Sensitivity of fatty acid desaturation and elongation to plasma zinc concentration: a randomised controlled trial in Beninese children

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Abstract

Zn status may affect fatty acid (FA) metabolism because it acts as a cofactor in FA desaturase and elongase enzymes. Zn supplementation affects the FA desaturases of Zn-deficient rats, but whether this occurs in humans is unclear. We evaluated the associations between baseline plasma Zn (PZn) concentration and plasma total phospholipid FA composition, as well as the effect of daily consumption of Zn-fortified water on FA status in Beninese children. A 20-week, double-blind randomised controlled trial was conducted in 186 school age children. The children were randomly assigned to receive a daily portion of Zn-fortified, filtered water delivering on average 2·8 mg Zn/d or non-fortified filtered water. Plasma total phospholipid FA composition was determined using capillary GLC and PZn concentrations by atomic absorption spectrometry. At baseline, PZn correlated positively with dihomo-γ-linolenic acid (DGLA, r 0·182; $P = 0·024$) and the DGLA:linoleic acid (LA) ratio (r 0·293; $P < 0·000$), and negatively with LA (r −0·211; $P = 0·009$) and the arachidonic acid:DGLA ratio (r −0·170; $P = 0·036$). With the intervention, Zn fortification increased nervonic acid (*B*: 0·109; 95 % CI 0·001, 0·218) in all children (n 186) and more so in children who were Zn-deficient (n 60) at baseline (*B*: 0·230; 95 % CI 0·023, 0·488). In conclusion, in this study, Zn-fortified filtered water prevented the reduction of nervonic acid composition in the plasma total phospholipids of children, and this effect was stronger in Zn-deficient children. Thus, Zn status may play an important role in FA desaturation and/or elongation.

Key words: Fatty acid: Plasma zinc: Zinc fortification: Fatty acid desaturase

Zn and essential fatty acids (FA) are required for optimal growth, immune response, gene expression, visual development, neurotransmission and cognition[1–3]. The high prevalence of Zn deficiency is a public health concern: 17 % of the world's population may be at risk of inadequate Zn intake. According to data derived from food balance sheets and demographic data, 17–25 % of the Benin population is at risk of Zn deficiency. Overall, the Beninese population is classified as moderately Zn deficient[4–6].

Zn acts as a cofactor of the FA desaturase and elongase enzymes[7,8]. The desaturase and elongase enzymes convert the essential linoleic acid (LA) and α-linolenic acid (ALA, 18 : 3*n*-3) to their long-chain PUFA (LCPUFA) metabolites, as well as the SFA palmitic acid (16 : 0) to nervonic acid (24 : 1*n*-9)[9]. A relationship between Zn and FA metabolism has been confirmed in several animal studies. Zn deficiency altered the FA composition of

phospholipids in rat liver, plasma and erythrocytes[8,10–14]. Some studies have reported higher levels of LA (18 : 2*n*-6)[12,15–17] whilst some have reported lower levels of arachidonic acid (AA, 20 : 4*n*-6)[11,15,16,18–20] and others high or normal levels of AA in tissue phospholipid of Zn-deficient animals. This relationship was also shown in a recent human study[21]. It has also been shown that Zn facilitates the incorporation of FA into phospholipids[22].

Plasma Zn (PZn) is the biomarker of choice for the assessment of Zn status in populations, however the search for a sensitive and specific biomarker for assessing body Zn concentrations at the individual level is still ongoing[23]. Although PZn responds well to supplementation, as assessed in depletion-repletion studies[24], PZn has been found to vary due to factors that are unrelated to Zn intake or status, such as infection/inflammation, time of sample collection, sample handling, among others, and

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might in fact not reflect individual Zn status[25]. Recent studies in chickens (*Gallus gallus*) and in humans have suggested the ratio of LA:dihomo-γ-linolenic acid (DGLA; 20 : 3*n*-6) or the similarly used DGLA:LA ratio, to be a potential biomarker of Zn status[21,26]. This is because the conversion of LA to DGLA requires desaturase and elongase enzymes. Reed *et al.*[26] and Knez *et al.*[21], respectively, found that Zn deficiency was positively associated with the LA:DGLA ratio in erythrocyte and plasma membranes. Knez *et al.*[21] found that a lower dietary Zn intake was associated with a higher LA:DGLA ratio in apparently healthy 28–55-year-old adults, but this relationship has not yet been investigated in children.

