



## Changes in whole-blood PUFA and their predictors during recovery from severe acute malnutrition

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(Submitted 24 July 2015 – Final revision received 17 December 2015 – Accepted 4 February 2016 – First published online 21 March 2016)

### Abstract

Children with severe acute malnutrition (SAM) with complications require in-patient management including therapeutic feeding. Little attention has been given to the effects of these feeds on the essential fatty acid status of children with SAM. The objective of this study was to describe changes in the PUFA composition in whole blood in children with SAM during treatment and to determine predictors of change. This prospective study took place in a paediatric nutrition rehabilitation unit in Kampala, Uganda, and assessed whole-blood fatty acid composition of children with SAM at admission, transition, discharge and follow-up (8 and 16 weeks). ANCOVA was used to identify predictors of change in whole-blood PUFA. The study included 120 children with SAM and twenty-nine healthy control children of similar age and sex. Among the SAM children, 38% were female and 64% had oedema. Whole-blood *n*-6 PUFA proportions increased from admission to follow-up, except for arachidonic acid, which decreased by 0.79 (95% CI 0.46, 1.12) fatty acid percentage (FA%) from admission to transition and 0.10 (95% CI 0.23, 0.44) FA% at discharge. *n*-3 Long-chain (LC) PUFA decreased by 0.21 (95% CI 0.03, 0.40) FA% at discharge and 0.22 (95% CI 0.01, 0.42) FA% at 8 weeks of follow-up. This decrease was greater in children from families with recent fish intake and those with nasogastric tube feeding. Current therapeutic feeds do not correct whole-blood levels of LCPUFA, particularly *n*-3 LCPUFA, in children with SAM. Increased attention is needed to the contents of *n*-3 LCPUFA in therapeutic feeds.

**Key words:** PUFA: Severe acute malnutrition: Recovery: Children

Severe acute malnutrition (SAM) is a major health problem in children in low-income and middle-income countries<sup>(1)</sup>. Children with complicated SAM require in-patient management of presenting complications while therapeutic feeds are being initiated. The standard nutritional treatment is milk-based formulas, such as starter F-75, initiated as soon as possible after admission<sup>(2)</sup>. F-75 is designed to restore physiological and metabolic functions and electrolyte balance and to maintain body weight. After stabilisation, the feed is gradually changed to a formula (F-100) that provides sufficient energy and protein to support rapid catch-up growth or directly to ready-to-use therapeutic feed (RUTF)<sup>(3)</sup>. RUTF is typically continued after discharge and given at regular follow-ups until recovery. An average period of 8 weeks of nutritional rehabilitation is considered adequate for recovery<sup>(4)</sup>, after which good feeding practices are encouraged. During rehabilitation of children with SAM, emphasis is put on adequate intake of energy and protein,

as well as on micronutrients, whereas PUFA intake is given less attention.

Fatty acids are good sources of energy. However, effects of *n*-3 and *n*-6 fatty acids go far beyond their role as fuels, as they are essential nutrients with important physiological functions. The *n*-6 PUFA linoleic acid (LA, 18:2*n*-6) is incorporated in skin ceramides, whereas arachidonic acid (AA, 20:4*n*-6) in cell membranes acts as a precursor of eicosanoid<sup>(5)</sup>. A deficiency in these fatty acids can therefore result in scaly skin, reduced growth and increased infections among others. The main function of *n*-3 PUFA is exerted via DHA (22:6*n*-3) in central nervous system membranes, where they have a structural role, as well as a role in functional processes that are not completely clarified<sup>(5)</sup>. A lack of essential *n*-6 and *n*-3 fatty acids will result in increased production of Mead acid (20:3*n*-9) and *n*-6 docosapentanoic acid (DPA) (22:5*n*-6), the metabolic counter parts of AA and DHA, respectively, that do not fulfil

**Abbreviations:** AA, arachidonic acid; ALA,  $\alpha$ -linolenic acid; DPA, docosapentanoic acid; %E, percentage of energy intake; FA%, fatty acid percentage; LA, linoleic acid; LCPUFA, long-chain PUFA; NG, nasogastric; RUTF, ready-to-use therapeutic feed; SAM, severe acute malnutrition.

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their essential functions. These two fatty acids may therefore indicate *n*-3 and *n*-6 deficiency, sometimes reported as ratios with their respective *n*-6 and *n*-3 fatty acid counterparts.

A number of studies show associations between SAM and a low *n*-3 and *n*-6 PUFA status<sup>(6)</sup>. However, limited data exist on the effect of recovery from SAM on LA,  $\alpha$ -linolenic acid (ALA, 18:3*n*-3) and long-chain (LC) PUFA status (LCPUFA defined as PUFA with >18 carbon atoms and >3 double bonds). A study of Nigerian hospitalised children with SAM found significant increases in LA values and decreases in AA values after 2 weeks of treatment with a therapeutic high-energy and high-protein diet with maize and milk, eggs, beans, vegetables, fish, meat, vegetable and palm oil<sup>(7)</sup>. ALA values did not change during hospitalisation, but the DHA content of plasma phospholipids increased significantly<sup>(7)</sup>. A recent study of Kenyan children with SAM found that *n*-3 and *n*-6 PUFA requirements of children with SAM were not met by the current formulation of RUTF or by a RUTF with elevated levels of ALA but without *n*-3 LCPUFA<sup>(8)</sup>. Another study of Malawian children with SAM compared standard RUTF with a novel RUTF with relatively less LA and more oleic acid (HO-RUTF) to children and found positive changes in plasma phospholipid DHA and EPA (20:5*n*-3) in the HO-RUTF group compared with the RUTF group<sup>(9)</sup>. No studies have investigated the effects of F-75 and F-100 during in-hospital treatment of SAM on children's fatty acid status.

This prospective study investigates changes in whole-blood PUFA in Ugandan children with SAM from hospital admission to transition, discharge and follow-up after 8 and 16 weeks. We furthermore describe associations between changes in whole-blood PUFA values and clinical conditions during treatment.

## Methods

### Study design, site and standard treatment

This prospective observational study followed up children with in-hospital treatment of SAM between October 2012 and June 2013.

Mwanamugimu Nutrition Unit at Mulago Hospital is the main treatment centre for children with complicated SAM in Uganda. Children received standard in-patient treatment according to the Ugandan National Protocol for the *Integrated Management of Acute Malnutrition*<sup>(10)</sup>, based on recommendations from the World Health Organization<sup>(11)</sup>. Children were given therapeutic diets, F-75 and F-100 (Nutraset) and empirical parenteral antibiotics, usually ampicillin and gentamicin. Small, frequent feeds of F-75 were given at a rate of 100–130 ml/kg body weight per d, either orally or by the nasogastric (NG) tube. After stabilisation, the children were changed from F-75 to catch-up milk-based formula F-100<sup>(2)</sup>. After about 2–3 d of transition, the volume of F-100 was gradually increased to a maximum of 220 ml/kg per d. When the children were clinically well, had regained appetite and had no oedema, they were discharged for out-patient treatment with RUTF. The children were then followed up in the hospital's out-patient clinic every 2 weeks until recovery from SAM, as indicated by 20% weight gain, no oedema for two consecutive follow-up visits and increasing

mid-upper arm circumference (MUAC) according to recommendations at the time of study<sup>(12)</sup>. Biological mothers were offered routine counselling and testing for HIV antibodies according to WHO guidelines, using Determine™ rapid test as first-line<sup>(13)</sup>. If the mother was infected or absent, the child was tested.

A few local modifications of the protocol were practiced at the time of the study: if a child had diarrhoea and milk intolerance was suspected, the diet was fully or partially replaced by rice porridge for some days. Furthermore, most children were given a daily serving of maize-soya-porridge during and after the transition phase, and these maize-soya-based products' composition varied. In preparation for discharge, all mothers participated in cooking classes preparing energy-dense complementary foods using locally available ingredients, which was also served to the children.

### Selection criteria

Children 6–59 months of age with SAM, defined as weight-for-height z-score (WHZ) < -3 of the WHO Growth Standard, MUAC < 11.5 cm or bilateral pitting oedema, and who lived near the hospital were eligible for the study. Those who were in shock at admission, had severe respiratory distress requiring resuscitation, Hb concentration < 4 g/dl, body weight < 4.5 kg or significant disability such as cerebral palsy were excluded. Inclusion was not possible during weekends or public holidays.

A control group of healthy well-nourished children was recruited among siblings of the admitted children and children of hospital staff. The inclusion criteria for these children were being apparently healthy, aged 6–59 months old and having a WHZ > -1.

### Sample size

The sample size of 120 was based on the main outcome, plasma phosphate<sup>(14)</sup>. With 120 SAM children, we would have 80% power to detect a difference of 0.5 SD or more in any normally distributed variable between two groups of equal size, with a 5% significance level. Similarly, with a control group of thirty children, we would have 80% power to detect differences of 0.6 SD or more between SAM and control.

### Ethical issues

Approval of the study was obtained from the Makerere University School of Medicine Research Ethics Committee, and Uganda National Council of Science and Technology, and a consultative approval was obtained from the Danish National Board of Research Ethics. Informed consent was provided by parents or guardians of the children before enrolment into the study. Regardless of participation in the study, all children admitted with SAM received similar routine medical and nutritional treatment.

