Effects of bilateral uterine vessel ligation on skeletal growth in rats

E. E. Oyhenart[1], L. M. Guimarey[2], M. C. Fucini[3], F. A. Quintero[4], B. Orden[5]

[1]*Prof. in Biological Anthropology, University of La Plata, and Senior Researcher-National Board of Scientific and Technological Research (CONICET)*
[2]*Asst. Prof. in Biological Anthropology, University of La Plata, and Senior Researcher-Scientific Board of Buenos Aires Province (CIC)*
[3]*Asst. Prof. in Radiology, University of La Plata,*
[4]*Doctoral Fellow, Scientific Board of Buenos Aires Province (CIC)*
[5]*Asst. Prof. in Biological Anthropology, University of the Center of Buenos Aires Province, and Postdoctoral Fellow, National Board of Scientific and Technological Research - CONICET - (Argentina)*

Summary

The aim of the present study was to assess the catch-up growth in the postcranial skeleton of intrauterine growth retarded (IUGR) rats. Male and female Wistar rats were assigned to one of the following groups: *controls, sham-operated, IUGR.* The IUGR was produced by uterine vessels bending (day 14th of pregnancy). Trunk, pelvis, femur and humerus were measured on Rx of each animal, from 1 to 84 days of age. Data were processed by repeated analysis of variance and LSD post hoc test. The reduced placental blood flow disturbed the skeletal growth in pups, with the axial skeleton relatively more affected than the bones of the extremities. The catch up only took place in femur length of both sexes. The widths of long bones remained significantly retarded. We concluded that nutritional rehabilitation during the postnatal period might not be enough to allow a complete growth recovery.

Key words: IUGR; Catch-up growth; Nutritional rehabilitation.

Introduction

The intrauterine environment plays a critical role on childhood growth [1]. Hales [2] suggested that intrauterine growth retardation (IUGR) has a nutritional basis, which produces a long-term impact on a fetus potential for development and survival. Both the timing and duration of the intrauterine insult determine the physical condition and body composition of children at birth [3].

Uterine vessel ligation is an extreme example of uteroplacental insufficiency, resulting in severe alterations of fetal growth and physiology [4, 5]. Several studies found that changes in uteroplacental blood supply lead to IUGR. Pups are comparatively small at birth and they do not always attain the normal size after suckling [6, 7].

The postnatal period is an opportunity to recover any growth deficit [8]. However, the factors predicting postnatal growth in IUGR infants are poorly understood because of the high heterogeneity of this population [9].

According to Williams [10] catch up may be complete or incomplete and this seems to depend on the amount of time available for recovery and the degree growth retardation that occurs. On the other hand, while catch up appears to be a whole body response, the parts of the body seem to respond in an individual way. Most of the studies about catch-up growth are focused on body weight and length, whereas the long bone growth remain largely unknown. The purpose of this work was to evaluate the growth pattern in the postcranial skeleton of intrauterine growth retarded rats and to determine if catch-up growth can be appreciated.

