Original Paper

Cells Tissues Organs

Cells Tissues Organs 2003;174:129–135
DOI: 10.1159/000071153

Accepted after revision: March 26, 2003

Effect of Undernutrition on the Cranial Growth of the Rat

An Intergenerational Study

María F. Cesani Bibiana Orden Mariel Zucchi María C. Muñe
Evelia E. Oyhenart Héctor M. Pucciarelli

Centro de Investigaciones en Genética Básica y Aplicada (CIGEBA), Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata (UNLP) y Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina

Key Words
Transgenerational undernutrition · Skull growth · Rat

Abstract
The cumulative effect of undernutrition on successive generations was tested. The cranial growth of three generations of undernourished rats (F1, F2, F3) was compared to that of the parental generation (P), in order to (1) measure the extent to which the growth of each facial and neurocranial functional component was retarded when animals were undernourished and (2) determine whether any cumulative effect between generations can be found. The P generation was fed ad libitum, and the undernourished generations were fed 50% (F1) and 75% (F2 and F3) of the parental diet. Nine radiographs were taken from the age of 20–100 days. The length, width and height of the neurocranial and facial components were measured on each radiograph. Neurocranial (VNI), facial (VFI), and neurofacial (NFI) indices were calculated. Data were processed by the Kruskal-Wallis and Kolmogorov-Smirnov tests. An impairment in neurocranial and facial growth was found, the latter being more affected than the former in F1. At variance, the neurocranium was more affected than the face in F2 and F3, resulting in variations of the shape of the skull. A cumulative effect of moderate transgenerational undernutrition was evident and points to the need for further analysis on this topic.

Copyright © 2003 S. Karger AG, Basel

Abbreviations used in this paper

Fh	facial height
Fl	facial length
Fw	facial width
NFI	neurofacial index
Nh	neurocranial height
Nl	neurocranial length
Nw	neurocranial width
PDM	percent differences between means
VFI	volumetric facial index
VNI	volumetric neurocranial index

Introduction

Moderate undernutrition strongly affects the growth of individuals when it is present chronically, i.e. through several generations of the same population. According to Kenney and Barton [1975], the effects of undernutrition may go beyond the generation under stress. Such intergenerational or transgenerational effects were defined by Emanuel [1986] as an entirety that influences the health, growth and development of the following generation.

KARGER
Fax + 41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

© 2003 S. Karger AG, Basel
1422–6405/03/1743–0129$19.50/0

Accessible online at:
www.karger.com/cto

María Florencia Cesani
Centro de Investigaciones en Genética Básica y Aplicada (CIGEBA)
Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata
Calles 60 y 118, B1900AVW, La Plata, Buenos Aires (Argentina)
Tel./Fax +54 221 4211799, E-Mail mfcesani@museo.fcnym.unlp.edu.ar

There is considerable support for the intergenerational hypothesis. Several studies found that the mother's birth weight, adult stature, and her health during growth are strong predictors of the birth weight of the offspring [Emanuel et al., 1992; Sanderson et al., 1995]. Garn and Clark [1975] pointed out that the poor in the United States were not suffering from acute undernutrition, but moderate chronic undernutrition resulting in a cumulative growth deficit. Similarly, experimental studies in rats showed a cumulative transgenerational effect on physical growth as well as on reproductive and behavioral parameters [Stewart et al., 1973; Zamenhof and van Marthens, 1978; Galler and Propert, 1981; Galler and Seelig, 1981; Pessoa et al., 2000; Pucciarelli et al., 2001].

It is well known that the craniofacial skeleton is critically affected by malnutrition [Miller and German, 1999]. It is necessary to know the mammalian skull growth to understand how undernutrition affects both its major components: neurocranium and face. They both have different functional requirements and growth patterns [Miller and German, 1999]. The face is involved in feeding and breathing, and its growth can be explained by the volumetric expansion of the oronasopharyngeal cavity [Moss, 1973], whereas the neurocranium supports and protects the brain, and its growth follows the brain expansion [Cheverud, 1982]. Facial components appear to be more susceptible to epigenetic factors than those of the neurocranium [Pucciarelli, 1981; Fields, 1991]. Accordingly, a nonproportional change – which results in shape variation – between these components is expected.

