

Nutritional status of preschool Senegalese children: long-term effects of early severe malnutrition

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The prevalence of malnutrition remains high in many developing countries. However, data relating to the long-term effects of severe malnutrition, specifically, serum levels of biochemical indicators of nutritional status, are still scarce in the literature. Hence the present study aimed to investigate the nutritional, biological and growth status of Senegalese preschool children previously hospitalised for severe malnutrition. The study involved twenty-four 7-year-old children who had suffered from marasmus 5 years earlier, twenty-four siblings living in the same household, and nineteen age-matched children living in the centre of Dakar. The siblings were of similar age to the post-marasmic children. Anthropometry, serum biochemical indicators of nutritional status, growth factors, and haematological and mineral parameters were measured. The prevalence of stunting and wasting was the same in the post-marasmic children as in the siblings. Body-fat and fat-free-mass (FFM) deficits in both groups were corroborated by abnormally low concentrations of transthyretin, osteocalcin, insulin-like growth factor (IGF)-1, and insulin-like growth factor-binding protein (IGFBP)-3. FFM was positively and significantly correlated with concentrations of IGF-1 and IGFBP-3. In the post-marasmic children, height for age was also correlated with IGF-1. Of the post-marasmic children, 53 % had Fe-deficiency anaemia, as did 35 % of the siblings and 29 % of the controls. No significant associations were found between the serum concentrations of Ca, Cu, K, Mg, Na, P, Se, Zn and growth retardation. At 5 years after nutritional rehabilitation, the post-marasmic children remained stunted with nutritional indices significantly lower than the control children. However, these children were doing as well as their siblings except for minor infections.

Stunting: Biochemical markers: Mineral status: Anaemia: West Africa

Severe protein–energy malnutrition in early childhood leads to linear growth retardation (stunting), a state that is associated with increased morbidity and mortality as well as a reduced physical work capacity in adulthood (Waterlow, 1994). It has been suggested that the poor linear growth is a result of inadequate intake of protein, vitamins and minerals (Golden & Golden, 1991; Prentice & Bates, 1994). However, many dietary intervention studies conducted in developing countries resulted only in weight gain but had little or no effect on height (Allen, 1994). Martorell *et al.* (1994) reported that children who continue to live in the same environment in which they became stunted experience little or no catch-up in growth later in life. On the other hand, there are children

in whom catch-up occurred in some environments. As reported by Golden (1994), successful reversal is observed but requires removal of the retarding factors of the environment. Nevertheless, early malnutrition has subsequent consequences on mortality (Hennart *et al.* 1987), motor performance and coordination (Bénéfice *et al.* 1999), cognitive function, and time of menarche (Grantham-McGregor, 1984; Galler *et al.* 1987*a,b*). In Senegal, Simondon *et al.* (1998) have shown that preschool stunted children have significant delays in sexual maturation suggesting the possibility of catch-up growth later in adolescence. Although one would assume or expect that dietary intervention corrects indicators of nutritional status, the long-term effects of severe malnutrition on the biological indices related

Abbreviations: Apo A1, apolipoprotein A1; Apo B, apolipoprotein B; FFM, fat-free mass; ht/age, height for age; IGF, insulin-like growth factor; IGFBP, insulin-like growth factor-binding protein; MUAC, mid-upper arm circumference; wt/age, weight for age; wt/ht, weight for height.

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† In memoriam.

to the growth and nutritional status of children previously hospitalised for severe protein–energy malnutrition are still scarce in the literature. Therefore, the present study was designed to evaluate the nutritional status of Senegalese preschool-age children who were previously hospitalised for the treatment of severe malnutrition. Various indicators of nutritional status including anthropometry and blood concentrations of biochemical and haematological markers, trace elements, minerals, and growth factors were measured. Previously malnourished children were compared with the following two groups of controls: siblings living in the same household; age-matched preschool children living in Dakar (the capital of Senegal).

Subjects and methods

Subjects

Previously hospitalised children and their siblings were recruited in Pikine, a suburb of North Dakar. Most residents of this suburb are from the low socio-economic class. In a preliminary survey, it was shown that only 16.2% of Pikine residents were wage earners compared with 29.4% of those from the inner city of Dakar (Sarr, 1996). Age-matched children were selected from families living in the centre of Dakar who had better socio-economic conditions.

Inclusion criteria of the study

The first group was a post-marasmic group comprising children who had suffered from protein–energy malnutrition and who had been treated at the Unité de Récupération Nutritionnelle, Hôpital Roi Baudouin, in Pikine at least 5 years before the study; the children were residents of Pikine and between 6 to 8 years of age. Twenty-four children were identified and studied.

The second group was a sibling group. The sibling closest in age to each post-marasmic child living in the same household since birth, and with no history of being hospitalised for malnutrition, was selected. Twenty-four children of 7 to 8.5 years of age were identified.

The third group was an age-matched group. Healthy children matched for age to the post-marasmic children and living in the centre of Dakar from a higher socio-economic area served as the control subjects. Nineteen children were included.

The study was approved by the ethics committee of Dakar University and Hôpital Roi Baudouin and was conducted according to the guidelines set out in the Helsinki declaration. The protocol was explained in detail to each parent before his or her child was enrolled in the study and his or her informed consent was obtained after clarifying that their refusal would have no bearing on his or her child's care.

Clinical examination and anthropometry

One physician (C. S. S.) examined all children. Following the complete physical examination, a medical file was established for each child. The child's wrist radiography

was performed in the Radiology Department of the Centre Hospitalier Universitaire de Fann. Weight, height, and age were recorded. Anthropometric indices were calculated using the National Center for Health Statistics/WHO reference values (Organisation Mondiale de la Santé, 1983), and expressed in SD units (Z scores). The anthropometric indices were weight for height (wt/ht) Z score, height for age (ht/age) Z score, and weight for age (wt/age) Z score. Body weights were measured to the nearest 0.02 kg on a mechanical scale (CPIP, Paris, France). Heights were measured using a fixed measuring stick to the nearest 1 mm. Mid-upper arm circumference (MUAC) and skinfold thicknesses were measured by the same investigator. For MUAC, each subject stood with his or her left arm flexed at 90° and arm circumference was measured midway between the acromion and the olecranon with the tape in contact with the skin. A reading was taken to the nearest 0.05 mm. Skinfold thicknesses were determined on the left side of the body according to Durnin & Rahman (1967) to the nearest 0.2 mm using a Holtain skinfold calliper (Holtain Ltd, Crymich, UK). To minimise the intra-operator variability, the average of three consecutive measurements was recorded.

