Effect of catfish supplementation on the fatty acid status and growth of undernourished rural preschool children under 6 years of age: an intervention trial in Lebowa, South Africa

Original Article

Effect of catfish supplementation on the fatty acid status and growth of undernourished rural preschool children under 6 years of age: An intervention trial in Lebowa, South Africa

HY Tichelaar[1] PhD, NP Steyn[2] PhD, JH Nel[3] DSc, CM Smuts[1] PhD, PJ van Jaarsveld[1] PhD, JF Prinsloo[4] DSc, J van Rooyen[5] PhD, CJ Lombard[6] PhD, MA Dhansay[1] FCP(SA) and AJS Benadé[1] DSc

[1]National Research Programme for Nutritional Intervention, Medical Research Council, Parrow Valley, South Africa
[2]Department of Human Nutrition, University of the North, Sovenga, South Africa
[3]Department of Computer Science, University of the North, Sovenga, South Africa
[4]Aquaculture Unit, University of the North, Sovenga, South Africa
[5]Department of Human and Animal Physiology, University of Stellenbosch, Stellenbosch, South Africa
[6]Centre for Epidemiological Research, Medical Research Council, Parrow Valley, South Africa

A group of 102 preschool children aged 13–69 months from a rural area of Lebowa were selected from a cross-sectional study of 659 children for an intervention trial (12 months) to study the effect of catfish (*Clarias gariepinus*) supplementation on their plasma phospholipid fatty acid status and growth. They were classified into undernourished and control groups according to their weight-for-age. The undernourished children (n = 52) received 43 g fish and 7.5 g sunflower cooking oil per day, whereas a matched (age and sex) well-nourished control group (n = 50) was not supplemented. At baseline, after 6 months and after 12 months of the study, anthropometry, haematology, blood biochemistry and plasma phospholipid fatty acid analyses were done. In the undernourished group, high baseline oleic acid (18:1ω9) levels in plasma phosphatidylcholine (PC) were replaced by docosahexaenoic acid (22:6ω3) with supplementation. In plasma PC, this reduction in 18:1ω9 and increase in 22:6ω3 was associated with significant increases in weight-for-age Z-scores, P = 0.0378 and P = 0.0415, respectively. The fish supplement and cooking oil that supplied additional 7% energy (7% E) and nutrients promoted growth of undernourished children, although this was inadequate for sustained growth during the second 6 months of intervention.

Key words: children, fatty acids, fish supplementation, growth, undernutrition.

Introduction

The causes of malnutrition are numerous and are multi-sectoral in nature. It is difficult to single out dietary components since an inadequate dietary intake and diseases are affected by basic health services, the environment, household food security as well as maternal and child care.[1] Various nutritional factors are usually implicated in undernutrition and include low energy and calcium intakes,[2] deficiencies of proteins,[3] zinc,[4,5] iron,[6] iodine[7] and vitamin A,[8] or the presence of dietary 'trans' fatty acids.[9] An optimal dietary and tissue balance between ω6 and ω3 fatty acids seems to be required for normal growth and development.[10] The aetiology and mechanisms responsible for this growth retardation remain unclear.

Undernutrition associated with the low socioeconomic status of rural black children is a major problem in South Africa.[11] Lebowa, situated in the Northern Province of South Africa, has a high prevalence of undernutrition with 23% of children under 6 years of age being underweight, 40% stunted and 2% wasted.[12] A survey of primary school children has shown an association between undernutrition and low dietary intakes of ω3 fatty acids.[13] Earlier reported results of a cross-sectional study on a selected group of undernourished rural preschool children in Lebowa suggest that increased endogenous synthesis of oleic acid (OA; 18:1ω9) might be a consequence of low energy intakes, and that the intake of ω3 fatty acids was reduced relative to that of ω6 fatty acids.[14]

In view of the high prevalence of undernutrition in Lebowa,[2,15] and the reduced dietary intake of ω3 fatty acids, the objectives of this study were: (i) to determine the nutritional status of rural Lebowa preschool children under 6 years of age; and (ii) to determine the effect of African sharp-

Correspondence address: Dr HY Tichelaar, National Research Programme for Nutritional Intervention, PO Box 19070, Tygerberg 7505, South Africa.
Tel: 27 21 938 0409; Fax: 27 21 938 0321.
Email: henk.tichelaar@mrc.ac.za
Accepted 3 September 1998.

tooth catfish **(***Clarias gariepinus*) supplementation on the nutritional status of undernourished preschool children, after a 6 month and 12 month intervention trial was subsequently evaluated, in order to compare the fatty acid status and growth of the supplemented children with those of a control group of 'normally' growing children. We hypothesized that fish supplementation would promote growth of the undernourished children**.**

Subjects and methods

Subjects

The present study formed part of an aquaculture intervention study on a low socioeconomic community in Monyamane, Lebowa, from April 1993 to May 1994. Initial screening of 659 children aged 6–72 months was done during March 1993 to identify those with weight-for-age measurements below minus two Standard Deviations (< –2 SD) of the reference median according to the National Center for Health Statistics (NCHS)[16,17] and a mid-upper-arm circumference (MUAC) below the 5th percentile of Frisancho's standards.[18]

A total of 52 children aged 13–69 months were diagnosed as being undernourished, with 35 meeting both selection criteria. The remaining 17 children were only underweight, but were nevertheless included, as 35 children was considered to be too small a number for the proposed intervention study. A control group of children was selected from the initial study sample and comprised well-nourished children (n = 50) with weights > –2 SD of the NCHS median and normal MUAC measurements, matched for age and sex. Permission to conduct this survey was obtained from the *Kgosi* (chief), and the health and school authorities from each area. Informed consent was obtained from the children's parents or guardians. The procedures and protocol were reviewed and approved by the Ethics Committee of the Medical Research Council.

Anthropometry and blood sampling

Anthropometric measurements and blood samples were collected in the local clinic at baseline, after 6 months intervention and after 12 months intervention. During each survey, all of the undernourished and control children were subjected to anthropometric measurements by the same observers. Anthropometric measurements were taken in the morning by two trained dietitians and a nurse according to standard methodology.[19] A movable headboard and a measuring tape fixed to a table were used to measure the recumbent length of children under 2 years of age. Heights of older children were measured without their shoes to the nearest 0.1 cm using a right-angled headboard and a non-stretchable measuring tape fastened to a flat vertical wall.

Weights were recorded on a digital scale to the nearest 0.01 kg. Mid-upper-arm circumference was measured at the midpoint between the lateral projection of the acromion process of the scapula and the inferior margin of the olecranon process of the ulna with an inflexible plastic tape to the nearest 0.1 cm. A flexible non-stretchable measuring tape was used to record head circumference (HC). The lower edge of the tape was positioned slightly above the supra-orbital ridges, above the ears and around the head so that a maximum circumference could be recorded to the nearest 0.1 cm. A paediatrician collected blood samples by peripheral venipuncture during the early morning. A 6 mL venous blood sample was obtained, of which 2.5 mL was transferred into ethylenediaminetetraacetic acid (EDTA) tubes and 3.5 mL into plain tubes without anticoagulant. Blood samples were immediately kept at 4°C. Precautions were taken to prevent contamination with zinc during blood sampling and storage.