So far there have hardly been any intergenerational studies on cranial growth. With the present study we intend to fill this gap. The aims were: (1) to determine to what extent an intergenerational moderate undernutrition delays growth of each cranial component (neurocranium and face) and (2) to show whether a cumulative effect between generations can be found.

Materials and Methods

One hundred Wistar rats *(Rattus norvegicus albinus)* were inbred for ten generations in order to reduce genetic noise as much as possible. The animals were kept free of pathogens and treated in compliance with standardized institutional guidelines. The animals were divided into two treatment groups: (1) control: parental generation (P) received a stock diet ad libitum and (2) undernutrition: filial generations received restricted amounts of the daily food intake of a control animal of the same sex and age ('pair-feeding technique'). Two kinds of undernutrition were applied:

(1) Severe intragenerational undernutrition: the F1 animals were submitted to a 50% food restriction from weaning (20 days of age) to sampling (100 days of age). One group of females was mated to give birth to the F2 animals.

(2) Moderate intergenerational undernutrition: F2 and F3 dams were submitted to a 25% food restriction during pregnancy. Since it is well known that diet restriction during lactation substantially alters the mother's behavior, the 'overcrowding method' was adopted during this period (twelve pups per litter instead of the eight normally studied). This method has frequently been employed so as to produce body growth retardation [Widdowson and McCance, 1963; Srivastava et al., 1974; Park and Nowosielski-Slepowron, 1983; Rajanna et al., 1984]. Pups were submitted to a 25% food restriction from weaning to sampling.

About 20 males and 20 females of each generation were separated from the main group and X-rayed every 10 days (table 1). The following measurements were taken on each radiograph: (1) neurocranial length (Nl) from nasion to opisthocranion, (2) neurocranial width (Nw) from euryon to euryon, (3) neurocranial height (Nh) from the sphenooccipital synchondrosis to vertex, (4) facial length (Fl) from nasion to rhinion, (5) facial width (Fw) from zygion to zygion, and (6) facial height (Fh) from palate (just before the first molars) to nasion. Volumetric neurocranial (VNI), volumetric facial (VFI), and neurofacial (NFI) indices were calculated as follows:

$$VNI = \sqrt[3]{(Nl \cdot Nw \cdot Nh)}$$

$$VFI = \sqrt[3]{(Fl \cdot Fw \cdot Fh)}$$

$$NFI = VNI/VFI$$

The first two indices are called volumetric because they express the geometric mean of the three main dimensions, reflecting the size variation of both major functional components (neurocranium and face). The third index is morphometric because it measures the shape

Table 1. Samples and treatments

Generation	Treatment	Food restriction %	Males	Females
Generation P	normal nutrition (controls)		20	21
Generation F1	severe intragenerational undernutrition	50	22	24
Generation F2	moderate intergenerational undernutrition	25	22	20
Generation F3	moderate intergenerational undernutrition	25	20	22
Total			84	87

in terms of neurocranial volume per unit of facial variation [for details, see Pucciarelli et al., 1990].

The normality of the distributions was tested by the one-sample Kolmogorov-Smirnov test. It indicated that 37% of the variables were asymmetrical, so that we employed the nonparametric Kruskal-Wallis test for the factor significance, and the two-sample Kolmogorov-Smirnov test for the comparison between ages and generations. Statistical work was done with the Systat 7.0 and SPSS 7.5 programs.

For graphical design, percent differences between means (PDM) were calculated in order to obtain a standardized difference between generations, according to the formula: $PDM = 100 \cdot (X_1 - X_2)/X_1$, where X_1 = mean value of P and X_2 = mean value of F1, F2 or F3. For instance, if we compare P-F1 and $PDM_{IVF} = 10$, it means that IVF in P is 10% greater than in F1. This standardization method has been frequently employed [see Pucciarelli et al., 1990]. In its current form, it reduces any difference to a percent value which is not affected by the magnitude of the variables or by the sense of the differences.