Body composition was assessed by calculating BMI for age (BMI Z score) using the National Center for Health Statistics/WHO reference values (Organisation Mondiale de la Santé, 1983), and the body density was estimated from the average log sum of the four skinfold thicknesses (biceps, triceps, supra-iliac and sub-scapular) using Brook's equations for children under 12 years (Brook, 1971). Body fat was calculated from the body density according to Siri (1956).

Blood sampling and analytical methods

Blood samples (5 ml) were drawn from overnight-fasted children; 2 ml were put into plain tubes, 1 ml into heparin tubes, and 2 ml into EDTA tubes. The samples were transported on ice to the laboratory within 4 h. After the haematological indices were assayed in EDTA-containing blood samples, the remaining blood and heparin blood samples were immediately centrifuged at 1735 g (4000 rpm, Sigma 3K20; B. Braun, Laboratory Centrifuges GmbH, 3360 Osterode/Harz, Germany) for 5 min at 4°C. The plasma was sampled and stored at –20°C. The plain tube samples were allowed to clot overnight at 4°C before centrifugation at 1735 g (4000 rpm) for 20 min at 4°C. Serum was sampled and stored at –20°C until analysis. Serum was used for the albumin, transthyretin, acute-phase protein, growth factor, ferritin, and mineral determinations. The heparin-containing blood samples were used for plasma folate analysis and the EDTA blood samples for plasma apolipoprotein determinations.

Protein determinations

The antibodies, protein standards, control serum, and the buffers used for the transthyretin and inflammatory markers, and the reagents for apolipoprotein determinations were purchased from Dako Diagnostics (Dako A/S, Glostrup, Denmark). The reagents for albumin analyses were purchased from Randox (Randox Laboratories Ltd,

Crumlin, UK). Serum insulin-like growth factor (IGF)-1 was determined by immunoradiometric assay (IGF-1 IRMA kit; Nichols Institute Diagnostics, San Juan Capistrano, CA, USA); serum insulin-like growth factor-binding protein (IGFBP)-3 was quantified using the IGFBP-3 RIA kit (Nichols Institute Diagnostics, San Juan Capistrano, CA, USA); serum osteocalcin was quantified using the RIA ELSA-OSTEO kit (Cis Bio International, Gif-sur-Yvette, France).

The concentrations of transthyretin, α_1 -acid glycoprotein, and C-reactive protein were quantified by radial immunodiffusion (Mancini *et al.* 1965). Serum albumin was measured by a colorimetric method using bromocresol green as the chromogen (Doumas *et al.* 1971), and plasma apolipoprotein A1 (Apo A1) and apolipoprotein B (Apo B) were determined by an immunoturbidimetry assay. Both determinations were done with a Cobas Fara Autoanalyser (Hoffman LaRoche, Basel, Switzerland).

Iron and folate determinations

Haemoglobin, packed cell volume and erythrocyte indices were determined with a Coulter Counter (T-660 Coultronics, Margency, France). Erythrocyte protoporphyrin was measured with a Protofluor-Z haematofluorometer (Helena-France Labs, Saint-Leu-la-Forêt, France) and serum ferritin was determined by radioimmunoassay (Behring nephelometer, Behring, Frankfurt, Germany). Erythrocyte folate was determined by the *Lactobacillus casei* method (Christides & Potier de Courcy, 1987).

Mineral determination

Serum Na, Ca, K and P were measured by colorimetric assay with a Hitachi 737 autoanalyzer (Boehringer-Mannheim, Mannheim, Germany) and serum Mg, Cu, and Zn by flame atomic absorption spectrophotometry (model 2380; Perkin Elmer, Norwalk, CT, USA). Serum Se was determined using an electrothermal atomic absorption spectrophotometer equipped with Zeeman background correction (model 5100; Perkin Elmer, Norwalk, CT, USA).

Reference values

Children were classified as stunted when their ht/age Z scores were below -2 , and wasted when their wt/ht Z or BMI Z scores were below -2 . With regard to biochemical parameters, undernutrition was diagnosed when the concentrations of any of the plasma proteins were below the following cut-off points: transthyretin < 160 mg/l; albumin < 35 g/l (Haider & Haider, 1984); Apo A1 < 1 g/l; Apo B < 0.7 g/l (Lentner, 1984). Inflammation and/or infection states were defined by α_1 -acid glycoprotein concentration > 1.20 g/l and/or detectable C-reactive protein > 5 mg/l (Engler, 1984). Anaemia was diagnosed when haemoglobin concentration was < 110 g/l (Organisation Mondiale de la Santé, 1975); erythrocyte folate deficiency was diagnosed when blood concentration was < 160 μ g/l (Herbert, 1987). The prevalence of Fe-deficiency anaemia was defined by the presence of at least two abnormal indicators: serum ferritin < 12 μ g/l; erythrocyte protoporphyrin

> 3 μ g/g; haemoglobin and/or mean corpuscular volume < 75 fl (Looker *et al.* 1991). Mean serum IGFBP-3 values in children aged 6–8 years, according to Nichols Institute Diagnostics, are 2.11 (95% CI 1.32, 3.38) mg/l for males and 2.37 (95% CI 1.21, 4.66) mg/l for females. According to Merimee *et al.* (1987), mean IGF-1 values in healthy children aged between 7 and 8 years are approximately 120 μ g/l.

Statistical analysis

The results are expressed as mean values and standard deviations. Paired *t* tests and ANOVA were performed with the use of Systat 8.0 (SPSS Inc., Chicago, IL, USA). Tukey's *post hoc* comparison test was used to identify group differences and proportions were compared by chi-square (χ^2) tests. The relationships between the anthropometric and biochemical indicators of nutritional status were calculated by Pearson's linear correlation test. The level of significance was set at $P < 0.05$.