Fish supplementation

The undernourished children and their family members each received 100 g catfish fillets (*Clarias gariepinus*) three times a week during the 12 month intervention period. Each family also received 750 mL sunflower oil every 2 weeks for cooking purposes. A shallow fat frying procedure was used where gut fat from the catfish fillets was melted and used as the oil source in addition to the sunflower oil to increase energy availability.[20] Fish was supplied to all family members to ensure that participating undernourished children received and consumed their fish portions. A control group of children received no fish. Fish used in the study were either produced by the Aquaculture Research Intensive Fish Production Unit of the University of the North or purchased from local commercial catfish farmers. Despite environmental differences, all the fish used for this intervention study were fed the same commercial diets (6.6% moisture, 34.6% protein, 3.9% fat, 2.1% ash; Brennco Feeds, Louis Trichardt, South Africa) to ensure consistency of the fish fillet fatty acid profiles (Table 1) as analysed by Hoffman *et al*.[21]

The undernourished families visited the local clinic three times a week to collect their raw fish portions. Two nutrition

Table 1. Approximate composition and mineral content of fillets of the African sharptooth catfish (*Clarias gariepinus*) and the calculated supplemented nutritional intake of undernourished children during this intervention study

	Fish fillet composition			
	100 g portion	42.86 g daily portion	% RDA	300 g intake/ week
Nutrients				
Protein (g)	16.6	7.1		49.8
Fat (g)	5.2	2.2		15.6
SFA (mg)	1913.6	820.1		5740.8
MUFA (mg)	1918.8	822.3		5756.4
PUFA (mg)	967.2	414.5		2901.6
ω3 (mg)	268.7	115.2		806.1
ω6 (mg)	698.5	299.4		2095.5
ω6/ω3 ratio	2.6	2.6		2.6
22:6ω3/20:5ω3 ratio	2.5	2.5		2.5
Energy of fish (kJ)	475	204		1425
Energy of fish + SO (kJ)	1122	481		3366
Minerals				
Calcium (µg)	16.9	7.2	<1	50.7
Phosphorus (µg)	97.5	41.8	<1	292.5
Magnesium (µg)	21.6	9.3	<1	64.8
Potassium (µg)	177.0	75.9	<1	531.0
Manganese (µg)	265.0	113.6	6	795.0
Iron (µg)	8545.0	3662.4	37	25635.0
Copper (µg)	520.0	222.9	22	1560.0
Zinc (µg)	3085.0	1322.2	13	9255.0

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SO, sunflower oil; RDA, Recommended Dietary Allowance.

educators who were recruited from the same village and thoroughly trained distributed the prepacked fish fillets, which were stored at –20°C. They were responsible for demonstrations regarding the preparation of fish and advised the mothers/guardians on the portion sizes for each participant. The educators were also responsible for keeping accurate records of the fish that was collected during the 12 month intervention period, and they meticulously followed up all families who neglected to collect their fish portions regularly. The mothers/guardians of both groups of children received the same nutritional counselling during the intervention period of 12 months.

Blood analyses

Haematology and biochemistry. Initial haematological determinations were done on EDTA samples, and have been discussed elsewhere.[15] Plasma for biochemistry was prepared by centrifugation and stored in 1.5 mL Eppendorf tubes at –80°C in a Forma bio-freezer until analyses could be done. The following concentrations were determined with commercially available kits from Boehringer Mannheim GmbH Diagnostics (Mannheim, Germany): triacylglycerol (TAG, Cat. no. 701 904), cholesterol (Cat. no. 290 319), albumin (Cat. no. 263 869), transferrin (Cat. nos. 1360 752 and 1852 686) and alkaline phosphatase (ALP; Cat. no. 1442 236). Ferritin (^{125}I; Cat. nos. 263 907, 263 991 and 263 915), vitamin B_{12} (^{57}Co) and folate (^{125}I) (Becton Dickinson, New York, USA, Cat. no. 264 806) were determined by immunoradiometric assays. Vitamins A (retinol) and E (α-tocopherol) were determined by liquid chromatography.[22] Plasma zinc was measured by electrothermal atomic absorption spectroscopy.

Fatty acid and lipid analysis. Frozen plasma samples were thawed at room temperature and processed within 1 week. Lipids were extracted from plasma with chloroform/methanol (2:1; v/v) containing butylated hydroxytoluene (454×10^{-6} mol/L) as antioxidant, and then separated by thin layer chromatography (TLC).[23] Plasma phosphatidylcholine (PC) was analysed as described previously.[14] Areas on TLC plates containing PC were identified by comparison with known response factor values, scraped into glass stoppered tubes and transmethylated.[14] Fatty acid analysis was performed on a model 3700 Varian gas-liquid chromatograph using fused silica megabore DB-225 columns (J & W Scientific, Folsom, USA, Cat. no. 125–2232).[14,24] Gas flow rates were: hydrogen (carrier gas), 5–8 mL/min; medical air, 250 mL/min; and hydrogen, 25 mL/min. Temperature programming was linear at 3°C/min, initial temperature 165°C, final temperature 220°C, injection temperature 240°C, and detector temperature 250°C. A fatty acid mixture standard was prepared from individual fatty acids (Sigma, St Louis, MO, USA). Fatty acid methyl esters of PC were identified by comparison of the retention times to those of the standard mixture of free fatty acids from 14:0 to 22:6ω3.

Statistical analysis

Results are expressed as means and standard deviations (SD). Differences between the undernourished and control groups were determined by using the Wilcoxon Signed Rank Test, as the variables were not distributed normally according to the Shapiro-Wilk test.[25] In spite of all children selected being matched for age and sex, groups were unequal in numbers because all selected children were not always available for blood collections. This may be attributed to control children and their guardians being less motivated to participate in this intervention study, because they did not receive fish.

A mixed-effects regression model was used to test the hypothesis that changes in fatty acid status after fish supplementation were associated with growth.[26] Regression analysis were assessed on all children (n = 102) who participated in this study, irrespective of group, and comprised Z-scores on both sides of the median. This model was preferred for its detailed analysis of longitudinal data that allows the use of data from individuals with missing data and the estimation of individual patterns of change in the same analysis. It includes random effects to account for the natural heterogeneity in growth patterns of children. This heterogeneity is expected due to the interaction of various factors that can affect growth. The model consisted of fixed effects for intervention status, time, intervention–time interaction, fatty acid (18:1ω9 or 22:6ω3), ferritin, ALP and a random intercept for each child. The growth outcomes modelled were height-for-age Z-scores (HAZ), weight-for-age Z-scores (WAZ) and weight-for-height Z-scores (WHZ). Ferritin[27,28] and ALP[29] are known to be influenced by growth, and were therefore included in the model, because they responded to the intervention treatment in concert with 18:1ω9 and 22:6ω3.

Results

Table 1 provides the approximate composition and mineral content of the catfish fillets and calculated supplemented daily intakes of protein, fat, energy and minerals of the undernourished children.[21] Assuming complete compliance, each undernourished child received 7.1 g animal protein, 2.2 g fat and 204 kJ energy daily from the fish. An additional 277 kJ energy was supplied daily by the sunflower cooking oil. No dietary information was collected in this study. A previous study of Pedi (a Sotho-speaking people, originally part of the Kgatla tribe of Botswana, which broke away and settled in Sekhukhune, a part of Lebowa in the north-east of South Africa) preschool children from the same area revealed low mean daily energy intakes of 4541 kJ,[15] but adequate daily protein (40 g *vs* RDA of 24 g) intakes, albeit low in animal proteins (chicken and eggs), which only contributed to 40% of the total protein intake. The fish (43 g/day) consequently provided an additional 481 kJ energy, which increased the daily energy intake of the undernourished ≠children from 60% to 67% of the recommended dietary allowance (RDA) for children of this age group.[30] In addition, the fish provided micronutrients[31] such as iron (3.7 mg/day; 37% of RDA), zinc (1.3 mg/day; 13% of RDA) and polyunsaturated fatty acids (PUFA) that consisted of ω6 and ω3 fatty acids (respectively 299 mg/day and 115 mg/day). This represents a 34% increase in iron intake and a 7% increase in PUFA intake. The sunflower cooking oil (7.5 g/day) provided an additional 4.8 g PUFA per day, which represents an 80% increase in PUFA intake.