Table 2. Kruskal-Wallis test

Variable	Treatment	Sex	Age
VNI	246.2**	267863**	1,157.4**
VFI	80.04**	258454**	1,344.4**
NFI	160.4**	328386**	1,071.8**

** $p < 0.01$.

Results

The Kruskal-Wallis test showed significant values for treatment, sex, and age in all the indices (table 2). The two-sample K-S test showed significant differences between P and filials (F1/F3) in both sexes. In males, such differences were seen in VNI and VFI. In all cases, the PDM values were positive, meaning that P was greater than F1, F2 and F3. On the other hand, VNI was significantly greater in F1 than in F2 and F3. The same was observed in VFI except for the comparison of F1-F2, whose PDM indicated that F2 was greater than F1. The NFI showed significant differences between P and filials with negative (P-F1) and positive (P-F2 and P-F3) PDM. When F1 was compared to F2 and F3, significant differences were observed with positive PDM values (table 3).

Similar results for VNI and VFI were found in females, when P was compared with F1/F3. The differences in VNI between F1 and F2/F3 were also similar to those

Table 3. PDM and Kolmogorov-Smirnov test for two samples in males

Variable	Age, days								
	20	30	40	50	60	70	80	90	100
VNI									
P-F1	1.18	2.95**	3.89**	5.15**	5.31**	5.67**	5.79**	6.56**	6.44**
P-F2	3.27**	4.78**	5.33**	7.45**	7.75**	8.78**	9.18**	9.85**	10.43**
P-F3	1.39	3.30**	4.10**	7.45**	8.56**	9.59**	10.31**	11.98**	13.09**
F1-F2	2.06**	1.77**	1.38**	2.18**	2.31**	2.95**	3.20**	3.09**	3.75**
F1-F3	0.21	0.34	0.20	2.18**	3.09**	3.71**	4.27**	5.08**	6.24**
VFI									
P-F1	2.04	2.80	6.91**	9.32**	10.08**	11.53**	12.14**	12.49**	11.45**
P-F2	5.46**	3.24*	4.71**	5.50**	4.75**	5.50**	5.72**	4.93**	3.70**
P-F3	6.38**	3.92**	6.59**	7.28**	6.32**	11.04**	8.70**	8.40**	6.44**
F1-F2	3.35**	0.43	−2.06	−3.49**	−4.89**	−5.41**	−5.72**	−6.72**	−6.95**
F1-F3	0.87**	0.65	1.76	1.65*	1.47**	5.00**	2.74**	3.21**	2.57**
NFI									
P-F1	−1.16	0.00	−2.60**	−3.31**	−4.70**	−5.41**	−5.48**	−5.48**	−4.86**
P-F2	−2.29*	1.26	0.67	2.10**	2.90**	2.94**	3.76**	4.55**	6.20**
P-F3	−5.00**	−0.62	−2.60	0.69	2.16	−2.78	1.47*	2.99**	6.20**
F1-F2	−1.14	1.26	3.36**	5.59**	7.97**	8.82**	9.77**	10.61**	11.63**
F1-F3	−3.89**	−0.62	0.01	4.14**	7.19**	2.78**	7.35**	8.96**	11.63**

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

found in males. However, the VFI was significantly greater in F2 and F3 than in F1 (negative PDM). The NFI showed the same pattern as in males, although interfilial differences were more erratic (table 4).

Discussion

Functional cranial components can be differentially altered, i.e. the face appears to be more susceptible to environmental factors than the neurocranium [Pucciarelli, 1981; Fields, 1991]. In agreement, it was found that facial growth was more affected than neurocranial growth in severely undernourished rats (9.7 and 6.5%, respectively) (fig. 1, 2). Such differences may result primarily from differences in time of growth: the face grows at a faster rate and for a shorter time than the neurocranium [Dressino and Pucciarelli, 1997]. On average, the braincase attains 93% of its adult size by the age of 1 month, whereas at this stage, the face has attained only 75% of its adult size [Moore, 1966].