Results

Health status and anthropometry

The post-marasmic children and their siblings were breastfed for 18 months and the age-matched controls for 21 months. Complementary food was introduced at 6, 5 and 3 months of age for the post-marasmic, siblings, and control children respectively.

Anthropometric data of the post-marasmic children at the time they were hospitalised in the Unité de Récupération Nutritionnelle are shown in Table 1. The mean wt/ht Z score increased from -3.0 (SD 0.6) at admission to -1.4 (SD 0.7) at discharge (t 15.0; $P < 0.001$). The wt/age Z score also increased (-3.3 (SD 0.7) to -2.2 (SD 0.5), t 8.9; $P < 0.001$), from admission to discharge. The data show that the wt/age and the ht/age Z scores also increased significantly (t 3.0; $P = 0.006$ and t 2.6; $P = 0.015$ respectively) since discharge, but the mean wt/ht Z value was not different from the discharge mean value (t 0.2; $P = 0.87$).

Physical examination of the age-matched children found them to be healthy and free of acute or chronic diseases, although 10% (n 2) did present signs of cutaneous infections. In contrast, all post-marasmic children (n 24) and 75% of their siblings (n 18) showed at least one symptom of infection and the difference between these two groups was significant (Fisher's exact test, $P = 0.02$; post-marasmic children *v.* siblings for any symptom). The main symptoms were cutaneous infections (n 4 and n 6 respectively), conjunctivitis (n 8 and n 3 respectively), signs of respiratory diseases such as rhinitis or asthma (n 4 and n 2 respectively), and signs of gastrointestinal infection such as abdominal pain, intermittent diarrhoea or anorexia (n 8 and n 7 respectively).

Information on immunisation was collected from the children's health-record charts. There were significant differences in immunisation rates between the three groups (χ^2 11.2; $P = 0.004$); 67% of the post-marasmic children and 63% of their siblings were not immunised

Table 1. Anthropometric indices of the post-marasmic children (*n* 24) when hospitalised 5 years previously* (Mean values and standard deviations)

	Age (months)		Wt/age Z score†		Ht/age Z score‡		Wt/ht Z score§	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Admission	20	6	-3.3	0.7	-1.8	0.9	-3.0	0.6
Discharge	20	6	-2.2	0.5	-1.8	1.0	-1.4	0.4
Current	86	12	-1.8	0.7	-1.3	0.9	-1.4	0.7

Wt/age, weight for age; Ht/age, height for age; Wt/height, weight for height.

* The mean duration of hospitalisation was 13.3 d with a mean weight gain of 15.1 g/kg per d.

† The mean Z score at admission was significantly lower than that at discharge (*t* 8.9; *P*<0.001); the mean Z score at discharge was significantly lower than the current mean Z score (*t* 3.0; *P*=0.006) (paired *t* tests).

‡ The mean Z score at admission was not significantly lower than that at discharge (*t* 0.5; *P*=0.65); the mean Z score at discharge was significantly lower than the current mean Z score (*t* 2.6; *P*=0.02) (paired *t* tests).

§ The mean Z score at admission was significantly lower than that at discharge (*t* 15.0; *P*<0.001); the mean Z score at discharge was not significantly lower than the current mean Z score (*t* 0.2; *P*=0.87) (paired *t* tests).

while 82% of the age-matched children had been immunised against all usual antigens (BCG (tuberculosis), measles, yellow fever, three boosters of diphtheria, tetanus, pertussis, and poliomyelitis). No difference was found between the post-marasmic children and their siblings.

The data on anthropometric measurements are summarised in Table 2. The mean anthropometric measurements of the control children were significantly higher than those of the post-marasmic and siblings groups (*P*<0.001). However, wt/ht and BMI Z scores were not different between the controls and siblings. None of the age-matched control children were stunted or wasted. Stunting was observed in 29% (*n* 7) of the post-marasmic children and in 17% (*n* 4) of their siblings and wasting was observed in 17% (*n* 4) and 4% (*n* 1) of these groups, respectively. The differences in stunting and wasting prevalence between these two groups were not significant (χ^2 1.06, *P*=0.30 for stunting; Fisher's exact test, *P*=0.35 for wasting).

The post-marasmic children and their siblings had significantly lower body composition indices (MUAC, kg body fat, fat-free mass (FFM)) than the age-matched control children (*P*<0.001). Percentage body fat and, hence, percentage FFM was similar in the siblings and control children but different in the post-marasmic and control children (*P*<0.001) (Table 3). However, for all these indices, no differences were found between the post-marasmic children and their siblings.

Radiography measurements of the wrist were performed for twelve, fourteen, and ten children from the post-marasmic children, siblings and age-matched controls, respectively. Mean bone age and chronological age were not different between the groups but mean bone age was low compared with chronological age in all groups (Table 4). None of the children had significantly abnormal bone-age values except for one in the post-marasmic group who showed a late appearance in the middle ulna. Also one sibling exhibited an irregularity of the metaphyses of the ulna and radius.

Biochemical parameters

Biochemical parameters of the nutritional status of the three study groups are summarised in Table 5. Although the three groups had mean serum albumin concentrations within the normal range, their transthyretin concentrations were below normal. No differences were found between the groups for either protein. Mean concentrations of acute-phase proteins (α_1 -acid glycoprotein and C-reactive protein) were within the normal range and, in fact, C-reactive protein was undetectable in all children. Of the age-matched controls, 5.5% (*n* 1) compared with 17% for siblings (*n* 4) and 17% (*n* 4) for the post-marasmic children had abnormal α_1 -acid glycoprotein values (≥ 1.20 g/l).