Table 2 presents anthropometric data of the undernourished and control children at baseline, after 6 months and 12 months of intervention. Mean weight and MUAC values remained significantly different between the undernourished and control children throughout the study. The undernour-

Table 2. Anthropometry of children under 6 years of age with low and normal anthropometric indices (NCHS) at baseline, 6 months intervention and 12 months intervention (mean (SD))

	Undernourished						Controls					
	Baseline $n = 52$		6 months $n = 44–52$		12 months $n = 34–35$		Baseline $n = 50$		6 months $n = 42–49$		12 months $n = 31–32$	
Height (cm)	89.65	(11.27)	94.81	(10.15)	96.43	(10.19)	93.97	(10.27)	99.50	(9.10)	100.89	(10.35)
Weight (kg)	11.91**	(2.37)	13.29*	(2.47)	13.91*	(2.55)	14.31	(2.83)	15.62	(2.84)	16.33	(3.04)
MUAC (mm)	142.69**	(7.81)	147.04*	(8.07)	150.50**	(9.35)	160.24	(10.78)	163.42	(10.37)	164.81	(12.95)
HC (cm)	48.75*	(2.06)	49.76*	(1.82)	49.93	(1.88)	49.85	(1.80)	50.71	(1.00)	50.66	(1.75)
HAZ	–2.45**	(0.86)	–2.31**	(0.78)	–2.24**	(0.70)	–1.18	(1.22)	–1.18	(1.05)	–1.12	(1.06)
WAZ	–2.23**	(0.41)	–1.94**	(0.53)	–1.89**	(0.65)	–0.73	(0.95)	–0.66	(0.81)	–0.62	(0.82)
WHZ	–1.03**	(0.49)	–0.79**	(0.70)	–0.69**	(0.90)	0.06	(0.93)	0.15	(0.72)	0.18	(0.77)

Significantly different from controls: *$P < 0.01$; **$P < 0.0001$. MUAC, mid-upper-arm circumference; HC, head circumference; HAZ, height-for-age Z-score; WAZ, weight-for-age Z-score; WHZ, weight-for-height Z-score.

ished children achieved a rate of weight gain (0.68 g/kg/day) that was 1.33 times faster than the rate of the control children (0.51 g/kg/day) during the 0–6 months period. Undernourished children had lower ($P \leq 0.01$) HC at baseline and after 6 month of fish supplementation compared with unsupplemented control children. This difference was, however, not significant after 12 months of intervention. Because children would have grown despite the intervention, the use of HAZ, WAZ and WHZ indices were considered to be more appropriate.

Figure 1 presents the mean change (SD) in anthropometry of children under 6 years of age with low and normal anthropometric indices (NCHS) after 0–6 months, 6–12 months and 0–12 months intervention. Changes in HAZ in the supplemented undernourished children compared with the control children over the 0–12 months intervention period were statistically significant ($P \leq 0.01$). Intervention had no effect on the prevalence of stunting that remained at 69.2% for the undernourished children versus 16% for the control children. Changes in WAZ were different ($P \leq 0.05$) in the supplemented undernourished children when compared with the control children during the 0–6 months intervention period. Changes in WAZ were, however, not significantly different between the two groups during the 6–12 months and 0–12 months intervention periods. Changes in WHZ were not significantly different between the two groups during the intervention study.

Table 3 presents biochemical data of the undernourished and control children at baseline, after 6 months and 12 months of intervention. Fish supplementation appeared to have no effect on plasma TAG, total cholesterol, albumin and transferrin concentrations. Although the undernourished children had lower plasma ALP activities at baseline than the control children, this was not significant. A considerable number of the undernourished children (57%) and control children (67.5%) had low plasma ferritin concentrations (<12 ng/mL) before intervention (normal range: 12–220 ng/mL), reflecting low iron stores.[12] After 0–6 months of intervention, 61.5% of the supplemented undernourished children and 72.1% of the control children had low ferritin concentrations. Plasma ferritin concentrations increased ($P \leq 0.001$) more in the supplemented undernourished children after 12 months of intervention compared with the control children. After 0–12 months of intervention, 4.7% of the supplemented undernourished children and 11.8% of the control children had low ferritin concentrations. Plasma folate and vitamin B_{12} concentrations were normal in both groups (normal range: 4–22 ng/mL and 200–800 pmol/L, respectively) during the intervention study.[32]

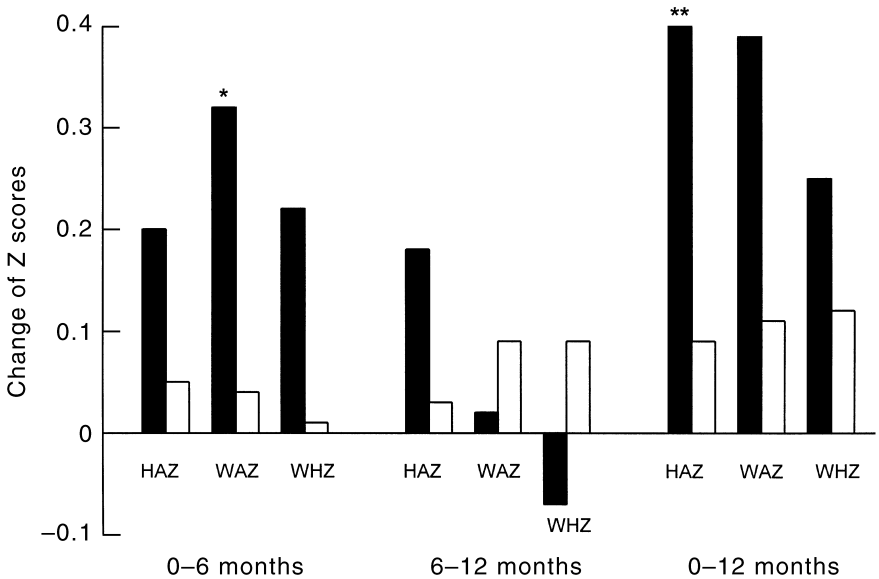

Figure 1. Mean change (SD) in anthropometry of (■) undernourished and (□) control children under 6 years of age with low and normal anthropometric indices (NCHS) after 0–6 months, 6–12 months and 0–12 months intervention. HAZ, height-for-age Z-scores; WAZ, weight-for-age Z-scores; WHZ, weight-for-height Z-scores; *$P \leq 0.05$; **$P \leq 0.01$.

Table 3. Biochemistry of children under 6 years of age with low and normal anthropometric indices (NCHS) at baseline, 6 months intervention and 12 month interventions (mean (SD))

	Undernourished						Controls					
	Baseline n = 50–52		6 months n = 50–52		12 months n = 38–44		Baseline n = 31–35		6 months n = 45–49		12 months n = 38	
Triacylglycerol (mmol/L)	1.24	(0.76)	1.02	(0.47)	1.02	(0.77)	0.98	(0.42)	0.87	(0.30)	0.96	(0.43)
Total cholesterol (mmol/L)	3.24	(0.76)	3.09	(0.73)	3.55	(0.83)	3.19	(0.66)	3.01	(0.65)	3.28	(0.66)
Albumin (g/L)	45.76	(3.19)	43.60	(4.20)	47.90	(2.54)	46.09	(3.37)	43.54	(5.55)	47.97	(2.67)
Transferrin (µmol/L)	42.65	(2.86)	40.96	(2.97)	41.06	(2.26)	43.15	(4.32)	40.38	(2.42)	40.68	(2.27)
Alkaline phosphatase (U/L)	577.77	(181.11)	661.14	(193.90)	641.84	(193.09)	647.17	(189.34)	655.70	(148.13)	683.00	(171.11)
Ferritin (ng/mL)	16.71	(17.39)	13.67	(19.10)	37.52**	(25.75)	12.21	(16.17)	11.02	(8.45)	22.91	(10.56)
Folate (ng/mL)	5.61	(2.97)	6.08	(2.64)	5.54	(1.89)	6.11	(3.40)	6.00	(2.31)	5.49	(1.75)
Vitamin B_{12} (pmol/L)	584.24	(216.89)	619.46	(275.87)	602.50	(177.38)	607.06	(211.64)	519.08	(198.27)	553.43	(146.30)
Haemoglobin (g/dL)	11.59	(1.91)	11.59	(1.20)	11.97	(1.81)	11.33	(1.37)	11.81	(1.18)	12.03	(1.69)
Vitamin A (µg/dL)	26.38	(6.94)	23.73	(6.27)	22.33	(6.26)	25.56	(7.24)	24.63	(6.64)	23.06	(5.18)
Vitamin E (mg/L)	7.79	(1.96)	7.26	(1.52)	–	–	7.44	(2.22)	7.12	(1.32)	–	–
Zinc (µmol/L)	12.54	(1.80)	10.78	(1.91)	11.59*	(1.62)	12.50	(1.86)	11.42	(1.88)	12.48	(1.81)

Significantly different from controls: *P < 0.01; **P < 0.001; –, no values.

