



MORPHOFUNCTIONAL CHANGES IN LONG BONES OF 14-DAY-OLD OFFSPRING OF MOTHERS EXPOSED TO PRENATAL STRESS

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Abstract: Prenatal maternal stress is considered an important environmental factor capable of modifying fetal development through neuroendocrine and metabolic pathways. Activation of the hypothalamic–pituitary–adrenal axis during maternal stress increases glucocorticoid exposure, which may affect osteoblast proliferation, osteoclast activity, chondrocyte differentiation, extracellular matrix formation, and bone mineralization. The present article summarizes a proposed experimental assessment of morphofunctional changes in the long bones of 14-day-old offspring born to mothers exposed to prenatal stress. The study framework is based on the supplied dissertation annotation and integrates histological examination, morphometry, bone mineral density assessment, and osteogenic/resorptive molecular markers. Femur and humerus are considered target bones. Particular attention is given to epiphyseal plate thickness, proliferative and hypertrophic zones, trabecular architecture, cortical thickness, bone mineral density, osteocalcin, RANKL, OPG, and type I collagen. The expected pattern is a reduction in growth-plate organization, trabecular thickness, cortical thickness and mineral density, together with changes in markers reflecting reduced osteogenesis and increased bone resorption. These findings may help clarify the morphological substrate of developmental programming associated with prenatal stress.

Keywords: *prenatal stress; maternal stress; long bones; offspring; epiphyseal plate; bone mineral density; osteogenesis; RANKL; OPG; osteocalcin.*



Introduction

Prenatal stress has attracted increasing attention because adverse maternal conditions may influence fetal development beyond the immediate period of exposure. The supplied research annotation emphasizes that maternal stress activates the hypothalamic–pituitary–adrenal axis and increases glucocorticoid concentrations. Glucocorticoids can cross the placental barrier and influence organogenesis, including skeletal development. Previous literature cited in the annotation associates prenatal stress with disturbances of osteogenesis and bone mineralization (Weinstock, 2017).

Bone is a metabolically active tissue that undergoes continuous modeling and remodeling. Its growth depends on coordinated proliferation and differentiation of chondrocytes within the epiphyseal growth plate, osteoblast-mediated matrix formation, osteoclast-mediated resorption, vascular support, and mineral deposition. According to the annotation, elevated glucocorticoid exposure may suppress osteoblast proliferation and enhance osteoclast activity, potentially accelerating bone resorption and disturbing chondrocyte differentiation (Sapolsky, 2015).

Long bones are particularly informative for evaluating developmental effects because their longitudinal growth depends on the organized architecture of the epiphyseal plate. Alterations in the proliferative and hypertrophic zones may reflect impaired endochondral ossification. In addition, reduced trabecular thickness, increased inter-trabecular distance, and thinning of the cortical layer may indicate changes in bone microarchitecture. Prenatal stress may also affect calcium–phosphorus metabolism and osteoid mineralization, potentially resulting in reduced bone mineral density (Bose et al., 2020).

The annotation proposes a comprehensive approach combining histology, morphometry, densitometry, and molecular-biochemical markers. This integrated design is important because a single histological parameter cannot fully characterize the functional state of developing bone. Osteocalcin, RANKL, OPG, and type I



collagen provide complementary information concerning osteogenesis, resorption, and extracellular matrix formation (Kovacs, 2016).

Aim of the Study

To characterize the expected morphofunctional changes in the long bones of 14-day-old offspring born to mothers exposed to experimental prenatal stress, with emphasis on growth-plate organization, bone microarchitecture, mineral density, and markers of bone formation and resorption.

Materials and Methods

The experimental framework follows the methodology described in the supplied dissertation annotation. The original protocol uses sexually mature healthy female Wistar rats weighing 180–220 g and maintains them under standard vivarium conditions ($22\pm 2^{\circ}\text{C}$, relative humidity 55–60%, and a 12-hour light/dark cycle). Animals are divided into control, experimental stress, and an observation group for dynamic melatonin analysis. The experimental stress model is immobilization during gestational days 7–19 for 2 hours daily. The annotation states that this procedure is intended to activate the hypothalamic–pituitary–adrenal axis and increase glucocorticoid secretion.

For the present article, the postnatal assessment point is specified as 14 days of age, as requested. This represents a modification of the time point listed in the supplied annotation, which describes fetal day 20, one-day-old offspring, and 30-day-old offspring. Therefore, the 14-day time point should be regarded as a proposed study time point rather than an already reported experimental result.

The femur and humerus are selected as representative long bones. Following fixation in 10% neutral formalin, bone specimens are processed using EDTA decalcification, paraffin embedding, and 5–7 μm sectioning. Hematoxylin–eosin, Masson trichrome, and Van Gieson staining are proposed for evaluation of growth-plate structure, osteon architecture, trabecular organization, osteoblast and osteoclast activity, collagen matrix, and vascular components.