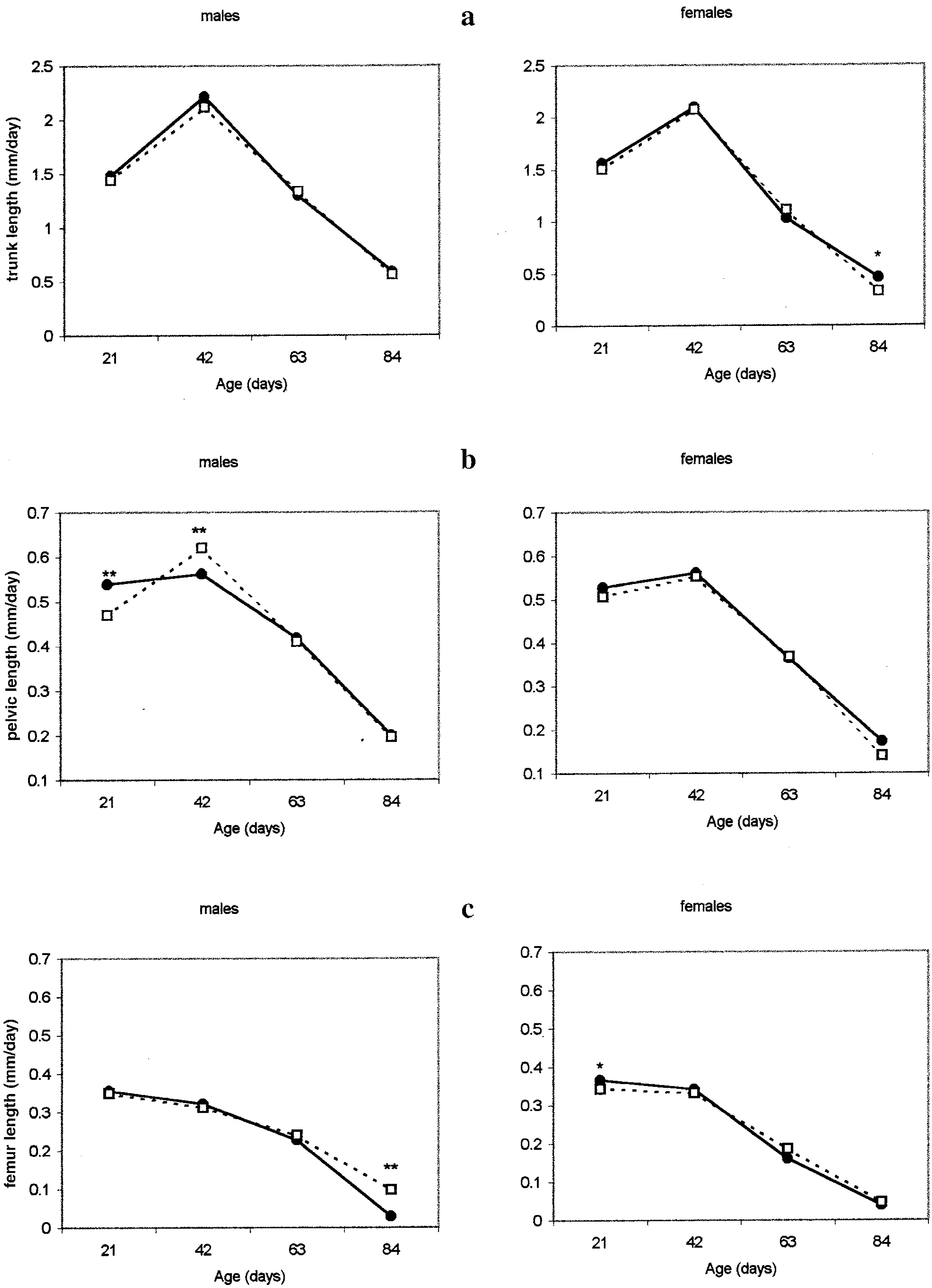
Revised manuscript accepted for publication February 20, 2002

Materials and Methods

Wistar rats had been brought up at the Centro de Investigaciones en Genética Básica y Aplicada (CIGEBA). Forty females (200-250 g body weight) were mated overnight with ten adult males. The beginning of pregnancy was determined by the presence of spermatozoa in the vaginal smear. Pregnant rats were housed in individual steel boxes and fed on a stock diet *ad libitum*. They were assigned to one of three experimental groups: *Control:* the dams did not receive any special treatment (C); *IUGR:* the dams were submitted to a lower midline laparotomy at day 14 of gestation. A light-ether anesthesia was given during surgery. The uterine vessels near the lower end of each uterine horn were partially ligated with a 3-0 silk suture [11]; *Sham-operated* (SH): the dams were submitted to a laparotomy, but vessels were non bending in order to measure the effect of the surgical procedure.

Pregnancy was allowed to proceed until delivery in all groups. During suckling, the IUGR and SH pups were cross-fostered to control dams. After weaning, they were fed ad libitum. Each 21 days, from 1 to 84 days of age, the animals were X-rayed. Pelvic and trunk lengths, and humerus and femur lengths and widths were measured on each Rx.

