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Effect of Undernutrition on the Cranial Growth of the Rat

An Intergenerational Study

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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.

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.

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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**

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

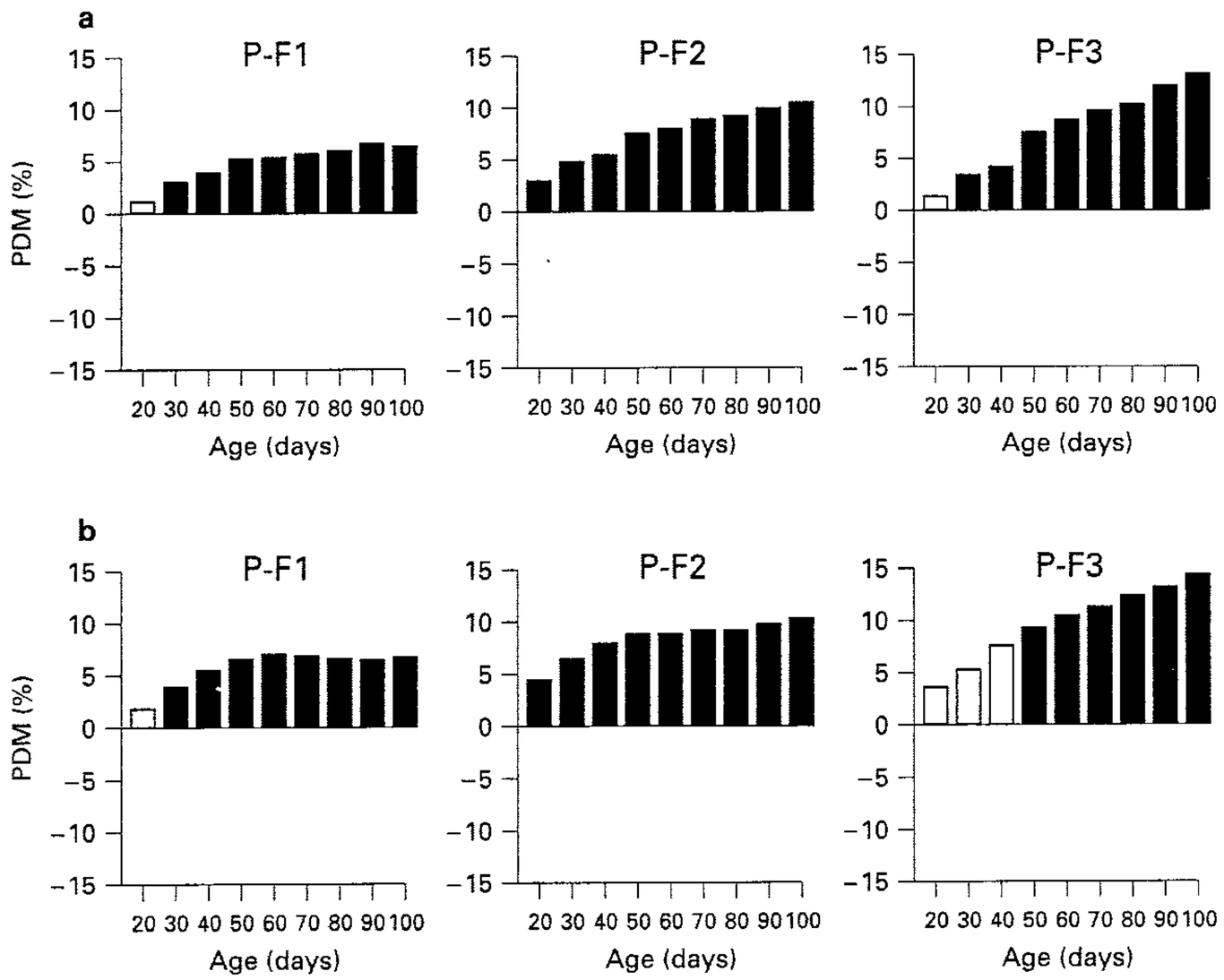

Fig. 1. PDM for VNI in males (**a**) and females (**b**). ■ = $p < 0.05$; □ = nonsignificant differences.

