Original Paper

Cells Tissues Organs

Cells Tissues Organs 2001;168:272–284

Accepted after revision: June 7, 2000

Effect of Undernutrition on Cranial Components and Somatotroph-Lactotroph Pituitary Populations in the Squirrel Monkey *(Saimiri sciureus boliviensis)*

G.M. Cónsole[a] E.E. Oyhenart[b] S.B. Jurado[c] F.L. Riccillo[a] H.M. Pucciarelli[b] C.L.A. Gómez Dumm[a]

[a]Cátedra de Histología-Embriología 'B', Facultad de Ciencias Médicas, UNLP, [b]Centro de Investigaciones en Genética Básica y Aplicada (CIGEBA), Facultad de Ciencias Veterinarias, UNLP y [c]Servicio Central de Microscopía Electrónica, Facultad de Ciencias Veterinarias, UNLP, La Plata, Argentina

Key Words

Undernutrition · Craniology, functional · Somatotrophs · Lactotrophs · Squirrel monkey

Abstract

The aim of the present study was to investigate in monkeys the effects of undernutrition on neurocranial and facial components, correlated with a histometric and ultrastructural analysis of somatotroph (growth hormone, GH) and lactotroph (prolactin, PRL) pituitary populations. Twenty *Saimiri sciureus boliviensis* (Cebidae) of both sexes were employed. The monkeys were born in captivity and when they reached 1 year of age, they were separated into two groups: control and undernourished animals. They were fed ad libitum a 20% and 10% protein diet, respectively. The monkeys were radiographed when they were 3 years old in order to measure the length, width and height of the anterior, middle and posterior components of the neurocranium, as well as those of the masticatory, respiratory and optic components of the face. The volumetric and morphometric indices were then calculated. After the sacrifice, pituitary glands were processed for light and electron microscopy. The quantitative immunohistochemistry revealed a decrease in the volume density and cell density of both GH and PRL cells from malnourished animals when compared to control ones. The ultrastructural study showed changes suggestive of cellular hyperfunction for both types of cells in the former experimental group. Under-

Abbreviations used in this paper

ANMI, ANVI	anteroneural (morphometric, volumetric indices)
BSA	bovine serum albumin
CD	cell density
FAVI	facial (volumetric index)
GH	growth hormone
GHRH	growth hormone-releasing hormone
IGF-I	insulin-like growth factor
MAMI, MAVI	masticatory (morphometric volumetric indices)
MNMI, MNVI	midneural (morphometric, volumetric indices)
NEVI	neurocranial (volumetric index)
NFMI	neurofacial (morphometric index)
OPMI, OPVI	optic (morphometric, volumetric indices)
PNMI, PNVI	posteroneural (morphometric, volumetric indices)
PRL	prolactin
RDM%	percent relative difference between means
REMI, REVI	respiratory (morphometric, volumetric indices)
VD	volume density

KARGER

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

1422–6405/01/1684–0272$17.50/0

Accessible online at:
www.karger.com/journals/cto

Gloria Cónsole
Cátedra de Histología-Embriología B
Facultad de Ciencias Médicas, UNLP
CC 455, La Plata 1900 (Argentina)
Tel. +54 221 425 6735, Fax +54 221 425 8989, E-Mail gconsole@atlas.med.unlp.edu.ar

nutrition also affected the size of the cranial components, with males being more affected than females; brain weight was, however, nonmodified by stress, with the brain/body ratio difference being the same for both sexes. We conclude that in monkeys, experimental undernutrition produces a decrease in the pituitary GH and PRL cell populations, in some way related to changes in the cranio-facial morphometric patterns.

Introduction

The regulation of growth hormone (GH) secretion is a complex process that comprises stimulatory and inhibiting hypothalamic mechanisms. Neuroendocrine mechanisms modulating GH secretion are sensitive to nutritional status since the normal pulsatile pattern is altered under conditions of malnutrition. Food deprivation decreased somatostatin and GH-releasing hormone (GHRH) immunostaining in sections of the median eminence of the hypothalamus, and mRNA levels for somatostatin, GHRH and GH were also reduced [Brogan et al., 1997]. These data suggest that GH secretion in food-deprived rats may reflect a downregulation. It is well known that a low protein diet induces various changes in the pituitary gland. GH is described as an anabolic, lipolytic and insulin antagonist that promotes an optional use of nutrients during deprivation periods [Snyder et al., 1988]. Age and the nutritional state determine variations in the secretion patterns. Pulsatile GH administration to GH-deficient adults causes significant alterations in the protein, carbohydrate and lipid metabolism, hence supporting the hypothesis that GH has a specific role in the use of nutrients [Lee Vance et al., 1992].

Experimental animal models of undernutrition on the basis of the administration of a low-protein diet have allowed an extensive morphometric study by measuring neurocranial and facial functional components [Pucciarelli et al., 1990; Oyhenart and Pucciarelli, 1992; Pucciarelli and Dressino, 1996].

Quantity and quality of food intake have a marked biologic influence on the hormones, especially as regards GH and prolactin (PRL) [Cheek and Hill, 1974; Pimstone, 1976]. The regulation of pulsatile GH secretion, at least the changes linked with malnutrition, appears to be dependent upon a leptin signal noticed at the level of neuroendocrine axis, possibly through a neuropeptide Y neurotransmission. The leptin can rescue normal pulsatile secretion, which had been altered after 3 days of fasting, by preventing the inhibitory action of neuropeptide Y [Vuagnat et al., 1998].

Insulin-like growth factor (IGF-I) may determine GH responses both to malnutrition and refeeding. The structure studies of the IGF-I receptor from somatotrophs provide useful tools to understand the metabolic control of the GH axis [Melmed et al., 1996].

The postweaning low-protein diet [Gómez Dumm et al., 1982] as well as malnutrition throughout the nursing period [Gómez Dumm et al., 1987] have rendered ultrastructural changes in the somatotroph cells of the rat pituitary, suggesting a decreased secretory activity.

The aim of the present study was to investigate in monkeys *(Saimiri sciureus boliviensis)* the effects of experimental undernutrition on (1) the structure of the skull components and (2) the immunohistochemistry and ultrastructure of the GH and PRL pituitary-cell populations, as well as to establish the possible relationship between changes of these two parameters.

Material and Methods

Animals and Specimen Collection

Ten male and 10 female *S. sciureus boliviensis* (Cebidae) were employed. The monkeys were born in captivity at the Centro Argentino de Primates (CAPRIM) and after weaning (7 months) they were raised at the Facultad de Ciencias Veterinarias de la Universidad Nacional de La Plata (CIGEBA). When the animals were 1 year of age, they were divided into a control group and an undernourished group, both comprising 5 monkeys of each sex. The animals were fed ad libitum a 20% and a 10% protein diet (table 1), respectively.

Table 1. Composition of control (20%) and low protein (10%) diets

Component	Control diet	Low protein diet
Soybean meal	28.0	9.9
Wheat meal	14.7	8.0
Glucose	0.0	6.7
Skimmed milk	10.6	4.9
Wheat bran	5.6	5.6
Saccharose	3.5	3.5
Rice meal	3.3	6.6
Corn starch	3.0	21.4
Margarine	4.2	6.7
Egg	7.0	3.2
Vitamin mixture	1.5	1.5
Salt mixture	1.5	1.5
Water	17.1	20.5
Total	100.0	100.0

Values are expressed in grams.