Zn supplementation can increase dietary Zn intake and improve Zn status[27]. Although the results from fortification studies are inconsistent[28], Zn fortification of staple food grain flours such as wheat, millet and maize is a recommended strategy to improve dietary Zn intake[29]. However, the bioavailability of Zn from many fortified grain flours is low as they have high phytate:Zn molar ratios[30]. Phytic acid is an inhibitory factor that reduces Zn absorption[31]. Fortified water as a fortification vehicle for Zn has the benefit of being free of inhibitors of absorption, making it a potentially good source of bioavailable Zn[32]. In a randomized, controlled trial, Galetti *et al.*[33] established that consumption of water from a filter able to fortify water with Zn at physiological concentrations, maintained PZn concentration of Beninese school children who were in the treatment group at baseline levels, whereas the PZn concentration of children in the control group was decreased over the 20-week study period.

In this secondary analysis of data from the Benin study[33], our study aims were to assess: (1) whether PZn and plasma total phospholipid FA composition are correlated in Beninese children; and (2) whether Zn fortification affects the LA:DGLA or DGLA:LA ratio, as a potential Zn status biomarker. Our hypotheses were: (1) that there are baseline associations between PZn and plasma total phospholipid FA composition; and (2) that children in the intervention group have better desaturase and elongase enzyme activities, estimated by product to precursor FA ratios, compared with children in the control group after a 20-week intervention period.

Methods

Study design, site and subjects

This study was part of a 20-week double-blind randomised controlled trial conducted in rural Benin in school age children between the months of February and June 2013. Its main objectives were to determine Zn bioavailability from Zn-fortified drinking water providing a mean of 2·8 mg Zn/d, and assess the efficacy of the Zn-fortified water in improving Zn status over a 20-week period[33]. At baseline, midpoint, and endpoint we measured PZn and inflammatory markers that may confound PZn, α1-acid glycoprotein (AGP) and C-reactive protein (CRP). The primary outcome of the main study was PZn concentration. We conducted the study at the primary school of Kotopounga, a rural town in north-western Benin, where the climate is a sub-equatorial/tropical savanna with one rainy

season (May–June to September–October) and one dry season (October–November to April–May). Study procedures were translated into the local Waama and Otamari languages. Children were excluded from the study if they had: (1) severe anaemia (defined as Hb < 70 g/l), (2) major chronic diseases, (3) long-term medication use or (4) recent Zn supplementation. We randomised children (*n* 277) at enrolment to one of three treatment arms: one group received Zn-fortified, filtered drinking water (Zn + filter group, 2·8 mg Zn/d), one group received non-fortified filtered drinking water (filter group, virtually no Zn) and the third group received local drinking water from a pumped well. In this study, we included only the Zn + filter (*n* 89) and filter (*n* 97) groups. Plasma total phospholipid FA composition was determined in 186 subjects (Fig. 1).

Sample size

To our knowledge, no similar study has been previously reported. We therefore based the sample size calculation for the intervention hypothesis of this secondary study on an independent *t* test analysis in G * power[34]. A sample size of fifty-two per group was needed to give the study 80 % power to detect a standardised effect size (0·6) of the intervention if significance was set at *P* = 0·05.

Intervention

Water was purified daily either with a filter equipped with a Zn fortification chamber (Zn + filter) or with a placebo chamber (filter), and hygienically stored in tanks until consumption. The Zn concentration in water was monitored daily, both with a rapid assessment method (Aquaquant; Merck) in the field, and after the intervention by flame atomic absorption spectrometry (Varian Inc.) at the Laboratory of Human Nutrition at ETH Zurich.