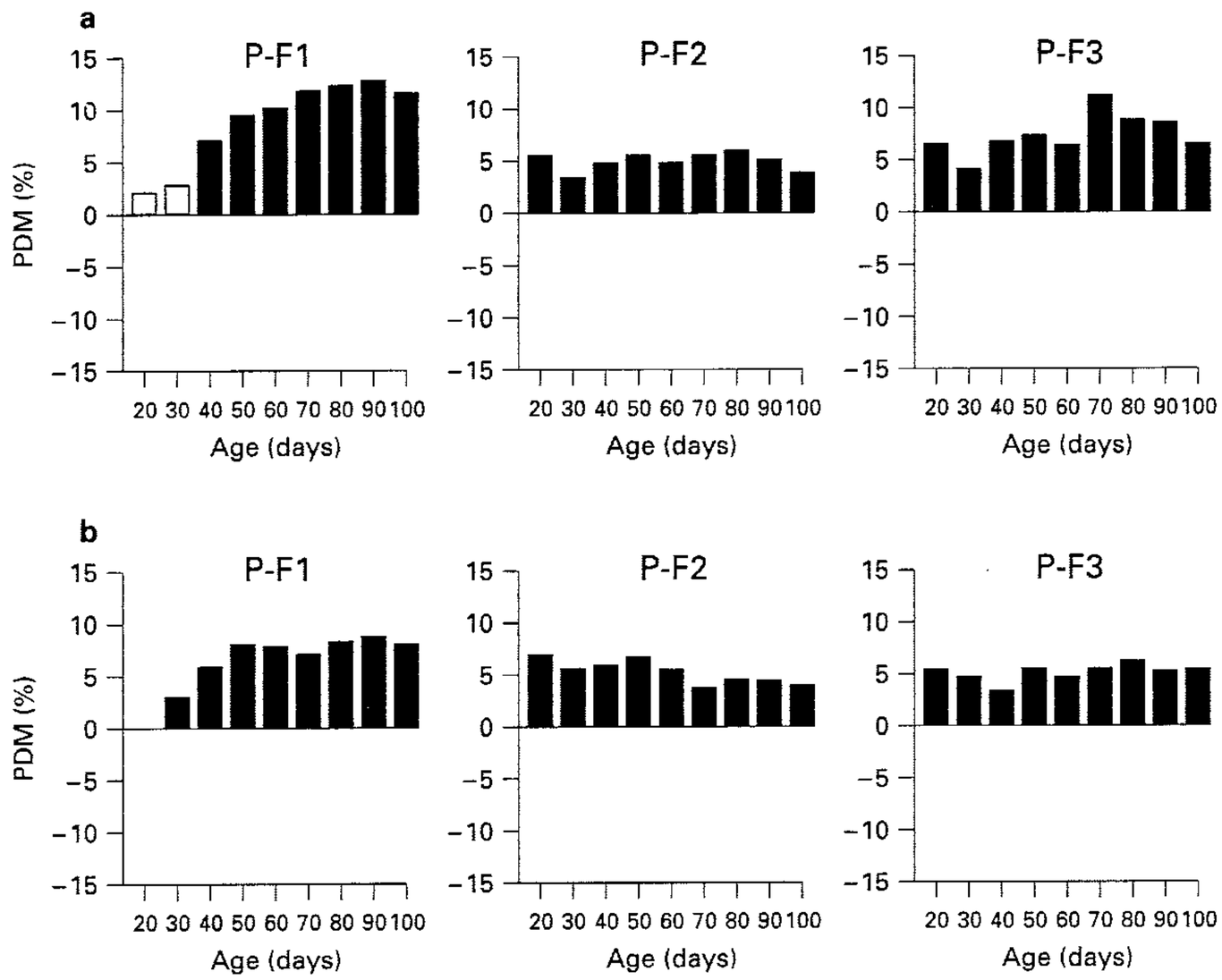

Fig. 2. PDM for VFI in males (**a**) and females (**b**). ■ = $p < 0.05$; □ = nonsignificant differences.