After applying the Kolmogorov-Smirnov test for goodness of fit for the frequency distributions, data were processed by repeated measures analysis of variance (ANOVA). In significant cases, post hoc comparisons were made by means of the Least Square Difference (LSD) multiple range tests. The tests were designed to assess the effects of surgery (C-SH) and bilateral uterine ligation (SH-IUGR). Between-group differences in growth velocity were estimated by the Mann-Whitney nonparametric tests.


a
males
females
trunk length (mm/day)
2.5
2
1.5
1
0.5
0
*
21
42
63
84
Age (days)
b
males
females
pelvic length (mm/day)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
**
**
21
42
63
84
Age (days)
c
males
females
femur length (mm/day)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
**
*
21
42
63
84
Age (days)

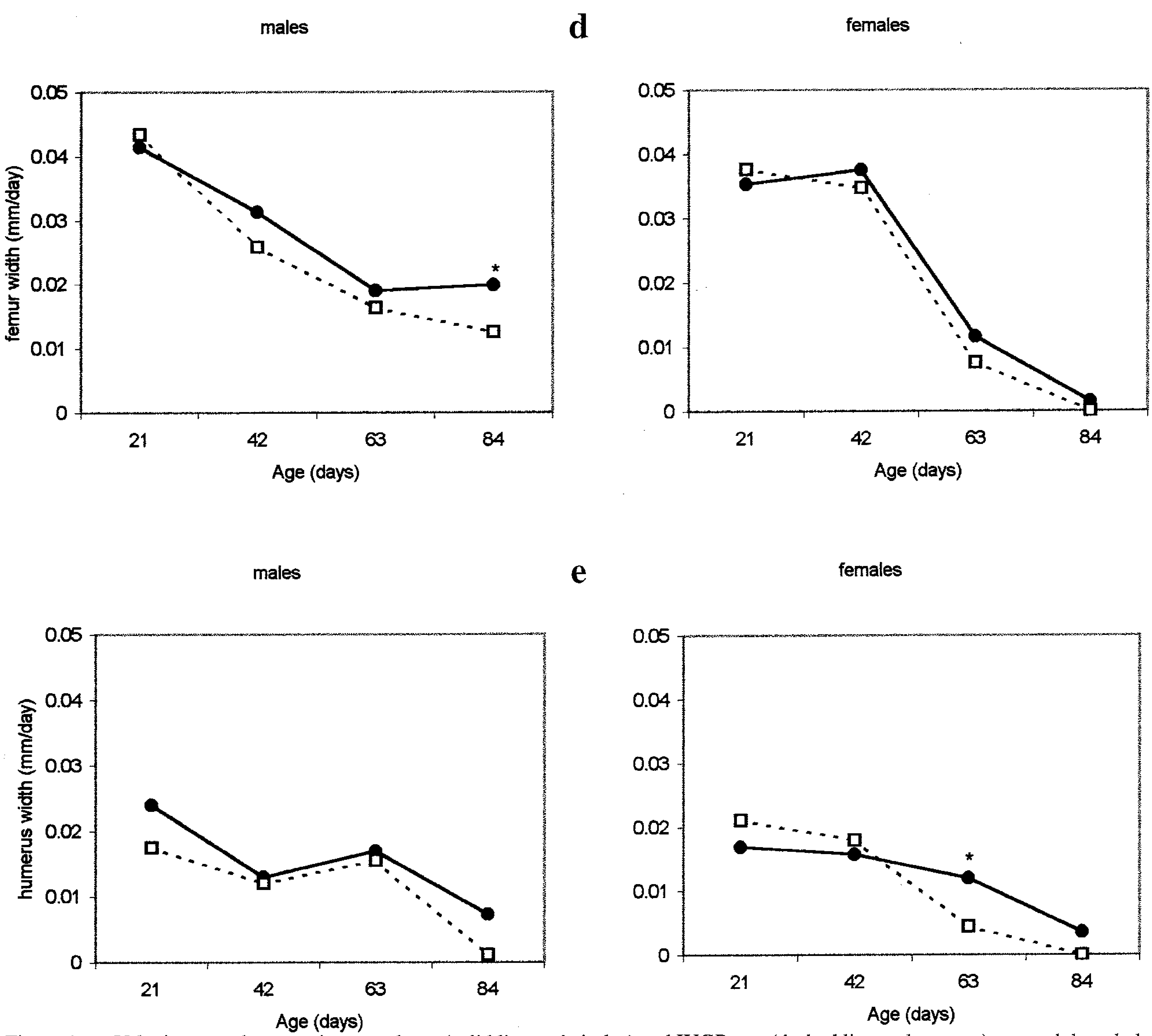


Figure 1. — Velocity growth curves in control rats (solid line and circles) and IUGR rats (dashed line and squares); a: trunk length, b: pelvic length, c: femur length, d: femur width, and e: humerus width. * $p < 0.05$; ** $p < 0.01$.