Table 2. Anthropometric indices for the post-marasmic children (*n* 24), their siblings (*n* 24) and control children (*n* 19) at the time of the study† (Mean values and standard deviations)

	Post-marasmic		Siblings		Age-matched controls	
	Mean	SD	Mean	SD	Mean	SD
Age (months)	85.6	11.5	83.2	17.3	92.7	8.9
Weight (kg)	17.8*	2.6	19.2*	3.1	25.4	3.0
Height (m)	1.148*	0.77	1.156*	0.97	1.286	0.44
Wt/age Z score	-1.8*	0.7	-1.3*	1.0	+0.2	0.6
Ht/age Z score	-1.3*	0.9	-1.0*	1.2	+0.6	0.5
Wt/ht Z score	-1.4*	0.7	-1.0	0.8	-0.4	0.7
BMI Z score	-1.5*	0.9	-1.0	1.1	-0.2	0.9

Wt/age, weight for age; Ht/age, height for age; Wt/ht, weight for height.

* Mean values were significantly different from those of the age-matched controls (ANOVA, Tukey *post hoc* test) (*P*<0.0001).

† For details of subjects and procedures, see p. 1124.

Table 3. Mid-upper arm circumference (MUAC) and body composition for the post-marasmic children (*n* 24), their siblings (*n* 24) and control children (*n* 19) at the time of the study†
(Mean values and standard deviations)

	Post-marasmic		Siblings		Age-matched controls	
	Mean	SD	Mean	SD	Mean	SD
MUAC (mm)	157*	9	164*	8	189	14
Weight (kg)	17.8*	2.6	19.2*	3.1	25.4	3.0
Body fat (kg)	1.8*	0.6	2.3*	0.6	3.6	1.4
Body fat (%)	9.8*	3.2	12.1	3.4	14.1	3.6
Fat-free mass (kg)	16.1*	2.3	16.9*	3.0	21.7	2.0
Fat-free mass (%)	90.2*	3.2	88.0	3.4	86.0	3.6

* Mean values were significantly different from those of the age-matched controls (ANOVA, Tukey *post hoc* test) ($P < 0.001$).

† For details of subjects and procedures, see p. 1124.

The difference was not significant between the three groups.

While plasma Apo A1 concentrations were significantly higher in the age-matched controls than in the two other groups ($P < 0.001$), those of Apo B concentrations were not different between the three groups. The post-marasmic children and their siblings did not have different concentrations of serum osteocalcin, IGF-1 or IGFBP-3, but both groups had significantly lower mean concentrations (except for serum osteocalcin) compared with the age-matched controls ($P < 0.001$ for IGF-1; $P < 0.01$ for IGFBP-3).

Correlations between ht/age *Z* score, FFM and MUAC with the different biochemical variables, IGF-1, IGFBP-3, and osteocalcin, were analysed to determine differences

in the relationship between these variables among the three groups. The results showed that only the post-marasmic children had a significant correlation between ht/age *Z* score and IGF-1 (r 0.464; $P = 0.026$). The post-marasmic children and their siblings had significant correlations between FFM and IGF-1 (r 0.503; $P = 0.014$ and r 0.476; $P = 0.019$ respectively). Finally, the only two groups to have significant correlations between FFM and IGFBP-3 were the siblings and control children (r 0.616; $P = 0.002$ and r 0.473; $P = 0.047$ respectively).

In Table 6, all the children were grouped according to whether they were stunted (ht/age *Z* < -2; *n* 11), marginally stunted ($-2 \leq$ ht/age *Z* \leq -1; *n* 16) or non-stunted (ht/age *Z* > -1; *n* 40). There were significant differences among the groups in wt/age *Z* score ($P < 0.001$), but not

Table 4. Bone and chronological age of the post-marasmic children (*n* 12), their siblings (*n* 14) and control children (*n* 10) at the time of the study*
(Mean values and standard deviations)

	Post-marasmic		Siblings		Age-matched controls	
	Mean	SD	Mean	SD	Mean	SD
Bone age (months)	45.2	11.6	56.1	21.0	62.0	16.0
Chronological age (months)	79.6	6.0	82.0	16.6	83.6	5.9

* For details of subjects and procedures, see p. 1124. There were no significant differences between the groups.

Table 5. Biochemical markers of nutritional and inflammatory status for the post-marasmic children (*n* 24), their siblings (*n* 24) and control children (*n* 18) at the time of the study†
(Mean values and standard deviations)

	Post-marasmic		Siblings		Age-matched controls	
	Mean	SD	Mean	SD	Mean	SD
Albumin (g/l)	45.0	1.9	43.8	2.5	45.4	1.7
Transferrin (mg/l)	124.4	23.8	120.7	26.6	139.9	38.3
Apolipoprotein A1 (g/l)	1.01*	0.19	0.96***	0.12	1.15	0.17
Apolipoprotein B (g/l)	0.6	0.2	0.7	0.2	0.7	0.1
Osteocalcin (μ g/l)	50.9	14.8	52.5	17.2	63.9	23.6
IGF-1 (μ g/l)	70.9***	42.0	78.5***	47.8	129.9	39.3
IGFBP-3 (mg/l)	1.7**	0.6	1.8*	0.8	2.4	0.5
α_1 -Acid glycoprotein (g/l)	0.8	0.3	0.9	0.4	0.8	0.3
C-reactive protein (mg/l)		nd		nd		nd

IGF-1, insulin-like growth factor-1; IGFBP-3, insulin-like growth factor-binding protein-3; nd, not detectable for values < 5 mg/l.

Mean values were significantly different from those of the age-matched controls * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ (ANOVA, Tukey *post hoc* test).

† For details of subjects and procedures, see p. 1124.

Table 6. Anthropometric and growth status of the stunted (*n* 11), marginally stunted (*n* 16) and non-stunted (*n* 40) children‡
(Mean values and standard deviations)

	Stunted (Ht/age <i>Z</i> < -2)		Marginally stunted (-2 ≤ Ht/age <i>Z</i> ≤ -1)		Non-stunted (Ht/age <i>Z</i> > -1)	
	Mean	SD	Mean	SD	Mean	SD
Wt/age <i>Z</i> score	-2.5***†	0.7	-1.7***	0.4	-0.4	0.8
Wt/ht <i>Z</i> score	-1.1	1.1	-1.2	0.6	-0.8	0.8
BMI <i>Z</i> score	-1.3	1.8	-1.4	0.7	-0.7	0.9
MUAC (mm)	155***	12	160***	7	176	16
Body fat (kg)	1.8*	0.7	1.9*	0.7	2.9	1.3
Fat-free mass (kg)	13.8***	2.0	16.2***	2.2	19.8	2.7
Osteocalcin (μg/l)	43.0*	12.5	54.9	20.2	58.6	19.0
IGF-1 (μg/l)	53.3**	37.5	83.3	51.0	103.1	47.4
IGFBP-3 (mg/l)	1.5*	0.5	1.9	0.8	2.1	0.6

Ht/age, height for age; Wt/age, weight for age; Wt/ht, weight for height; MUAC, mid-upper arm circumference; IGF-1, insulin-like growth factor-1; IGFBP-3, insulin-like growth factor-binding protein-3.