### Data collection

A questionnaire was used to obtain socio-demographic information, as well as feeding and medical history. Physical



examination including vital signs and checking of bilateral pitting oedema was performed. Anthropometric measurements involved taking MUAC using a tape (MUAC Child Red/Pac-50) and measurement of length to the nearest 1 mm using an infant length board (Infant/Child ShorrBoard®). Body weight was measured daily to the nearest 100 g using a digital scale (Seca 813; Seca GmbH & Co.). Anthropometric *z*-scores were computed using WHO Growth Standards<sup>(15)</sup> adjusting for the fact that length was measured even in children <2 years, and using the lowest weight recorded during admission, to determine weight free from oedema.

### Blood sampling

On admission, Hb was measured in venous blood collected in heparinized vacutainer tubes (Becton Dickinson) using a Hb 201+ (HemoCue). At admission, transition and discharge, approximately 40 µl of heparinized whole blood was applied to a chromatography paper strip (grade 3MM; Whatman), from which potential lipid contaminants had previously been removed with chloroform and methanol, and treated with antioxidants (1000 µg deferoxamine and 50 µg butylated hydroxy toluene)<sup>(16)</sup> so that approximately 1 cm<sup>2</sup> of the strip was saturated with blood. At 8 and 16 weeks of follow-up, blood samples were obtained by finger-prick and applied to antioxidant-treated chromatography paper strip. Results from analysis of dried blood spots collected from fingertips have been compared with venous blood samples and found to give substantially identical information<sup>(17)</sup>. The blood spots were allowed to dry completely at room temperature and were then stored in a sealed container (polypropylene 'ziplock' bag) in a refrigerator for up to 2 months<sup>(18)</sup> until they were shipped to University of Waterloo, Department of Kinesiology, Canada, for fatty acid analysis.

Whole-blood spots were chosen as the method of sample preparation because of the simplified method for blood collection, storage and shipment to the analytical laboratory where samples are easily processed for analysis<sup>(17)</sup>. Furthermore, whole-blood fatty acid analysis includes all lipid fractions and is most representative of the body fatty acid status, when compared with fatty acids in plasma, blood cells such as erythrocytes and platelets<sup>(19,20)</sup>.

Plasma was obtained from a vacutainer with citrate (Cell-Preparation Tube; Becton Dickinson) by centrifugation at 1300–2200 *g* for 20 min, and it was stored at –80°C until shipped on dry ice to the University of Copenhagen, Department of Nutrition, Exercise and Sports, Denmark, where plasma level of C-reactive protein (CRP) was measured using the high-sensitivity kit on a ABX Pentra 400 (Horiba, no. A11A01611 and A11A01696).

### Fatty acid analysis

Fatty acid methyl esters were prepared from the whole-blood spots by direct trans-esterification, and they were analysed by high-throughput GLC<sup>(21,22)</sup>. Briefly, the whole-blood spot was directly trans-esterified with the addition of 1 ml of 14% boron trifluoride in methanol (Pierce Chemicals), 300 µl of hexane and

3 µg of an internal standard (22:3 *n*-3 ethyl ester; Nu-Chek Prep) and heating at 95°C for 1 h<sup>(22)</sup>. Samples were allowed to cool to room temperature, after which water and hexane were added (1 ml each). The samples were vortexed for 1 min, and then centrifuged for 5 min at 3000 rpm to separate the organic and aqueous phases. The organic upper hexane layer containing fatty acid methyl esters was collected, dried under a stream of N and reconstituted in hexane. Fatty acid methyl esters were analysed on a Varian 3900 gas chromatograph equipped with a capillary column of 15 m × 0.10 mm i.d. × 0.10 µm thick film of nitroterephthalic acid-modified polyethylene glycol (DB-FFAP from J & W Scientific, Agilent Technologies) and hydrogen as the carrier gas<sup>(22)</sup>. Samples (1 µl) were introduced by a Varian CP-8400 autosampler into the 250°C injector with a split ratio of 100:1. The initial temperature was 150°C, which was held for 0.25 min, followed by a 35°C/min ramp to 200°C, an 8°C/min ramp to 225°C, where it was kept for 3.2 min, and then an 80°C/min ramp up to 245°C, with a 15-min hold at the end<sup>(22)</sup>. The flame ionisation detector temperature was 300°C, with air and N make-up gas flow rates of 300 and 25 ml/min, respectively, and a sampling frequency of 80 Hz. Peaks were identified (thirty-four fatty acids) by comparing retention times with an external mixed standard sample (GLC-462, Nu-Chek Prep Inc.). Concentrations of individual fatty acids were determined by comparing peak areas to the response of the known concentration of the internal standard. Individual fatty acids were also expressed as the weight percentage of total fatty acids (FA%). Unknown peaks were included in the calculations for weight percentage, and for each sample the identified fatty acids accounted for over 95% of the total fatty acid concentration. Various sums and ratios of fatty acids were calculated from the weight percentage fatty acid data.

### Statistics

Double data entry was done into Epidata, and analysis was performed using Stata 12 (StataCorp LP). *t* Test and  $\chi^2$  tests were used to test for differences in means and proportions between children with SAM and controls. Paired *t* test was used to analyse change in fatty acid values at different times during follow-up. ANCOVA in the changes of mean values from admission was done to identify predictors of changes in PUFA with adjustment for age and sex. *P*-values <0.05 were considered statistically significant.

### Results

The study recruited 120 children with a median age of 15.9 months. In all, 38% were female, 64% had oedema and 19% were HIV infected (Table 1). Control children and the children with SAM were similar in age and sex. Other data from the same children have been published previously<sup>(23,24)</sup>.

The median duration of hospital admission was 19 d. At admission, blood samples were obtained from 108 of the 120 recruited children with SAM. Before transition, eight children died, five self-discharged and one was excluded because of very low blood Hb, leaving 106 children going through

**Table 1.** Baseline characteristics of children with severe acute malnutrition (SAM) and healthy controls\* (Medians and 25, 75 percentiles; numbers and percentages; mean values and standard deviations)

|   | Children with SAM (n 120) |     | Control children (n 29) |     | P      |
|---|---------------------------|-----|-------------------------|-----|--------|
|   | n                         | %   | n                       | %   |        |
| Age (months)                                      |                           |     |                         |     | 0.194  |
| Median  | 15.9                      |     | 16.3                    |     |        |
| 25; 75 percentiles                                | 12.6; 21.9                |     | 11.3; 24.6              |     |        |
| Sex   |                           |     |                         |     | 0.521  |
| Male  | 74                        | 62  | 16                      | 55  |        |
| Female  | 46                        | 38  | 13                      | 45  |        |
| Weight-for-height z-score†                        | -3.4                      | 1.4 | 0.8                     | 0.9 | <0.001 |
| Weight gain at discharge (g/kg per d)†            | 5.8                       | 3.2 | N/A                     |     |        |
| Weight gain at 8 of weeks follow-up (g/kg per d)† | 4.9                       | 2.3 | N/A                     |     |        |
| Height-for-age z-score†                           | -3.1                      | 1.5 | -0.9                    | 1.1 | <0.001 |
| Height gain at discharge (cm)†                    | -0.2                      | 1.6 | N/A                     |     |        |
| Height gain at 8 weeks of follow-up (cm)†         | 0.8                       | 1.4 | N/A                     |     |        |
| Mother's education                                |                           |     |                         |     | 0.410  |
| No school   | 6                         | 6   | 1                       | 4   |        |
| Primary school                                    | 42                        | 43  | 10                      | 35  |        |
| Secondary school                                  | 42                        | 43  | 14                      | 48  |        |
| More than secondary school                        | 7                         | 7   | 3                       | 10  |        |
| Mother's BMI (kg/m <sup>2</sup> )†                | 21.7                      | 2.7 | 22.0                    | 4.3 | 0.753  |
| Breast-feeding                                    |                           |     |                         |     | <0.001 |
| Breast-feeding                                    | 19                        | 17  | 15                      | 52  |        |
| Not breast-feeding                                | 92                        | 83  | 14                      | 48  |        |
| Fish served in household in last 2 weeks          |                           |     |                         |     | 0.072  |
| Yes   | 62                        | 62  | 21                      | 81  |        |
| No  | 38                        | 38  | 5                       | 19  |        |
| Hb (g/dl)†  | 9.0                       | 2.3 | 10.2                    | 1.5 | 0.015  |
| Serum CRP (mg/l)                                  |                           |     |                         |     | 0.006  |
| Median  | 19.0                      |     | 10.8                    |     |        |
| 25; 75 percentiles                                | 7.3; 36.7                 |     | 0.2; 2.9                |     |        |
| Oedema  |                           |     | N/A                     |     |        |
| Present   | 77                        | 64  |                         |     |        |
| Absent  | 43                        | 36  |                         |     |        |
| HIV status  |                           |     | -                       |     |        |
| Infected  | 20                        | 19  |                         |     |        |
| Not infected                                      | 84                        | 81  |                         |     |        |

CRP, C-reactive protein; -, data not available.