Results

In the between-subjects analysis, sex and treatment – except for humerus length – were statistically significant. The interaction between both factors was significant for femur length and width and humerus length (Table 1).

Effect of the surgical procedure

There were significant differences between C and SH males for trunk (ages 1-21, 63 days) and pelvic lengths (age 63 days), femur length (age 1, 84 days) and width (age 84 days), and humerus width (age 42 days). In females, the differences were non-significant, except for trunk (age 1 day), pelvic (age 63 days), and femur lengths (age 21 days). Thus, the sham-operated animals were taken as the reference point (Table 2).

Effect of uterine ligation

When sham-operated and IUGR males were compared, significant differences were seen in trunk length and femur width at all ages. Other significant differences were found in pelvic length (ages 1-21, 63-84 days), femur length (ages 1, 42, 84 days), and humerus width (ages 21-84 days). In females only pelvic length showed significant differences at all ages. Significant differences were also found in trunk length (ages 1-42, 84 days), femur length (ages 21-42 days) and width (ages 63-84 days), and humerus width (ages 63-84 days) (Table 2).

Growth velocity

The curves for growth velocity in males and females are shown in Figure 1. There were a few significant differences between groups, being – except for pelvic and femur length in males (42 and 84 days of age, respectively) – significantly greater in sham-operated.

Table 1. — *Effects of treatment, sex, and their interaction.*

Variable	Treatment	Factors Sex	Interaction
Trunk length	25.0 **	46.1 **	0.7
Pelvic length	21.3 **	67.0 **	0.3
Femur length	13.8 **	5.3 *	3.2 *
Femur width	24.0 **	15.4 **	4.0 *
Humerus length	0.3	18.9 **	3.1 *
Humerus width	14.4 **	9.8 **	1.2

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

Table 2. — *Comparisons between groups for surgical and uterine ligation factors.*

Variables	Control-sham operated Males	Control-sham operated Females	Sham operated - IUGR Males	Sham operated - IUGR Females
Trunk Length				
1	1.05**	1.48**	1.81**	1.79**
21	1.93**	1.60	2.68**	2.92**
42	1.29	1.69	4.67**	3.42**
63	-3.49**	0.54	3.87**	1.71
84	-2.06	0.01	4.55**	4.47**
Pelvic Length				
1	0.12	0.01	0.48**	0.51**
21	-0.51	-0.26	1.87**	0.93**
42	0.20	-0.33	0.64	1.11**
63	-1.38**	-0.80*	0.82*	1.06**
84	-0.01	0.18	0.91**	1.76**
Femur Length				
1	0.28**	0.10	0.22**	0.14
21	0.32	0.50*	0.33	0.60**
42	0.34	0.48	0.55*	0.81**
63	-0.54	0.39	0.30	0.28
84	0.76*	0.55	-1.15**	0.13
Femur Width				
1	0.03	-0.01	0.20**	0.09
21	-0.08	0.07	0.16*	0.05
42	-0.06	0.06	0.27**	0.11
63	-0.13	0.06	0.33**	0.19*
84	-0.44**	0.02	0.48**	0.29**
Humerus Width				
1	-0.03	0.04	0.01	0.13
21	-0.04	-0.01	0.14**	0.05
42	-0.13*	0.08	0.16**	-0.01
63	-0.03	-0.04	0.19**	0.16*
84	-0.12	0.04	0.32**	0.25**

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

Discussion

Disturbed pregnancies have been associated with retarded ossification and modification of the biochemical composition in bones during fetal and perinatal periods [12-14]. In agreement with these findings, we found that – except for humerus length – impaired placental blood supply produced significant skeletal growth retardation in the newborn rat. However, two aspects should be noted: a - the relative sensitivity of particular bones, and b - the sex differences. It was found that the axial skeleton was relatively more affected than the bones of the extremities. As an example, humerus length was unaffected by the experimental stress. These results are in accordance with those reported by Nakamoto *et al.* [15] and Woodall *et al.* [16], who found that the skeletal regions were differently affected by prenatal malnutrition, possibly because of differences in the critical period of growth. That differential sensitivity led to allometric changes in the newborn IUGR rat.