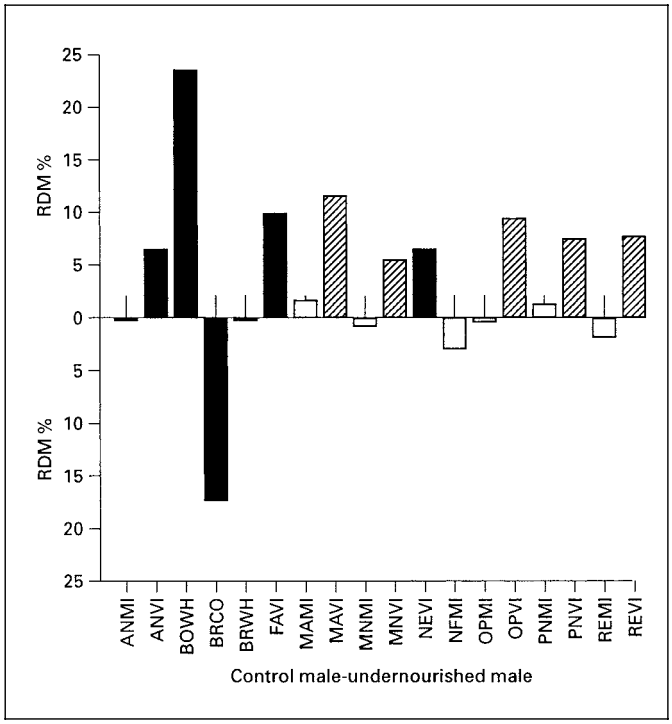


Fig. 1. RDM and the Jonckheere-Terpstra exact significances in the male control-treated comparison. ■ = Highly significant differences ($p < 0.01$); ▨ = significant differences ($p < 0.05$), □ = nonsignificant differences ($p > 0.05$). Bars above zero = control mean greater than treated ones. Bars below zero = control mean lower than treated ones. Index symbols are depicted on the x-axis.

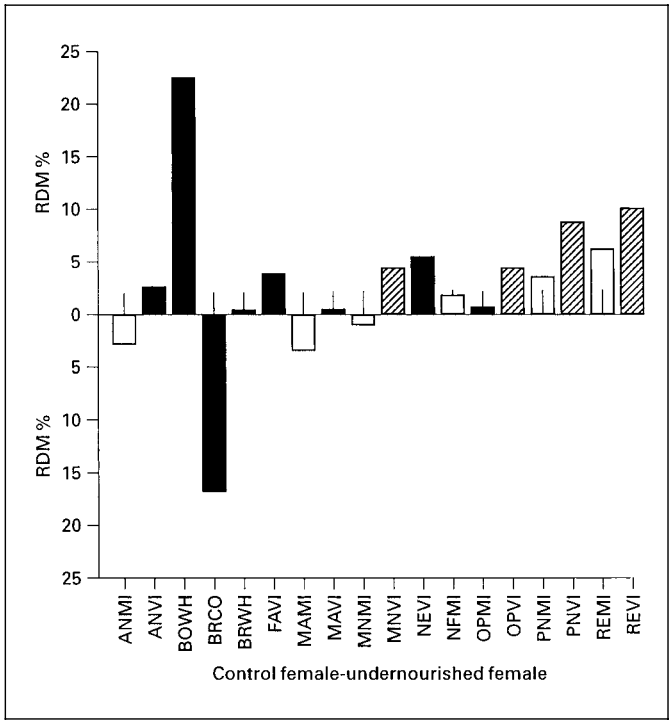


Fig. 2. RDM and the Jonckheere-Terpstra exact significances in the female control-treated comparison. ■ = Highly significant differences ($p < 0.01$); ▨ = significant differences ($p < 0.05$); □ = nonsignificant differences ($p > 0.05$). Bars above zero = control mean greater than treated ones. Bars below zero = control mean lower than treated ones. Index symbols are depicted on the x-axis.

When they were 3 years old, radiographs were taken in order to measure the neurocranial and facial components. Thereafter, the monkeys were weighed and sacrificed by decapitation under light ether anesthesia. The skulls were opened and the brains were removed and weighed. The pituitary glands were dissected out and processed for microscopical study. Maintenance and treatment of the animals were according to the National Institute of Health (NIH) Guide for Care and Use of Laboratory Animals.

Immunohistochemistry and Morphometry

Pituitaries from all the animals were fixed in Bouin's fluid and embedded in paraffin. Serial sections of 4 μm were obtained at different levels of the blocks, following a ventral-to-dorsal sequence. Stated in brief, sections were incubated for 1 h at room temperature with primary antibodies: anti-GH and anti-PRL (mouse-Dako) diluted 1:200. Thoroughly washed sections were treated for 30 min with a ready-to-use EnVision reaction system (Dako). The peroxide-sensitive chromogen was diaminobenzidine. The specificity of the primary antiserum was monitored either by the ability to block the immunocytochemical reaction by preadsorption of the antibody with an excess of the related antigen or by replacing the first antiserum by normal rabbit serum or PBS. Measurements of cell parameters were made by means of an image analysis system (Imaging Technology, Optimas 5.2). The immunostained cells and reference area were analyzed for each field for an average of ten micrographs taken from different levels (ventral, medial and dorsal). These measurements were recorded, processed automatically, and finally used to calculate the following parameters: volume density (VD = cell area/reference area) and cell density (CD = number of cells/reference area).

Electron Microscopy

Pars distalis from 5 animals of each group were processed for ultrastructural examination. They were dissected away and fixed with 2% (v/v) glutaraldehyde in 0.05 *M* cacodylate buffer. The material was cut into small pieces (1 × 1 mm), postfixed in 1% (w/v) osmium tetroxide, and embedded in Araldite. Thin sections (about 1 μm) were stained with toluidine blue and inspected under light microscopy to select fields. Ultrathin sections were mounted on 200-mesh copper grids stained with uranyl acetate and lead citrate, and examined in a JEM-1200 EX transmission electron microscope at 80 kV.

Immunoelectron Microscopy

Ultrathin sections were mounted on 200 mesh nickel grids. The tissues were etched with 10% (v/v) hydrogen peroxide for 10 min. For GH-PRL detection, the grids were incubated in anti-GH and anti-PRL (mouse-Dako) at a dilution of 1:200 in phosphate buffer containing 1% (v/v) bovine serum albumin (BSA) at 4°C for 24 h.

Table 2. Tests for normality of distributions (K-S) and homogeneity of variances (L) for measurements and indices in the entire sample

Indices	K-S	Probability	L	Probability
Soft tissues				
Body weight (BOWH)	0.18	0.77	4.94	0.03*
Brain weight (BRWH)	0.15	0.91	2.68	0.12
Brain coefficient (BRCO)	0.14	0.94	2.06	0.18
Volumetric indices				
Neurocranial (NEVI)	0.17	0.81	0.91	0.48
Anteroneural (ANVI)	0.13	0.96	0.95	0.46
Midneural (MNVI)	0.18	0.76	1.91	0.21
Posteroneural (PNVI)	0.21	0.58	2.11	0.18
Facial (FAVI)	0.11	1.00	2.09	0.18
Optic (OPVI)	0.09	1.00	0.58	0.65
Respiratory (REVI)	0.22	0.54	4.91	0.03*
Masticatory (MAVI)	0.14	0.95	8.85	0.01**
Morphometric indices				
Neurofacial (NFMI)	0.12	0.98	14.96	0.01**
Anteroneural (ANMI)	0.23	0.50	2.68	0.12
Midneural (MNMI)	0.17	0.81	2.35	0.15
Posteroneural (PNMI)	0.25	0.38	5.85	0.02*
Optic (OPMI)	0.22	0.52	0.04	0.99
Respiratory (REMI)	0.14	0.95	1.01	0.44
Masticatory (MAMI)	0.22	0.54	1.85	0.22

$0.01 < *p < 0.05$; ** $p < 0.01$. K-S = Kolmogorov-Smirnov tests for one sample; L = Levene tests.