\* Numbers in categories may not sum up because of missing data. Data on rate of weight gain at discharge were available for eighty-two SAM children and on weight gain at 8 weeks of follow-up on sixty-six children. Data on height gain at discharge and follow-up were available on eighty-three and sixty-five SAM children, respectively. Data on mothers BMI were available for eighty-one SAM and seven control children. Similarly, data were available on Hb from 112 SAM and twenty-five control children, on CRP from eighty-three SAM and twenty control children and on HIV from 104 SAM children. † Test or  $\chi^2$  tests were used to compare children with SAM and healthy controls.

†Mean and standard deviation.

transition, and blood samples were obtained from seventy-six children. From transition to hospital discharge, nine children died and thirteen self-discharged or withdrew their consent to participate. Accordingly, eighty-three children were discharged, and blood samples were obtained from seventy-two children. At first follow-up 8 weeks after admission, sixty-six children turned up, and blood samples were obtained from forty-three children, whereas forty-nine children came for follow-up at 16 weeks after admission and blood samples were obtained from forty-four children.

The children with SAM presented with lower LCPUFA values at admission compared with healthy controls (Table 2). The mean values of most SFA were lower in children with SAM until 8 weeks of follow-up (for total SFA, 44.7 FA% at transition and 44.4 FA% at discharge *v.* 47.7 FA% for the control children, both  $P < 0.001$ ). The proportion of most MUFA remained higher in children with SAM throughout the follow-up period (for total MUFA, 31.0 FA% at admission, 33.5 FA% at transition, 30.7 FA% at discharge, 27.2 FA% at 8 weeks and 27.8 FA% at 16 weeks

follow-up *v.* 24.5 FA% for the controls,  $P < 0.001$  for all time periods except 8 weeks of follow-up, which was 0.001). The total values of *n*-6 PUFA in children with SAM increased to values similar to the controls at 8 weeks of follow-up, but it again decreased at 16 weeks of follow-up (20.0 FA% for SAM children *v.* 23.1 FA% for controls,  $P < 0.001$ ). At discharge, all individual *n*-6 PUFA became similar to values in the control children, except AA, which remained lower (4.42 FA% for SAM children compared with 5.95 FA% in controls,  $P < 0.001$ ). *n*-6 LCPUFA were similar to the controls by 8 weeks of follow-up. Individual and total *n*-3 PUFA values decreased after admission, apart from ALA, which was similar to healthy children throughout follow-up. The ratio of *n*-6:*n*-3 PUFA was lower in children with SAM than in the healthy controls at admission (7.9 *v.* 8.9,  $P = 0.043$ ), but it became higher in children with SAM at all times after initiation of the treatment. The Mead acid:AA ratio remained higher in children with SAM throughout the entire period of treatment compared with the control children.

**Table 2.** Fatty acid values during treatment of children with severe acute malnutrition (SAM) and healthy controls† (Mean values and standard deviations)

| Fatty acid                     | Children with SAM |      |                   |      |                  |      |                |      |                 |      |                         |      |
|--------------------------------|-------------------|------|-------------------|------|------------------|------|----------------|------|-----------------|------|-------------------------|------|
|                                | Admission (n 108) |      | Transition (n 76) |      | Discharge (n 72) |      | 8 weeks (n 43) |      | 16 weeks (n 44) |      | Control children (n 24) |      |
|                                | Mean              | SD   | Mean              | SD   | Mean             | SD   | Mean           | SD   | Mean            | SD   | Mean                    | SD   |
| <b>Saturated</b>               |                   |      |                   |      |                  |      |                |      |                 |      |                         |      |
| 12:0                           | 0.20*             | 0.20 | 0.24              | 0.26 | 0.19*            | 0.23 | 0.23           | 0.21 | 0.22*           | 0.19 | 0.37                    | 0.41 |
| 14:0                           | 1.41*             | 0.60 | 1.20**            | 0.51 | 1.09**           | 0.67 | 1.42           | 0.80 | 1.33*           | 0.67 | 1.84                    | 1.01 |
| 16:0                           | 28.8*             | 1.8  | 28.9**            | 1.9  | 27.5             | 1.9  | 27.1           | 1.6  | 27.7            | 1.8  | 27.4                    | 1.5  |
| 17:0                           | 0.34**            | 0.09 | 0.25**            | 0.07 | 0.23**           | 0.06 | 0.53           | 1.18 | 0.39            | 0.12 | 0.41                    | 0.10 |
| 18:0                           | 13.0              | 2.3  | 11.4**            | 2.1  | 12.0*            | 2.1  | 13.6           | 2.8  | 13.6            | 2.9  | 13.5                    | 1.3  |
| 20:0                           | 0.27**            | 0.06 | 0.29**            | 0.07 | 0.31*            | 0.05 | 0.38*          | 0.09 | 0.36            | 0.07 | 0.34                    | 0.06 |
| 22:0                           | 0.59**            | 0.16 | 0.55**            | 0.17 | 0.66**           | 0.15 | 0.97*          | 0.23 | 0.89            | 0.24 | 0.86                    | 0.17 |
| 23:0                           | 0.10**            | 0.05 | 0.09**            | 0.04 | 0.11**           | 0.03 | 0.15*          | 0.04 | 0.15            | 0.05 | 0.17                    | 0.05 |
| 24:0                           | 0.94**            | 0.33 | 0.83**            | 0.30 | 1.02**           | 0.39 | 1.50           | 0.37 | 1.49            | 0.41 | 1.44                    | 0.33 |
| SFA                            | 46.7              | 2.6  | 44.7**            | 3.0  | 44.4**           | 3.3  | 46.8           | 4.1  | 47.2            | 3.8  | 47.7                    | 1.6  |
| <b>Unsaturated</b>             |                   |      |                   |      |                  |      |                |      |                 |      |                         |      |
| 16:1n-7                        | 1.41              | 0.56 | 1.68              | 0.86 | 1.21             | 0.57 | 1.28           | 0.71 | 1.75            | 0.76 | 1.41                    | 0.56 |
| 18:1n-7                        | 2.10**            | 0.36 | 1.70              | 0.38 | 1.57             | 0.42 | 1.49*          | 0.27 | 1.59            | 0.29 | 1.70                    | 0.36 |
| 18:1n-9                        | 23.9**            | 3.1  | 27.9*             | 3.8  | 25.3**           | 3.2  | 21.6*          | 3.0  | 21.8**          | 2.9  | 19.0                    | 2.5  |
| 20:1n-9                        | 0.25*             | 0.44 | 0.24*             | 0.04 | 0.30             | 0.28 | 0.32**         | 0.05 | 0.27**          | 0.06 | 0.21                    | 0.45 |
| 22:1n-9                        | 0.96*             | 0.60 | 0.78              | 0.54 | 0.83             | 0.99 | 0.95**         | 0.36 | 0.87*           | 0.48 | 0.64                    | 0.18 |
| 24:1n-9                        | 1.19              | 0.34 | 1.06*             | 0.32 | 1.30             | 0.40 | 1.39           | 0.30 | 1.31            | 0.30 | 1.29                    | 0.27 |
| MUFA                           | 31.0**            | 3.3  | 33.5**            | 3.7  | 30.7**           | 3.7  | 27.2*          | 3.1  | 27.8**          | 3.0  | 24.5                    | 2.8  |
| Mead acid (20:3n-9)            | 0.14              | 0.10 | 0.15              | 0.15 | 0.19*            | 0.13 | 0.13*          | 0.06 | 0.15*           | 0.07 | 0.11                    | 0.06 |
| <b>n-6 PUFA</b>                |                   |      |                   |      |                  |      |                |      |                 |      |                         |      |
| LA (18:2n-6)                   | 10.4**            | 2.5  | 12.1**            | 2.0  | 13.7             | 2.5  | 13.9           | 3.0  | 12.4**          | 2.2  | 14.8                    | 2.2  |
| 18:3n-6                        | 0.07**            | 0.04 | 0.11              | 0.06 | 0.12             | 0.06 | 0.12           | 0.05 | 0.16*           | 0.08 | 0.12                    | 0.07 |
| 20:2n-6                        | 0.16*             | 0.04 | 0.13**            | 0.04 | 0.17             | 0.04 | 0.22*          | 0.06 | 0.18            | 0.04 | 0.18                    | 0.04 |
| 20:3n-6                        | 0.57**            | 0.13 | 0.58**            | 0.14 | 0.85             | 0.23 | 0.93           | 0.19 | 0.88            | 0.20 | 0.90                    | 0.18 |
| AA (20:4n-6)                   | 4.67**            | 1.07 | 3.54**            | 0.90 | 4.42**           | 1.07 | 5.09*          | 1.17 | 5.07*           | 1.47 | 5.95                    | 1.01 |
| 22:4n-6                        | 0.62*             | 0.20 | 0.54**            | 0.17 | 0.72             | 0.23 | 0.90           | 0.27 | 0.84            | 0.34 | 0.77                    | 0.33 |
| 22:5n-6                        | 0.26*             | 0.08 | 0.21**            | 0.06 | 0.28             | 0.09 | 0.32           | 0.09 | 0.31            | 0.11 | 0.31                    | 0.09 |
| n-6 LCPUFA                     | 6.2**             | 1.3  | 4.9**             | 1.1  | 6.3**            | 1.4  | 7.3            | 1.6  | 7.2             | 2.0  | 8.0                     | 1.4  |
| n-6 Fatty acids                | 16.8**            | 2.7  | 17.3**            | 2.5  | 20.3**           | 3.0  | 21.5           | 3.9  | 20.0**          | 2.6  | 23.1                    | 3.0  |
| <b>n-3 PUFA</b>                |                   |      |                   |      |                  |      |                |      |                 |      |                         |      |
| ALA (18:3n-3)                  | 0.17              | 0.07 | 0.14*             | 0.05 | 0.17             | 0.11 | 0.16           | 0.07 | 0.20            | 0.11 | 0.19                    | 0.11 |
| EPA (20:5n-3)                  | 0.18              | 0.08 | 0.11**            | 0.05 | 0.12**           | 0.05 | 0.12**         | 0.05 | 0.14**          | 0.06 | 0.20                    | 0.09 |
| 22:5n-3                        | 0.49              | 0.16 | 0.37**            | 0.13 | 0.41*            | 0.14 | 0.41*          | 0.10 | 0.43*           | 0.15 | 0.51                    | 0.11 |
| DHA (22:6n-3)                  | 1.40**            | 0.45 | 1.08**            | 0.42 | 1.22**           | 0.51 | 1.06**         | 0.46 | 1.14**          | 0.56 | 1.78                    | 0.54 |
| n-3 LCPUFA                     | 2.10*             | 0.57 | 1.59**            | 0.52 | 1.77**           | 0.64 | 1.64**         | 0.52 | 1.74**          | 0.71 | 2.53                    | 0.65 |
| n-3 Fatty acids                | 2.26*             | 0.58 | 1.73**            | 0.54 | 1.94**           | 0.68 | 1.80**         | 0.54 | 1.94**          | 0.71 | 2.72                    | 0.65 |
| PUFA                           | 19.0**            | 2.9  | 19.0**            | 2.8  | 22.2**           | 3.5  | 23.3*          | 4.1  | 21.9**          | 2.9  | 25.8                    | 3.2  |
| Total fatty acid concentration | 277               | 86   | 326*              | 118  | 307*             | 100  | 238            | 86   | 238             | 66   | 246                     | 52   |
| n-6:n-3 PUFA ratio             | 7.9*              | 2.3  | 10.8*             | 2.9  | 11.4*            | 3.3  | 12.9**         | 4.1  | 11.6*           | 4.3  | 8.9                     | 2.2  |
| Mead:AA ratio                  | 0.03*             | 0.02 | 0.05*             | 0.06 | 0.05**           | 0.03 | 0.03*          | 0.01 | 0.03*           | 0.01 | 0.02                    | 0.01 |
| EPA:AA ratio                   | 0.04              | 0.02 | 0.03              | 0.01 | 0.03*            | 0.01 | 0.03*          | 0.01 | 0.03*           | 0.01 | 0.04                    | 0.02 |
| 22:5n-6:DHA ratio              | 0.20              | 0.08 | 0.22              | 0.08 | 0.26*            | 0.10 | 0.34**         | 0.14 | 0.31**          | 0.13 | 0.19                    | 0.08 |

