Craniofacial catch-up growth in intrauterine growth retarded rats following postnatal nutritional rehabilitation

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Summary

Purpose: The aim of the study was to analyze the effect of postnatal nutritional rehabilitation on the craniofacial growth in rats with intrauterine growth retardation (IUGR). *Materials and Methods:* Wistar rats were assigned to one of the following groups: *control, Sham-operated, and IUGR.* The IUGR was produced by uterine vessels bending (day 14 of pregnancy). At days 1, 21, 42, 63, and 84 of postnatal life, each animal was X-rayed, and neural and facial length, width and height were measured. Volumetric and morphometric indices were calculated. *Results:* The decreased maternal-fetal blood flow during the last-third of the gestation period modified cranial size and shape of both sexes at birth. *Discussion:* Postnatal nutritional rehabilitation is not fully sufficient to reverse the prenatal growth retardation. There are specific responses depending on the sex and the age of the IUGR pups. Regardless of the changes in size, the shape is not modified during all the postnatal period.

Key words: Intrauterine growth retardation; Postnatal nutritional rehabilitation.

Introduction

Fetal growth is a dynamic process that involves a balance between mechanisms that control the entry of substrates, fetal synthesis of proteins and lipids, and energy production to their metabolic requirements. In analogy with postnatal life, intrauterine growth is determined by the interaction of exogenous factors (nutritional, toxic, infectious), and endogenous (genetic) [1].

It is assumed that most prenatal growth restriction is due to interference in the placental contribution of nutrients, which can be at the entrance of maternal nutrients, placental blood flow or function of the placenta [2, 3]. In this sense, the authors have reported the impact of the reduction of maternal-fetal blood flow on fetal development, with direct consequences in determining intrauterine growth retardation (IUGR) in body weight and skeletal dimensions at birth [4, 5]. Epidemiological and experimental studies have reported that individual tissues and organ systems as a whole are programmed in the uterus during critical periods of development, and in stressful situations they have adverse functional consequences in postnatal life [6, 7]. Thus, children with IUGR have low nutritional reserves and feeding difficulties, and often 10% of them remain vulnerable during their growth [8, 9].

Morphological variation emerges from complex interactions between genetic and environmental factors that are modulated by sequential and interacting developmental processes [10]. In the postnatal period, different mechanisms may act to reverse the morphological modification leading to what is called "catch-up" [11, 12]. The degree of growth retardation may determine the ability to catch-up. In this regard, each organ or system has its own growth pattern which generates different responses to the prenatal stress. So, the skull of all vertebrates is not a single developing unit but a complex structure that comprises recognizable parts that are coherent according to their developmental origin, structure, and function. These parts can be thought of as modules in the sense that they are highly integrated by numerous and usually strong interactions, while the interactions among them are relatively weaker [13, 14, 15].

To date, however, it is still necessary to understand how the modifications of craniofacial growth as a consequence of a prenatal perturbation may affect the postnatal pattern of interaction between cranial traits. On this basis, the authors propose to analyze the effect of postnatal nutritional rehabilitation on the craniofacial growth in rats with intrauterine growth retardation.

Materials and Methods

The animals involved in this study were *Rattus norvegicus albinus*, var. Wistar, from the Instituto de Genética Veterinaria (IGEVET, UNLP- CONICET). The animals were kept free of pathogens and treated in compliance with standardized institutional guidelines. They were fed on a pelleted and sterilized commercial stock diet.