After several washings in PBS-BSA, the protein A-colloidal gold (EY Laboratories, San Mateo, Calif., USA) labeled immunoreactive sites for 1 h at room temperature (20-nm particles). The grids were stained with uranyl acetate and lead citrate. The specificity of staining was checked as follows: (1) incubation of normal rabbit serum instead of antimouse GH-PRL serum, and (2) preabsorption of the antiserum with mouse GH-PRL. No immunolabeling was observed in these controls.

Craniofacial Morphometric Measurements

Two tele-radiographs per animal – one from strict dorsal-ventral and the other from strict laterolateral views – were taken. The length (Lx) and the height (Hx) from the first, as well as the width (Wx) from the second, were measured for each cranial component. Then, the volumetric and the morphometric indices were calculated according to formulas previously developed [Pucciarelli et al., 1990]. The following differences in size were estimated by the volumetric indices: anteroneural (ANVI), midneural (MNVI), posteroneural (PNVI), optic (OPVI), respiratory (REVI) and masticatory (MAVI) for the minor components, and neurocranial (NEVI) and facial (FAVI) for the major ones. Shape differences – in terms of relative growth pattern alterations – were estimated by the morphometric indices: anteroneural (ANMI), midneural (MNMI), posteroneural (PNMI), optic (OPMI), respiratory (REMI) and masticatory (MAMI) for the minor components, and neurofacial (NFMI) for the major ones.

In order to estimate the difference between treatments on the same variable or index, the percent relative difference between means (RDM%) was employed in the graphic comparisons (fig. 1, 2). This formula measures which proportion of the control variables is affected by treatment [Pucciarelli et al., 1990].

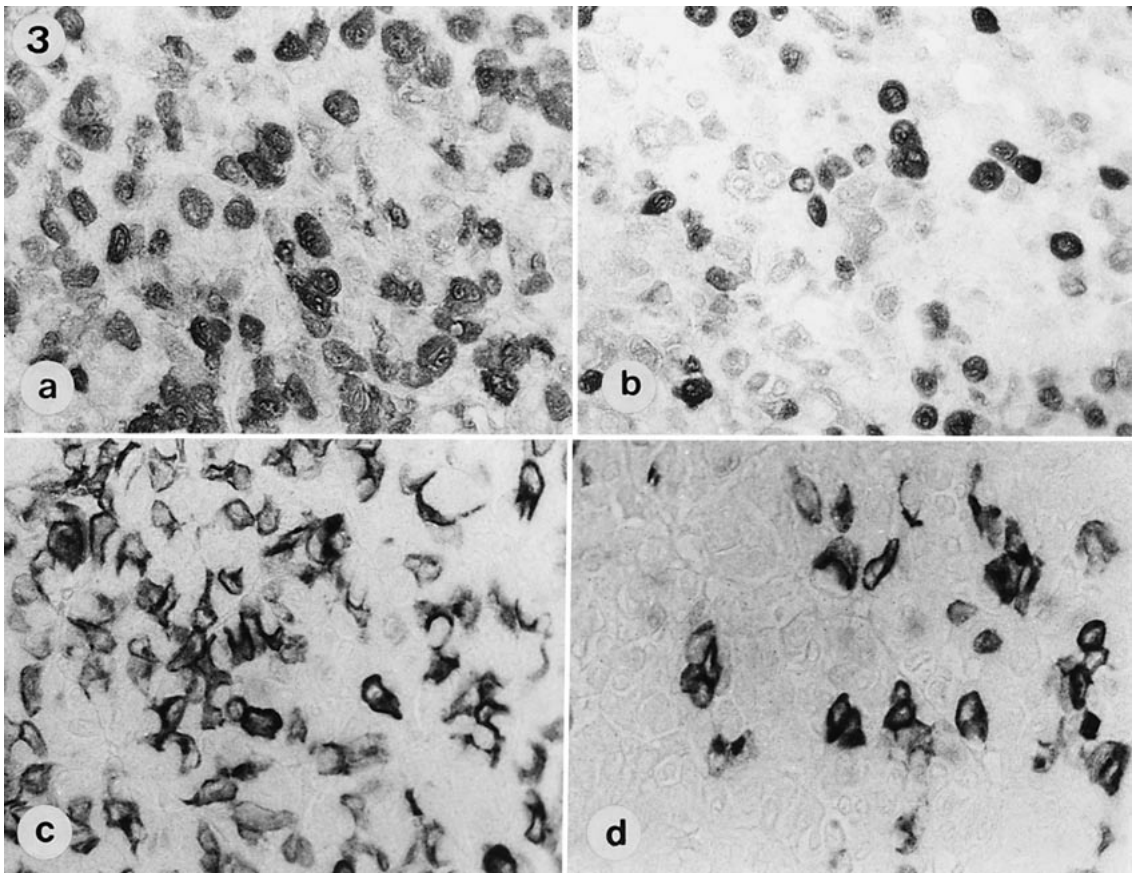
Statistical Analysis

Data distributions of the volumetric and the morphometric indices were evaluated by both the one-sample Kolmogorov-Smirnov test for testing normality and the Levene test for testing homogeneity of variances. All variables were normally distributed, but some of them showed nonhomogeneous variances (table 2). Consequently, for the comparisons, we adopted the Jonckheere-Terpstra (J-T) nonparametric test with the exact method of significance, especially devised for small sample sizes [Mehta and Patel, 1996]. For histological studies, statistical evaluation was tested by means of one-way analysis of variance, followed by LSD test for multiple comparisons. Data were expressed as mean ± SEM; the statistical work was mainly done by the Systat 7.0 package.

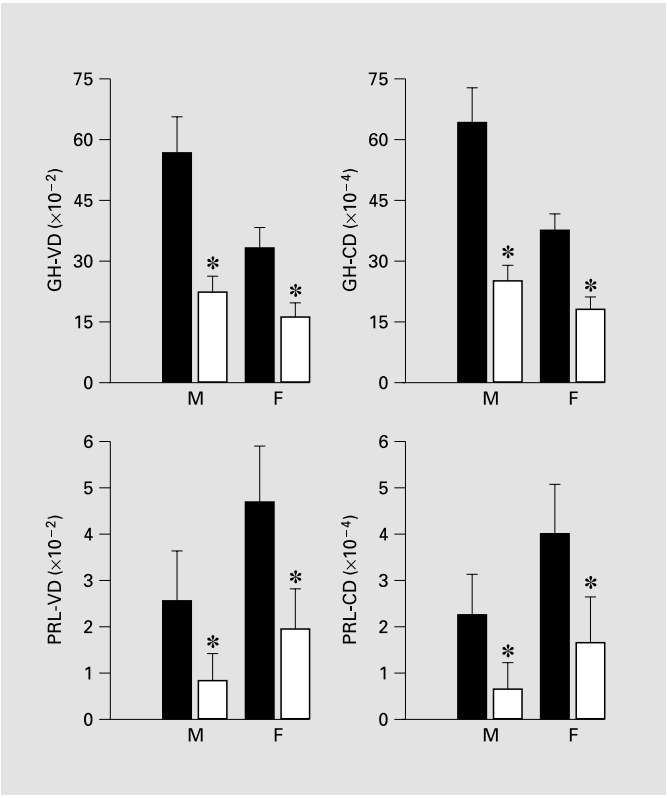
Results

Quantitative Immunohistochemical Studies

Immunostained somatotroph and lactotroph cells stood out in sharp relief, exhibiting a definite granular cytoplasmic pattern. In all the sections of the pars distalis,



3



4

these cells showed a homogeneous distribution in all groups. A decline in the number of somatotroph and lactotroph cells of male and female control and undernourished (fig. 3a–d) monkeys was evident even before the quantitative evaluation. The analysis of morphological parameters (fig. 4) revealed a significant ($p < 0.05$) decrease in VD and CD of malnourished animals with respect to control ones of both sexes.

Fig. 3. Representative fields of specifically immunostained pituitary cells. Somatotrophs from male control (**a**) and male undernourished (**b**) groups: Lactotrophs from female control (**c**) and female undernourished (**d**) groups. Micrographs taken with a × 40 objective.