n, Number of children in whom information was available; LA, linoleic acid; AA, arachidonic acid; LCPUFA, long-chain PUFA; n-6:n-3 PUFA ratio, ratio of total n-6 PUFA:total n-3 PUFA.

\*  $P < 0.05$  and \*\*  $P < 0.001$  relative to control children. Paired *t* test analyses were done to test for differences in fatty acids between children with SAM at different times of follow-up and healthy controls.

† Data for individual fatty acids and fatty acid classes are given as weight percentage of total fatty acids (FA%) and total whole-blood fatty acid concentration is expressed as  $\mu\text{g}/100\mu\text{l}$ .

The mean changes in fatty acid values from admission to transition, discharge and follow-up are shown in Table 3. The mean of total SFA values decreased by 1.4 (95% CI 0.16, 2.19) FA% during transition ( $P = 0.001$ ) and by 1.6 (95% CI 0.18, 2.41) FA% at discharge ( $P < 0.001$ ). This decrease was initially matched by an increase in the mean of total MUFA by 1.53 (95% CI 0.43, 2.63) FA% at transition ( $P = 0.007$ ) but followed by a decrease at 8 and 16 weeks of follow-up (3.6; 95% CI 2.39, 4.88 FA% and 3.2; 95% CI 2.0, 4.47 FA%, respectively,  $P < 0.001$ ). Generally, values of n-6 PUFA increased by 16 weeks of follow-up, whereas n-3 PUFA decreased.

Fig. 1 shows changes in values of PUFA classes and selected PUFA during treatment and follow-up. LA values increased initially at transition and discharge, remained high at 8 weeks of follow-up and then decreased at 16 weeks. AA values reached a nadir at transition, followed by a small increase at discharge through to 16 weeks of follow-up. The percentage contribution of total n-3 PUFA dropped already at transition and never returned to admission values.

Predictors of changes in fatty acid values of children with SAM from admission to discharge adjusted for age and sex are summarised in Table 4. Weight gain during hospital treatment

**Table 3.** Mean changes in fatty acid values during treatment in children with severe acute malnutrition† (Regression coefficients (*b*) and 95 % confidence intervals)