The number of children who presented with anaemia before intervention was considerable: a prevalence of 28.7% using a cut-off point of <11 g/dL.[33] Considerably fewer undernourished children (23.6%) than control children (35.9%) were anaemic before intervention.[12,34] Both groups had similar Hb concentrations during the intervention study. After 0–6 months of intervention, 26.8% of the supplemented undernourished children and 17.7% of the control children were anaemic. This relates to a change of 20% for the total group of children (undernourished and controls). After 0–12 months of intervention, 18.9% of the supplemented undernourished children and 18% of the control children were anaemic. Both groups had normal mean serum vitamin A concentrations (normal: >20 µg/dL) during the intervention study.

Surprisingly, the undernourished children did not appear to be vitamin A deficient before intervention, despite a reported prevalence of about 44% in the Northern Province.[35] Averages, however, can be misleading, because 20% (n = 11) of the undernourished children and 21% (n = 8) of the control children had vitamin A concentrations <20 µg/dL at baseline. After 0–6 months of intervention, 30.8% of the supplemented undernourished children and 21.7% of the control children were vitamin A deficient. After 0–12 months of intervention, 42.9% of the supplemented undernourished children and 35% of the control children were vitamin A deficient. Both groups had normal serum vitamin E concentrations (normal range: 0.5–1.6 mg/L) at baseline and after 6 months of intervention.[36] No samples could be analysed from the 12 month intervention batch due to inadequate volumes. Plasma zinc concentrations were reduced in both groups after 6 months of intervention. After 12 months of intervention, the supplemented undernourished children had lower ($P \leq 0.01$) zinc concentrations than the control children.

The change in ALP is shown in **Fig. 2**. Mean ALP activity tended to be increased after 0–6 months of intervention, but was significantly ($P \leq 0.05$) reduced by 22.16 U/L in the undernourished children during the 6–12 months intervention period compared with the increase of 52.43 U/L in the control children.

The change in ferritin is shown in **Fig. 3**. The mean change of ferritin concentrations was higher in the supplemented undernourished children during the 6–12 months (P

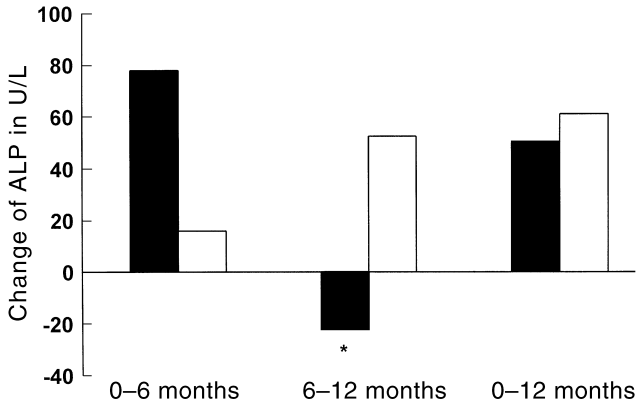

Figure 2. Mean change (SD) of plasma alkaline phosphatase (ALP) of (■) undernourished and (□) control children children under 6 years of age with low and normal anthropometric indices (NCHS) after 0–6 months, 6–12 months and 0–12 months intervention. *$P \leq 0.05$.

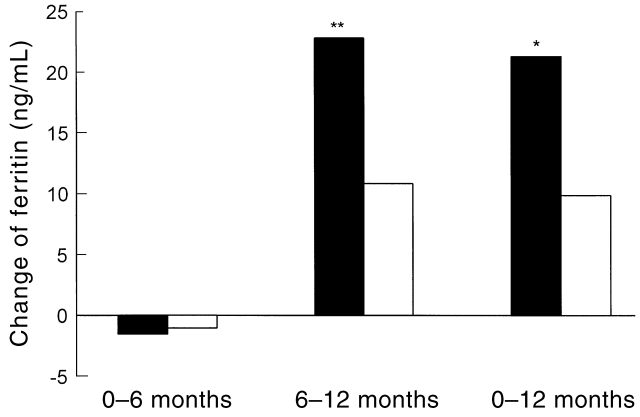

Figure 3. Mean change (SD) in ferritin of (■) undernourished and (□) control children under 6 years of age with low and normal anthropometric indices (NCHS) after 0–6 months, 6–12 months and 0–12 months intervention. *$P \leq 0.05$; **$P \leq 0.01$.

≤ 0.01) and 0–12 months ($P \leq 0.05$) intervention periods compared with that of the control children.

Table 4 presents the fatty acid composition of plasma PC for the undernourished and control children at baseline, after 6 months and 12 months of intervention. Baseline OA levels were higher ($P \leq 0.05$) in the undernourished children compared with the control children before supplementation with concomitantly lower ($P \leq 0.05$) Σω6 and Σ(ω6 + ω3) fatty acids. Eicosatrienoic acid (ETA; 20:3ω3) was higher ($P \leq 0.05$) in the supplemented undernourished children compared with the control children after 6 months of intervention. Docosapentaenoic acid (DPA; 22:5ω3) was lower ($P \leq 0.05$) in the supplemented undernourished children compared with the control children after 12 months of intervention. Mean docosahexaeoic acid (DHA; 22:6ω3) levels were maintained at the levels that were reached after 6 months of fish supplementation.

Table 5 presents the effect of fish supplementation on the mean change of plasma PC fatty acids of undernourished children after 0–6 months, after 6–12 months and after 0–12 months compared with unsupplemented controls. Eicosapentaeonic acid (EPA; 20:5ω3), DHA, post Δ5 ω3 and Σ ω3 fatty acids were increased ($P \leq 0.05$) in the supplemented undernourished children during the 0–6 months intervention period compared with those of the control children. Docosapentaenoic acid (DPA; 11:5ω3) was decreased ($P \leq 0.05$) in the supplemented undernourished children during the 6–12 months intervention period compared with that of the control children. The mean change of fatty acids was not significantly different between groups during the 0–12 months intervention period.

Two mixed-effects regression models (**Table 6**) estimated the regression coefficients of OA and DHA on growth outcomes that were adjusted for experimental design, ferritin and ALP. Outcomes are coefficients (standard error). The model of OA in plasma PC predicted that HAZ would shift to the left by 0.0026 (0.0012) Z-scores for every unit that ALP increased ($P = 0.0302$) or that HAZ would shift to the right by 0.0002 (0.0001) Z-scores for every 1% increase of OA (range 7–15%) in combination with every unit that ALP increased ($P = 0.0359$). This model also predicted that WAZ would shift to the left by 0.1221 (0.0582) Z-scores for every 1% that OA increased ($P = 0.0378$).