From Monday to Friday, each child received a morning and an afternoon serving of 300 ml filtered water (during the 10.00 hours and the 15.00 hours breaks), with the exception of Wednesdays, when they consumed only the morning serving. The 300-ml Zn-fortified water serving contained a median Zn dosage of 2·4 (interquartile range (IQR) 2·0, 3·2) mg, which resulted in an average daily Zn intake of 4·3 (IQR 3·5, 5·2) mg and 2·8 (IQR 0·0, 4·5) mg on school days and over the entire study, respectively.

Dietary Zn intake was evaluated in a subsample of thirty-six children; mean Zn intake was 8·1 (SD 62·3) mg/d[4]. Since the main contributing sources of Zn were maize (38 %), sorghum (21 %), beans and legumes (16 %), vegetables (5 %) and cassava, yam and rice (2·7 % each), the estimated daily intake of absorbable Zn was 1·5 (SD 0·5) mg[35] according to the WHO algorithm for unrefined cereal-based diet and 1·9 (SD 0·3) mg according to the algorithm by Miller *et al.*[36].

Blood sampling, biochemical analysis and anthropometry

Two morning (mean time of day: 10:24 (SD 1) h 18 min) fasting (subjects in non-fasting state were 5 and 3·3 % at baseline and endpoint sampling, respectively) venous whole-blood samples from each study participant were collected directly into one trace element–free lithium heparin tube for PZn analysis and
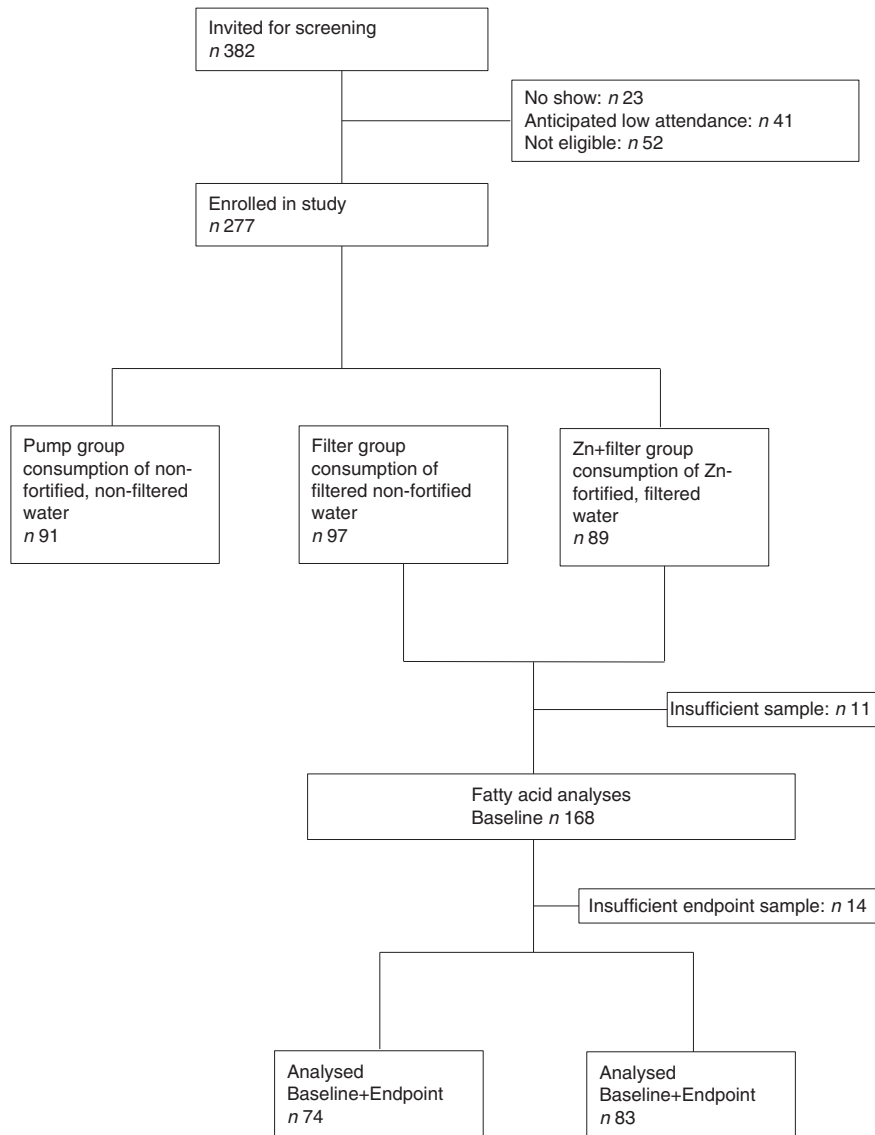
Fig. 1. Flow diagram of the secondary study in context of the main study.