Fig. 4. VD and CD of GH and PRL cell populations in male (M) and female (F) monkeys from control (■) and undernourished (□) groups. * $p < 0.05$.

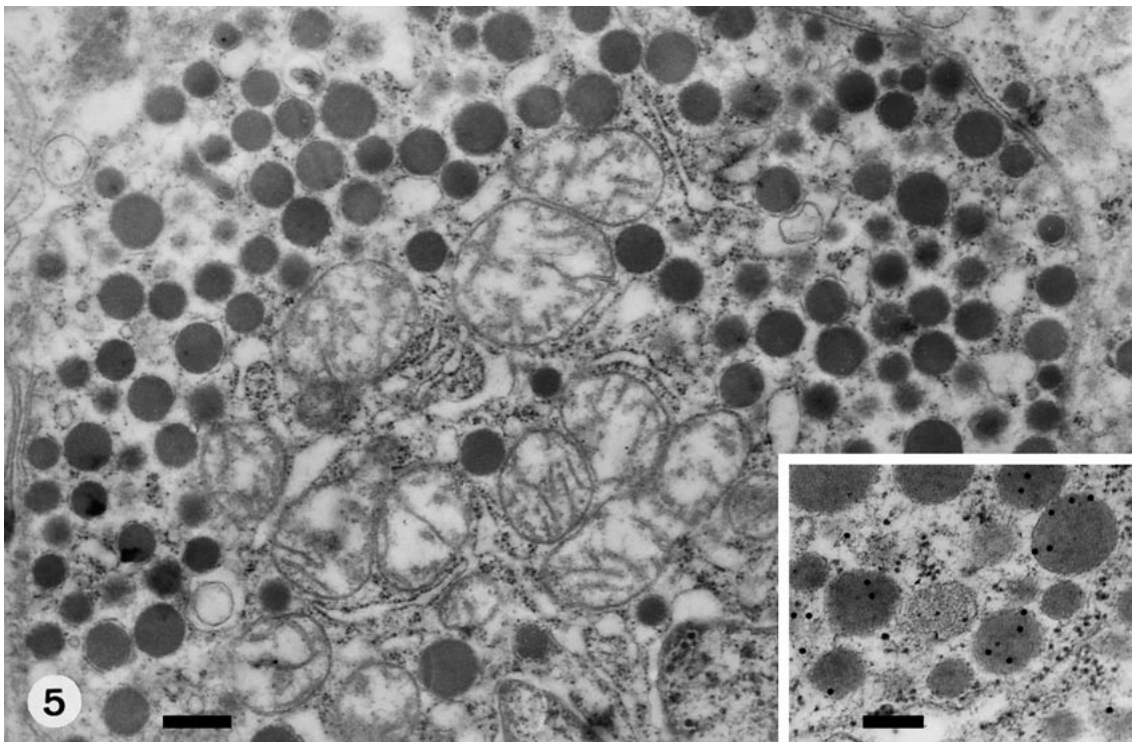


Fig. 5. Electron micrograph of a GH cell from a control animal exhibiting round, large (250–400 nm) and numerous secretory granules. Bar scale: 500 nm. Inset: Immunocytochemical analysis revealed the colloidal gold particles labeling the secretory granules. Bar scale: 200 nm.

Ultrastructural Study

The electron microscopy revealed that many GH and PRL cells from undernourished monkeys exhibited changes, when compared to control animals. In the control group, the GH cells contained round, large (250–400 nm) and numerous secretory granules. The immunocytochemical analysis revealed the colloidal gold particles labeling them (fig. 5). The PRL cells of the control group exhibited pleomorphic secretory granules ranging from 100 to 600 nm with colloidal gold particles selectively located (fig. 6). In the undernourished group, electron microscopy of PRL cells showed an extended and dilated Golgi complex, while the secretory granules were less numerous (fig. 7). A well-developed rough endoplasmic reticulum with a high number of attached and free ribosomes was observed, along with marginated secretory granules showing exocytotic profiles (fig. 8). In addition, only in the undernourished animals, a number of PRL cells showed cytoplasmic groups of long crystalloids related to lipid inclusions. Heterogeneous lysosomes containing multilaminated profiles were also frequently seen (fig. 9). The GH cells from undernourished animals exhibited a dilated Golgi complex and peripheral secretory granules. Some of them were evidently contacting the plasma membrane (fig. 10), and an irregular dilated rough endoplasmic reticulum as well as peripheral secretory granules were seen under an exocytosis process (fig. 11).

Craniometrical Study

The descriptive statistics for both sexes is shown in table 3, while the Jonckheere-Terpstra comparative results are given in table 4. In both sexes, undernutrition did not affect either the brain weight or the morphometric indices. In males, the body weight, the brain/body ratio, and all the volumetric indices were significantly delayed (fig. 1). In females, undernutrition affected the same indices as it did in males, although with a lower intensity. The brain/body ratio was an exception, since its percentual control-treatment difference (17%) was the same in both sexes (fig. 2).

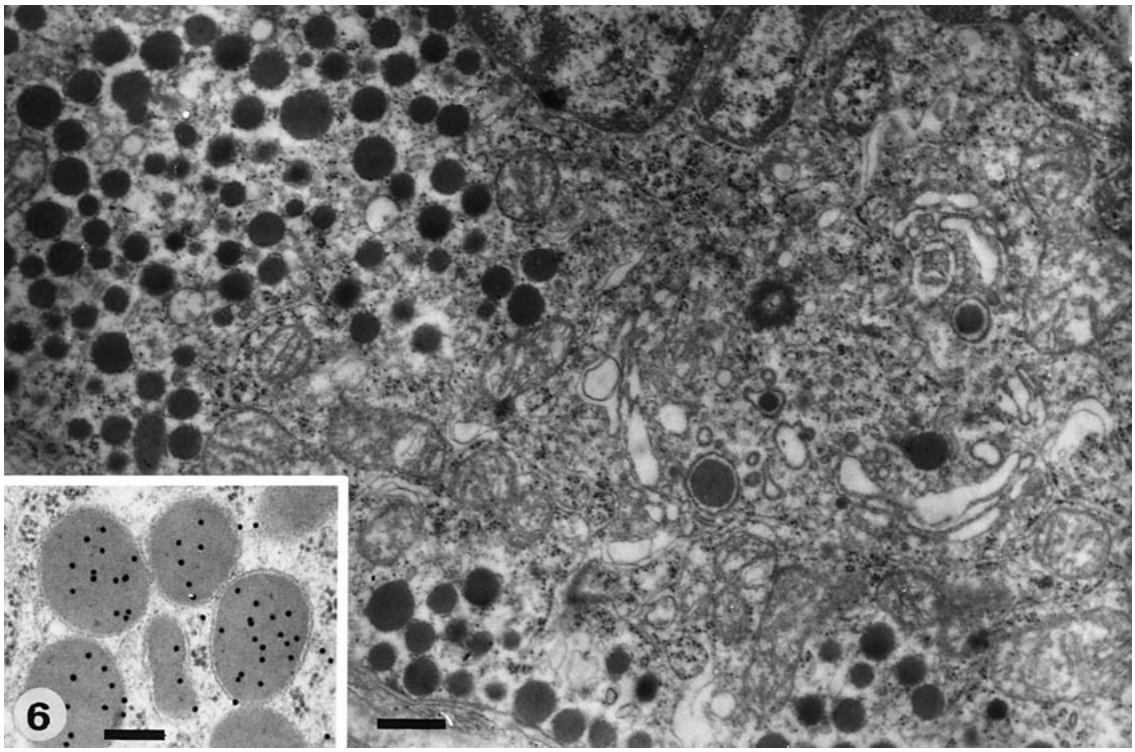


Fig. 6. Electron micrograph of a PRL cell from a control animal showing pleomorphic secretory granules ranging from 100 to 600 nm. Bar scale: 500 nm. Inset: Colloidal gold particles are selectively located in the secretory granules. Bar scale: 200 nm.