| Fatty acid                                 | Transition ( <i>n</i> 76) |                | Discharge ( <i>n</i> 72) |                | 8 weeks ( <i>n</i> 43) |                | 16 weeks ( <i>n</i> 44) |                |
|--|---------------------------|----------------|--------------------------|----------------|------------------------|----------------|-------------------------|----------------|
|  | <i>b</i>                  | 95 % CI        | <i>b</i>                 | 95 % CI        | <i>b</i>               | 95 % CI        | <i>b</i>                | 95 % CI        |
| <b>Saturated</b>                           |                           |                |                          |                |                        |                |                         |                |
| 12:0                                       | 0.04                      | -0.04, 0.11    | 0.01                     | -0.07, 0.08    | 0.05                   | -0.04, 0.13    | 0.04                    | -0.04, 0.13    |
| 14:0                                       | -0.14                     | -0.34, 0.07    | -0.21                    | -0.42, -0.01*  | 0.08                   | -0.15, 0.31    | 0.02                    | -0.21, 0.25    |
| 16:0                                       | 0.00                      | -0.47, 0.47    | -1.07                    | -1.54, -0.60** | -1.35                  | -1.88, -0.83** | -0.93                   | -1.46, -0.41*  |
| 17:0                                       | -0.06                     | -0.16, 0.04    | -0.06                    | -0.16, 0.04    | 0.14                   | 0.03, 0.25*    | 0.05                    | -0.06, 0.16    |
| 18:0                                       | -1.14                     | -1.71, -0.57** | -0.68                    | -1.26, -0.11*  | 0.51                   | -0.14, 1.15    | 0.49                    | -0.15, 1.13    |
| 20:0                                       | 0.01                      | -0.00, 0.03    | 0.03                     | 0.02, 0.05**   | 0.08                   | 0.06, 0.10**   | 0.07                    | 0.05, 0.09**   |
| 22:0                                       | -0.01                     | -0.06, 0.04    | 0.07                     | 0.02, 0.12*    | 0.29                   | 0.23, 0.35**   | 0.24                    | 0.18, 0.30**   |
| 23:0                                       | -0.00                     | -0.02, 0.01    | 0.01                     | -0.00, 0.02    | 0.04                   | 0.03, 0.06**   | 0.05                    | 0.03, 0.06**   |
| 24:0                                       | -0.06                     | -0.16, 0.05    | 0.09                     | -0.01, 0.19    | 0.44                   | 0.33, 0.56**   | 0.44                    | 0.32, 0.55**   |
| SFA  | -1.40                     | -2.19, -0.61*  | -1.61                    | -2.41, -0.81** | 0.27                   | -0.62, 1.17    | 0.50                    | -0.40, 1.39    |
| <b>Unsaturated</b>                         |                           |                |                          |                |                        |                |                         |                |
| 16:1 <i>n</i> -7                           | -0.64                     | -0.83, -0.44** | -0.99                    | -1.19, -0.79** | -0.92                  | -1.14, -0.70** | -0.62                   | -0.84, -0.40** |
| 18:1 <i>n</i> -7                           | -0.33                     | -0.43, -0.24** | -0.43                    | -0.52, -0.33** | -0.46                  | -0.57, -0.36** | -0.40                   | -0.51, -0.29** |
| 18:1 <i>n</i> -9                           | 2.77                      | 1.74, 3.79**   | 0.68                     | -0.36, 1.71    | -2.37                  | -3.53, -1.21** | -2.20                   | -3.36, -1.05** |
| 20:1 <i>n</i> -9                           | -0.00                     | -0.04, 0.03    | 0.04                     | 0.01, 0.07*    | 0.04                   | 0.00, 0.08*    | 0.01                    | -0.02, 0.05    |
| 22:1 <i>n</i> -9                           | -0.16                     | -0.31, -0.01*  | -0.12                    | -0.27, 0.03    | -0.06                  | -0.23, 0.11    | -0.12                   | -0.29, 0.06    |
| 24:1 <i>n</i> -9                           | -0.09                     | -0.18, -0.01*  | 0.09                     | -0.00, 0.17    | 0.14                   | 0.05, 0.24*    | 0.09                    | -0.00, 0.19    |
| MUFA                                       | 1.53                      | 0.43, 2.63*    | -0.72                    | -1.84, 0.39    | -3.63                  | -4.88, -2.39** | -3.23                   | -4.47, -2.00** |
| Mead acid (20:3 <i>n</i> -9)               | 0.01                      | -0.02, 0.03    | 0.03                     | 0.01, 0.06*    | -0.01                  | -0.04, 0.03    | -0.00                   | -0.03, 0.03    |
| <b><i>n</i>-6 PUFA</b>                     |                           |                |                          |                |                        |                |                         |                |
| LA (18:2 <i>n</i> -6)                      | 1.59                      | 0.91, 2.28**   | 2.77                     | 2.07, 3.46**   | 3.01                   | 2.23, 3.78**   | 2.10                    | 1.33, 2.87**   |
| 18:3 <i>n</i> -6                           | 0.04                      | 0.02, 0.05**   | 0.04                     | 0.02, 0.05**   | 0.04                   | 0.03, 0.06**   | 0.07                    | 0.05, 0.08**   |
| 20:2 <i>n</i> -6                           | -0.02                     | -0.03, -0.01*  | 0.01                     | -0.00, 0.02    | 0.04                   | 0.03, 0.06**   | 0.02                    | 0.01, 0.04*    |
| 20:3 <i>n</i> -6                           | 0.03                      | -0.02, 0.08    | 0.23                     | 0.18, 0.29**   | 0.29                   | 0.23, 0.35**   | 0.26                    | 0.20, 0.32**   |
| AA (20:4 <i>n</i> -6)                      | -0.79                     | -1.12, -0.46** | -0.10                    | -0.44, 0.23    | 0.50                   | 0.12, 0.87*    | 0.48                    | 0.11, 0.85*    |
| 22:4 <i>n</i> -6                           | -0.06                     | -0.13, 0.01    | 0.08                     | 0.01, 0.15*    | 0.21                   | 0.13, 0.28**   | 0.16                    | 0.09, 0.24**   |
| 22:5 <i>n</i> -6                           | -0.03                     | -0.06, -0.01*  | 0.02                     | -0.00, 0.04    | 0.05                   | 0.02, 0.07**   | 0.04                    | 0.01, 0.07*    |
| <i>n</i> -6 LCPUFA                         | -0.85                     | -1.28, -0.43** | 0.23                     | -0.20, 0.66    | 1.05                   | 0.57, 1.53**   | 0.95                    | 0.47, 1.43**   |
| <i>n</i> -6 Fatty acids                    | 0.76                      | -0.15, 1.67    | 3.04                     | 2.12, 3.96**   | 4.14                   | 3.11, 5.17**   | 3.13                    | 2.10, 4.16**   |
| <b><i>n</i>-3 PUFA</b>                     |                           |                |                          |                |                        |                |                         |                |
| ALA (18:3 <i>n</i> -3)                     | -0.02                     | -0.04, 0.01    | 0.01                     | -0.02, 0.03    | 0.00                   | -0.03, 0.03    | 0.03                    | 0.00, 0.05*    |
| EPA (20:5 <i>n</i> -3)                     | -0.05                     | -0.07, -0.03** | -0.04                    | -0.06, -0.02** | -0.04                  | -0.06, -0.01*  | -0.03                   | -0.05, -0.01*  |
| 22:5 <i>n</i> -3                           | -0.09                     | -0.13, -0.05** | -0.06                    | -0.10, -0.02*  | -0.05                  | -0.09, -0.00*  | -0.04                   | -0.08, 0.00    |
| DHA (22:6 <i>n</i> -3)                     | -0.22                     | -0.36, -0.07*  | -0.11                    | -0.26, 0.04    | -0.15                  | -0.31, 0.02    | -0.10                   | -0.26, 0.06    |
| <i>n</i> -3 LCPUFA                         | -0.36                     | -0.54, -0.18** | -0.21                    | -0.40, -0.03*  | -0.22                  | -0.42, -0.01*  | -0.16                   | -0.36, 0.05    |
| <i>n</i> -3 Fatty acids                    | -0.38                     | -0.56, -0.19** | -0.21                    | -0.39, -0.02*  | -0.22                  | -0.43, -0.01*  | -0.13                   | -0.34, 0.08    |
| PUFA                                       | 0.38                      | -0.63, 1.39    | 2.84                     | 1.81, 3.86**   | 3.93                   | 2.78, 5.07**   | 3.00                    | 1.86, 4.14**   |
| Total fatty acid concentration (µg/100 µl) | 35.5                      | 12.5, 58.5*    | 20.6                     | -2.6, 43.9     | -29.9                  | -55.1, -3.9*   | -30.1                   | -56.0, -4.2*   |
| <i>n</i> -6: <i>n</i> -3 PUFA ratio        | 2.3                       | 1.5, 3.1**     | 2.7                      | 1.9, 3.5**     | 3.4                    | 2.5, 4.3**     | 2.6                     | 1.7, 3.5**     |
| Mead acid:AA ratio                         | 0.01                      | 0.00, 0.02*    | 0.01                     | 0.00, 0.02*    | -0.00                  | -0.01, 0.01    | -0.00                   | -0.01, 0.01    |
| EPA:AA ratio                               | -0.01                     | -0.01, -0.00** | -0.01                    | -0.01, -0.01** | -0.01                  | -0.02, -0.01** | -0.01                   | -0.01, -0.01** |
| 22:5 <i>n</i> -6:DHA ratio                 | 0.01                      | -0.02, 0.04    | 0.04                     | 0.02, 0.07*    | 0.09                   | 0.06, 0.12**   | 0.07                    | 0.04, 0.10**   |

*n*, Number of children in whom information was available; LA, linoleic acid; AA, arachidonic acid; LCPUFA, long-chain PUFA; *n*-6:*n*-3 PUFA ratio, ratio of total *n*-6 PUFA:total *n*-3 PUFA.

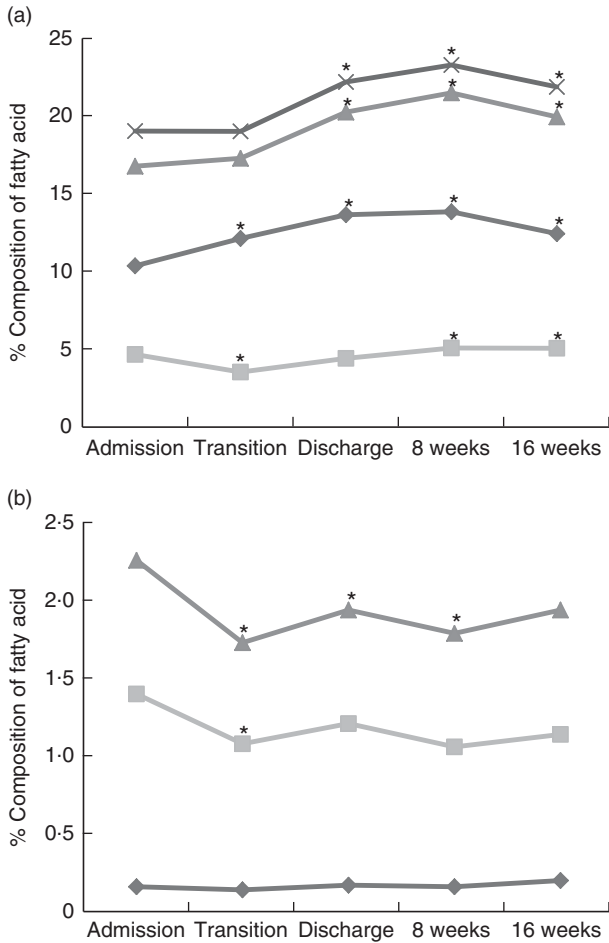
\* *P* < 0.05 and \*\* *P* < 0.001 relative to admission values.

† Paired *t* test analyses were done to test for differences in fatty acids at different times of follow-up compared with admission values.

was associated with a higher increase in LA values (*P* = 0.021), whereas an increase in height was associated with a smaller increase in LA (*P* = 0.043). Breast-fed children had a smaller increase in LA (*P* < 0.001) and a smaller decrease in EPA (*P* = 0.043) compared with non-breast-fed children. Children from families with recent fish intake had a smaller increase in *n*-6 LCPUFA (*P* = 0.045) and a greater decrease in *n*-3 LCPUFA values (*P* = 0.045). At discharge, children admitted with oedema had a smaller decrease in *n*-3 DPA than those without oedema (*P* = 0.038). High Hb values on admission predicted a higher increase in LA (*P* = 0.028) but a smaller increase in *n*-6 LCPUFA values (*P* = 0.042). HIV-infected children had a smaller increase in LA values than those not infected (*P* = 0.001). CRP > 5 mg/l predicted a more pronounced increase in LA (*P* = 0.049) and a

smaller decrease in *n*-3 LCPUFA values (*P* = 0.031). Children in whom fever was reported at admission also had a greater increase in LA values compared with those without fever (*P* = 0.033).

