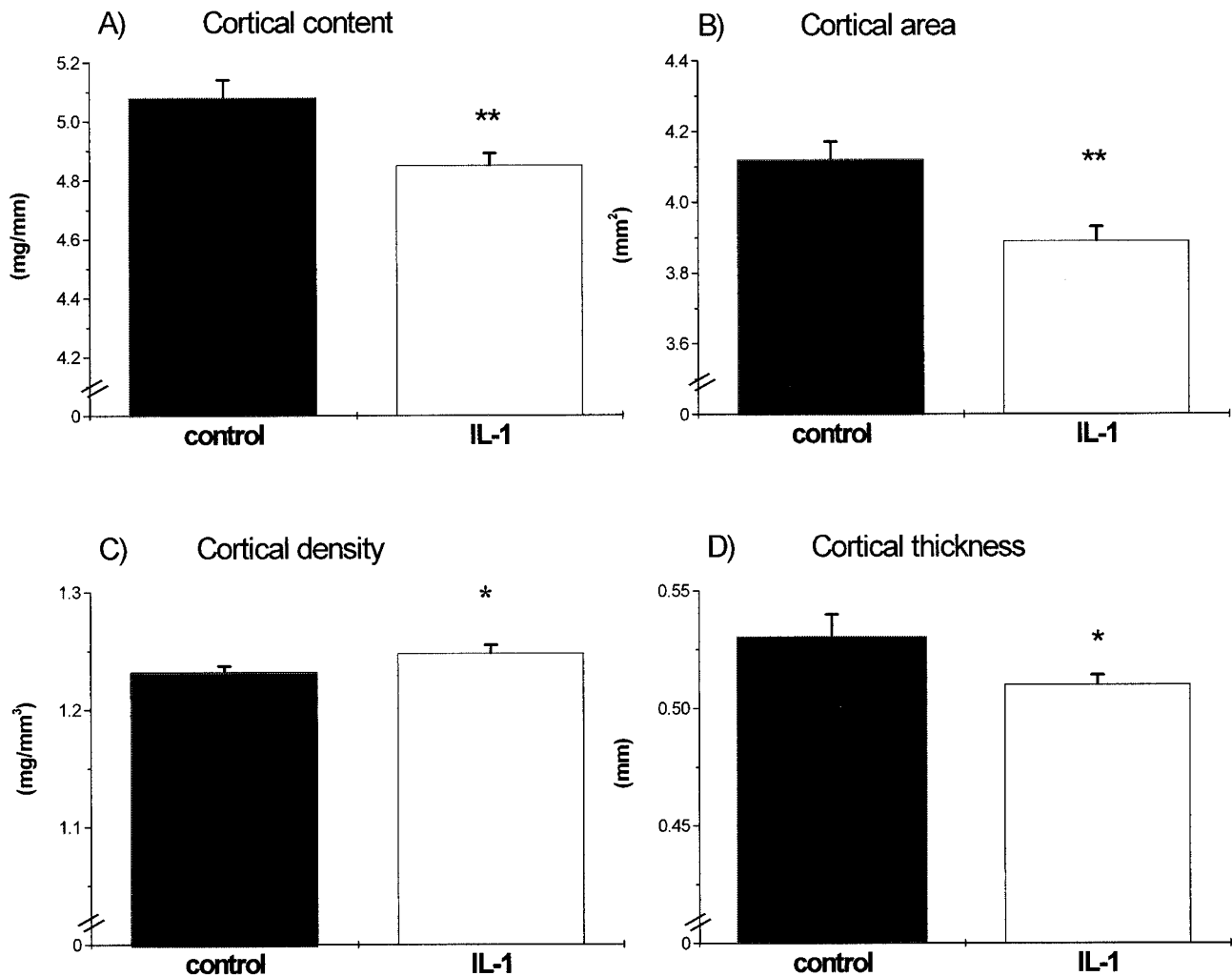

Figure 2. Cortical bone variables of the tibia as measured by pQCT for IL-1–treated male offspring (*open bars*, $n = 10$) and control (*solid bars*, $n = 10$). *A,* cortical BMC. *B,* cortical cross-sectional area. *C,* cortical BMD. *D,* cortical thickness. The analyses were performed at 10 wk of age. Values are given as mean ± SEM. Data were analyzed with Mann-Whitney nonparametric *U* test. *$p < 0.05$, **$p < 0.01$; IL-1 *vs* controls.

Table 4. *Serum variables*

	Male		Female	
	Control	IL-1	Control	IL-1
Osteocalcin (ng/mL)	222 ± 11	228 ± 9	222 ± 18	192 ± 12
TRAP 5b (U/L)	0.99 ± 0.11	0.62 ± 0.07	0.36 ± 0.05	0.61 ± 0.07*
C-terminal type I collagen (ng/mL)	55.6 ± 4.0	55.7 ± 4.8	74.6 ± 12.2	48.3 ± 2.7**
IGF-I (ng/mL)	603 ± 50	735 ± 67	832 ± 70	719 ± 69
Corticosterone (ng/mL)	287 ± 23	316 ± 51	374 ± 48	287 ± 51
Leptin (ng/mL)	2.36 ± 0.44	1.77 ± 0.18	1.25 ± 0.11	1.38 ± 0.11
Testosterone (nM)	1.57 ± 0.55	1.83 ± 0.79	0.106 ± 0.007	0.131 ± 0.009*
Progesterone (nM)	3.45 ± 0.85	6.46 ± 1.39	25.23 ± 3.7	25.9 ± 3.7
Estradiol (pM)	Not detectable	Not detectable	15.8 ± 1.9	14.2 ± 1.2

Biochemical serum variables measured between 5 and 12 wk of age for male and female rats ($n = 9$–12). Values are given as mean ± SEM. Data were analyzed with Mann-Whitney nonparametric *U* test. *$p < 0.05$, **$p < 0.01$; IL-1 *vs* control.

life, is supported by human epidemiologic data and animal models. Several studies have demonstrated that changes in the nutritional, metabolic, and hormonal environment during both prenatal and postnatal periods of life may have profound effects on body composition, glucose tolerance, blood pressure, and cardiovascular diseases (2, 3, 28, 29). A recent clinical study suggests that a low birth weight is associated with decreased bone and muscle mass in adults, indicating that the risk of osteoporosis in later life might be programmed by genetic or environmental influences during gestation (6). The notion that skeletal growth may be programmed during intrauterine or early postnatal life is supported by a study by Cooper *et al.* (30), demonstrating a relationship between adult bone mass and weight at 1 y of age.