Morphometric measurements are performed using digital microscopy and ImageJ software. The principal parameters include total epiphyseal-plate thickness, heights of the proliferative and hypertrophic zones, trabecular thickness, inter-trabecular distance, and cortical thickness. The annotation specifies analysis of at least 10 standardized microscopic fields per preparation.

Bone mineral density (BMD) and bone mineral content (BMC) are proposed to be assessed by dual-energy X-ray absorptiometry (DEXA). Molecular-biochemical assessment includes osteocalcin, RANKL, OPG, and type I collagen using ELISA. Statistical processing in the original protocol is planned using SPSS 26.0, with Student's t-test and one-way ANOVA for group comparisons and Pearson correlation for relationships among variables; $p < 0.05$ is considered statistically significant.

Table 1. Main parameters for assessment of long-bone development in 14-day-old offspring

Domain	Parameter	Expected direction in prenatal-stress offspring
Histology	Epiphyseal plate thickness	Decrease
Histology	Organization of chondrocyte columns	Disorganization
Histology	Proliferative/hypertrophic zones	Reduced thickness
Microarchitecture	Trabecular thickness	Decrease
Microarchitecture	Inter-trabecular distance	Increase
Microarchitecture	Cortical thickness	Decrease
Mineralization	Bone mineral density (BMD)	Decrease
Osteogenesis	Osteocalcin	Decrease
Resorption	RANKL	Increase
Resorption regulation	OPG	Decrease



Matrix formation

Collagen type I

Decrease

Expected Results

Because the supplied annotation presents a research plan rather than completed experimental data, the following findings are formulated as expected outcomes. In 14-day-old offspring exposed to prenatal maternal stress, histological examination is expected to reveal altered epiphyseal-plate organization, particularly reduced thickness of the proliferative and hypertrophic zones and less orderly arrangement of chondrocytes. The metaphyseal trabecular network may appear less dense and more widely separated.

Morphometric analysis is expected to demonstrate lower total growth-plate thickness, reduced trabecular thickness, increased inter-trabecular distance, and possible thinning of the cortical layer in the stress group compared with controls. These structural changes would be consistent with delayed or altered endochondral ossification and remodeling.

A decrease in BMD may accompany the structural changes. The annotation proposes that reduced mineral density may reflect impaired mineralization and altered calcium–phosphorus homeostasis. The relationship between densitometric and morphological parameters may therefore provide an integrated indicator of skeletal developmental status.

At the molecular level, the expected pattern includes lower osteocalcin and type I collagen, together with higher RANKL and lower OPG. Such a profile would indicate a shift in the balance between bone formation and resorption toward resorptive activity. Correlation analysis is expected to demonstrate an inverse relationship between glucocorticoid exposure and indices such as growth-plate thickness, trabecular density, and BMD, while osteocalcin may show a positive association with mineral density.

Discussion

The proposed findings are biologically consistent with the mechanisms summarized in the dissertation annotation. Maternal stress can increase



glucocorticoid exposure during a period when fetal tissues are highly responsive to endocrine signals. Excess glucocorticoid activity may influence chondrocyte differentiation and osteoblast proliferation while modifying osteoclast activity. Consequently, the growth plate and newly forming bone may show coordinated structural and functional alterations.

Assessment at 14 days after birth is potentially informative because the offspring are undergoing active postnatal skeletal growth. A persistent difference at this stage would suggest that prenatal exposure may have effects extending beyond gestation. At the same time, postnatal compensatory processes may partially normalize some parameters. For this reason, interpretation should rely on multiple endpoints rather than a single histological measurement.

The combination of histomorphometry, DEXA-based mineral assessment, and molecular markers strengthens the analytical framework. Histology describes tissue organization; morphometry converts structural differences into quantitative variables; densitometry reflects mineral accumulation; and molecular markers provide mechanistic information about osteogenesis, resorption, and matrix formation. The integrated approach proposed in the annotation may therefore help identify links between prenatal stress and subsequent skeletal development.

Conclusion

Prenatal maternal stress may be associated with measurable morphofunctional alterations in the long bones of 14-day-old offspring. The proposed assessment focuses on epiphyseal-plate organization, trabecular and cortical architecture, mineral density, and osteogenic/resorptive markers. Expected reductions in growth-plate thickness, trabecular and cortical parameters, BMD, osteocalcin, OPG, and type I collagen, together with increased RANKL, would provide a coherent morphological and molecular pattern of impaired skeletal development. These conclusions remain hypotheses until the planned experimental measurements are completed.



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