The neurocranium was progressively affected by age, showing a greater effect on F3 (13.7%) than on F2 (10.3%). The face behaved similarly: F3 had a greater impact (5.9%) than F2 (3.8%) (fig. 1, 2). In synthesis, moderate undernutrition evoked a transgenerational cumulative effect on both major cranial components, adding support to the results reported by several authors [Cowley and Griesel, 1966; Stewart et al., 1973; Bresler et al., 1975; Resnick and Morgane, 1984], who found a gradual impairment of body growth through successive generations. A biologically plausible mechanism, which could account for the intergenerational phenomenon, was suggested by Emanuel [1997] based both on human and animal data. According to him, poorly grown human infants have small organs, primarily because of a reduced cytoplasmic/nuclear ratio and/or a reduced cell number, having long-term physiologic consequences. Additionally, maternal growth factors – birth weight and adult stature – have a strong relation to fetal growth. Furthermore, the association of the maternal grandmother's stature with the grandchild's fetal growth in two studies indicated a multigenerational process [Emanuel et al., 1992; Klebanoff et al., 1997]. Intergenerational biological changes are not substantially different from the secular trend in human beings. The trend we observed is a negative change of growth reflected by a reduction of volumetric indices across generations.

Table 4. PDM and Kolmogorov-Smirnov test for two samples in females

Variable	Age, days								
	20	30	40	50	60	70	80	90	100
VNI									
P-F1	1.75	3.78**	5.24**	6.37**	0.83**	6.80**	6.48**	6.30**	6.60**
P-F2	4.22**	6.15**	7.63**	8.59**	8.65**	9.06**	9.21**	9.56	10.07**
P-F3	3.63	5.20	7.41	9.23**	10.40**	11.20**	12.08**	12.95**	14.27**
F1-F2	2.43**	2.28	2.28**	2.08**	1.71**	2.11**	2.56**	3.06**	3.26**
F1-F3	1.85	1.37**	2.07**	2.68**	3.34**	4.11**	5.26	6.26	7.20*
VFI									
P-F1	0.01	2.95**	5.97**	7.80**	7.76**	7.05**	8.20**	8.63**	7.89**
P-F2	6.90**	5.48**	5.86**	6.67**	5.32**	3.63**	4.47**	4.35**	3.84**
P-F3	5.28**	4.55**	3.25**	5.36**	4.57**	5.36**	6.16**	5.04**	5.27**
F1-F2	6.90	2.46	−0.10	−1.05**	−2.26**	−3.20**	−3.45**	−3.94**	−3.76*
F1-F3	5.28	1.55	−2.56	−2.26**	−2.96	−1.58**	−1.88**	−3.31**	−2.43*
NFI									
P-F1	1.75	0.62	−1.29**	−1.32**	−0.67**	0.01**	−2.04**	−2.72**	−1.38**
P-F2	−2.79	0.62	1.32	1.36**	3.50**	5.76**	4.35**	4.38**	5.93**
P-F3	−1.69	0.62	4.08	3.47**	5.71**	5.76**	5.11**	7.52**	8.33**
F1-F2	−4.47	0.01	2.65	2.72	4.20**	5.76**	6.52	7.30*	7.41**
F1-F3	−3.39	0.01	5.44**	4.86	6.43**	5.76	7.30	10.53*	9.85**