Table 5 represents a summary of predictors of changes in fatty acid values from admission to 8 weeks of follow-up in children with SAM. Boys had a smaller decrease in values of *n*-3 LCPUFA than girls (*P* = 0.016). Weight gain at 8 weeks of follow-up was associated with a higher increase in LA values (*P* = 0.049), whereas an increase in height was associated with a smaller decrease in DHA values (*P* = 0.036). Breast-fed children had a smaller decrease in *n*-3 LCPUFA compared with non-breast-fed children (*P* = 0.013). High Hb concentration was associated with a greater decrease in DHA values (*P* = 0.044).



**Fig. 1.** Trend of changes in fatty acid values during treatment. (a) ◆, linoleic acid; ■, arachidonic acid; ▲, total *n*-6 PUFA; ×, total PUFA. (b) ◆,  $\alpha$ -Linolenic acid; ◆, DHA; ■, total *n*-3 PUFA ▲. \* Significant increase or decrease from admission values.

Children with oral thrush had a smaller increase in *n*-6 LCPUFA ( $P=0.035$ ), whereas those who had NG tube feeding in the first 2d of admission had a smaller increase in *n*-6 LCPUFA ( $P=0.027$ ) and a greater decrease in *n*-3 LCPUFA ( $P=0.047$ ).

Children who died or were otherwise lost to follow-up had slightly higher CRP values on admission than those who remained in the study ( $P=0.068$ ), and were not different with respect to age, sex, breast-feeding, nutritional status and admission Hb concentration (data not shown). Admission values of LA, ALA, AA and DHA were also not different in children who were followed up and those not followed up.

**Discussion**

This study shows that in-hospital treatment of SAM with conventional F-75 and F-100 was associated with decreases in whole-blood *n*-3 LCPUFA proportions at discharge. As we demonstrate that the children with SAM already at admission had significantly lower proportions of LCPUFA than controls, this is a cause for concern. There could be many reasons for decreases in *n*-3 LCPUFA, one being that F-75 and F-100 contain

**Table 4.** Predictors of change in fatty acid values of children with severe acute malnutrition at discharge† (Regression coefficients (b) and 95% confidence intervals)

|                    | n  | LA    |               |        | AA            |       |               | n-6 LCPUFA |              |       | EPA          |       |             | DPA   |             |       | n-3 PUFA    |       |             | DHA   |             |       | n-3 LCPUFA  |       |             |
|--------------------|----|-------|---------------|--------|---------------|-------|---------------|------------|--------------|-------|--------------|-------|-------------|-------|-------------|-------|-------------|-------|-------------|-------|-------------|-------|-------------|-------|-------------|
|                    |    | b     | 95% CI        | b      | 95% CI        | b     | 95% CI        | b          | 95% CI       | b     | 95% CI       | b     | 95% CI      | b     | 95% CI      | b     | 95% CI      | b     | 95% CI      | b     | 95% CI      | b     | 95% CI      |       |             |
| Age (years)        | 66 | 0.81  | -2.17, 0.56   | -0.34  | -0.94, 0.26   | -0.45 | -1.24, 0.35   | 0.01       | -0.03, 0.05  | -0.05 | -0.13, 0.03  | -0.08 | -0.29, 0.14 | -0.12 | -0.42, 0.19 | -0.08 | -0.29, 0.14 | -0.08 | -0.29, 0.14 | -0.08 | -0.29, 0.14 | -0.08 | -0.29, 0.14 | -0.08 | -0.29, 0.14 |
| Sex                | 66 | 1.12  | -0.56, 2.81   | 0.59   | -0.15, 1.33   | 0.60  | -0.38, 1.57   | 0.01       | -0.03, 0.06  | 0.04  | -0.06, 0.14  | 0.21  | -0.06, 0.47 | 0.25  | -0.12, 0.63 | 0.04  | -0.06, 0.47 | 0.04  | -0.06, 0.47 | 0.04  | -0.06, 0.47 | 0.04  | -0.06, 0.47 | 0.04  | -0.06, 0.47 |
| WHZ                | 66 | -0.11 | -0.86, 0.65   | -0.03  | -0.36, 0.31   | -0.06 | -0.50, 0.38   | -0.01      | -0.03, 0.01  | 0.02  | -0.03, 0.06  | 0.04  | -0.08, 0.16 | 0.05  | -0.12, 0.22 | 0.04  | -0.08, 0.16 | 0.04  | -0.08, 0.16 | 0.04  | -0.08, 0.16 | 0.04  | -0.08, 0.16 | 0.04  | -0.08, 0.16 |
| Weight gain (kg/d) | 66 | 2.19  | 0.35, 4.03*   | 0.35   | -0.50, 1.19   | 0.80  | -0.32, 1.91   | 0.001      | -0.05, 0.05  | -0.02 | -0.14, 0.09  | -0.02 | -0.14, 0.09 | -0.02 | -0.45, 0.41 | -0.02 | -0.14, 0.09 | -0.02 | -0.14, 0.09 | -0.02 | -0.14, 0.09 | -0.02 | -0.14, 0.09 | -0.02 | -0.14, 0.09 |
| Height gain (cm)   | 59 | -0.56 | -1.11, -0.02* | -0.002 | -0.25, 0.25   | -0.05 | -0.38, 0.28   | -0.01      | -0.03, 0.04  | -0.01 | -0.04, 0.02  | -0.01 | -0.04, 0.02 | -0.01 | -0.14, 0.11 | -0.01 | -0.04, 0.02 | -0.01 | -0.04, 0.02 | -0.01 | -0.04, 0.02 | -0.01 | -0.04, 0.02 | -0.01 | -0.04, 0.02 |
| Breast-feeding     | 59 | 4.56  | 6.84, -2.29** | 0.14   | -0.92, 1.20   | -0.02 | -1.42, 1.39   | 0.07       | -0.02, 0.13* | 0.06  | -0.04, 0.20  | 0.06  | -0.04, 0.20 | 0.06  | -0.04, 0.20 | 0.06  | -0.04, 0.20 | 0.06  | -0.04, 0.20 | 0.06  | -0.04, 0.20 | 0.06  | -0.04, 0.20 | 0.06  | -0.04, 0.20 |
| Fish intake        | 57 | -0.95 | -2.75, 0.86   | -0.81  | -1.54, -0.07* | -1.01 | -1.99, -0.03* | -0.03      | -0.08, 0.01  | -0.09 | -0.19, 0.00  | -0.09 | -0.19, 0.00 | -0.09 | -0.19, 0.00 | -0.09 | -0.19, 0.00 | -0.09 | -0.19, 0.00 | -0.09 | -0.19, 0.00 | -0.09 | -0.19, 0.00 | -0.09 | -0.19, 0.00 |
| Oedema             | 66 | 1.46  | -0.27, 3.18   | 0.45   | -0.32, 1.21   | 0.67  | -0.34, 1.68   | 0.02       | -0.02, 0.07  | 0.11  | -0.01, 0.21* | 0.15  | -0.12, 0.42 | 0.15  | -0.12, 0.42 | 0.15  | -0.12, 0.42 | 0.15  | -0.12, 0.42 | 0.15  | -0.12, 0.42 | 0.15  | -0.12, 0.42 | 0.15  | -0.12, 0.42 |
| Hb (g/dl)          | 62 | 0.44  | -0.05, 0.83*  | -0.16  | -0.33, 0.01   | -0.23 | -0.44, -0.01* | -0.01      | -0.02, 0.01  | -0.02 | -0.04, 0.01  | -0.02 | -0.04, 0.01 | -0.02 | -0.04, 0.01 | -0.02 | -0.04, 0.01 | -0.02 | -0.04, 0.01 | -0.02 | -0.04, 0.01 | -0.02 | -0.04, 0.01 | -0.02 | -0.04, 0.01 |
| HIV status         | 66 | -4.33 | -6.77, -1.89* | 0.88   | -0.27, 2.04   | 1.14  | -0.39, 2.67   | 0.06       | -0.01, 0.13  | 0.12  | -0.04, 0.27  | 0.14  | -0.04, 0.27 | 0.14  | -0.04, 0.27 | 0.14  | -0.04, 0.27 | 0.14  | -0.04, 0.27 | 0.14  | -0.04, 0.27 | 0.14  | -0.04, 0.27 | 0.14  | -0.04, 0.27 |
| CRP >5 (mg/l)      | 48 | 2.20  | 0.01, 4.40*   | 0.66   | -0.33, 1.65   | 0.98  | -0.31, 2.27   | 0.06       | 0.01, 0.12*  | 0.16  | 0.04, 0.29*  | 0.28  | 0.04, 0.29* | 0.28  | 0.04, 0.29* | 0.28  | 0.04, 0.29* | 0.28  | 0.04, 0.29* | 0.28  | 0.04, 0.29* | 0.28  | 0.04, 0.29* | 0.28  | 0.04, 0.29* |
| Fever              | 62 | 1.82  | -0.16, 3.49*  | -0.11  | -0.89, 0.67   | -0.03 | -0.99, 1.06   | 0.01       | -0.04, 0.06  | -0.08 | -0.18, 0.02  | -0.11 | -0.04, 0.06 | -0.11 | -0.04, 0.06 | -0.11 | -0.04, 0.06 | -0.11 | -0.04, 0.06 | -0.11 | -0.04, 0.06 | -0.11 | -0.04, 0.06 | -0.11 | -0.04, 0.06 |
| Oral thrush        | 50 | -1.41 | -3.58, 0.76   | -0.11  | -1.04, 0.82   | -0.14 | -1.38, 1.10   | 0.01       | -0.05, 0.07  | 0.07  | -0.07, 0.20  | 0.01  | -0.05, 0.07 | 0.01  | -0.05, 0.07 | 0.01  | -0.05, 0.07 | 0.01  | -0.05, 0.07 | 0.01  | -0.05, 0.07 | 0.01  | -0.05, 0.07 | 0.01  | -0.05, 0.07 |
| NG tube            | 62 | -0.02 | -2.11, 2.08   | -0.33  | -1.24, 0.57   | -0.51 | -1.72, 0.70   | 0.03       | -0.02, 0.09  | 0.01  | -0.12, 0.13  | 0.01  | -0.12, 0.13 | 0.01  | -0.12, 0.13 | 0.01  | -0.12, 0.13 | 0.01  | -0.12, 0.13 | 0.01  | -0.12, 0.13 | 0.01  | -0.12, 0.13 | 0.01  | -0.12, 0.13 |
| Diarrhoea          | 62 | 0.48  | -1.20, 2.15   | -0.36  | -1.11, 0.38   | -0.48 | -1.48, 0.51   | -0.001     | -0.05, 0.05  | -0.03 | -0.13, 0.07  | -0.03 | -0.13, 0.07 | -0.03 | -0.13, 0.07 | -0.03 | -0.13, 0.07 | -0.03 | -0.13, 0.07 | -0.03 | -0.13, 0.07 | -0.03 | -0.13, 0.07 | -0.03 | -0.13, 0.07 |