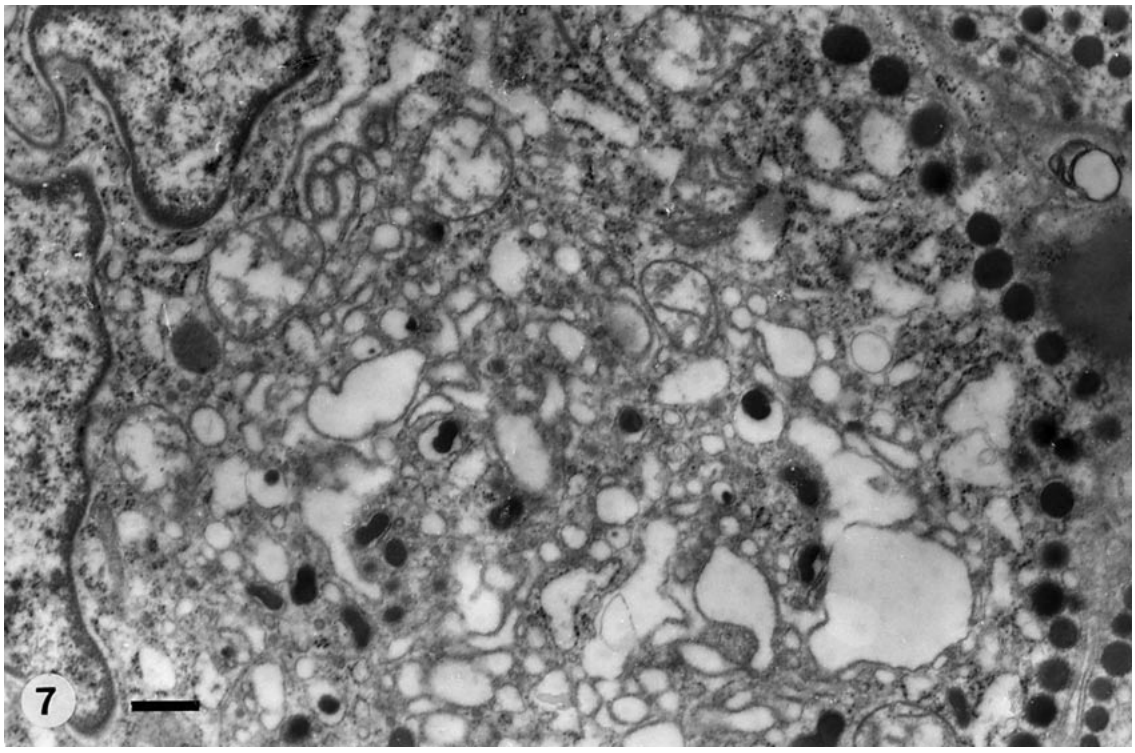
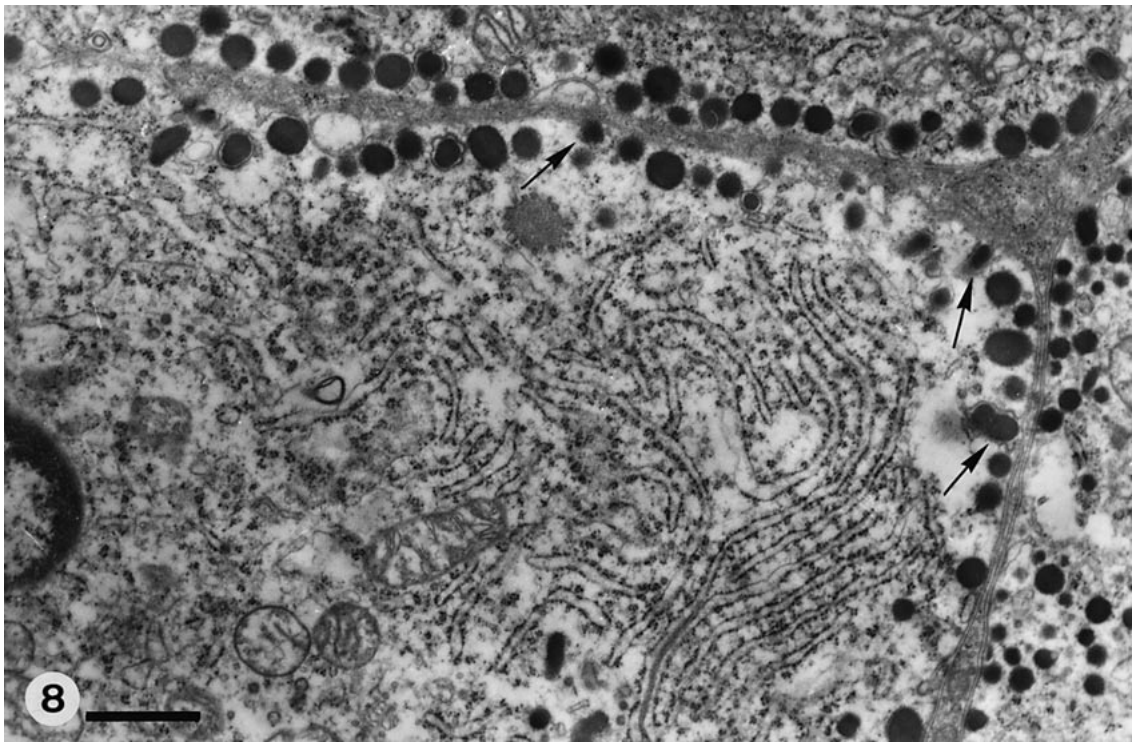


Fig. 7. Electron micrograph of a PRL cell from an undernourished monkey showing an extended and dilated Golgi complex. Bar scale: 500 nm.