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

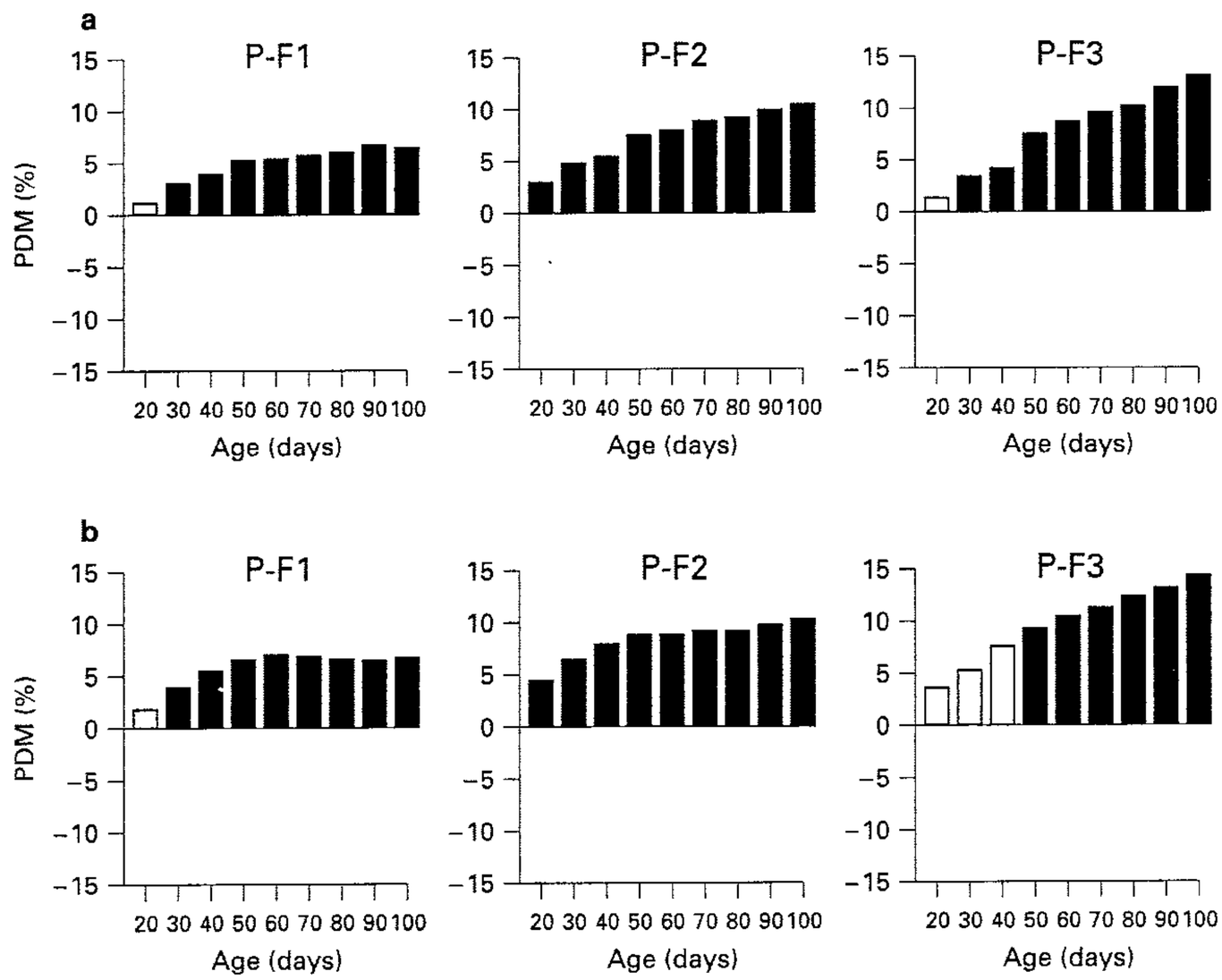

Fig. 1. PDM for VNI in males (**a**) and females (**b**). ■ = $p < 0.05$; □ = nonsignificant differences.