n, Number of children in whom information was available; LA, linoleic acid; AA, arachidonic acid; LCPUFA, long-chain PUFA; DPA, *n*-3 docosapentaenoic acid; WHZ, weight-for-height z-score; CRP, C-reactive protein; NG, nasogastric. \*  $P < 0.05$ , \*\*  $P < 0.001$ . † ANCOVA was done to identify predictors of change in fatty acid values at discharge from admission values, adjusting for age and sex. Data are weight percentage of total fatty acids after adjusting for age and sex.

**Table 5.** Predictors of change in fatty acid values of children with severe acute malnutrition at 8 weeks follow-up† (Regression coefficients (b) and 95% confidence intervals)

|                    | n  | n-6 PUFA |             |       |               |            |               | n-3 PUFA |               |       |             |       |               |            |               |
|--------------------|----|----------|-------------|-------|---------------|------------|---------------|----------|---------------|-------|-------------|-------|---------------|------------|---------------|
|                    |    | LA       |             | AA    |               | n-6 LCPUFA |               | EPA      |               | DPA   |             | DHA   |               | n-3 LCPUFA |               |
|                    |    | b        | 95% CI      | b     | 95% CI        | b          | 95% CI        | b        | 95% CI        | b     | 95% CI      | b     | 95% CI        | b          | 95% CI        |
| Age (years)        | 37 | -2.66    | -6.23, 0.91 | 0.07  | -1.24, 1.38   | 0.21       | -1.48, 1.89   | 0.03     | -0.05, 0.11   | 0.03  | -0.11, 0.18 | 0.00  | -0.48, 0.49   | 0.06       | -0.56, 0.68   |
| Sex                | 37 | -1.50    | -4.25, 1.24 | 0.84  | -0.17, 1.84   | 0.96       | -0.33, 2.25   | 0.10*    | 0.04, 0.17    | 0.13* | 0.02, 0.24  | 0.36  | -0.02, 0.73   | 0.59       | -0.12, 1.06*  |
| WHZ                | 37 | -0.51    | -1.72, 0.71 | -0.19 | -0.63, 0.26   | -0.26      | -0.83, 0.31   | 0.01     | -0.02, 0.03   | 0.01  | -0.04, 0.06 | -0.02 | -0.18, 0.15   | 0.01       | -0.20, 0.22   |
| Weight gain (kg/d) | 37 | 1.57     | 0.01, 3.12* | 0.14  | -0.47, 0.74   | 0.27       | -0.52, 1.06   | -0.04    | -0.07, 0.0003 | -0.06 | -0.12, 0.01 | -0.04 | -0.27, 0.18   | -0.14      | -0.42, 0.14   |
| Height gain (cm)   | 37 | 0.09     | -1.09, 1.26 | 0.25  | -0.17, 0.67   | 0.19       | -0.36, 0.74   | -0.01    | -0.03, 0.02   | -0.02 | -0.07, 0.02 | 0.16  | 0.01, 0.31*   | 0.13       | -0.07, 0.33   |
| Breast-feeding     | 34 | -1.42    | -5.21, 2.37 | 0.66  | -0.75, 2.07   | 0.53       | -1.32, 2.38   | 0.07     | -0.02, 0.15   | 0.12  | -0.02, 0.26 | 0.59  | 0.11, 1.08*   | 0.79       | 0.18, 1.40*   |
| Fish intake        | 34 | -0.84    | -3.67, 1.98 | -0.70 | -1.71, 0.32   | -0.92      | -2.23, 0.39   | -0.03    | -0.08, 0.03   | -0.06 | -0.18, 0.05 | 0.13  | -0.50, 0.24   | -0.20      | -0.68, 0.28   |
| Oedema             | 37 | -0.01    | -2.76, 2.73 | 0.07  | -0.93, 1.08   | 0.26       | -1.03, 1.55   | 0.03     | -0.03, 0.09   | 0.05  | -0.06, 0.16 | -0.18 | -0.55, 0.18   | -0.09      | -0.56, 0.39   |
| Hb (g/dl)          | 36 | 0.18     | -0.47, 0.83 | -0.21 | -0.44, 0.02   | -0.21      | -0.51, 0.09   | -0.01    | -0.02, 0.01   | -0.01 | -0.04, 0.02 | -0.09 | -0.17, -0.00* | 0.10       | -0.21, 0.10   |
| HIV status         | 37 | -3.46    | -9.80, 2.88 | -0.47 | -2.83, 1.89   | -0.98      | -4.00, 2.04   | -0.01    | -0.15, 0.14   | 0.03  | -0.23, 0.29 | 0.02  | -0.85, 0.90   | 0.06       | -1.06, 1.17   |
| CRP > 5 (mg/l)     | 29 | 1.17     | -2.26, 4.61 | -0.12 | -1.32, 1.09   | 0.02       | -1.53, 1.58   | 0.00     | -0.06, 0.07   | 0.01  | -0.13, 0.14 | -0.06 | -0.50, 0.38   | -0.04      | -0.60, 0.51   |
| Fever              | 36 | 1.28     | -1.54, 4.10 | 0.35  | -0.67, 1.37   | 0.51       | -0.80, 1.83   | 0.01     | -0.05, 0.08   | -0.02 | -0.14, 0.09 | 0.11  | -0.28, 0.49   | 0.08       | -0.41, 0.57   |
| Oral thrush        | 25 | -0.83    | -5.07, 3.41 | -1.49 | -2.90, -0.09* | -1.98      | -3.82, -0.15* | -0.07    | -0.16, 0.02   | -0.01 | -0.19, 0.17 | -0.47 | -0.98, 0.05   | -0.55      | -1.22, 0.12   |
| NG tube            | 36 | -1.85    | -5.18, 1.48 | -1.28 | -2.45, -0.11* | -1.69      | -3.18, -0.21* | 0.00     | -0.08, 0.08   | -0.02 | -0.16, 0.12 | -0.55 | -0.97, -0.13* | -0.56      | -1.12, -0.01* |
| Diarrhoea          | 36 | -1.82    | -0.87, 4.51 | -0.73 | -1.69, 0.23   | -1.01      | -2.28, 0.26   | -0.04    | -0.10, 0.02   | -0.09 | -0.20, 0.02 | -0.13 | -0.50, 0.24   | -0.26      | -0.73, 0.20   |

n, Number of children in whom information was available; LA, linoleic acid; AA, arachidonic acid; LCPUFA, long-chain PUFA; DPA, n-3 docosapentaenoic acid; WHZ, weight-for-height z-score; CRP, C-reactive protein; NG, nasogastric; \* P < 0.05; † ANCOVA was done to identify predictors of change in fatty acid values at 8 weeks follow-up from admission values, adjusting for age and sex. Data are weight percentage of total fatty acids after adjusting for age and sex.

low levels of ALA (0.25 percentage of energy intake (%E) and 0.3%E, respectively). Another reason may be that F-75 and F-100 contain only ALA and LA and thus require an ability in the child to convert these into the functionally important LCPUFA, AA, EPA and DHA. Several studies suggest a compromised ability to elongate and desaturate in children with SAM<sup>(5,25)</sup>. Most recently, a study demonstrated that Kenyan children could only improve their DHA status when fish oil was added directly to the feeds, not with extra ALA<sup>(8)</sup>. Another important observation in this study was that the children with SAM had persistent lower whole-blood levels of PUFA, especially of n-3 PUFA, during recovery compared with healthy control children. The treatment resulted in normalisation in the percentage contribution of LA at discharge, but LA values had decreased to less than the control values again by 16 weeks of follow-up. The mean values of AA initially reduced at transition, but then increased during treatment, although it remained lower than that of control children at the end of recovery. These data agree with Koletzko *et al.*<sup>(25)</sup>, who also found increasing levels of LA in Nigerian children with SAM and decreasing AA levels during treatment. In the current study, DHA values decreased during the entire follow-up period. Similarly, other studies have found a lack of increases in DHA values during dietary intervention of malnourished children<sup>(6,25)</sup>.