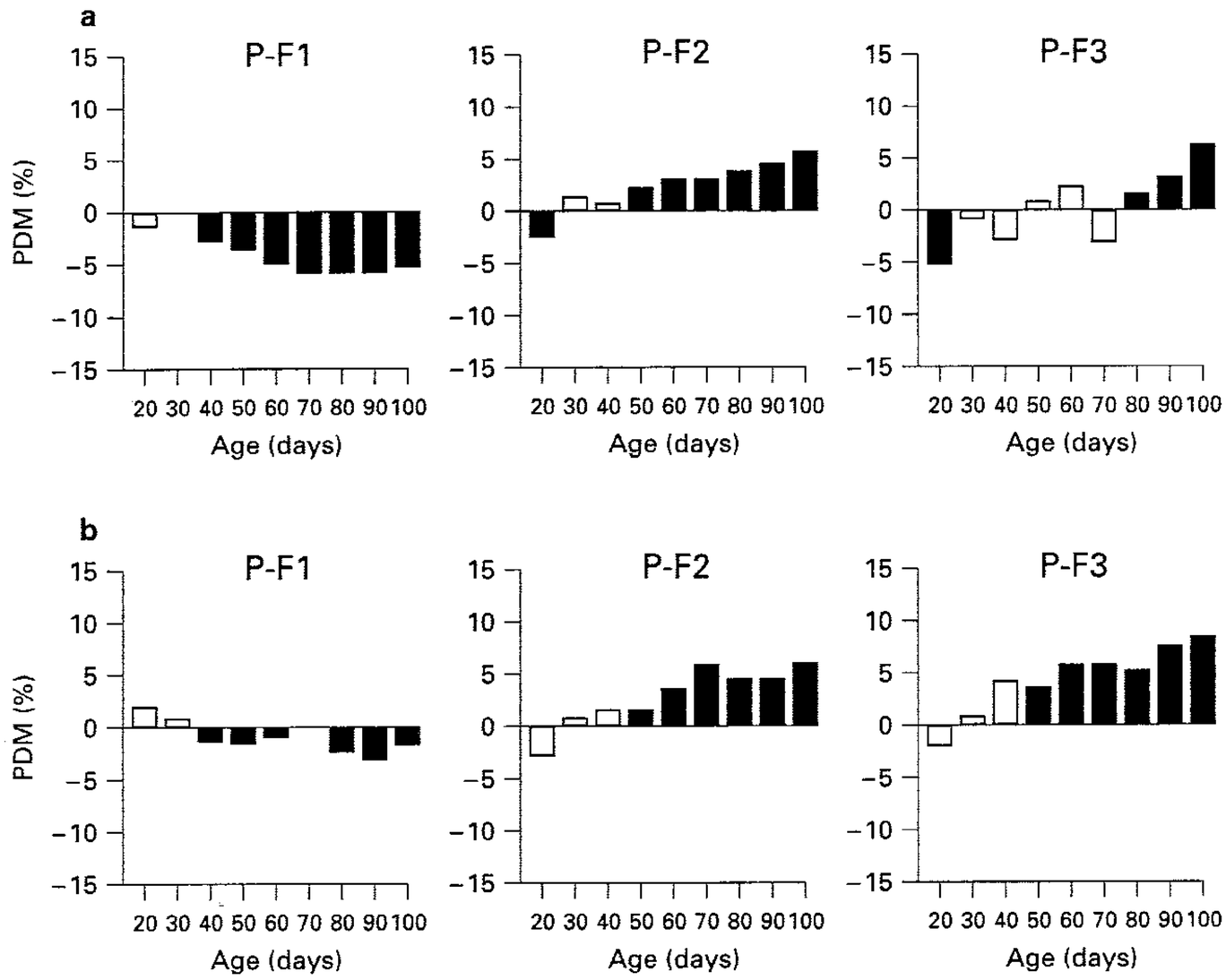

Fig. 3. PDM for NFI in males (**a**) and females (**b**). ■ = $p < 0.05$; □ = nonsignificant differences.

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.

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