

Skeletal maturation in intrauterine growth-retarded rats treated with growth hormone

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Summary

The objective of this study was to analyse the effects of intrauterine growth retardation (IUGR) and growth hormone (Gh) therapy on skeletal maturation in growth retarded rats. One-hundred and thirty-five rats constituted the groups: Control (C), Sham-operated (SH), IUGR and IUGR+Gh: injected with Genotropin[®] 3.0 mg/kg/day) from 21 to 60 days of age. SH was injected only with saline solution. The thickness of tibial cartilage was assessed on X-ray at the ages 1, 21, 42, 63 and 84 days and categorised according to three levels. L₁: maximal thickness, L₂: reduction of 50% and L₃: absence. The percentual differences between frequencies for each level were compared and clustered by simple ligation in Euclidean distance. The results lead us to conclude that skeletal maturation does not appear to be modified by IUGR, while it is accelerated by growth hormone in growth-retarded rats.

Key words: IUGR; Bone maturation; Growth hormone.

Introduction

Intrauterine growth retardation has been involved with long-term consequences, affecting pubertal development and maturation in rats and humans [1]. Although some studies indicate a low variation range for the onset of puberty in small for gestational age (SGA) children [2], the association between IUGR and maturity development appears to be controversial. Engelbregt *et al.* [3] found that IUGR during late gestation resulted in a delayed onset of puberty in male and female rats suggesting that the perinatal period may be a critical time for the maturational process.

Ossification also seems to be retarded in disturbed pregnancies as well as modification of the biochemical composition of bones during fetal and perinatal periods [4, 5]. In humans Walther *et al.* [6] found significant skeletal retardation in infants malnourished in utero. At three years old, those children below the national 10th percentile for body length, still showed growth retardation.

Previous results indicate that IUGR offspring failed to reach the control values, although they grew at a similar velocity [7]. The mechanisms underlying this phenomenon have yet to be explained. One of the most important causes has been attributed to an inadequate synthesis of growth hormone (Gh). According to Towses and Lee [8] there is no evidence that growth hormone had any effect on the skeletal development in control or IUGR offspring. Others authors have found similar effects in control animals, while Gh-deficient rats appear to be responsive to Gh treatment [9, 10].

The present paper assesses the effects of intrauterine growth retardation (IUGR) and growth hormone (Gh) therapy on skeletal maturation in growth-retarded rats.

Material and Methods

Adult male and female Wistar rats were mated overnight. The beginning of pregnancy was determined by the presence of spermatozoa in the vaginal smear. Pregnant rats were fed a stock diet *ad libitum* and assigned to one of three experimental groups: Control (C, 17 males and 16 females), IUGR (35 males and 36 females) and Sham-operated (SH, 15 males and 16 females). Control dams did not receive any treatment. A lower midline laparotomy was done in the mothers of the IUGR group on the 14th day of gestation. Uterine vessels near the lower end of each uterine horn were partially ligated according to the method of Oyhenart *et al.* [11]. Pregnancy was allowed to proceed until delivery.

The SH dams were submitted to laparotomy without vessel bending in order to isolate the effects of surgery from those of vessel ligation. After delivery, IUGR and SH pups were cross-fostered to well-nourished control dams. A standard diet was available "ad libitum" to mothers and offspring. IUGR rats were divided into subgroups: non-treated (IUGR, 17 males and 18 females), and injected subcutaneously with Gh (3.0 mg/kg/days of Genotropin[®], Pharmacia) from 21 (weaning) to 60 days old (IUGR+Gh, 18 males and 18 females).

Each animal was X-rayed at 1, 21, 42, 63 and 84 days of age. The skeletal development was assessed by the thickness of tibial cartilage. The degree of ossification was scored in three levels: L₁: maximal thickness, L₂: 50% thickness, and L₃: absence of cartilage or complete ossification. The percentual frequencies of each level were compared and clustered by simple ligation in Euclidean distance. The SH group was taken as the reference since there were significant differences between the C and SH groups.