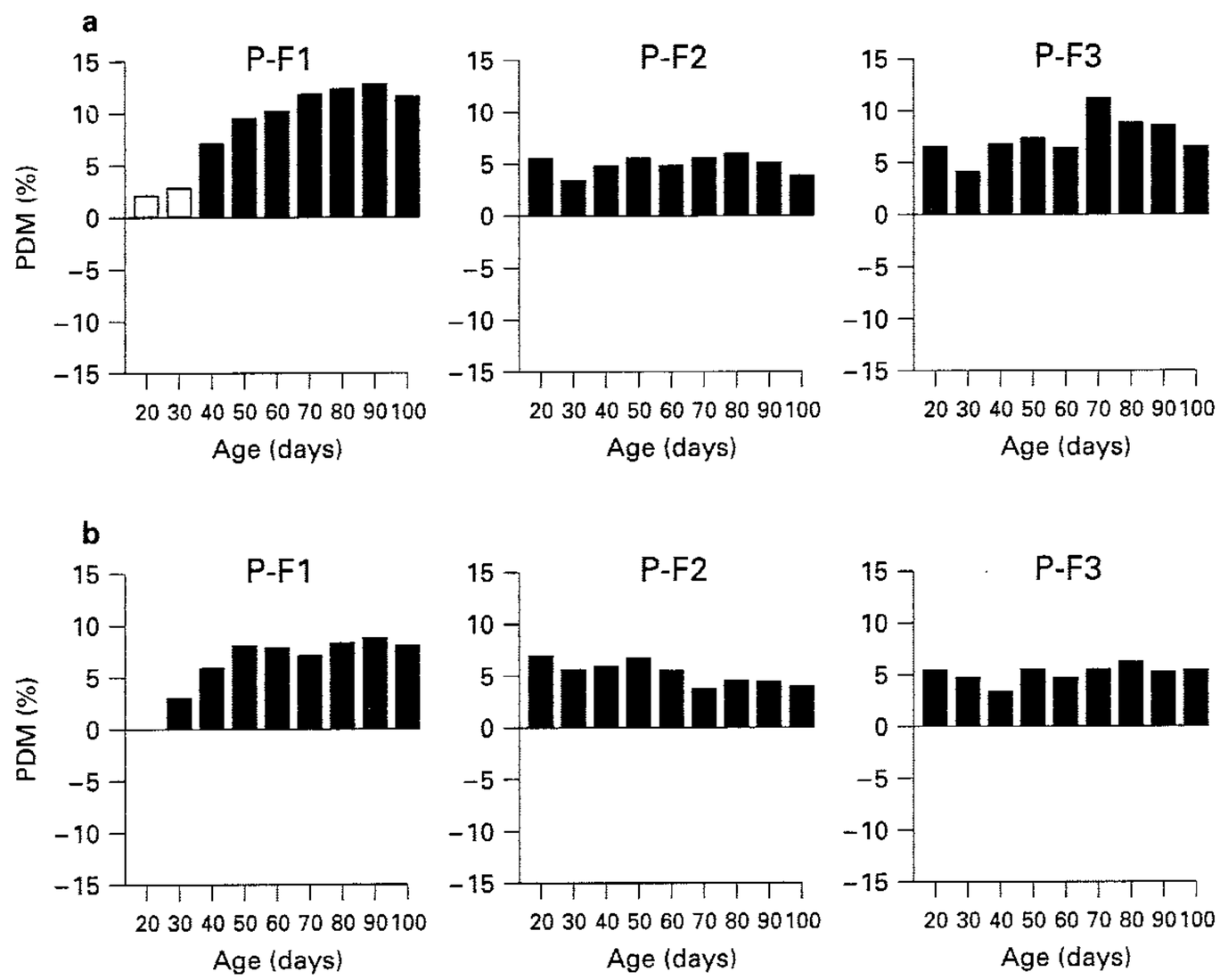

Fig. 2. PDM for VFI in males (**a**) and females (**b**). ■ = $p < 0.05$; □ = nonsignificant differences.