Fifty females (200–250 g body weight) were mated overnight with ten adult males. Pregnancy was assumed to commence after spermatozoa were found in the vaginal smear. Pregnant rats were housed in individual steel boxes and fed on stock diet, with water *ad libitum*, and assigned to one of three experimental groups: (a) *control* (C) = control dams and pups did not receive any treatment; (b)

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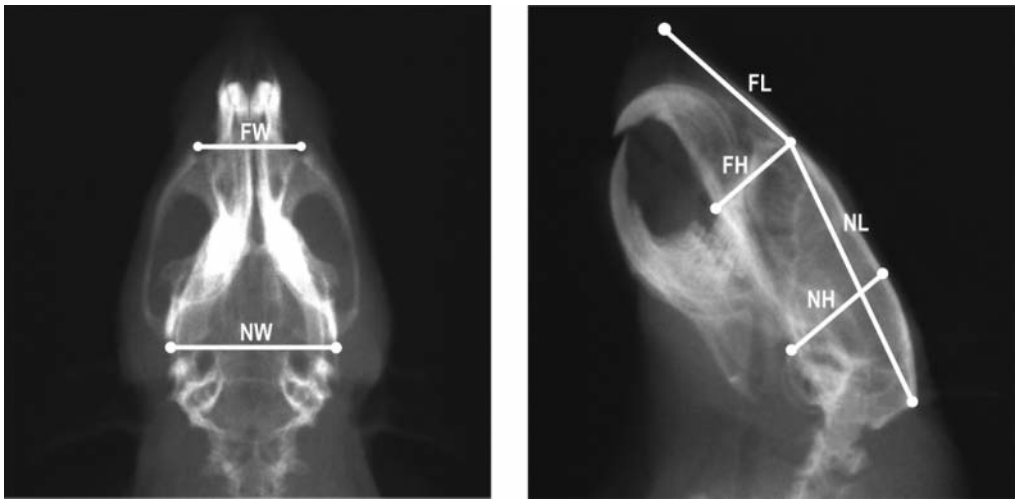

Figure 1. — Radiography of rat skull showing measurement used in this study: neurocranial length (NL), neurocranial width (NW), neurocranial height (NH), facial length (FL), facial width (FW), and facial height (FH).

IUGR = a lower midline laparotomy was performed in the mothers of the IUGR group at day 14 of gestation. Animals were anesthetized intramuscularly with ketalar (0.005 ml 100^{-1} g body weight). Complementary light-ether anesthesia was administered during surgery. After opening the peritoneal cavity, the uterus was exposed. The uterine vessels near the lower end of each uterine horn were bent and fastened with a 3–0 silk suture. Pregnancy was allowed to go on until delivery [16]. (c) *Sham-operated* (SH) = The procedure applied to sham-operated animals was similar to that used for IUGR ones. However, the uterine vessels were not obstructed in order to separate the effects of surgery from that of vessel bending.

During lactation (1 to 21 days of age) IUGR and SH pups were cross-fostered to control dams. Litters were reduced to four males and four females each, to render lactation uniform across the groups. Pups suckled *ad-libitum*. During the postlactation period (from 21 days of age onwards) a standard diet was available *ad-libitum* to offsprings.

Each animal was X-rayed in dorsal and lateral position at 1, 21, 42, 63, and 84 days of age. In each X-ray, neurocranial length (NL), width (NW), and height (NH), and facial length (FL), width (FW), and height (FH) were measured (Figure 1).

To estimate the size variations of neural and facial components by age and sex, volumetric indices were calculated as follows: neural index = (VNI: 3ÖNL x NW x NH); facial index= (VFI: 3ÖFL x FW x FH). Finally, to evaluate changes in the skull shape, a morphometric neurofacial index (MNFI) was calculated as follows: (MNFI= VNI/VFI).

The normality of distributions was assessed by the one-sample Kolmogorov–Smirnov test. This test indicated no significant differences in all the indices, compelling the authors to use an ANOVA analysis to examine the factors significance, and the Least Square Difference test (LSD) for the comparison between groups. Percentage differences between means (PDM) were calculated in order to obtain standardized differences between treatments, according to the formula: PDM = 100 * (X1 – X2)/X1. For instance, X1= mean value of SH and X2= mean value of IUGR.

Results

The ANOVA test showed significant differences for age, sex and treatment factors in VNI, VFI and MNFI. The interaction between factors did not indicate any significant difference.