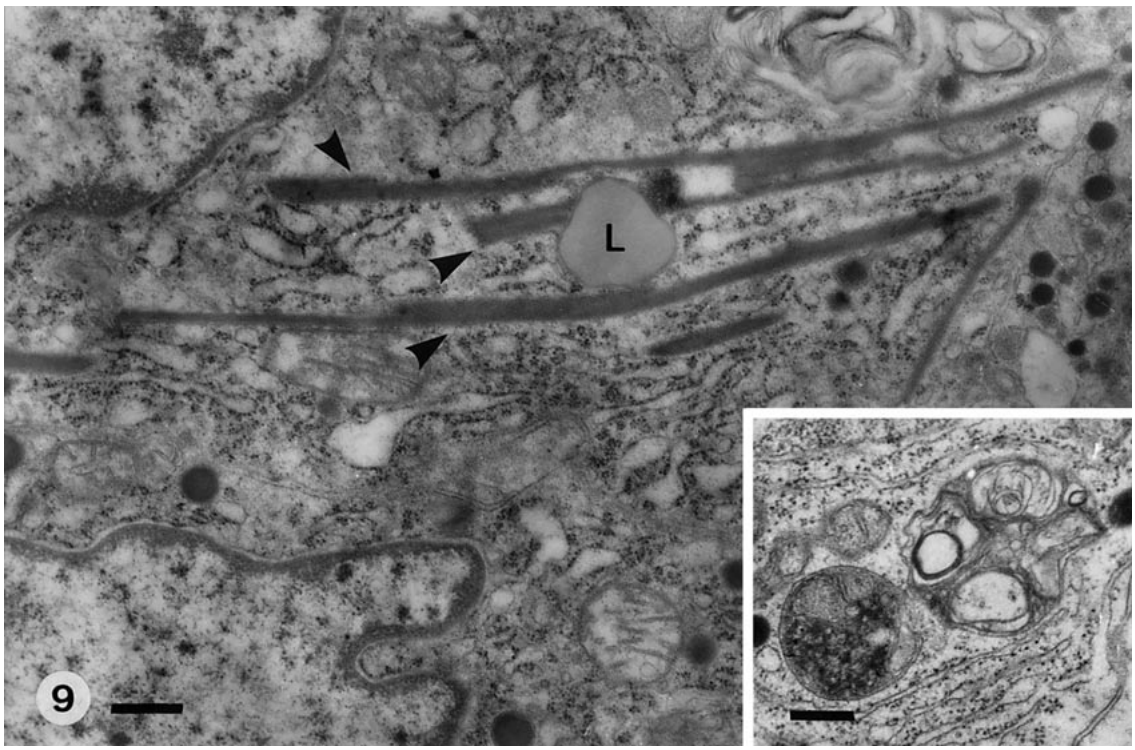
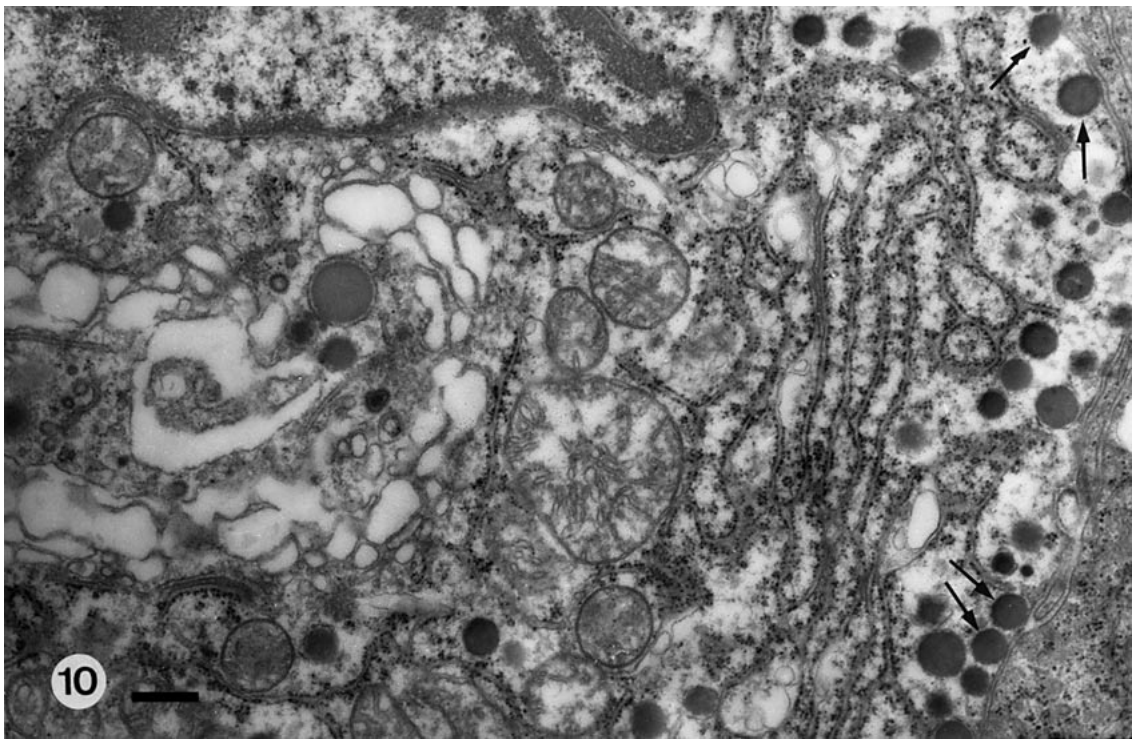


Fig. 8. Electron micrograph of a PRL cell from an undernourished animal. An area exhibiting a well-developed rough endoplasmic reticulum where a high number of attached and free ribosomes is observed. Marginated secretory granules showing exocytosis (arrows) are also seen. Bar scale: 1 μm.

Fig. 9. Electron micrograph of a PRL cell from an undernourished monkey containing long crystalloids (arrowheads) related to a lipid droplet (L). Bar scale: 500 nm. Inset: Heterogeneous lysosome with multilaminated profiles. Bar scale: 500 nm.



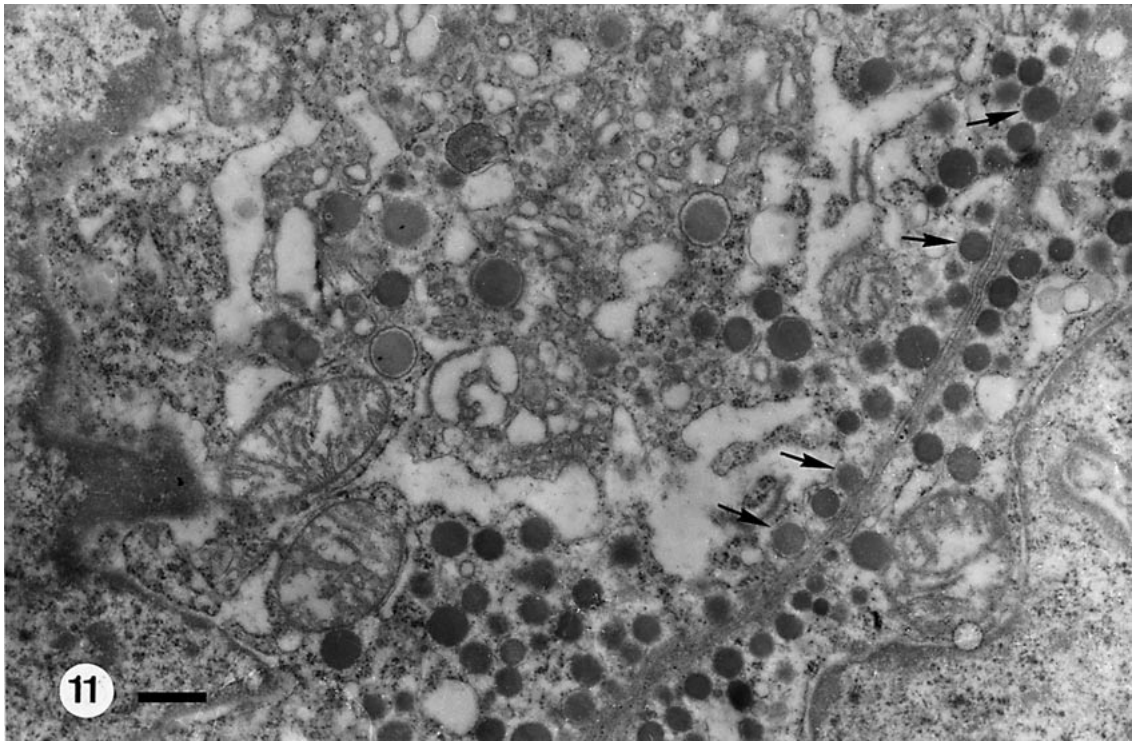


Fig. 10. Electron micrograph of a GH cell from an undernourished animal showing a dilated Golgi complex (left). Peripheral secretory granules, some of them contacting the plasma membrane (arrows), are also evident. Bar scale: 500 nm.

Fig. 11. Electron micrograph of a GH cell from an undernourished animal exhibiting an irregular dilated rough endoplasmic reticulum as well as peripheral secretory granules in contact with the plasma membrane (arrows). Bar scale: 500 nm.