Mean values were significantly different to those of the non-stunted children (ANOVA, Tukey *post hoc* test): **P*<0.05, ***P*<0.01, ****P*<0.001.

† Mean value was significantly different from that of the marginally stunted children (ANOVA, Tukey *post hoc* test) (*P*<0.05).

‡ For details of subjects and procedures, see p. 1124.

in wt/ht or BMI *Z* scores. No differences were observed in body fat, MUAC, or FFM between the severely and marginally stunted children. However, both groups of the stunted children had significantly lower FFM, MUAC and body fat than the non-stunted children (*P*<0.001 to 0.05). The severely stunted children also had significantly lower serum osteocalcin, IGF-1 and IGFBP-3 than the non-stunted children.

Haematological status

No significant differences were observed among the three study groups in mean haematological measurements (Table 7). However, all three groups had mean levels of haemoglobin and packed cell volume below normal. Using the WHO cut-off point of haemoglobin <110 g/l, anaemia was present in 71% (*n* 17), 87% (*n* 20) and 39% (*n* 7) of the post-marasmic, siblings and control children, respectively. The prevalence of anaemia was not different between the post-marasmic children and siblings (Fisher's exact test; *P*=0.29) but the prevalence of anaemia was significantly higher in these former groups than

in the control children (χ^2 10.84, *P*=0.004; controls *v.* post-marasmic children and siblings). Among the anaemic children, 53, 35 and 29% of the post-marasmic children, siblings and age-matched controls, respectively, had at least two indicators of Fe status below normal (mean corpuscular volume, erythrocyte protoporphyrin and/or serum ferritin). Erythrocyte folate levels were elevated in all children suggesting the absence of megaloblastic anaemia. In the post-marasmic children, there were positive and significant correlations between haemoglobin and ht/age *Z* score (*r* 0.726; *P*<0.001), mean corpuscular volume and ht/age *Z* score (*r* 0.633; *P*<0.001), and serum ferritin and ht/age *Z* score (*r* 0.515; *P*<0.05). There was a negative and significant correlation between erythrocyte protoporphyrin and ht/age *Z* score (*r* -0.704; *P*<0.001). These correlations were not found in the other groups.

Mineral status

There were no significant differences in the mean concentrations of various minerals and trace elements between the groups as shown in Table 8. Although the mean levels of

Table 7. Haematological parameters of the post-marasmic children (*n* 24), their siblings (*n* 23) and control children (*n* 18) at the time of the study*
(Mean values and standard deviations)

	Post-marasmic		Siblings		Age-matched controls	
	Mean	SD	Mean	SD	Mean	SD
Haemoglobin (g/l)	98	20	101	14	108	13
Packed cell volume (%)	29.3	4.7	30.1	3.3	31.7	3.3
Mean corpuscular volume (fl)	72.4	10.1	75.0	7.9	77.5	8.1
Erythrocyte protoporphyrin (μg/g haemoglobin)	4.1	3.9	7.1	22.7	2.5	2.8
Serum ferritin (μg/l)	20.8	17.0	20.3	12.5	27.5	15.1
Erythrocyte folate (μg/l)	416	203	388	322	328	130

* For details of subjects and procedures, see p. 1124. There were no significant differences between the groups.

Table 8. Mineral status of the post-marasmic children (*n* 23), their siblings (*n* 24) and control children (*n* 18) at the time of the study*
(Mean values and standard deviations)

	Post-marasmic		Siblings		Age-matched controls	
	Mean	SD	Mean	SD	Mean	SD
Na (mmol/l)	139	3	138	6	138	2
K (mmol/l)	6.7	1.0	6.9	1.1	6.8	1.1
Ca (mmol/l)	2.3	0.1	2.3	0.2	2.4	0.1
P (mmol/l)	1.6	0.1	1.6	0.1	1.7	0.1
Mg (mmol/l)	0.7	0.1	0.7	0.1	0.7	0.1
Cu (μ mol/l)	24.5	3.1	24.5	5.0	23.0	3.5
Zn (μ mol/l)	13.8	3.0	13.0	3.8	14.5	3.5
Se (μ mol/l)	1.1	0.1	1.1	0.10	1.1	0.1

* For details of subjects and procedures, see p. 1125. There were no significant differences between the groups.

these minerals were within the normal range for age, 43, 42 and 28 % of the post-marasmic children, siblings and age-matched controls, respectively, had low serum Ca values (<2.34 mmol/l). About one-third of the post-marasmic children (32 %) and siblings (33 %) and 11 % of the age-matched control children had low serum Mg values (<0.68 mmol/l). However, the differences in the prevalence of low serum Ca and Mg were not significant between the groups (χ^2 1.22, $P=0.54$ for low serum Ca; Fisher's exact test, $P=0.12$ for low serum Mg).

Discussion

The prevalence of malnutrition has been declining in some countries throughout the world, but continues to remain high in many developing countries. While considerable research has explored the best routes by which malnutrition may be prevented or treated, little or no data exist on the long-term effects of severe malnutrition after recovery, specifically serum levels of the biochemical indicators of nutritional status. Thus, the present study was undertaken to investigate the nutritional, biological and growth status of Senegalese preschool children previously hospitalised for severe malnutrition.