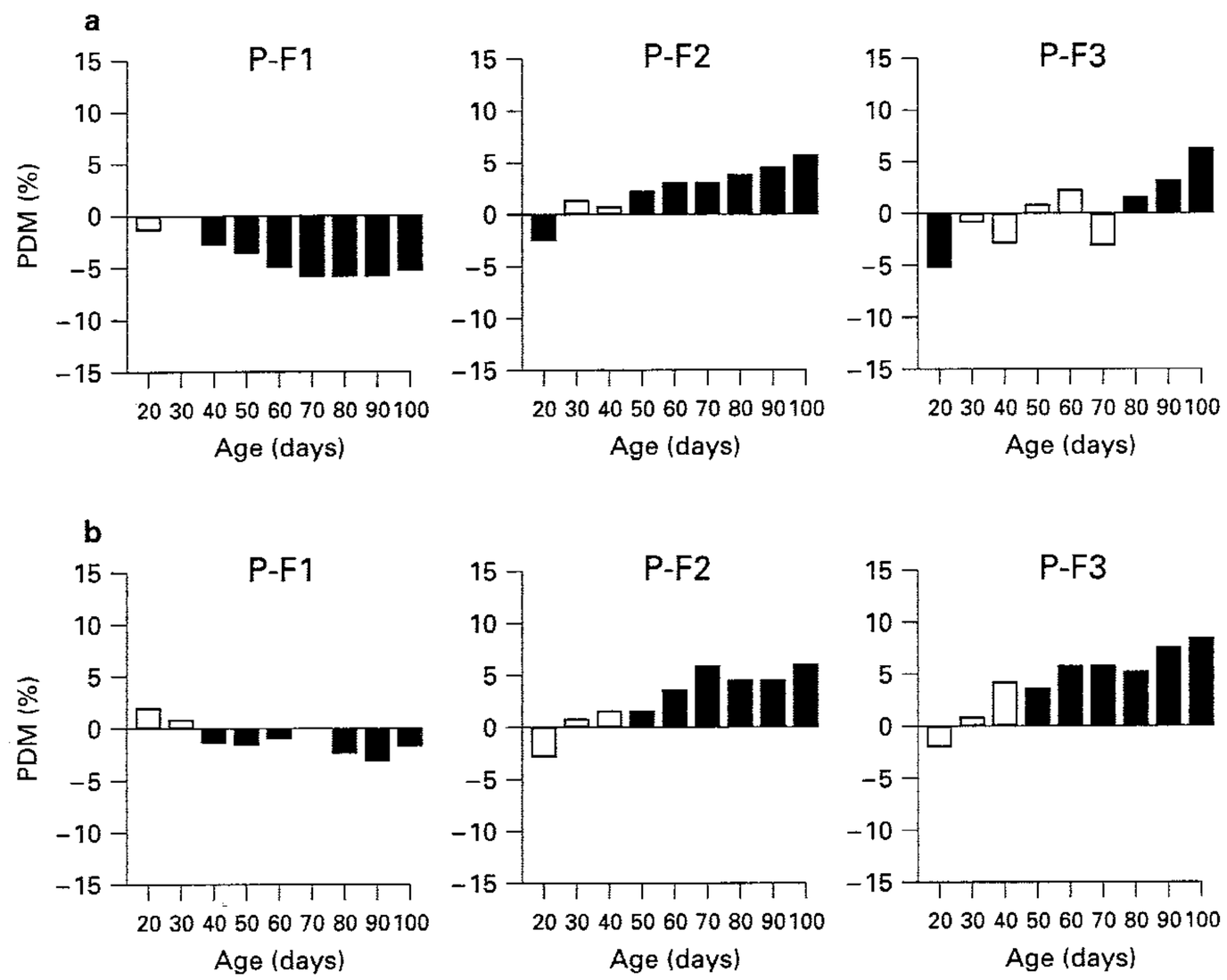

Fig. 3. PDM for NFI in males (**a**) and females (**b**). ■ = $p < 0.05$; □ = nonsignificant differences.

Undernutrition also evoked changes in shape by altering the relative growth between the two major functional components. This fact was explained by the variation of the NFI. At first, the NFI increased, F1 being greater than P. Then, the NFI was greater in P than in F2 and F3. This means that, while severe intragenerational undernutrition affected the development of the face relatively more than that of the neurocranium, the moderate transgenerational undernutrition impaired neurocranial growth more than that of the face (fig. 3). This may be explained by the fact that moderate undernutrition – in contrast to severe undernutrition – was applied during gestation, affecting the brain before its growth would be finished. Resnick and Morgane [1984] found that a protein restriction in the first generation becomes a more severe protein restriction in the second generation, based on several parameters including brain weight at birth. These results, as well as our findings, would be in contrast to the well-known brain-sparing effect, which means an adaptive mechanism for neural growth and development in stressing conditions.

Although we cannot explain the significance of morphological evidence for the function of the central nervous system, further experimental analyses on this topic need to be done.

Acknowledgements

The authors are grateful to Mrs. Damiana C. Pucciarelli for her highly valuable help with the language corrections and to Fernando Feller for the graphical design. This study was partially supported by the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Agencia Nacional de Promoción Científica y Tecnológica (ANPCYT) and Universidad Nacional de La Plata (UNLP).

References

Bresler, D.E., et al. (1975) Learning deficits in rats with malnourished grandmothers. Dev Psychobiol *8:* 315–323.

Cheverud, J.M. (1982) Phenotypic, genetic, and environmental morphological integration in the cranium. Evolution *36:* 449–516.

Cowley, J.J., R.D. Griesel (1966) The effect on growth and behavior of rehabilitation of first and second generation low protein rats. Anim Behav *14:*506–517.