one EDTA tube (Sarstedt) for analysis of inflammation markers. Hb was assessed with a portable HemoCue 201+ photometer (HemoCue AB). The blood samples were refrigerated at 4–8°C immediately after collection, centrifuged within 1 h (3000 **g** for 10 min at room temperature), divided into aliquots in acid-washed plastic vials (Eppendorf AG), and stored at −20°C (within 5 h) until analysis. The samples were shipped to ETH Zurich on dry ice. PZn concentrations were measured at ETH Zurich as described previously[33]. Briefly, PZn was measured by flame atomic absorption spectrometer (AA240FS; Varian Inc.) using a commercial aqueous standard (Titrisol 1.009953.0001; Merck) for external calibration, and Seronorm Trace Elements Serum L-2 (Sero AS) as reference material, which delivered values within acceptable ranges as specified by the manufacturer. Standardised protocols by the International Zn Nutrition Consultative Group (IZiNCG) were followed during sample collection, handling, and analysis in order to avoid exogenous or endogenous Zn contamination[37].

CRP (Code A0073; Dako Denmark) and AGP (Code Q032605-2; Dako Denmark) were analysed by using a sandwich ELISA[38]. Liquicheck Trilevel (Bio-Rad Laboratories Inc.) was used as control material with each run of analysis, and measured values were within acceptable ranges as specified by the manufacturer.

PZn values were adjusted with correction factors according to the subjects' respective phases of inflammation: Subjects in incubation (CRP > 5 mg/l and/or AGP > 1·0 g/l) with 1·08, those in early convalescence (CRP > 5 mg/l and AGP > 1·0 g/l) with 1·17 and those in late convalescence (CRP < 5 mg/l and AGP > 1·0 g/l) with 1·06[39]. The prevalence of Zn deficiency was calculated by using sex- and age-specific PZn cut-offs suggested by the IZiNCG[40]. Low PZn concentration was defined as PZn <9·9 µmol/l for children aged <10 years (morning sampling), <8·7 µmol/l for children aged <10 years (afternoon sampling), <10·7 µmol/l for females aged ≥10 years (morning fasting sampling), <10·1 µmol/l for females aged ≥10 years (morning

non-fasting sampling), <9·0 µmol/l for females aged ≥10 years (afternoon sampling), <11·3 µmol/l for males aged ≥10 years (morning fasting sampling), <10·7 µmol/l for males aged ≥10 years (morning non-fasting sampling), and <9·3 µmol/l for males aged ≥10 years (afternoon sampling)[41]. Subclinical inflammation was defined ≥5 mg/l for CRP and/or 1 g/l for AGP. Severe anaemia was defined as Hb ≤ 70 g/l[42].