IL-1 levels might be increased in pregnant women as well as in newborns as a result of, for instance, infections and inflammatory diseases. Previous studies regarding prenatal exposure to IL-1 have focused on alterations in psychomotor development, behavior, and the neuroendocrine system (12, 13). The aim of the present study was to investigate whether prenatal IL-1 exposure affects skeletal growth or adult bone metabolism in rat offspring.

We demonstrate that prenatal IL-1 exposure results in decreased axial as well as appendicular skeletal growth. One possible explanation for the reduced skeletal growth could be that the food intake of dams was reduced after each IL-1 injection. It is well known that low birth weight is associated with insulin resistance in children and adolescents (4, 5). Furthermore, intrauterine exposure to a maternal low-protein diet has recently been shown to reduce adult bone mass and alter growth plate morphology in rats (31). However, as the reduction in food intake in the dams that occurred the day after the injection affected neither the total body weight of the dam nor the birth weight of the IL-1–exposed offspring, it is unlikely that the decreased food intake is involved in the disturbed skeletal growth in the offspring. To definitively rule out differences in food intake of the dams as being involved in mechanism behind the skeletal phenotype in the offspring, additional studies including pair feeding of untreated animals are required. Interestingly, the weights of several different organs and tissues, including muscles, fat depots, gonads, thymus, spleen, and heart, were unchanged in the IL-1–treated offspring, indicating that the skeletal effect was specific and did not just reflect a general growth disturbance.

The weight of the adrenals was, however, decreased in male offspring. Similarly, in a previous study by Götz *et al.* (12), it was demonstrated that exposure to IL-1 late in pregnancy (days 17–21) resulted in decreased adrenal weight in the offspring. Furthermore, both in the present study and in the study by Götz *et al.* (12), it was found that serum levels of androgens were increased in IL-1–treated female offspring.

In this study we found evidence that the skeletal tissue is another target for the programming theory. DXA measurements demonstrated that areal BMD was decreased in the spine. The pQCT technique and histomorphometry were used to separate the effects on cortical bone and trabecular bone. Both the pQCT technique and histomorphometry demonstrated that trabecular bone was unaffected in the IL-1–treated offspring. However, male IL-1–treated offspring showed a clear reduction in cortical BMC, caused by a decreased cortical cross-sectional area. The reduced cortical cross-sectional area was the result of decreased cortical thickness. Interestingly, serum levels of the osteoclast bone marker TRAP 5b were decreased in male and increased in female IL-1–treated offspring. The mechanism behind this effect remains to be elucidated.

In the present study a clear effect was seen on the axial skeletal (vertebrae) growth whereas only a modest effect was seen on the appendicular skeletal (tibia) growth. There are a limited number of previous reports regarding fetal programming of the skeleton (32, 33). However, the effect on the appendicular skeletal growth in the present study was of the same magnitude as previously reported in studies using prenatal exposure with leptin and dexamethasone, and the effect on the axial skeletal growth was of the same magnitude as prenatal leptin exposure but more pronounced than for prenatal dexamethasone exposure (32, 33).

Our study clearly demonstrates that exposure to IL-1 results in decreased skeletal growth and affects cortical bone variables in rat offspring. However, the mechanism behind this effect is unclear and might include both central and peripheral effects. One possible mechanism is that IL-1 might exert a direct central effect on the brain, supported by the fact that the IL-1 treatment in the present study corresponds to an early period when fetal brain development occurs in the rat (34). It is well known that IL-1 increases glucocorticoid secretion by enhancing synthesis and release of corticotropin-releasing hormone and ACTH (35). We have previously demonstrated that treat-

ment with dexamethasone results in disturbed skeletal growth in the offspring (32). A disturbed HPA axis in the treated offspring is supported by the fact that the weight of the adrenal gland was decreased in male offspring. However, basal serum levels of corticosterone were unchanged. Dysregulation of the HPA axis, however, cannot be excluded, as a reduced corticosterone response to stress has been seen after postnatal IL-1 administration (13).

IL-1 exerts direct effects on skeletal cells (19, 20). Thus the effect of IL-1 on skeletal programming might be a direct effect on skeletal cells, including chondrocytes, osteoblasts, and osteoclasts. An effect at the cellular level has recently been reported by Oreffo *et al.* (36). They found reduced osteoblast activity and affected bone marrow stromal cells in offspring from mothers on a low-protein diet. A third pathway by which IL-1 might influence skeletal programming in the offspring is an indirect effect via the placenta or via hormones and growth factors in the pregnant dam. A limitation with the present study is that the exact mechanism for the described skeletal effects is not identified, and therefore future additional mechanistic studies are required.

CONCLUSIONS

Our results demonstrate that prenatal exposure of IL-1 can induce specific programming of skeletal tissue. In conclusion, prenatal IL-1 exposure results in decreased skeletal growth and reduced amount of cortical bone but unchanged trabecular BMD in rat offspring.

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