The model for DHA in plasma PC predicted the following: For every 1% that DHA (range: 2.22–7.68% in plasma PC) increased ($P = 0.0415$), WAZ would shift to the right by 0.1393 (0.0677) Z-scores. If ALP increased by one unit ($P = 0.0094$), WAZ would shift to the right by 0.0013 (0.0005) Z-scores. When DHA increased by 1% in combination with

Table 4. Fatty acid composition of plasma phosphatidylcholine (PC) in children under 6 years of age with low and normal anthropometric indices (NCHS) at baseline, 6 months intervention and 12 months intervention (mean (SD))

	Undernourished						Controls					
	Baseline		6 months		12 months		Baseline		6 months		12 months	
Fatty acids	*n* = 52		*n* = 52		*n* = 43		*n* = 33		*n* = 50		*n* = 38	
14:0	0.13	(0.06)	0.11	(0.05)	0.14*	(0.06)	0.15	(0.08)	0.13	(0.05)	0.16	(0.05)
16:0	26.85	(3.06)	25.85	(1.94)	27.92*	(1.76)	26.19	(2.43)	26.01	(1.74)	28.37	(1.48)
16:1ω7	0.28	(0.22)	0.28	(0.21)	0.28	(0.11)	0.24	(0.19)	0.25	(0.16)	0.35	(0.15)
18:0	16.96	(2.04)	16.84	(1.27)	17.50	(1.14)	16.55	(1.88)	16.93	(1.12)	17.50	(1.04)
18:1ω9 (OA)	12.08*	(2.44)	10.73	(1.50)	10.10	(1.16)	10.73	(1.55)	10.49	(1.37)	10.13	(1.13)
18:2ω6 (LA)	21.79	(4.37)	22.55	(3.14)	20.07	(3.22)	23.02	(3.62)	22.91	(3.11)	21.82	(2.29)
18:3ω6 (GLA)	Trace		Trace		Trace		Trace		Trace		Trace	
18:3ω3 (ALA)	0.14	(0.11)	0.25	(0.04)	Trace		0.11	(0.08)	0.27	(0.05)	Trace	
20:3ω9 (ETA)	0.54	(0.19)	0.56*	(0.16)	0.57	(0.20)	0.52	(0.18)	0.52	(0.20)	0.62	(0.36)
20:3ω6 (DGLA)	2.81	(0.72)	2.94	(0.64)	2.74	(0.63)	2.93	(0.78)	2.92	(0.67)	2.74	(0.52)
20:4ω6 (AA)	12.47	(2.29)	13.12	(0.98)	12.19	(1.81)	13.34	(2.41)	13.14	(1.79)	12.01	(1.59)
20:5ω3 (EPA)	0.35	(0.21)	0.58	(0.60)	0.43	(0.46)	0.30	(0.17)	0.43	(0.32)	0.32	(0.18)
22:4ω6 (DTA)	0.61	(0.30)	0.58	(0.19)	0.50	(0.13)	0.64	(0.33)	0.65	(0.28)	0.51	(0.14)
22:5ω3 (DPA)	0.85	(0.34)	0.78	(0.25)	0.67*	(0.34)	0.83	(0.28)	0.82	(0.29)	0.81	(0.28)
22:6ω3 (DHA)	4.11	(1.53)	4.80	(1.10)	4.79	(1.09)	4.42	(1.29)	4.50	(1.28)	4.54	(1.13)
post Δ5ω6	13.08	(2.29)	13.70	(2.04)	12.69	(1.86)	13.98	(2.54)	13.79	(1.81)	12.52	(1.64)
post Δ5ω3	5.31	(1.80)	6.16	(1.36)	5.89	(1.46)	5.54	(1.50)	5.76	(1.59)	5.68	(1.40)
Σ ω6	37.71*	(4.65)	39.22	(2.30)	37.51	(2.17)	39.97	(2.56)	39.64	(2.27)	37.12	(1.71)
Σ ω3	5.45	(1.80)	6.41	(1.36)	5.98	(1.46)	5.65	(1.50)	6.03	(1.58)	5.76	(1.40)
Σ (ω6 + ω3)	43.16*	(5.15)	45.63	(2.41)	43.49	(1.76)	45.62	(3.01)	45.67	(2.47)	42.88	(1.80)

Significantly different from controls: *$P \leq 0.05$. Trace ≤ 0.09.

Table 5. The effect of fish supplementation on the mean (SD) percentage change of plasma phosphatidylcholine (PC) fatty acids of undernourished children under 6 years of age after 0–6 months, after 6–12 months and after 0–12 months compared with unsupplemented controls

	Undernourished						Controls					
	0–6 months		6–12 months		0–12 months		0–6 months		6–12 months		0–12 months	
Fatty acids	*n* = 38		*n* = 38		*n* = 38		*n* = 24		*n* = 24		*n* = 24	
14:0	–0.03	(0.07)	0.03	(0.08)	0.00	(0.09)	–0.03	(0.09)	0.02	(0.08)	–0.01	(0.10)
16:0	–0.16	(3.32)	2.43	(1.72)	1.27	(3.79)	–0.40	(2.68)	2.36	(2.70)	1.96	(2.90)
16:1ω7	–0.00	(0.23)	0.04	(0.16)	0.03	(0.20)	0.03	(0.23)	0.05	(0.18)	0.09	(0.15)
18:0	–0.03	(2.02)	0.43	(1.17)	0.40	(2.02)	0.42	(2.06)	0.41	(1.41)	0.83	(2.07)
18:1ω9 (OA)	–1.26	(2.31)	–0.35	(1.40)	–1.61	(2.32)	–0.21	(2.12)	–0.48	(1.57)	–0.69	(2.15)
18:2ω6 (LA)	–0.04	(4.31)	–0.28	(2.30)	–0.31	(4.28)	–0.40	(4.46)	–0.85	(4.06)	–1.25	(3.78)
18:3ω6 (GLA)	–0.01	(0.07)	–0.00	(0.04)	–0.02	(0.07)	–0.04	(0.06)	0.03	(0.05)	–0.01	(0.07)
18:3ω3 (ALA)	0.12	(0.10)	–0.16	(0.08)	–0.05	(0.12)	0.16	(0.09)	–0.19	(0.09)	–0.03	(0.09)
20:3ω9 (ETA)	0.01	(0.21)	0.01	(0.24)	0.02	(0.27)	–0.03	(0.23)	0.07	(0.19)	0.03	(0.24)
20:3ω6 (DGLA)	0.10	(0.85)	–0.23	(0.71)	–0.13	(0.86)	0.02	(1.01)	–0.30	(0.75)	–0.29	(0.63)
20:4ω6 (AA)	1.01	(2.15)	–1.25	(1.66)	–0.24	(2.20)	0.40	(2.17)	–1.06	(1.79)	–0.66	(2.20)
20:5ω3 (EPA)	0.26*	(0.52)	–0.14	(0.74)	0.12	(0.49)	0.03	(0.23)	–0.06	(0.24)	–0.03	(0.25)
22:4ω6 (DTA)	–0.03	(0.30)	–0.09	(0.18)	–0.12	(0.32)	0.09	(0.43)	–0.16	(0.36)	–0.06	(0.28)
22:5ω3 (DPA)	0.00	(0.41)	–0.16*	(0.35)	–0.16	(0.44)	–0.03	(0.41)	0.02	(0.28)	–0.01	(0.36)
22:6ω3 (DHA)	1.05*	(1.54)	–0.27	(1.19)	0.77	(1.76)	–0.02	(1.48)	0.14	(1.07)	0.13	(1.54)
post Δ5ω6	0.98	(2.13)	–1.34	(1.71)	–0.36	(2.20)	0.50	(2.25)	–1.22	(1.79)	–0.72	(2.34)
post Δ5ω3	1.31*	(2.01)	–0.58	(1.78)	0.73	(2.25)	–0.02	(1.90)	0.11	(1.31)	0.09	(1.92)
Σ ω6	1.03	(4.41)	–1.84	(2.67)	–0.82	(4.57)	0.08	(3.54)	–2.35	(3.57)	–2.27	(3.02)
Σ ω3	1.43*	(2.00)	–0.74	(1.79)	0.69	(2.24)	0.14	(1.91)	–0.08	(1.33)	0.06	(1.92)
Σ (ω6 + ω3)	2.46	(4.99)	–2.59	(1.83)	–0.13	(5.22)	0.22	(3.96)	–2.43	(3.52)	–2.21	(3.57)

Significantly different from controls: $^{*}P \leq 0.05$.

every unit that ferritin increased ($P = 0.0290$), WAZ would shift to the right by 0.0017 (0.0008) Z-scores. For every unit that ferritin increased ($P = 0.0103$), WAZ would shift to the left by 0.0125 (0.0048) Z-scores. When DHA increased by 1% in combination with every unit that ALP increased ($P = 0.0283$), WAZ would shift to the left by 0.0002 (0.0001) Z-scores. Oleic acid was not associated with WHZ. DHA was not associated with either HAZ or WHZ.