Baseline and endpoint plasma samples for each subject were prepared for plasma total phospholipid FA analysis and analysed with GLC together in the same batch (pair-wise analysis) at the Non-Communicable Diseases Research Unit of the South African Medical Research Council. In short, lipids were extracted from plasma with chloroform: methanol (2:1 vol: vol; containing 0·01 % butylated hydroxytoluene) by using a modification of the Folch *et al.*[43,44] method. Lipid extracts were concentrated, the neutral lipids separated from the total phospholipid fraction by using TLC (silica gel 60 plates, 10 × 10 cm; Merck), and eluted with diethyl ether: petroleum ether: acetic acid (30:90:1; vol: vol: vol). The lipid band that contained total phospholipids (application origin) was removed from the TLC plate and *trans*-methylated with methanol: sulphuric acid (95:5 vol: vol) at 70°C for 2 h to yield fatty acid methyl esters (FAME). The resulting FAME were extracted with water and hexane. The organic layer was evaporated, redissolved in hexane, and analysed (sample injection volume 1 µl) by using GLC on a finnigan Focus Gas Chromatograph equipped with flame ionisation detector (Thermo Electron Corporation) and a 30-m capillary column of 0·32 mm internal diameter; BPX70 0·25 µm (SGE International Pty Ltd). Gas flow rates were: N_2 (make up gas), 25 ml/min; air, 250 ml/min; H_2 (carrier gas), 25 ml/min, with a split ratio of 20:1. GC-oven temperature programming was linear at 4·5°C/min, initial temperature 140°C (hold-time 1 min), final temperature 220°C (hold-time 5 min), injector temperature 220°C, and detector temperature 250°C. The FAME were identified by comparison of the retention times with those of a standard FAME mixture (27 FAME; NuChek Prep Inc.). Relative percentages of FA were calculated by taking the area count (AUC) of a given FAME as a percentage of the total area count of all FA identified in the sample.

Product:precursor FA ratios were used as a proxy to reflect enzyme activity. The GLA:LA (although not widely used as GLA is rapidly converted to DGLA), AA:DGLA and DGLA:LA ratios were used to estimate Δ-5- and Δ-6-desaturase activities[45,46]. Anthropometric measures were done as described previously[33]. Total *n*-6 LCPUFA was defined as DGLA plus AA.

Statistical analysis

The software package SPSS, version 22 (IBM), together with Excel, version 14 (Microsoft Office) were used to analyse the data of this secondary study. All data were checked for normality using histograms and the Shapiro–Wilk test and any value that departed more than 3 SD from the standardised mean were examined. When data were not normally distributed, values were either logarithmically or square root transformed before statistical analysis. Values in the text and in the tables are represented as mean values and standard deviations for normally distributed

data, medians and IQR for non-normal data, and percentages for categorical variables. Baseline differences in participant characteristics between the groups were tested by using independent *t* tests for continuous variables and Pearson's χ^2 test for categorical variables. Partial correlation coefficients, adjusted for sex, BMI and age were calculated to determine associations between FA and PZn. The effects of the Zn fortification on plasma total phospholipid FA composition were analysed with one-way ANCOVA by using respective baseline FA values, age, sex and BMI as individual level covariates in the total group, as well as in the children who were Zn deficient at baseline. Parameter estimates, unstandardised β-coefficients (β values) with 95 % CI, were obtained to indicate the size and direction of the intervention effects. Significance was set at $P < 0·05$.

Ethical considerations

The study was conducted according to the principles of the Declaration of Helsinki of 1975, as revised in 1983, and an independent Data Safety Monitoring Board was set up to monitor the intervention. Before the screening, parents gave informed consent by either a written signature or a fingerprint. The ethical committees of, the Beninese National Ethics Committee for Health Research (no. 029; 19 October 2012), and the ETH Zürich, Switzerland (EK-N-47), and the North-West University, Potchefstroom, South Africa (NWU-00086-15-A1) approved the study protocol. This trial was registered at ClinicalTrials.gov PRS with the identifier NCT01790321.