Table 1. — *Least Square Difference Test (LSD) for the comparison between SH and IUGR groups.*

Age (days)	Comparison SH-IUGR					
	Males			Females		
	VNI	VFI	MNFI	VNI	VFI	MNFI
1	0.62**	0.57**	0.11**	0.31**	0.33**	-0.08**
21	0.21*	0.19*	-0.02	0.11	-0.03	0.02
42	0.06	0.04	0.00	0.25**	-0.18	0.00
63	0.16	0.12	0.00	0.33**	0.29**	-0.01
84	0.24**	0.13	0.00	0.37**	0.20*	0.01

*$p < 0.05$; **$p < 0.01$

The post-hoc analysis between C and SH groups showed no significant differences in males, but significant differences in VNI, VFI, and MNFI at varying ages in females. Therefore, the last group was used as reference.

The comparison between SH-IUGR indicated, in both sexes, significant differences at birth in all the cranial indices. At weaning (21 days), there were significant differences in males for VNI and VFI. Nevertheless, in females there were no significant differences. At day 42 and 63, males showed no significant differences in any of the indices analyzed. However, significant differences were observed in females in VNI (42 days), and VNI and VFI (63 days). Finally, at day 84 there were significant differences in VNI (males), and VNI, and VFI (females). The MNFI showed no difference from day 21 onwards, in both females and males (Table 1).

Discussion

Birth

The intrauterine environmental perturbation during the last third of pregnancy altered skull growth. Both neural and facial components showed growth retardation. In this