Sexual dimorphism results from the differences in growth rates and/or growth spans belonging to each sex [17, 18]. Philip [19] observed that ossification was more frequently absent in male than female growth-retarded newborn infants. Coincidentally, Oyhenart *et al.* [11] reported that craniofacial growth at birth was more delayed in male rats as a consequence of impaired placental blood supply. In our study sexual differences in response to intrauterine stress were also seen at birth, since males were relatively less resistant than females. It does not indicate that females were more resistant at all, since at later ages they showed some significant differences compared to aged-control matches.

The lack of catch-up growth has been reported as a consequence of several factors, such as prolonged fetal starvation or placental insufficiency [20-22]. Fetal growth retardation has a lasting adverse effect on later physical growth [23]. Nevertheless, there is notable diversity in individual growth patterns: while some babies grow following the same percentile channel in which they were born, others show catch-up [8, 24]. Walther *et al.* [25] found a significant skeletal retardation in infants malnourished in utero. At three years old, those children below the national tenth percentile for body length, still showed growth retardation.

According to Hughes [26], catch-up is not possible in some regions where permanent structural and/or functional abnormalities have been induced. There is no single mechanism regulating catch-up. The parts of the body seem to respond in an individual manner [10]. Thus, while the axial skeleton (trunk and pelvis) did not show any growth recovery, a differential pattern was observed in the long bones: the catch-up took place only in the longitudinal growth of the femur in both sexes, while transversal growth – as reflection of appositional growth – remained strongly retarded. Such phenomenon was noted by Yahya and Millward [27], who also found differential growth in stressed animals: while epiphyseal cartilage width responded to a sensitive reduction in protein diet, the epiphysis length was only minimally affected.

The velocity growth indicated that IUGR rats grew at similar rates as non-stressed animals, and the basic growth pattern was kept constant. That similarity in growth rates did not allow IUGR rats to reach the control values in most of the variables.

In summary, bone growth in rats was modified by reductions in uteroplacental blood supply. The greater effect on the axial skeleton led to changes in body proportions in the newborn rats. Nutritional rehabilitation during the postnatal period might be not enough to allow a complete growth recovery.

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) and Fundación de Endocrinología, Nutrición Infantil y Crecimiento (FUNDENIC).

References

[1] Strauss R. S.: "Effects of the intrauterine environment on childhood growth". *Br. Med. Bull.*, 1997, *53*, 81.

[2] Hales C. N.: "Metabolic consequences of intrauterine growth retardation". *Acta Paediatr. Scand.*, 1997, *423*, 184.

[3] Bhatia B. D., Agarwal K. N., Jain N. P., Bhargava V.: "Growth pattern of intrauterine growth retarded (IUGR) babies in first nine months of life". *Acta Paediatr. Scand.*, 1984, *73*, 189.

[4] Simmons R. A., Gounis A. S., Bangalore S. A., Ogata E. S.: "Intrauterine growth retardation: fetal glucose transport is diminished in lung but spared in brain". *Pediatr. Res.*, 1992, *31*, 59.

[5] Tanaka M., Natori M., Ishimoto H., Miyazaki T., Nozawa S.: "Experimental growth retardation produced by transient period of uteroplacental ischemia in pregnant Sprague-Dawley rats". *Am. J. Obstet. Gynecol.*, 1994, *171*, 1231.

[6] Hohenauer L., Oh W.: "Body composition in experimental intrauterine growth retardation in the rat". *J. Nutr.*, 1969, *99*, 23.

[7] Levitsky L. L., Kimber A., Marchichow J. A., Uchara J.: "Metabolic response to fasting in experimental growth retardation induced by surgical and non-surgical maternal stress". *Biol. Neonate*, 1977, *31*, 311.

[8] Davies D. P.: "Growth of "small-for-dates" babies". *Early Hum. Dev.*, 1981, *5*, 95.

[9] Leger J., Czernichow P.: "Retardation of intrauterine growth. Prognosis and therapeutic perspectives". *Presse Med.*, 1994, *23*, 969.