Results from the present study indicate that 5 years after hospitalisation for nutritional rehabilitation, post-marasmic children showed a significant improvement in their wt/age and ht/age *Z* scores since discharge. In contrast, their wt/ht *Z* scores did not change during the same time period, suggesting that the weight gain of the post-marasmic children during rehabilitation was not lost, but was not accelerated during the post-hospitalisation period. The post-marasmic children and their siblings were stunted to the same degree and had lower FFM compared with the control group. Growth deficits and reduced body fat as well as FFM of the post-marasmic children and their siblings could possibly be due to low protein, energy and/or mineral intakes and/or chronic repeated infections in early life (Sigman *et al.* 1989; Golden, 1994).

Thus, as expected from previous reports (Grantham-McGregor, 1984; Golden, 1994), a poor social environment appears to be a major determinant of stunting. This implies that complete recovery of children who are hospitalised for

severe protein–energy malnutrition does not occur if they continue to live in the environment in which they became stunted. In the present study, without changes in their environment, the post-marasmic children were doing as well as their non-hospitalised siblings. They appear to have somewhat lower nutritional indices than their siblings and perhaps with larger numbers this difference would have become significant.

Although both poor nutrition and repeated infections have been shown to lead to poor growth (Waterlow, 1994; Frongillo *et al.* 1997), the present data on acute-phase proteins do not suggest that recent infection and/or inflammation is a major influence on growth in stunted children. At the time of the study, mean levels of acute-phase proteins of the stunted children were within the normal range and no differences were observed between the three study groups. However, all post-marasmic children showed minor infections such as cutaneous infections, conjunctivitis, rhinitis or asthma and signs of gastrointestinal infection. The high prevalence of stunting and Fe-deficiency anaemia in the post-marasmic children might be the consequence of their prior status and other aggravating factors such as these infections. This hypothesis is supported by the fact that siblings are stunted and anaemic to the same degree as the post-marasmic children.

Animal studies have shown that undernutrition in early life can induce permanent morphological, metabolic and hormonal changes that are detected in adulthood even after dietary intake becomes normal. Some of these changes are reduced number of adipocytes, impaired insulin response and altered energy metabolism in muscle, liver and adipose tissue (Aubert *et al.* 1980). In the present study, osteocalcin, IGF-1, transthyretin and apolipoproteins were used as indicators of nutritional status, as they appear to be regulated by energy intake (Clemmons *et al.* 1985; Wade *et al.* 1988a; Gouache *et al.* 1989; Guiro & Sall, 1991; Ndiaye *et al.* 1992, 1995; Feillet *et al.* 1993). IGFBP-3 was also assayed because it is thought to be regulated by protein and energy intakes (Jeevanandam, 1996). In laboratory animals, specifically rats, energy restriction was shown to be associated with reduced serum osteocalcin concentration due to decreased bone mineralisation by osteoblasts (Ndiaye *et al.* 1992). In young children with growth retardation, Colle *et al.* (1988) have reported low osteocalcin concentrations compared with age-matched control children. This is in agreement with the tendency of delayed bone age observed more in the post-marasmic children and with their short stature than in the two other children groups. IGF are major growth factors that mediate many of the growth-hormone functions by endocrine, autocrine and paracrine mechanisms in a great number of different tissues and organs. Reduced IGF blood levels will therefore have direct and negative consequences on tissue growth. The significant and positive correlation between IGF-1 and ht/age *Z* score and between IGF-1 and FFM in the post-marasmic children suggests a possible effect of IGF-1 on children's linear and ponderal growth.

In both the post-marasmic children and siblings, Apo A1 concentrations were significantly lower than those of the controls. Since these proteins are involved in lipoprotein

transport, their reduced serum concentrations imply altered lipid metabolism (Feillet *et al.* 1993). The present results are in agreement with those of Mahu *et al.* (1989), who found that children with low transthyretin levels also had significantly reduced concentrations of Apo A1 and B compared with those with normal levels. Hoffman *et al.* (2000) showed, using metabolic studies in children, recovered from undernutrition, but still growth retarded, an apparent impairment in fat oxidation in stunted children. Therefore, it would have been interesting to assess lipid metabolism in such stunted children.

Biochemical indicators of malnutrition were not different between the post-marasmic children and their siblings. When all children were classified according to the degree of stunting, serum concentrations of osteocalcin, IGF-1 and IGFBP-3 were lowest in those with the greatest height deficits, and highest in those without deficits. Therefore, the present results imply that nutritional status is impaired in the stunted children regardless of their being hospitalised for malnutrition. The low concentrations of osteocalcin and IGF-1 in stunted children may explain the delay between bone age and chronological age observed particularly in the post-marasmic children, who had the highest prevalence of stunting. But on the other hand, as suggested by Lewis *et al.* (2002), using the atlas of Greulich and Pyle as the standard reference text, bone age could not be assessed accurately in sub-Saharan African children.

In the present study, the age-matched control children showed reduced concentrations of serum transthyretin and osteocalcin compared with those found in children from industrialised countries or Senegalese children living in France (Wade *et al.* 1988b; Ndiaye *et al.* 1995). These (control) children had anthropometric measurements within the normal range for age and sex and they had no sign of inflammation or infection, as judged by serum levels of α_1 -acid glycoprotein and C-reactive protein. Thus, the decreased transthyretin concentration was probably not a result of an acute-phase response but rather due to inadequate energy and protein intake.

No association was found between marasmus during early childhood and haematological indices. The prevalence of Fe-deficiency anaemia was 53, 35 and 29% in the post-marasmic children, siblings and age-matched control children, respectively. A χ^2 test was not performed because of the small size of the samples. Since ht/age Z score correlated significantly with mean erythrocyte corpuscular volume, serum ferritin and mean erythrocyte protoporphyrin, it is possible that Fe-deficiency contributed to stunting. In fact, some studies suggest that chronic Fe-deficiency anaemia can affect growth rate since the Fe supplementation of preschool- and school-age children improved the growth rates of undernourished children (Allen, 1994; Adish *et al.* 1999).