Revised manuscript accepted for publication October 1, 2003

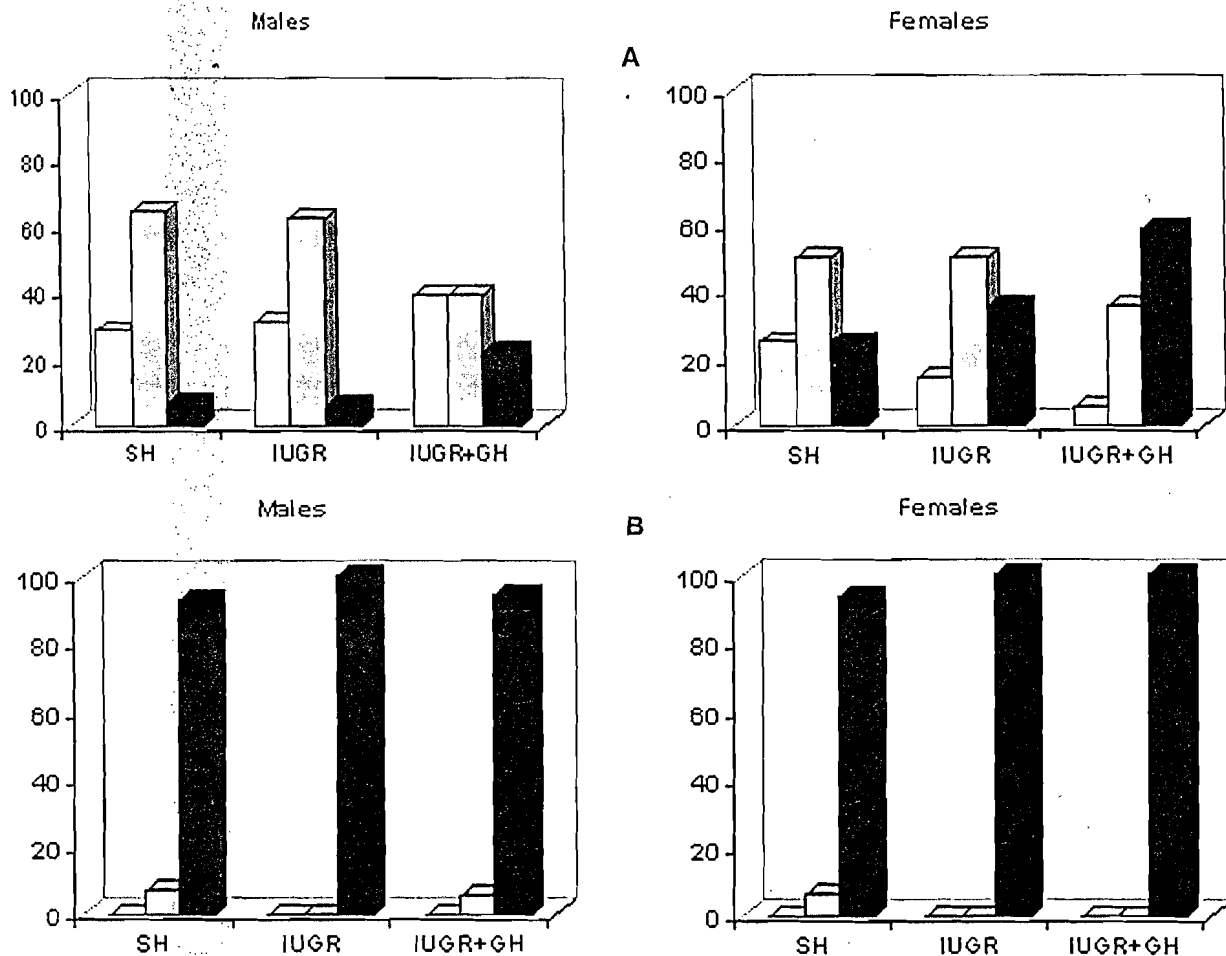


Figure 1. — Percentage of individuals with variable levels of ossification at 63 (A) and 84 (B) days of age. L₁: white bars; L₂: gray bars; L₃: black bars.

Results and Discussion

A complete presence of cartilage (L₁ = 100%) was observed up to 63 days of age in all groups. The interval between 63 and 84 days of age was characterised as the period in which each experimental group showed its own rate of ossification.

In addition to its effect on skeletal growth, prenatal stress has been shown to influence the progress of bone maturation [8, 12]. However, our data do not suggest any effect of intrauterine growth retardation on bone maturation rates, since control and IUGR animals showed — on average — a similar pattern. These differences may be attributed to a model of gestational stress. As was expected females matured earlier than males (Figure 1A). At the final age almost all control and IUGR animals (93–100%) had closure of the growth cartilage (Figure 1B).

The role of Gh-treatment on skeletal maturation in IUGR children has been largely discussed. According to some studies hormonal therapy causes arrested growth because it promotes greater bone maturation than linear growth [13–15]. In agreement our results showed that — compared to IUGR — the animals treated with Gh had an

increased rate of ossification, with females being relatively more advanced than males. This fact was particularly evident at 63 days of age. At 84 days ossification was completed in Gh-injected animals as was seen for the other groups (Figures 1A & B).

Figure 2 shows the differentiation among groups expressed by Euclidean distances. A closer association can be seen between the SH and IUGR groups, suggesting that these groups mature more similarly compared to the IUGR+GH group.

It can be concluded that skeletal maturation does not appear to be modified by IUGR, while it is accelerated by Gh therapy in growth-retarded rats.

Acknowledgement for financial support

This work was partially supported by grants from the Comisión de Investigaciones Científicas de la Provincia de Buenos Aires (CICPBA), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Universidad Nacional de Las Plata and Fundación de Endocrinología, Nutrición Infantil y Crecimiento (FUNDENIC).

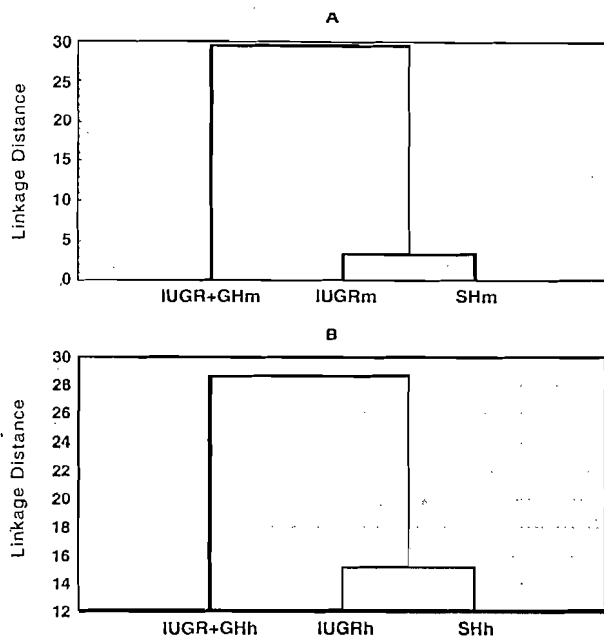


Figure 2. — Single linkage by Euclidean distance in males (A) and females (B)

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