The decrease in n-3 LCPUFA was less pronounced in breast-fed children. Breastmilk is a recognised source of n-3 LCPUFA, the composition being dependent on maternal nutrition<sup>(26,27)</sup>. Children from families with recent fish intake had a smaller increase in n-6 LCPUFA and a greater decrease in n-3 LCPUFA during treatment. This may imply that they had better LCPUFA levels at admission probably because of a better supply of LCPUFA than the therapeutic feeds given at the hospital. Furthermore, the changes in LCPUFA levels ceased to be significant at 8 weeks of follow-up, which may be related to a gradual return to a home diet. This emphasizes a need to revisit the PUFA content in therapeutic feeds.

Children with lower Hb at admission had a less pronounced increase in LA at discharge and a higher increase in total n-6 LCPUFA. We anticipate that there may be a build-up of Hb and erythrocytes during rehabilitation. It could be speculated that the increased dietary supply of LA during treatment may have been converted to n-6 LCPUFA for deposition in the erythrocyte cell membrane as SAM subsides and growth and build of tissues restarts. Hence, the increase in whole-blood n-6 LCPUFA may be more pronounced in anaemic children in an effort to restore Hb levels. Children infected with HIV also had a smaller increase in LA by the time of discharge than HIV-negative children. Low levels of LA have previously been described in HIV-infected children, and they have been attributed to high PUFA turn-over<sup>(28)</sup>.

A number of children with SAM have infections when first admitted<sup>(29,30)</sup>, and they all receive empirical antibiotics according to guidelines<sup>(10)</sup>. Infections in general, as indicated by CRP >5 mg/l at admission, predicted a more pronounced increase in whole-blood LA values and a smaller decrease in the level of n-3 LCPUFA at discharge, but the association ceased to be significant by 8 weeks of follow-up. When infections are controlled, the need for AA for eicosanoid synthesis may



decrease, which could explain this observed increase in LA values. In addition, as inflammatory processes subside, it is plausible that less *n*-3 LCPUFA is catabolised for the production of anti-inflammatory mediators, which may explain the observed smaller decrease in *n*-3 LCPUFA. However, children with oral thrush at admission and those who had been fed through the NG tube within the first 2 d of admission had a smaller increase in values of AA and total *n*-6 LCPUFA at 8 weeks of follow-up and NG tube fed children also had a larger decrease in DHA and total *n*-3 LCPUFA values at 8 weeks of follow-up. Oral thrush is a fungal infection of oral cavity mucous membrane, and children who were NG tube fed were quite sick and prone to severe infections. All these processes could perhaps lead to marked derangement in metabolism, increased production of inflammatory eicosanoids and thus contribute to the use of LCPUFA.

One limitation to this study is its observational nature limiting causal inference of the decreased values of LCPUFA in children with SAM. Second, as the aim was to explore predictors of changes in essential fatty acids, a large number of tests were performed, which may have increased the risk of chance findings. Furthermore, we had a high loss of patients, particularly at 8 and 16 weeks of follow-up, and as many of these dropouts were because of children dying, it is likely that the children lost were sicker than those who remained in the study. However, when assessing changes over time, we only compared the children for whom data were available at both admission and later. The high loss to follow-up of children reduces the statistical power of our study. Besides losing children in follow-up, it was not possible to get blood samples from all the children we were able to follow-up. In addition, whole-blood fatty acid analysis limits our ability to determine fatty acid distribution in the different lipid classes as compared with detailed sub analyses. Having said this, our results are in line with previous results. Another limitation may be that control children were only studied once, whereas study children were followed up over time. It is possible that some change would also have occurred in the fatty acid proportions in control children over time, because of the seasonal change in diet. However, the change in study children during in-patient treatment is unlikely to be explained by seasonal variation, as the diet was standardised, with almost no local foods included.

There is an increasing concern whether therapeutic feeds are able to correct essential fatty acid status of children recovering from SAM<sup>(31)</sup>. As the children were treated according to the Uganda Ministry of Health guidelines, PUFA levels at transition reflect the impact of F-75, at discharge the impact of F-100 and at 8 weeks of follow-up the impact of RUTF. During recovery, energy- and nutrient-dense foods are encouraged at home to achieve good catch-up growth; hence, 16 weeks of follow-up reflects the impact of the home diet. Thus, the low levels of particularly *n*-3 LCPUFA at discharge and 8 weeks of recovery from SAM suggest that the F-100 and RUTF cannot correct pre-existing compromised essential fatty acid status. Furthermore, the ratio of *n*-6:*n*-3 PUFA at admission was lower in children with SAM, but then became higher than in the healthy controls following treatment, thus reflecting an overall pronounced lack of *n*-3 PUFA in all therapeutic diets. For RUTF, WHO and the

World Food Programme recommend a fat content of 45–60%E, of which 3–10%E should come from *n*-6 fatty acids and 0.3–2.5%E from *n*-3 fatty acids<sup>(11)</sup>. F-100 follows these same specifications for essential fatty acids. Standard RUTF contains ALA corresponding to 0.3–2.5%E and 3–10%E LA. Both RUTF and F-100 meet the WHO minimum recommendations for ALA and LA and none of the recommendations mention anything about content of LCPUFA relative to the shorter-chain PUFA in therapeutic diets.

Hsieh *et al.*<sup>(9)</sup> demonstrated a worsening of *n*-3 LCPUFA status in children with uncomplicated SAM after 4 weeks of treatment with conventional RUTF compared with children treated with HO-RUTF with decreased LA contents. Furthermore, Jones *et al.*<sup>(8)</sup> noted that provision of RUTF with elevated ALA had minimal impact on *n*-6 and *n*-3 PUFA status, but that addition of fish oil was associated with marked increases in *n*-3 LCPUFA. HO-RUTF on the other hand led to relative increases of +29% and +87% for DHA and EPA, respectively<sup>(9)</sup>. The strategy of increasing oleic acid and decreasing LA to a 1:1 ratio with ALA may therefore be a promising way to improve the *n*-3 LCPUFA status of children treated for SAM without having to add fish oil to the therapeutic feeds.

## Conclusion

Whole-blood *n*-6 PUFA proportions increased from admission over discharge to follow-up, except for AA, whereas *n*-3 LCPUFA proportions of EPA and DHA decreased during in-hospital treatment. Furthermore, both *n*-3 and *n*-6 PUFA proportions remained lower than those of the healthy controls at the end of treatment. Thus, the current recommended therapeutic feeds for rehabilitating children with SAM are not able to correct their whole-blood LCPUFA compromised status. Although there were significant increases in *n*-6 LCPUFA values during treatment, the decreases in *n*-3 LCPUFA and AA proportions from admission to discharge pose particular concern, as AA and DHA play important functional roles in the brain, retina and immune system. F-75 and F-100 formulations with higher *n*-3 PUFA contents to decrease *n*-6:*n*-3 ratio and preformed LCPUFA may need to be considered in therapeutic diets for children with SAM.

## Acknowledgements

The authors are grateful to Elizabeth Kiboneka, head of Mwanamugimu Nutrition Unit, for guidance and facilitating the study; to Sofine Heilskov, Amira Catharina Khatar Sørensen and Kia Hee Schultz for data collection; to Julian Eyotaru, Loice Atuhaire, Susan Awori, Justine Naggayi and Joseph Mbabazi for data collection and skilled care of the patients; and to Christian Ritz for statistical help.

The study was funded by a PhD grant from University of Copenhagen, and received support from Augustinus Fonden, Brødrene Hartmanns Fond, Arvid Nielsens Fond, Axel Muusfeldts Fond, Aase and Einar Danielsens Fond and Torkild Steenbecks Legat. The funding sources had no influence on design of the study, data collection and analysis, or interpretation of the results.

H. F., M. J. H. R., C. G. M. and H. N. designed the study; E. B.-I., M. J. H. R. and C. G. M. collected data; E. B.-I. analyzed data and drafted the initial manuscript; L. L. contributed to the interpretation of the data; E. B.-I. had primary responsibility for final content. All above authors plus E. M., K. F. M., A. B. and K. D. S. reviewed and revised the manuscript. All authors read and approved the final manuscript.

The authors declare that there are no conflicts of interest.

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