Results

Study participant characteristics

Table 1 summarises the baseline anthropometric and biochemical characteristics of the study participants. Hb was significantly higher in the Zn + filter group than in the filter group at baseline ($P = 0·030$). Anthropometric measures between groups at baseline did not differ. Median PZn concentration was 10·8 µmol/l (IQR 9·7, 12·1) for the Zn + filter group and 10·5 µmol/l (IQR 9·6, 11·8) for the filter group at baseline, and a prevalence of Zn deficiency in all the children was 32 % (*n/N* 60/186). There were no differences at baseline between the plasma total phospholipid FA compositions of the treatment groups except for LA ($P = 0·040$) that was higher in the Zn + filter group and the AA:DGLA ratio ($P = 0·030$) that was lower in the Zn + filter group as seen in Table 3.

Baseline associations of plasma zinc with plasma total phospholipid fatty acid composition

Table 2 shows the corrected correlation coefficients at baseline for the associations between PZn and plasma total phospholipid FA composition, adjusted for age, sex, and BMI. Positive correlations were found between PZn and the DGLA:LA (r 0·293, $P ≤ 0·000$), and the GLA:LA (r 0·359, $P = 0·032$) ratios. PZn also correlated positively with DGLA (r 0·182, $P = 0·024$), and negatively with LA (r −0·211, $P = 0·009$), and the AA:DGLA ratio (r −0·170, $P ≤ 0·032$). There were no significant differences at baseline in plasma total phospholipid FA composition between

Table 1. Baseline anthropometric measurements and biochemical characteristics of Beninese children by treatment group*
(Percentages and numbers; medians and interquartile ranges (IQR); mean values and standard deviations)

Characteristics	Zn + filter (n 89)		Filter (n 97)	
	%	n	%	n
Male	52	46*	58	56
Female	48	43	42	41
Moderate stunting (HAZ < 2)	13	12	3	3
Severe stunting (HAZ < 3)	6	5	8	8
Moderate underweight (WAZ < 2)	18	18	23	23
Severe underweight (WAZ < 3)	8	8	10	10
Moderate thinness (BAZ < 2)	19	17	25	24
Severe thinness (BAZ < 3)	8	7	6	6
Low PZn†	30	26	35	34
Inflammation (CRP ≥ 5 mg/l)	10	9	6	6
Anaemia	4	4	10	10
Inflammation (AGP > 1 g/l)	34	30	32	31
	Median	IQR	Median	IQR
Age (years)	8·10	6·64, 9·59	8·50	7·33, 9·58
Height (cm)				
Mean		121·94		121·03
SD		10·75		9·91
Weight (kg)	21·00	18·00, 23·00	19·25	17·46, 23·38
BMI (kg/m²)‡				
Mean		13·77		13·77
SD		1·11		1·07
HAZ	−1·22	−1·84, −0·41	−1·47	−2·01, −0·74
WAZ				
Mean		−1·76		−1·92
SD		1·05		0·97
BAZ	−1·41	−2·21, −0·97	−1·62	−2·21, −0·99
MUAC (mm)				
Mean		171·98		170·09
SD		16·12		15·25
PZn (μmol/l)	10·77	9·67, 12·05	10·51	9·61, 11·75
Hb (g/l)§				
Mean		133·02		129·61
SD		10·10		11·11
CRP (mg/l)	0·29	0·15, 1·29	0·48	0·19, 1·11
AGP (g/l)	0·87	0·69, 1·12	0·88	0·71, 1·09

HAZ, height-for-age z score; WAZ, weight-for-age z score; BAZ, BMI-for-age z score; MUAC, mid upper arm circumference; PZn, plasma Zn; CRP, C-reactive protein; AGP, α1-acid glycoprotein.

* Differences between the Zn + filter group and filter groups were examined by using independent t tests for continuous variables and by using the χ^2 test for categorical variables.

† Percentage (%, n/N× 100) (all such values). Low PZn concentration defined as PZn <9·9 μmol/ for children aged <10 years (morning sampling), <8·7 μmol/l for children aged <10 years (afternoon sampling), <10·7 μmol/l for females aged ≥10 years (morning fasting sampling), <10·1 μmol/l for females aged ≥10 years (morning non-fasting sampling), <9·0 μmol/l for females aged ≥10 years (afternoon sampling), <11·3 μmol/l for males aged ≥10 years (morning fasting sampling), <10·7 μmol/l for males aged ≥10 years (morning non-fasting sampling), and <9·3 μmol/l for males aged ≥10 years (afternoon sampling).[40]

‡ BMI = weight (kg)/height² (m²).