Discussion

This study provides an insight into changes that occurred in the biochemistry and lipid metabolism of undernourished preschool children in Lebowa in response to a habitual diet that was supplemented with catfish and sunflower cooking oil. The results indicate that fish supplementation could play an important role in reducing growth retardation of preschool children. Undernourished children are particularly at risk of developing degenerative diseases with rising affluence.[37] This risk of increased morbidity can, however, be prevented by improving the diets and lifestyles of such children.

Weight-for-age Z-scores improved more in the supplemented undernourished children during the 0–6 months period compared with that of the control children, which questions compliance to the fish supplement during the last 6 months of this intervention study. Possible explanations are seasonal growth changes,[38] or that the supplemented undernourished children became tired of eating the fish for longer than 6 months. However, significant increases in the ferritin concentrations of the supplemented undernourished children compared with those of the control children during the 6–12 months period, suggests that ferritin increased in addition to any seasonal changes. The increased ferritin levels in both groups after 12 months may be an intervention effect, as mothers/guardians could have been influenced to give their children more food, viz. both groups of mothers/guardians received the same nutritional counselling during the intervention period of 12 months.

Another reason for ferritin increases in both groups could be underlying infections.[39] It is also possible that the undernourished children reached a new plateau of growth in weight after 6 months of supplementation, as growth is known to be an oscillatory process.[40] DHA incorporation in the plasma PC of the undernourished children also reached a plateau after 6 months of fish supplementation and remained at these levels for the remaining 6 months. If the children did not eat the fish during the last 6 months of this intervention study, lower plasma PC DHA levels would have been expected at the 12 month period.[41] The results of this study therefore suggest compliance to the fish diet.

Significant improvement of HAZ (Fig. 1) after 1 year of intervention could be attributed to the increased consumption of animal proteins that was provided by the catfish,[3,42] since a previous survey indicated an inadequate intake of animal proteins that was associated with stunting.[14] Animal proteins increase the bioavailability of micronutrients such as zinc and iron, which are normally poorly absorbed with diets that have a high fibre and phytate content.[43] This makes undernourished children with higher requirements for nutrients especially vulnerable to stunting when they are exposed to marginal dietary intakes.[44] The improvement (increased change) in WAZ seen after 6 months of intervention was no longer significant after 12 months of intervention, which may indicate that the undernourished children received insufficient supplementation for sustained growth.[45]

Significant increases in the concentration of ferritin and content of long-chain ω3 fatty acids with concomitant decreases in ALP and OA of the undernourished children upon intervention clearly shows the complexity of the inter-

Table 6. Estimated regression coefficients of oleic acid (18:1ω9; OA) and of docosahexaenoic acid (22:6ω3; DHA) in plasma phosphatidylcholine (PC) on growth outcomes adjusted for the experimental design, ferritin and ALP in children under 6 years of age

Growth outcome	Coefficient	Standard error	*P*
HAZ intercept	–1.4142	0.8069	0.0822
18:1ω9	–0.1222	0.0742	0.1022
Ferritin	0.0070	0.0081	0.3920
ALP	–0.0026	0.0012	0.0302
18:1ω9 + Ferritin	–0.0006	0.0008	0.4530
18:1ω9 + ALP	0.0002	0.0001	0.0359
HAZ intercept	–2.7551	0.5081	0.0000
22:6ω3	0.0659	0.0846	0.4371
Ferritin	–0.0097	0.0061	0.1133
ALP	0.0003	0.0006	0.5993
22:6ω3 + Ferritin	0.0017	0.0010	0.0898
22:6ω3 + ALP	–0.0001	0.0001	0.3294
WAZ intercept	–1.4475	0.6367	0.0246
18:1ω9	–0.1221	0.0582	0.0378
Ferritin	0.0007	0.0066	0.9155
ALP	–0.0012	0.0009	0.2110
18:1ω9 + Ferritin	–0.0002	0.0006	0.7549
18:1ω9 + ALP	0.0001	0.0001	0.1024
WAZ intercept	–3.3228	0.3913	0.0000
22:6ω3	0.1393	0.0677	0.0415
Ferritin	–0.0125	0.0048	0.0103
ALP	0.0013	0.0005	0.0094
22:6ω3 + Ferritin	0.0017	0.0008	0.0290
22:6ω3 + ALP	–0.0002	0.0001	0.0283
WHZ intercept	–0.5289	0.8149	0.5176
18:1ω9	–0.0861	0.0750	0.2534
Ferritin	–0.0039	0.0083	0.6356
ALP	–0.0003	0.0012	0.7970
18:1ω9 + Ferritin	0.0001	0.0001	0.8873
18:1ω9 + ALP	0.0001	0.0001	0.6351
WHZ intercept	–1.8096	0.5119	0.0006
22:6ω3	0.0745	0.0874	0.3957
Ferritin	–0.0106	0.0062	0.0888
ALP	0.0008	0.0006	0.2202
22:6ω3 + Ferritin	0.0012	0.0010	0.2395
22:6ω3 + ALP	–0.0001	0.0001	0.2992

ALP, alkaline phosphatase; HC, head circumference; HAZ, height-for-age Z-score; WAZ, weight-for-age Z-score; WHZ, weight-for-height Z-score.

actions that occurred when growth was promoted by the nutritious fish. The meaning of a single measurement in isolation can accordingly be very difficult to interpret. Because linear and ponderal growth improved with fish supplementation, other factors besides energy and proteins need to be considered.[6] The increased intake of iron (as measured by changes in ferritin concentration) by the supplemented undernourished children may also have contributed to the growth improvement.[27,28,46] Although the supplemented fish had no effect on the plasma zinc concentration of the undernourished children,[4] an indirect effect on growth cannot be excluded. A low prevalence of vitamin A deficiency in both groups at baseline (20–21%), has important epidemiological implications which indicate that inference from provincial levels cannot be extrapolated to specific areas.[35]

A prominent feature of the undernourished children at baseline was their high OA content in plasma PC, which was reduced to the levels of the control children with intervention. Fish supplementation resulted in a decrease of OA that was compensated for by significant increases in plasma PC ω3 fatty acids after 0–6 months of intervention (Table 5). This was mainly due to increases ($P \leq 0.05$) of EPA and DHA and indicates dietary compliance to the supplemented fish.[47] Plasma PC DHA reflected fish intake better than EPA in this study, in contrast to the findings of Bønaa *et al*.,[48] probably due to the higher DHA content of catfish, which makes DHA a better marker for dietary compliance to long-term fish intake.[49]

The most significant finding of this study was the demonstration of significant associations between WAZ shifts to the right and OA decreases in plasma PC ($P = 0.0378$), suggesting that increased levels of plasma PC OA during undernutrition may have a negative effect on weight. This confirms the inverse association that we observed between plasma phospholipid OA levels and weight increases of growing rats.[50] Because the mixed-effects models take increases in ALP and ferritin into account, OA could be used as a marker to predict WAZ status.