Contrary to what was expected, the serum concentrations of eight minerals were not affected or reduced in previously malnourished children and their siblings. Deficiencies of Ca, P and Zn are known to induce rickets as well as growth deficits in children and primates (Prentice & Bates, 1994). According to Golden & Golden (1991), growth failure could be the clinical characteristic

of a diet deficient in Zn, Mg, P, K, or Na (type II nutrient). The present data do not suggest that the growth failure in post-marasmic children and their siblings is related to serum levels of any of these minerals. However it is also possible that the method used in the present study was not sensitive enough to detect sub-clinical deficits of the studied minerals. In fact, this is the case for Zn as it has been reported that growth-retarded Zambian children had normal serum Zn levels (Hautvast *et al.* 2000) and that Zn supplementation of children who had normal serum Zn levels improved their growth (Walravens *et al.* 1989; Nakamura *et al.* 1993).

In summary, post-marasmic children hospitalised 5 years earlier and their siblings have significantly lower nutritional indices than age-matched controls living in better socio-economic conditions. However the post-marasmic children were doing as well as their non-hospitalised siblings in all anthropometric and biochemical indices except for morbidity data. The serum concentrations of various minerals were not affected by their prior status. Based on the results of the present study it is concluded that children who were hospitalised for marasmus are able to recover up to the level of children living in the same environment but remain stunted compared with those living in better socio-economic conditions. In addition, it is concluded that while treatment for severe protein-energy malnutrition may be effective in weight recovery in the short term, continued treatment must be undertaken to ensure adequate nutrition in the long term and, also, the environmental conditions that promote undernutrition need to improve.

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References

- Adish AA, Ersey SA, Gyorkos TW, Jean-Baptiste J & Rojhani A (1999) Effect of consumption of food cooked in iron pots on iron status and growth of young children: a randomized trial. *Lancet* **353**, 712-716.
- Allen LH (1994) Nutritional influences on linear growth: a general review. *Eur J Clin Nutr* **48**, Suppl. 1, 75-89.

- Aubert R, Suquet JP & Lemonnier D (1980) Long-term morphological and metabolic effects of early under- and over-nutrition in mice. *J Nutr* **110**, 649–661.
- Bénéfice E, Fouéré T & Malina RM (1999) Early nutritional history and motor performance of Senegalese children, 4–6 years of age. *Ann Hum Biol* **26**, 443–455.
- Brook CGD (1971) Determination of body composition of children from skinfold measurements. *Arch Dis Child* **46**, 182–184.
- Christides JP & Potier de Courcy G (1987) Teneur en acide folique des aliments 2. Optimisation du dosage microbiologique des folates dans les aliments (Folic acid content of food 2. Optimisation of the microbiological proportioning of folates in food). *Sci Aliments* **7**, 7–22.
- Clemmons DR, Underwood LE, Dickerson RN, *et al.* (1985) Use of plasma somatomedin-C/insulin-like growth factor I measurements to monitor the response to nutritional repletion in malnourished patients. *Am J Clin Nutr* **41**, 191–198.
- Colle M, Ruffié A & Ruedas E (1988) Osteocalcin in children with short stature. *Acta Paediatr Scand* **343**, Suppl., 196–197.
- Doumas BT, Watson WA & Biggs HG (1971) Albumin standards and the measurement of serum albumin with bromocresol green. *Clin Chim Acta* **31**, 87–96.
- Durnin JVGA & Rahaman MN (1967) The assessment of the amount of fat in the human body from measurements of skinfold thickness. *Br J Nutr* **21**, 681–689.
- Engler R (1984) Bases méthodologiques: protéines de la réaction inflammatoire. Notion de profil protéique (Inflammatory reaction proteins. Concept of the protein profile). *Pédiatrie* **39**, 339–344.
- Feillet F, Parra HJ, Kamian K, Bard JM, Fruchart JC & Vidailhet M (1993) Lipoprotein metabolism in marasmic children of northern Mauritania. *Am J Clin Nutr* **58**, 484–488.
- Frongillo EA, de Onis M & Hanson KM (1997) Socioeconomic and demographic factors are associated with worldwide patterns of stunting and wasting of children. *J Nutr* **127**, 2302–2309.
- Galler JR, Ramsey FC, Forde V, Salt P & Archer E (1987b) Long-term effects of early kwashiorkor compared with marasmus. II. Intellectual performance. *J Pediatr Gastroenterol Nutr* **6**, 847–854.
- Galler JR, Ramsey FC, Salt P & Archer E (1987a) Long-term effects of early kwashiorkor compared with marasmus. I. Physical growth and sexual maturation. *J Pediatr Gastroenterol Nutr* **6**, 841–846.
- Golden BE & Golden MHN (1991) Relationships among dietary quality, children's appetites, growth stunting, and efficiency of growth in poor populations. *Food Nutr Bull* **13**, 105–109.
- Golden MHN (1994) Is complete catch-up possible for stunted malnourished children? *Eur J Clin Nutr* **48**, Suppl. 1, 58–71.
- Gouache P, Le Moulac B, Aubert R, Flament C & Bleiberg-Daniel F (1989) Evolution de la composition des lipoprotéines au cours d'une carence protéique chez le rat (Evolution of the composition of lipoproteins in the course of protein deprivation in the rat). In *Les Carences Nutritionnelles dans les PVD. 3èmes Journées du GERM*, pp. 130–137 [D Lemonnier and Y Ingenbleek, editors]. Paris, France: Karthala-ACCT.
- Grantham-McGregor S (1984) Chronic undernutrition and cognitive abilities. *Hum Nutr Clin Nutr* **38C**, 83–94.
- Guïro AT & Sall MG (1991) Transthyréline et apolipoprotéines A et B au cours de la malnutrition protéino-énergétique des enfants sénégalais (Transthyretin and apolipoproteins A and B in the course of protein-energy malnutrition in Senegalese children). In *Alimentation et Nutrition dans les Pays en Développement. 4èmes Journées du GERM*, pp. 304–309 [D Lemonnier, Y Ingenbleek and P Hennart, editors]. Paris, France: Karthala-ACCT-Aupelf.
- Haider M & Haider SQ (1984) Assessment of protein energy malnutrition. *Clin Chem* **30**, 1286–1299.
- Hautvast JLA, Tolboom JJM, Kafwembe EM, *et al.* (2000) Severe linear growth retardation in rural Zambian children: the influence of biological variables. *Am J Clin Nutr* **71**, 550–559.
- Hennart P, Beghin D & Bossuyt M (1987) Long-term follow-up of severe protein-energy malnutrition in Eastern Zaïre. *J Trop Pediatr* **33**, 10–12.
- Herbert V (1987) Signification des tests de laboratoire relatifs au statut en folates. Besoins quotidiens en folates (Significance of laboratory tests relating to folates status. Daily requirements of folates). In *Folates et Cobalamines: Collection Progrès en Hématologie no. 9*, pp. 115–122 [J Zittoun and BA Cooper, editor]. Paris, France: Pr. R. Zittoun.
- Hoffman DJ, Sawaya AL, Verreschi I, Tucker KL & Roberts SB (2000) Why are nutritionally stunted children at increased risk of obesity? Studies of metabolic rate and fat oxidation in shantytown children from Sao Paulo, Brazil. *Am J Clin Nutr* **72**, 702–707.
- Jeevanandam M (1996) IGF-1, marqueur nutritionnel (IGF-1, nutritional marker). *Nutr Clin Métabol* **10**, 265–274.
- Lentner C (editor) (1984) *Physical Chemistry, Composition of Blood, Hematology, Somatometric Data. Geigy Scientific Tables*, 8th ed. vol. 3, pp. 115–125. Basle, Switzerland: Ciba-Geigy Limited.
- Lewis CP, Lavy CBD & Harrison WJ (2002) Delay in skeletal maturity in Malawian children. *J Bone Joint Surg* **84B**, 732–734.
- Looker A, Johnson C & McDowell M (1991) Prevalence of impaired iron status and anemia in the United States. In *Aspects Actuels des Carences en Fer et en Folates dans le Monde*, pp. 29–38 [S Hercberg, P Galan and H Dupin, editors]. Colloque INSERM, vol. 197 Paris, France: INSERM.
- Mahu JL, Suquet JP, Bleiberg-Daniel F, Turquet M & Lemonnier D (1989) Enquête nutritionnelle chez les jeunes enfants à l'île de la Réunion: prévalence de la malnutrition protéino-énergétique, des infections et des parasitoses (Nutritional investigation of the young children of Reunion Island: the prevalence of protein-energy malnutrition, infections and parasitoses). In *Les Carences Nutritionnelles dans les PVD. 3èmes Journées du GERM*, pp. 114–126 [D Lemonnier and Y Ingenbleek, editors]. Paris, France: Karthala-ACCT.
- Mancini G, Carbonara AO & Hereman JF (1965) Immunochemical quantitation of antigens by single radial immunodiffusion. *Int J Immunochem* **2**, 235–259.
- Martorell R, Kettel Khan L & Schroeder DG (1994) Reversibility of stunting: epidemiological findings in children from developing countries. *Eur J Clin Nutr* **48**, Suppl. 1, 45–57.
- Merimee TJ, Zapf J, Hewlett B & Cavalli-Sforza LL (1987) Insulin-like growth factors in pygmies. The role of puberty in determining final stature. *N Engl J Med* **316**, 906–911.
- Nakamura T, Nishiyama S, Futagoishi-Suginohara Y, Matsuda I & Higashi A (1993) Mild to moderate zinc deficiency in short children: effect of zinc supplementation on linear growth velocity. *J Pediatr* **123**, 65–69.
- Ndiaye B, Lemonnier D, Sall MG, *et al.* (1995) Serum osteocalcin regulation in protein-energy malnourished children. *Pediatr Res* **37**, 606–610.
- Ndiaye B, Prudhon C, Guillozo H & Lemonnier D (1992) Rat serum osteocalcin concentration is determined by food intake and not by inflammation. *J Nutr* **122**, 1870–1874.
- Organisation Mondiale de la Santé (1975) *Lutte Contre les Anémies Nutritionnelles en Particulier la Carence en Fer. Série de Rapports Techniques, no. 580*. Geneva, Switzerland: OMS.
- Organisation Mondiale de la Santé (1983) *Mesure des modifications de l'état nutritionnel. Guide pour la mesure de l'impact nutritionnel des programmes d'alimentation complémentaire visant les groupes vulnérables*. Geneva, Switzerland: OMS.