Table 3. Mean (X) and standard deviation (SD) values

Indices	Control				Undernourished			
	males		females		males		females	
	X	SD	X	SD	X	SD	X	SD
Soft tissues								
Body weight (BOWH)	527.67	17.47	438.00	126.10	428.00	31.18	357.75	56.62
Brain weight (BRWH)	26.85	1.48	26.43	3.29	26.30	1.00	26.37	1.81
Brain coefficient (BRCO)	5.09	0.12	6.28	1.29	6.87	0.48	7.51	1.32
Volumetric indices								
Neurocranial (NEVI)	74.88	0.99	72.13	2.47	70.52	1.50	68.64	4.28
Anteroneural (ANVI)	20.59	0.41	19.35	0.69	19.39	0.04	18.88	0.64
Midneural (MNVI)	27.74	0.15	26.96	1.24	26.38	0.65	25.92	1.35
Posteroneural (PNVI)	26.55	0.48	25.83	0.57	24.75	0.90	23.84	2.48
Facial (FAVI)	50.16	1.36	51.07	2.38	50.16	1.36	49.31	2.18
Optic (OPVI)	22.55	0.81	21.05	1.34	20.68	0.92	20.24	0.85
Respiratory (REVI)	10.64	0.84	10.62	0.12	9.90	0.64	9.69	0.18
Masticatory (MAVI)	21.75	0.21	19.39	1.09	19.58	0.20	19.38	1.33
Morphometric indices								
Neurofacial (NFMI)	1.36	0.01	1.41	0.02	1.41	0.01	1.39	0.05
Anteroneural (ANMI)	27.5	0.26	26.82	0.22	27.50	0.64	27.55	0.97
Midneural (MNMI)	37.05	0.30	37.37	0.49	37.41	0.11	37.78	0.94
Posteroneural (PNMI)	35.45	0.23	35.81	0.44	35.09	0.53	34.67	1.54
Optic (OPMI)	41.04	0.89	41.20	0.94	41.21	0.71	41.05	0.82
Respiratory (REMI)	19.36	1.30	20.83	0.84	19.72	0.73	19.68	0.72
Masticatory (MAMI)	39.6	0.97	37.97	0.86	39.06	1.44	39.27	1.04

Table 4. Intergroup comparisons for body and cranial measurement and indices

Indices	J-T observed	J-T typified	J-T exact significance
Soft tissues			
Body weight (BOWH)	8.000	–2.645	0.004**
Brain weight (BRWH)	21.500	–1.718	0.247
Brain coefficient (BRCO)	43.000	2.359	0.010**
Volumetric indices			
Neurocranial (NEVI)	9.000	–2.502	0.006**
Anteroneural (ANVI)	8.000	–2.645	0.004**
Midneural (MNVI)	12.000	–2.073	0.021*
Posteroneural (PNVI)	14.000	–1.787	0.043*
Facial (FAVI)	8.000	–2.645	0.004**
Optic (OPVI)	11.000	–2.216	0.015*
Respiratory (REVI)	13.000	–1.930	0.031*
Masticatory (MAVI)	14.000	–1.787	0.043*
Morphometric indices			
Neurofacial (NFMI)	35.000	1.215	0.130
Anteroneural (ANMI)	24.000	–1.357	0.390
Midneural (MNMI)	37.000	1.501	0.078
Posteroneural (PNMI)	26.000	–1.071	0.500
Optic (OPMI)	26.000	–1.071	0.500
Respiratory (REMI)	28.000	0.214	0.445
Masticatory (MAMI)	25.000	–1.214	0.445

$0.01 < * p < 0.05$; ** $p < 0.01$. J-T = Jonckheere-Terpstra exact test for sex and treatment.

Discussion

Since in the squirrel monkey undernutrition retarded an increase in the body weight – though not the brain weight – the ratio expressed by the brain coefficient was largely greater in undernourished animals than in control ones. Although size decrements were seen in all the components, shape changes were not evident at a craniofacial level. The neurocranial reduction observed could not be the consequence of the softening of the exocranial insertion surfaces in the stressed animals – due to the head muscle mass reduction – since measurements were taken on the inner table of the bones. Consequently, an intrinsical brain modification is suggested: if the brain does not reduce its weight, but the neurocranial cavity reduces its volume, then this could be explained by an increment in the brain density caused by the stress.

Even though sex dimorphism was significant, taking into account that the RDM values of the volumetric indices were always greater in males than in females (fig. 1, 2), it was not evident either in the cranial morphometric indices or in the brain/body ratio. The delayed growth in size of all the functional components can be explained by allometry, considering that undernutrition mainly affects body weight and height. This effect was seen in both sexes, but females were quantitatively less affected than males. According to our results, sex dimorphism in the undernourished monkeys was less evident than in controls. Similar results were found in rats [Pucciarelli, 1980, 1981] and men [Pucciarelli et al., 1993]. This fact may be explained by the 'better canalization hypothesis' postulated by Tanner [1962], by which females resist better than males leaving their normal growth canals, although the nutritional environment is the same for both sexes.

Conditions of malnutrition are the cause of an alteration of growth despite an increase in circulating levels of GH. Growth includes the actions of somatomedins and IGF-I with anabolic effects on cartilage, fat and muscle. In malnutrition, mechanisms of growth impairment appear to show a decrease in GH-induced generation of somatomedins and a rise in GH secretion due to a decreased negative feedback from somatomedins [Phillips, 1986].

Polkowska et al. [1996] found that the chronic restriction of dietary proteins (8% for 20 weeks) in female lambs markedly decreased the content of hypothalamic somatostatin. Moreover, they detected an increased GH concentration in the peripheral blood with significant elevation of the pulse amplitude. In the pituitary gland, a marked increase of the somatotrophs was observed. These immunocytochemical findings are in disagreement with our results, but the period of malnutrition is very different from that in our study. It is probable that the long-term effects of a low-protein diet reflect a downregulation of the neuroendocrine axis. Hara et al. [1998], feeding rats a low protein diet (8% for 30 days), showed in a quantitative morphological analysis that the average sectional areas of both PRL-GH-secreting cells were smaller in size than those in controls. This diet diminished the cell numbers in the subpopulations of PRL mRNA-positive cells and somatotrophs.

In our material, the quantitative immunohistochemical parameters (VD and CD) revealed a significant decrease of the GH and PRL cell populations in malnourished monkeys, showing dimorphism according to sex. These observations correlated well with alterations described in the somatotroph cell population for malnourished rats [Shimokawa et al., 1996] and with those reported by Medvedev et al. [1995] on the morphometric analysis of the somatotrophs of mice submitted to protein energetic insufficiency (5% for 20 days). They detected a marked decrease in the cell number and volume of the cytoplasm.

Herbert et al. [1980, 1993] have shown the effects of protein-calorie malnutrition on the morphology of different populations of the pituitary gland. Male rats were fed a low protein diet (8%) from 20 to 50 days of age. The overall number of PRL cells appeared to be unaffected by the deficiency in dietary protein. According to our results, the number of somatotrophs, along with the cell areas, was significantly reduced as a result of the low protein diet [Herbert et al., 1993].

Furthermore, biochemical data have led to the detection of reduced GH levels in malnourished rats [Nitzan and Wilber, 1974], in relation to the lower serum activity of somatomedins [Price et al., 1979; Phillips, 1986]. Arce and Perdomo [1985] studied pituitary content, synthesis and secretion of PRL and GH after incubation of rat pituitaries in a solution containing labeled amino acids. Malnutrition resulted in decreased content and secretion of GH, and only reduced content and synthesis, but not secretion, of PRL were observed.

In malnourished rats, we have previously reported ultrastructural evidence of degranulation in GH cells [Gómez Dumm et al., 1982, 1987]. Medvedev et al. [1995] detected by electron microscopy a diminution of the medium diameter of the secretory granules of somatotrophs of mice on a low protein diet. From an ultrastructural point of view, Herbert [1980] described in undernourished male rats, a less extensive granular endoplas-

mic reticulum and Golgi complex. The number of secretory granules was not different; however, their size was markedly smaller and their shape was round to ovoid rather than pleomorphic. Our ultrastructural changes in GH and PRL cells from undernourished monkeys are suggestive of an attempt to release a higher amount of secretory material within the frame of a compensatory hyperstimulation model.