The mechanism of the interrelationship between OA and growth is unknown. Results from this study suggest that OA seems to be replaced by DHA in plasma PC. DHA was positively associated with WAZ, which was 107-fold greater than the positive association with ALP, and 82-fold greater than the combined positive association with ferritin. The DHA status of plasma PC in combination with ferritin therefore appears to be a powerful indicator that predicts WAZ status of children. The physiological significance of this association is not clear. The possibility of DHA's effect on WAZ could be mediated via EPA through prostaglandins of the 3-series, because of dietary DHA's ability to retroconvert to EPA.[51] As neither OA nor DHA was associated with HAZ in plasma PC, it can be concluded that these fatty acids had no effect on bone formation, because both are expected to be intracellular messengers of calcium mobilization.[52] Unlike a negative effect of a DHA-supplemented formula on growth of preterm infants and unaffected AA status,[53] the results of this study suggest that DHA had a positive effect on growth (WAZ that shifted to the right as DHA composition in plasma PC increased) and no effect on AA status. The catfish as well as the sunflower cooking oil (±65% ω6 fatty acids) in this study actually provided more ω6 than ω3 fatty acids (Table 1), which could have counteracted the inhibiting effect of the ω3 fatty acids. This might explain why the metabolism of AA was not affected.

Fish oil, rich in EPA and DHA, has been shown to improve calcium status in rats.[54] Children who consume a vegetarian diet are likely to have low calcium intakes, because a vegetarian diet typically tends to be low in calcium, zinc and iron.[55] Fish supplementation may therefore improve calcium availability of shorter children so that bone formation can occur.[56] Increases in the number and activity of ALP-producing cells of bone that stimulate bone growth are known to increase the levels of serum ALP in normally growing children,[57] which agrees with the results of this study in which ALP activity tended to increase in the supplemented undernourished children during the first 6 months of intervention that resulted in growth.

The sizeable intake of fish-derived micronutrients (iron and zinc), proteins and energy,[58] which may affect growth independently of fatty acid intake,[59] as well as shortcomings in the study design, undermine the ability to attribute growth increments to any specific nutrients that were consumed. An increase of fish protein, a major source of carnitine, could also explain the growth improvement, as carnitine facilitates the entry of long-chain fatty acids into mitochondria to be used in energy-generating processes that include muscle formation and bone growth.[60] An increase in ALP, a marker of bone formation, suggests that bone growth was promoted, as HAZ improved significantly in the supplemented undernourished children.[29]

Although regression to the mean effects are likely to occur where the children studied belonged to extreme anthropometric groups,[61] the use of a control group and intervention before baseline intervention commenced, minimized the effect of regression to the mean.[62] Natural regression might have been accelerated in the undernourished group that was supplemented and may question the response in relation to the control group that was not supplemented,[63] for consent to venipuncture also constitutes an intervention. Complete control of regression would, however, be impossible, as it would be unethical to use a control group of undernourished children who were denied supplementation.[61]

A supplement of 43 g catfish (*Clarias gariepinus*) and 7.5 g sunflower cooking oil per day to undernourished children (WAZ < –2 SD) resulted in significant improvements in WAZ after 6 months of intervention, and in HAZ after 12 months of intervention. Because the improved WAZ was only significant after the first 6 months of intervention, the fish supplement and cooking oil that supplied additional energy (7% E) and nutrients may have been adequate to promote growth of the undernourished children, but was inadequate to maintain this growth during the second 6 months of intervention. The study shows that fish can be an ideal vehicle to supplement undernourished children with good food that provides, in addition to energy, animal proteins, ω6 and ω3 fatty acids as well as micronutrients (iron and zinc). Long-term solutions are, however, dependent on easily available aquaculture ventures that rely on community participation.

Acknowledgements. The authors thank Mr C de Wet Marais, Mrs MP Marais, Mr GP Engelbrecht, Mr AM Tichelaar, Ms ME van Stuijvenberg, Ms J van Wyk and Mr EAR Harmse for technical assistance, Sister RA Mathete for the use of her Mamabolo Clinic facilities, Mr Z Mangena for assistance as interpreter and J Modiba and W Monyepa (Nutritional Advisors) for recruiting the participants of the study. The valuable suggestions and critical evaluation of this manuscript by Dr M Weight are appreciated.

References

1. UNICEF. Policy Review: Strategy for improved nutrition of children and women in developing countries. New York: UNICEF, 1990; 5–36.
2. Sanders TAB, Purves R. An anthropometric and dietary assessment of the nutritional status of vegan preschool children. J Hum Nutr 1981; 35: 349–357.
3. Guang-Jun Y. The nutrient intakes of Chinese children and adolescents and their impact on growth and development. Asia Pacific J Clin Nutr 1995; 4: 13–18.
4. Walravens PA, Hambidge KM, Koepfer DM. Zinc supplementation in infants with a nutritional pattern of failure to thrive: a double-blind, controlled study. Pediatr 1989; 83: 532–538.
5. Hongo T, Suzuki T, Ohba T *et al*. Nutritional assessment of a group of Japanese elementary school children in Tokyo: with special emphasis on growth, anemia, and obesity. J Nutr Sci Vitaminol (Tokyo) 1992; 38: 177–196.
6. Angeles IT, Schultink WJ, Matulessi P, Gross R, Sastroamidjojo S. Decreased rate of stunting among anemic Indonesian preschool children through iron supplementation. Am J Clin Nutr 1993; 58: 1–4.
7. Jooste PL, Badenhorst CJ, Schutte CHJ *et al.* Endemic goitre among undernourished schoolchildren in eastern Caprivi, Namibia. S Afr Med J 1992; 81: 571–574.
8. Kirkwood BR, Ross DA, Arthur P *et al.* Effect of vitamin A supplementation on the growth of young children in northern Ghana. Am J Clin Nutr 1996; 63: 773–781.
9. Koletzko B. Trans fatty acids may impair biosynthesis of long-chain polyunsaturates and growth in man. Acta Paediatr 1992; 81: 302–306.
10. Salem N, Ward GR. Are ω3 fatty acids essential nutrients for mammals? World Rev Nutr Diet 1993; 72: 128–147.
11. Cameron N, Kgamphe JS, Leschner KF, Farrant PJ. Urban–rural differences in the growth of South African black children. Ann Hum Biol 1992; 19: 23–33.
12. Steyn NP, Nel HJ, Tichelaar HY *et al.* Malnutrition in Pedi preschool children, their siblings and caretakers. S Afr J Clin Nutr 1994; 7: 12–18.
13. Tichelaar HY, Steyn NP, Badenhorst CJ *et al.* Epidemiological evidence of impaired fatty acid status of undernourished rural primary school children in Lebowa. S Afr J Food Sci Nutr 1994; 6: 60–65.
14. Tichelaar HY, Steyn NP, Nel JH *et al.* Fatty acid and nutritional status of undernourished rural Pedi preschool children under 6 years of age in Lebowa. S Afr J Food Sci Nutr 1995; 7: 27–32.
15. Steyn NP, Badenhorst CJ, Nel JH, Jooste PL. The nutritional status of Pedi preschool children in two rural areas of Lebowa. S Afr J Food Sci Nutr 1992; 4: 24–28.
16. WHO. Measuring change in nutritional status. Geneva: WHO 1983.
17. Hamill PV, Drizd TA, Johnson CL, Reed RB, Roche AF, Moore WM. Physical growth: National Center for Health Statistics percentiles. Am J Clin Nutr 1979; 32: 607–629.
18. Frisancho AR. New norms of upper limb fat and muscle area for the assessment of nutritional status. Am J Clin Nutr 1981; 34: 2540–2545.
19. Cameron N. The measurement of human growth. London: Croom Helm, 1984.
20. Hoffman LC, Prinsloo JF, Casey NH, Theron J. Effects of five cooking methods on the proximate, fatty acid and mineral composition of fillets of the African sharptooth catfish, *Clarias gariepinus*. S Afr J Food Sci Nutr 1994; 6: 146–152.
21. Hoffman LC, Casey NH, Prinsloo JF. A further investigation into the fatty acid composition of the lipids of the African catfish (*Clarias gariepinus*). S Afr J Food Sci Nutr 1993; 5: 41–42.
22. Catignani GL, Bieri JG. Simultaneous determination of retinol and α-tocopherol in serum or plasma by liquid chromatography. Clin Chem 1983; 29: 708–712.
23. Benadé AJS, Fincham JE, Smuts CM *et al*. Plasma low density lipoprotein composition in relation to atherosclerosis in nutritionally defined Vervet monkeys. Atherosclerosis 1988; 74: 157–168.
24. Smuts CM, Tichelaar HY, van Jaarsveld PJ *et al.* The effect of iron fortification on the fatty acid composition of plasma and erythrocyte membranes in primary school children with and without iron deficiency. Prostaglandins Leukot Essent Fatty Acids 1994; 51: 277–285.
25. Shapiro SS, Wilk MB. An analysis of variance test for normality (complete samples). Biometrika 1965; 52: 591–611.
26. Lindstrom MJ, Bates DM. Newton-Raphson and EM algorithms for linear mixed-effects models for repeated-measures data. J Am Stat Assoc 1988; 83: 1014–1022.
27. Chwang L-C, Soemantri AG, Pollitt E. Iron supplementation and physical growth of rural Indonesian children. Am J Clin Nutr 1988; 47: 496–501.