§ Hb was significantly higher in the Zn + filter group (P = 0·03).

the Zn-sufficient and Zn-deficient groups (data not shown). The adjustment of PZn values with correction factors for inflammation did not affect the main findings.

Effects of zinc fortification on plasma zinc and plasma total phospholipid fatty acid composition

As shown in Table 3, Zn fortification significantly prevented the reduction of PZn concentration in the Zn + filter group (P = 0·030) compared with the filter group. The effects of Zn fortification on the plasma total phospholipid FA composition of all the children are given in Table 3. When compared with the filter group, Zn fortification prevented reduction of nervonic acid (P = 0·047) in the Zn + filter group.

The effect of Zn fortification on the plasma total phospholipid FA composition in only the Zn-deficient children is shown in the online Supplementary Table S1. In these children, Zn fortification significantly increased nervonic acid (P = 0·030) and decreased ALA (P = 0·008).

Discussion

In rural Beninese school age children, PZn concentration at baseline was positively correlated with plasma total phospholipid FA composition for DGLA, DGLA:LA and GLA:LA ratios. Thus, confirming a previously reported relationship between PZn and the DGLA:LA ratio, which is a proxy marker for Δ-6-desaturase activity and has been proposed as a potential

Table 2. Baseline correlations of plasma zinc with plasma total phospholipid fatty acid composition*
(Mean values and standard deviations; medians and interquartile ranges (IQR))

	All children			
Fatty acids (% of total fatty acids)	Mean	SD	r	P
18 : 1n-9 (OA) (n 168)†			0·024	0·772
Median	13·44			
IQR	12·56, 14·46			
24 : 1n-9 (Nervonic) (n 168)	1·39	0·52	0·067	0·411
18 : 2n-6 (LA) (n 168)†			− 0·211	0·009
Median	15·68			
IQR	14·46, 17·1			
18 : 3n-6 (GLA) (n 42)	0·18	0·10	0·290	0·086
20 : 2n-6 (EPA) (n 139)†			0·014	0·874
Median	0·39			
IQR	0·34, 0·45			
20 : 3n-6 (DGLA) (n 168)‡			0·182	0·024
Median	2·31			
IQR	1·85, 2·70			
20 : 4n-6 (AA) (n 168)	7·60	1·84	0·008	0·924
22 : 4n-6 (Adrenic) (n 133)	0·46	0·15	0·061	0·512
22 : 5n-6 (n-6 DPA) (n 135)	0·50	0·18	0·136	0·138
Total n-6 LCPUFA (n 168)	9·94	2·17	0·061	0·454
18 : 3n-3 (ALA) (n 39)§	0·21	0·19	−0·193	0·281
20 : 5n-3 (EPA) (n 65)	0·19	0·07	−0·136	0·321
22 : 5n-3 (n-3 DPA) (n 123)†			0··079	0·407
Median	0·34			
IQR	0·26, 0·41			
22 : 6n-3 (DHA) (n 167)	1·63	0·58	0·092	0·260
GLA : LA ratio (n 42)	0·01	0·01	0·359	0·032
DGLA:GLA ratio (n 42)	17·71	7·79	−0·050	0·774
DGLA:LA ratio (n 168)	0·15	0·04	0·293	<0·000
AA:DGLA ratio (n 168)	3·37	0·89	−0·170	0·036
AA:LA ratio (n 168)	0·49	0·13	0·133	0·102
n-6 DPA:DHA ratio (n 135)	0·29	0·09	0·069	0·451