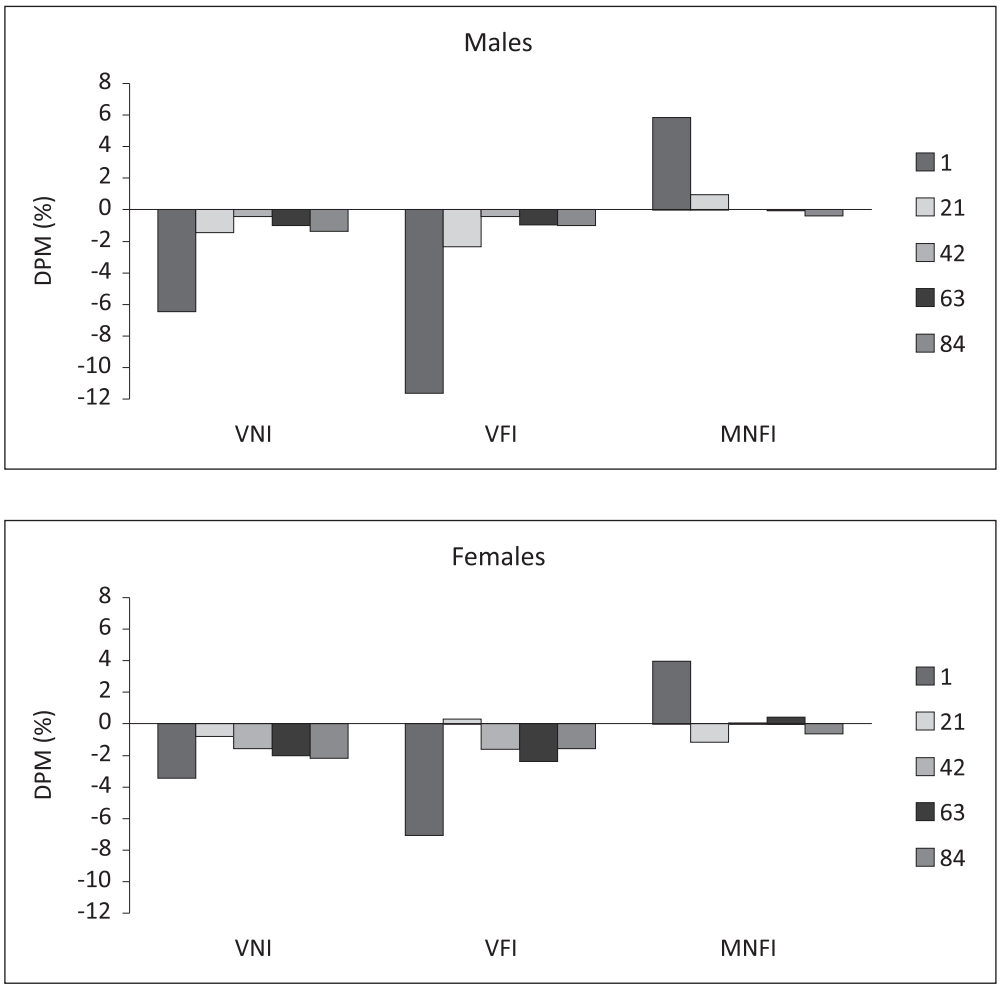

Figure 2. — Percentage differences between means (PDM) for the comparison between SH and IUGR groups; VNI: volumetric neural index; VFI: volumetric facial index; MNFI: morphometric neurofacial index.

sense, neural volume decreased about six percent and facial volume decreased 12% in males, while in females the reduction was of three and seven percent, respectively (Figure 2). Consequently, the different growth patterns of cranial structures led to shape changes. This non-proportional growth, in which the facial component was more affected than the neural one, was previously reported by Oyhenart *et al.* [17]. This can be explained because those cranial morphological features that are functionally related and development connected tend to co-vary with each other and to be independent of other characteristics, due to the modular organization of the craniofacial skeleton [18, 19].

Lactational period

Growth retardation observed in males pup at birth persisted even when they had a normal lactation. Thus, the catch-up growth in males was incomplete in both neural and facial components. Again, the facial volume was more affected than the neural one. Nevertheless, size variation was smaller than that found at birth (only 2%, approximately). In contrast, females had complete compensatory craniofacial growth. In this regard, Oyhenart *et al.* [17] reported an incomplete lactational catch-up growth in IUGR animals, since males reached control size only in neurocranial height, and females in neurocranial length, width and height, and facial height. Likewise, Jones *et al.* [20], in a model of gestational protein restriction, also reported compensatory growth in females after nutritional rehabilitation during lactation.

Furthermore, size changes were not accompanied by shape changes. This can probably be due to the fact that the variation in the skull shape decreases early postnatal growth [21, 10].

Postlactational period

It has been reported that compensatory growth can be associated not only with the intensity of stress but also with the time available for nutritional rehabilitation to produce an effect [17]. Both conditions were observed in the present study. At first, the male growth retardation seen in the neurocranial component from birth to the end of lactation continued during the postlactation period. In fact, the severity of the intrauterine stress acted in the formative period of neural structures and prevented its subsequent recovery.

Nevertheless, the facial component had a compensatory growth because the growth of the facial structures continued during the postnatal period [22]. However, the nutritional rehabilitation in the IUGR pups needed more time to achieve the control size.

Although IUGR females showed a catch-up growth in craniofacial size during lactation, a retarded growth was observed again during postlactation. These results may be explained mainly by the hypothesis of "fetal programming of life", suggesting that fetal malnutrition triggers endocrine adaptations with a permanent change in the morphology, physiology, and metabolism [23-25]. The current theoretical perspective regarding the adaptive significance of fetal life programming emphasizes the benefit of reducing the nutritional requirements through a lower growth trajectory in the uterus [26]. Thus, adult phenotype depends largely on the operating stressors during intrauterine growth [27].

As seen in the previous ontogenetic period, morphometric phenotypic variation in shape appears to be stable.

Conclusion

The decreased maternal-fetal blood flow during the last third of the gestation period modifies cranial size and shape of both sexes at birth. Postnatal nutritional rehabilitation is not fully sufficient to reverse the prenatal growth retardation. There are specifics responses depending on the sex and the age of the IUGR pups. Regardless of the changes in size, the shape is not modified during all the postnatal period.

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