28. Lawless JW, Latham MC, Stephenson LS, Kinoti SN, Pertet AM. Iron supplementation improves appetite and growth in anemic Kenyan primary school children. J Nutr 1994; 124: 645–654.
29. Wright NM, Renault J, Hollis B, Bell NH, Key LL. Effect of growth hormone on bone: bone mineral density, trabecular bone volume, and alkaline phosphatase improve or are restored in the dwarf rat treated with growth hormone. J Bone Miner Res 1995; 10: 127–131.
30. Monsen ER. The 10th edition of the Recommended Dietary Allowances: What's new in the 1989 RDAs? J Am Diet Assoc 1989; 89: 1748–1752.
31. Hoffman LC, Casey NH, Prinsloo JF. Fatty acid, amino acid and mineral contents of African sharptooth catfish (Clarias gariepinus) fillets. S Afr J Food Sci Nutr 1992; 4: 36–40.
32. Young DS. Implimentation of SI units for clinical laboratory data. Ann Intern Med 1987; 106: 114–129.
33. WHO. Iron deficiency: Indicators for assessment and strategies for prevention. WHO/NUT/96.12. Geneva: World Health Organization, 1999.
34. Yip R, Johnson C, Dallman PR. Age-related changes in laboratory values used in the diagnosis of anemia and iron deficiency. Am J Clin Nutr 1984; 39: 427–436.
35. South African Vitamin A Consultative Group (SAVACG). Children aged 6 to 71 months in South Africa, 1994: their anthropometric, vitamin A, and iron status. Medical Update. Isando: Vitamin Information Centre, 1996; 26 (Part I).
36. Meydani M. Vitamin E. Lancet 1995; 345: 170–175.
37. Scrimshaw NS. The new paradigm of public health nutrition. Am J Public Health 1995; 85: 622–624.
38. Panter-Brick C. Seasonal growth patterns in rural Nepali children. Ann Hum Biol 1997; 24: 1–18.
39. Elin RJ, Wolff SM, Finch CA. Effect of induced fever on serum iron and ferritin concentrations in man. Blood 1977; 49: 147–153.
40. Hermanussen M. The measurement of short-term growth. In: Tanner JM, ed. Auxology 88. Perspectives in the Science of Growth and Development. Nishimura: Smith-Gordon, 1989; 49–61.
41. Van Rooyen J, Swanevelder S, Morgenthal JC, Benadé AJS. Diet can manipulate the metabolism of EPA and GLA in erythrocyte membrane and plasma. Prostaglandins Leukot Essent Fatty Acids 1998; 59: 27–38.
42. Allen LH, Backstrand JR, Stanek EJ *et al*. The interactive effects of dietary quality on the growth and attained size of young Mexican children. Am J Clin Nutr 1992; 56: 353–364.
43. Neumann CG, Harrison GG. Onset and evolution of stunting in infants and children. Examples from the Human Nutrition Collaborative Research Support Program. Kenya and Egypt studies. Eur J Clin Nutr 1994; 48: S90–S102.
44. Walker SP, Powell CA, Grantham-McGregor SM. Dietary intakes and activity levels of stunted and non-stunted children in Kingston, Jamaica. Part 1. Dietary intakes. Eur J Clin Nutr 1990; 44: 527–534.
45. Golden MHN. Is complete catch-up possible for stunted malnourished children? Eur J Clin Nutr 1994; 48: S58–S71.
46. Kruger M, Badenhorst CJ, Mansvelt EPG, Laubscher JA, Benadé AJS. Effects of iron fortification in a school feeding scheme and anthelmintic therapy on the iron status and growth of six- to eight-year-old schoolchildren. Food Nutr Bull 1996; 17: 11–21.
47. Svensson B-G, Åkensson B, Nilsson A, Skerving S. Fatty acid composition of serum phosphatidylcholine in healthy subjects consuming varying amounts of fish. Eur J Clin Nutr 1993; 47: 132–140.
48. Bønaa KH, Bjerve KS, Nordøy A. Habitual fish consumption, plasma phospholipid fatty acids, and serum lipids: the Tromso Study. Am J Clin Nutr 1992; 55: 1126–1134.
49. Marckmann P, Lassen A, Haraldsdóttir J, Sandström B. Biomarkers of habitual fish intake in adipose tissue. Am J Clin Nutr 1995; 62: 956–959.
50. Tichelaar HY, Smuts CM, Gross R, Jooste PL, Faber M, Benadé AJS. The effect of dietary iron deficiency on the fatty acid composition of plasma and erythrocyte membrane phospholipids in the rat. Prostaglandins Leukot Essent Fatty Acids 1997; 56: 229–233.
51. Conquer JA, Holub BJ. Dietary docosahexaenoic acid as a source of eicosapentaenoic acid in vegetarians and omnivores. Lipids 1997; 32: 341–345.
52. Siafaka-Kapadai A, Hanahan DJ, Javors MA. Oleic acid-induced Ca^{2+} mobilization in human platelets: is oleic acid an intracellular messenger? J Lipid Mediat Cell Signal 1997; 15: 215–232.
53. Carlson SE, Werkman SH, Tollet EA. Effect of long-chain n-3 fatty acid supplementation on visual acuity and growth of preterm infants with and without bronchopulmonary dysplasia. Am J Clin Nutr 1996; 63: 687–697.
54. Kruger MC, Coetzer H, de Winter R, Claassen N. Eicosapentaenoic acid and docosahexaenoic acid supplementation increases calcium balance. Nutr Res 1995; 15: 211–219.
55. Jacobs C, Dwyer JT. Vegetarian children: appropriate and inappropriate diets. Am J Clin Nutr 1988; 48: 811–818.
56. Chusilp K, Somnasang P, Kirdpon W *et al*. Observations on the development of stunting in children of the Khon Kaen region of Thailand. Eur J Clin Nutr 1992; 46: 475–488.
57. Moss DW, Henderson A. Enzymes. In: Burtis CA, Ashwood ER, eds. Tietz Textbook of Clinical Chemistry. Philadelphia: WB Saunders Company, 1994; 735–896.
58. Hoffman LC, Prinsloo JF, Casey NH, Theron J. The anatomical heterogeneity in the proximate composition, amino acid, fatty acid and mineral concentrations of muscle of the African sharptooth catfish, Clarias gariepinus (Burchell). S Afr J Food Sci Nutr 1994; 6: 30–35.
59. Waterlow JC. Reflections on stunting. Int Child Health 1991; 2: 25–35.
60. Rebouche CJ. Carnitine function and requirements during the life cycle. FASEB J 1992; 6: 3379–3386.
61. Schofield WN. Treatment of malnutrition. Lancet 1995; 345: 787.
62. Yudkin PL, Stratton IM. How to deal with regression to the mean in intervention studies. Lancet 1996; 347: 241–243.
63. Johnston HJ, Jones M, Ridler-Dutton G, Spechler F, Stokes GS, Wyndham LE. Diet modification in lowering plasma cholesterol levels. Med J Aust 1995; 162: 524–526.