[10] Williams J. P. G.: "Catch-up growth". *J. Embryol. exp. Morph.*, 1981, *65*, 89.

[11] Oyhenart E. E., Muñe M. C., Pucciarelli H. M.: "Influence of intrauterine blood supply on cranial growth and sexual dimorphism at birth". *Growth, Dev & Aging*, 1998, *62*, 187.

[12] Miwa T., Shoji H., Solomonow M., Yazdani M., Nakamoto T.: "The effect of prenatal protein-energy malnutrition on collagen metabolism in fetal bones". *Orthopedics*, 1989, *12*, 973.

[13] Miwa T., Shoji H., Solomonow M., Nakamoto T.: "The effects of prenatal protein-energy malnutrition on ossification of fetal rat bones: a biochemical study". *Orthopedics*, 1990, *13*, 541.

[14] Glockner R., Schwartz S., Jahne F.: "Simple methods for the characterization of skeletal development of rats pups during the perinatal period as a parameter of maturity". *Anat. Anz.*, 1991, *173*, 209.

[15] Nakamoto T., Porter J. R., Winkler M. M.: "The effect of prenatal protein-energy malnutrition on the development of mandibles and long bones in newborn rats". *Br. J. Nutr.*, 1983, *50*, 75.

[16] Woodall S. M., Breier B. H., Johnston B. M., Gluckman P. D.: "A model of intrauterine growth retardation caused by chronic maternal undernutrition in the rat: effects on the somatotrophic axis and postnatal growth". *J. Endocrinol.*, 1996, *150*, 231.

[17] Leigh S. R., Cheverud J. M.: "Sexual dimorphism in the baboon facial skeleton". *Am. J. Phys. Anthropol.*, 1991, *84*, 193.

[18] Loth S. R., Henneberg M.: "Mandibular ramus flexure: a new morphologic indicator of sexual dimorphism in the human skeleton". *Am. J. Phys. Anthropol.*, 1996, *99*, 473.

[19] Philip A. G.: "Fetal growth retardation: femurs, fontanels, and follow up". *Pediatrics*, 1978, *62*, 446.

[20] Fancourt R., Campbell S., Harvey D., Norman A. P.: "Follow-up study of small-for-dates babies". *Br. Med. J.*, 1976, *1*, 1435.

[21] Villar J., Smeriglio V., Martorell R., Brown C. H., Klein R. E.: "Heterogeneous growth and mental development of intrauterine growth-retarded infants during the first 3 years of life". *Pediatrics*, 1984, *74*, 783.

[22] Henrichsen L., Skinhoj K., Andersen G. E.: "Delayed growth and reduced intelligence in 9-17 year old growth retarded children compared with their monozygous co-twin". *Acta Pædiatr. Scand.*, 1986, *75*, 31.

[23] Bhargava S. K., Ramji S., Srivastava U., Sachdev H. P., Kappani V., Datta V., Satyanarayana L.: "Growth and sexual maturation of low birth weight children: a 14 year follow up". *Indian Pediatr.*, 1995, *32*, 963.

[24] Martorell R., Ramakrishnan U., Schroeder D. G., Melgar P., Neufeld L.: "Intrauterine growth retardation, body size, body composition and physical performance in adolescence". *Eur. J. Clin. Nutr.*, 1998, *52*, S43.

[25] Walther F. J., Ramackers L. H., van Engelshoven J. M.: "Skeletal maturity at birth and at the age of 3 years of infants malnourished in utero". *Early Hum. Dev.*, 1981, *5*, 139.

[26] Hughes P. C.: "Morphometric studies of catch-up growth in the rat". *Prog. Clin. Biol. Res.*, 1982, *101*, 433.

[27] Yahya Z. A., Millward D. J.: "Dietary protein and the regulation of long-bone and muscle growth in the rat". *Clin. Sci. (Colch)*, 1994, *87*, 213.

Address reprint requests to:
E. E. OYHENART
Centro di Investigaciones en Genética Básica y Aplicada (CIGEBA)
Facultad de Ciencias Veterinarias (UNLP)
Calles 60 y 118 S/N cc 296
B 1900 Avw La Plata (Argentina)