Our results cannot be extrapolated to those obtained during a short-term food reduction period. The diminished activity of the target organ during short-term starvation is associated with a lowered hypothalamic and pituitary synthesis and secretion. However, little is known about prolonged malnutrition (10% for 2 years) in monkeys.

In summary, the immunohistochemical and ultrastructural findings in GH and PRL populations of malnourished monkeys are related to the craniofacial morphometric patterns reported elsewhere [Pucciarelli et al., 1990; Corner and Richtsmeier, 1992; Oyhenart and Pucciarelli, 1992; Pucciarelli and Dressino, 1996; Dressino and Pucciarelli, 1997].

Finally, we can conclude the following. (1) In squirrel monkeys, undernutrition due to a low protein diet produces a decrease in body and cranial sizes, but not in the brain weight. This suggests an increment of brain density. (2) The undernutrition also evoked a decrease in the pituitary GH and PRL cell mass due to a diminution of the cell number together with evidence of compensatory cellular hyperfunction. (3) A straight cause-effect relationship between neurocranial, cerebral and pituitary chances cannot be established although a general process by which different brain components evolve together would be evident.

Acknowledgements

The authors are grateful to Ms Celia Ferese, Ms Gabriela Simonetto, Ms Bibiana Orden and Ms María C. Muñe for technical assistance. This work was partially supported by grants from Comisión de Investigaciones Científicas de la Provincia de Buenos Aires (CICBA), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), and Universidad Nacional de La Plata (UNLP). G.M. Cónsole and C.L.A. Gómez Dumm are Members of the Researcher's Career, CICBA, Argentina. E.E. Oyhenart and H.M. Pucciarelli are Members of the Researcher's Career, CONICET, Argentina.

References

Arce, J.A., M.Z. Perdomo (1985) Synthesis and secretion of prolactin and growth hormone in rats with protein-calorie malnutrition and effect of administration of cortisol. Probl Endocrinol (Mosk) *31/1:* 55–57.

Brogan, R.S., S.K. Fife, L.K. Conley, A. Giustina, W.B. Wehrenberg (1997) Effects of food deprivation on the GH axis: Immunocytochemical and molecular analysis. Neuroendocrinology *65/2:* 129–135.

Cheek, D.B., D.E. Hill (1974) Effect of growth hormone on cell and somatic growth; in Greep R.O., E.B. Astwood (eds): Handbook of Physiology (Endocrinology). Baltimore, Waverley, vol 4, pp 159–183.

Corner, B.D., J.T. Richtsmeier (1992) Cranial growth in the squirrel monkey *(Saimiri sciureus):* A quantitative analysis using three-dimensional coordinate data. Am J Phys Anthropol *87:* 67–82.

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.

Gómez Dumm, C.L.A., H.M. Pucciarelli, V. Dressino (1987) Quantitative ultrastructural study of somatotropic cells in malnourished weanling rats. Acta Anat *129:* 200–202.

Gómez Dumm, C.L.A., H.M. Pucciarelli, M.C. Terreros (1982) Effects of a low-protein diet on the ultrastructure of somatotroph cells in the rat. Com Biol *1/2:* 165–170.

Hara, M., D.C. Herbert, T. Taniguchi, A. Hattori, R. Ohtani-Kaneko, M. Iigo, Y. Kato, K. Hirata (1998) Effects of a low-protein diet on prolactin- and growth hormone-producing cells in the rat pituitary gland. Anat Rec *251/1:* 37–43.

Herbert, D.C. (1980) Morphology of the mammotrophs and gonadotrophs in the anterior pituitary gland of rats with protein-calorie malnutrition. Am J Anat *158:* 521–531.

Herbert, D.C., T. Yashiro, T. Muraki, T. Okano, A. Hattori, T. Suzuki (1993) Quantitative morphological analysis of the pituitary gland in protein-calorie malnourished rats. Anat Rec *235/1:* 121–125.

Lee Vance, M., M.L. Hartman, M.O. Thorner (1992) Growth hormone and nutrition. Horm Res *38(suppl 1):* 85–88.

Medvedev, D.I., I.I. Babichenko, O.B. Savrova, I.Z. Eremia, A.I. Kravtsova (1995) The morphological state of the adenohypophyseal somatotrophs during body development under conditions of protein-energy insufficiency. Aviakosm Ekolog Med *29/3:* 28–31.

Mehta, C.R., N.R. Patel (1996) SPSS Exact Tests 7.0 for Windows. Illinois, SPSS Inc.

Melemd, S., S. Yamashita, H. Yamasaki, J. Fagin, H. Namba, H. Yamamoto, M. Weber, S. Morita, J. Webster, D. Prager (1996) IGF-I receptor signalling: Lessons from the somatotroph. Recent Prog Horm Res *51:* 189–215.

Nitzan, M., I.F. Wilber (1974) Effect of postnatal malnutrition on plasma proteins and growth hormone in the rat. Horm Res *5:* 167–172.

Oyhenart, E.E., H.M. Pucciarelli (1992) Sexual cranial dimorphism in malnourished rats treated with growth hormone. Growth Dev Aging *56:* 179–184.

Phillips, L.S. (1986) Nutrition, somatomedins, and the brain. Metabolism *35/1:* 78–87.

Pimstone, B.L. (1976) Endocrine function in protein calorie malnutrition. Clin Endocrinol *5:* 79–95.

Polkowska, J., P. Krejci, M. Snochowski (1996) The long-term effect of low protein diet on the somatostatin hypothalamic neuronal system and the pituitary growth hormone cells in growing ewe. Exp Clin Endocrinol Diabetes *104/1:* 59–66.

Price, D.A., J.M. Wit, S. Van Buul-Offers, A.M. Korteland-van Male, A.K.M. van Rooyen-Wehrneijer, C. Hoogerbrugge, J.L. Van den Brande (1979) Serum somatomedin activity and cartilage metabolism in acutely fasted, chronically malnourished, and refed rats. Endocrinology *105:* 851–861.

Pucciarelli, H.M. (1980) The effects of race, sex, and nutrition on craniofacial differentiation in rats. A multivariate analysis. Am J Phys Anthropol *53:* 359–368.

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

Pucciarelli, H.M., F.R. Carnese, L.V. Pinotti, L.M. Guimarey, A.S. Goicoechea (1993) Sexual dimorphism in schoolchildren of the Villa IAPI neighborhood (Quilmes, Buenos Aires, Argentina). Am J Phys Anthropol *92:* 165–172.

Pucciarelli, H.M., V. Dressino (1996) Orthocephalization in the postweaning squirrel monkey. Am J Phys Anthropol *101:* 173–181.

Pucciarelli, H.M., V. Dressino, M.H. Niveiro (1990) Changes in skull components of the squirrel monkey evoked by growth and nutrition: An experimental study. Am J Phys Anthropol *81:* 535–544.

Shimokawa, I., B.P. Yu, Y. Higami, T. Ikeda (1996) Morphometric analysis of somatotrophs: Effects of age and dietary restriction. Neurobiol Aging *17/1:* 79–86.

Snyder, D.K., D.R. Clemmons, L.E. Underwood (1988) Treatment of obese, diet-restricted subjects with growth hormone for 11 weeks: Effects on anabolism, lipolysis and body composition. J Clin Endocrinol Metab *67:* 54–61.

Tanner, J.M. (1962) Growth at Adolescence. Oxford, Blackwell.

Vuagnat, B.A., D.D. Pierroz, M. Lalaoui, P. Englaro, F.P. Pralong, W.F. Blum, M.L. Aubert (1998) Evidence for a leptin-neuropeptide Y axis for the regulation of growth hormone secretion in the rat. Neuroendocrinology *67/5:* 291–300.