Dressino, V., H.M. Pucciarelli (1997) Cranial growth in *Saimiri sciureus* (Cebidae) and its alteration by nutritional factors: A longitudinal study. Am J Phys Anthropol *102:* 545–554.

Emanuel, I. (1986) Maternal health during childhood and later reproductive performance. Ann NY Acad Sci *477:* 27–39.

Emanuel, I. (1997) Invited commentary: An assessment of maternal intergenerational factors in pregnancy outcome. Am J Epidemiol *146:* 820–825.

Emanuel, I., et al. (1992) Intergenerational studies of human birth weight from the 1958 birth cohort. 1. Evidence for a multigenerational effect. Br J Obstet Gynaecol *99:* 67–74.

Fields, H.W. (1991) Craniofacial growth from infancy to adulthood. Pediatr Clin North Am *38:* 1053–1088.

Galler, J.R., K.J. Propert (1981) Maternal behavior following rehabilitation of rats with intergenerational malnutrition. 1. Persistent changes in lactation-related behaviors. J Nutr *111:* 1330–1336.

Galler, J.R., C. Seelig (1981) Home-orienting behavior in rat pups: The effect of 2 and 3 generations of rehabilitation following intergenerational malnutrition. Dev Psychobiol *14:* 541–548.

Garn, S.M., D.C. Clarck (1975) Nutrition, growth, development, and maturation: Findings from the Ten-State Nutrition Survey of 1968–1970. Pediatrics *56:* 300–319.

Kenney, M.A., E.B. Barton (1975) Malnutrition and fetal development in two generations of rats. Nutr Rep Int *11:* 243–250.

Klebanoff, M.A., et al. (1997) Preterm and small-for-gestational-age birth across generations. Am J Obstet Gynecol *176:* 521–526.

Miller, J.P., R.Z. German (1999) Protein malnutrition affects the growth trajectories of the craniofacial skeleton in rats. J Nutr *129:* 2061–2069.

Moore, W.J. (1966) Skull growth in the albino rat *(Rattus norvegicus)*. J Zool Lond *149:* 137–144.

Moss, M.L. (1973) A functional cranial analysis of primate craniofacial growth. 4th International Congress of Primatology. Basel, Karger, vol 3, pp 191–208.

Park, A.W., B.J. Nowosielski-Slepowron (1983) Postnatal skull growth of the rat relative to environmental variance. Acta Morph Neerl Scand *21:* 141–163.

Pessoa, D.C., et al. (2000) Dietary proteins on reproductive performance in three consecutive generations of rats. Arch Latinoam Nutr *50:* 55–61.

Pucciarelli, H.M. (1981) Growth of the functional components of the rat skull and its alteration by nutritional effects. Am J Phys Anthropol *56:* 33–41.

Pucciarelli, H.M., et al. (1990) Changes in skull components of the squirrel monkey evoked by growth and nutrition: An experimental study. Am J Phys Anthropol *81:* 535–543.

Pucciarelli, H.M., et al. (2001) Relative food intake of rats submitted to a moderate transgenerational undernutrition. Growth Dev Aging *65:* 83–93.

Rajanna, B., et al. (1984) Experimental study on rats to find the usefulness of nutritional supplementation to undernourished offspring of parents undernourished life-long. Indian J Physiol Pharmacol *28:* 83–96.

Resnick, O., P.J. Morgane (1984) Generational effect of protein malnutrition in the rat. Brain Res *317:* 219–227.

Sanderson, M., I. Emanuel, V. Holt, (1995) The intergenerational relationship between birthweight, infant birthweight and infant mortality in black and white mothers. Paediatr Perinat Epidemiol *9:* 391–405.

Srivastava, U., et al. (1974) Maternal dietary deficiency and cellular development of progeny in the rat. J Nutr *104:* 512–520.

Stewart, R.J., et al. (1973) The long-term effects of marginal protein-energy deficiency. Proc Nutr Soc *32:* 102A–103A.

Widdowson, E.M., R.A. McCance (1963) The effect of finite periods of undernutrition at different ages on the composition and subsequent development on the rat. Proc R Soc B *158:* 329–342.

Zamenhof, S., E. van Marthens (1978) The effects of chronic undernutrition over generations on rat development. J Nutr *108:* 1719–1723.