- Prentice A & Bates CJ (1994) Adequacy of dietary mineral supply for human bone growth and mineralisation. *Eur J Clin Nutr* **48**, Suppl. 1, 161–177.
- Sarr CS (1996) *Evaluation du devenir d'enfants ayant souffert de malnutrition protéino-énergétique sévère à Dakar (résultats préliminaires)*. Medical thesis, Université Cheikh Anta Diop, Dakar, Sénégal.
- Sigman M, Neumann C, Baksh M, Bwibo N & McDonald MA (1989) Relationship between nutrition and development in Kenyan toddlers. *J Pediatr* **115**, 357–364.
- Simondon KB, Simondon F, Simon I, *et al.* (1998) Preschool stunting, age at menarche and adolescent height: a longitudinal study in rural Senegal. *Eur J Clin Nutr* **52**, 412–418.
- Siri WB (1956) The gross composition of the body. In *Advances in Biological and Medical Physics*, vol. 4, pp. 239–280 [CA Tobias and JH Lawrence, editors]. New York: Academic Press.
- Wade S, Bleiberg-Daniel F, Le Moullac B, *et al.* (1988a) Value of serum transthyretin measurements in the assessment of marginal protein energy malnutrition in rats. *J Nutr* **118**, 1002–1010.
- Wade S, Parent G, Bleiberg-Daniel F, *et al.* (1988b) Thymulin (Zn-FTS) activity in protein-energy malnutrition: new evidence for the interaction between malnutrition and infection in the thymic function. *Am J Clin Nutr* **47**, 305–311.
- Walravens PA, Hambidge KM & Koepfer DM (1989) Zinc supplementation in infants with a nutritional pattern of failure to thrive: a double-blind, controlled study. *Pediatrics* **83**, 532–538.
- Waterlow JC (1994) Introduction. Causes and mechanisms of linear growth retardation (stunting). *Eur J Clin Nutr* **48**, Suppl. 1, 1–4.