OA, oleic acid; LA, linoleic acid; GLA, γ-linolenic acid; DGLA, dihomo-γ-linolenic acid, AA, arachidonic acid; ALA, a-linolenic acid; LCPUFA, long-chain PUFA.
* Correlations were determined by using linear regressional analysis, adjusted for sex, BMI, age and partial correlation coefficients are shown. PZn values were corrected using adjusted and AGP correction factors[39]. Fatty acids with low sample sizes (<39), due to peak area counts below 0·1 % of the total chromatogram were not included in analysis.
† Data were log transformed to perform ANCOVA.
‡ Data were squared to perform ANCOVA.
§ Data were reciprocally transformed to perform ANCOVA.

biomarker for Zn status. The most unexpected finding, however, was that Zn fortification prevented reduction of the longer-chain n-9 MUFA, nervonic acid in plasma total phospholipids, particularly in children that were Zn-deficient at baseline.

The positive association of PZn concentration with the DGLA: LA ratio at baseline indicates an enhanced conversion of precursor FA to long-chain FA metabolites with increasing PZn concentration. Accordingly, we showed that PZn correlates with DGLA. An inverse relationship between PZn concentration and LA also suggests that a higher availability of circulating Zn results in a more efficient conversion of LA, an essential FA, to longer-chain metabolites. Zn is a cofactor for desaturase enzymes[47], and Zn deficiency may result in a disruption of the rate-limiting Δ-6-desaturase step in FA synthesis, which is necessary for the conversion of LA into DGLA[48]. Additionally, Zn deficiency and low dietary Zn intake affect the electron transport of the mitochondrial electron transport chain[21], as microsomal Zn levels directly determine electron transfer chain activity[49]. There was a weak positive association between PZn concentration and the GLA:LA ratio; this was likely due to the increased elongation of GLA from LA[45]. Subsequently, an improvement in Zn status, as previously shown in other studies,

would be expected to result in a positive association between PZn and the AA:DGLA ratio. The proportion of LCPUFA to their PUFA precursors is expected to be increased as the availability of circulating Zn results in an elevated desaturase activity. This study, however, found an inverse relationship between the two, which may indicate that only Δ-6-desaturase activity, but not Δ-5 activity necessary for the conversion of DGLA to AA, is affected by Zn status. This supports the value of the LA:DGLA or DGLA:LA ratio as an emerging biomarker for Zn status, as proposed by Knez et al.[21] and Reed et al.[26], although in the latter study it was not clear whether the ratio shift was due to a decrease in feed consumption or decrease in Zn intake.

Contrary to findings from the study conducted by Reed and colleagues, we found no significant difference in the DGLA:LA ratio in the Zn-deficient group when compared with the Zn-sufficient group (0·15 (SD 0·43) and 0·14 (SD 0·42), respectively, P = 0·217)[26]. However, it should be noted that the study conducted by Reed et al. was an animal study, and the animals were kept in a highly controlled environment and fed a standardised diet.

To our knowledge, this is one of the first human studies to investigate the effect of Zn intervention on FA status. Most previous studies used animal models and found higher LA and

Table 3. Effects of zinc fortification for 20 weeks on plasma total phospholipid fatty acid composition of 6- to 10-year-old Beninese children†
(Mean values and standard deviations; medians and interquartile ranges (IQR); β-coefficients and 95 % confidence intervals)

Zn (µmol/l) and fatty acids (% of total fatty acids)	Zn + filter Mean	SD	Filter Mean	SD	Intervention effect β	95 % CI
PZn	*n* 79		*n* 83			
Baseline						
Median	10·77		10·51			
IQR	9·67, 12·05		9·61, 11·75			
Endpoint					3·530	0·341, 6·718
Median	10·51		9·87*			
IQR	9·43, 11·60		8·69, 11·17			
18 : 1*n*-9 (OA)‡	*n* 74		*n* 81			
Baseline					0·002	−0·013, 0·016
Median	13·28		13·45			
IQR	12·47, 14·46		12·68, 14·46			
Endpoint						
Median	12·81		12·8			
IQR	11·7, 14·02		11·7, 13·95			
24 : 1*n*-9 (Nervonic)	*n* 73		*n* 79			
Baseline	1·41	0·53	1·36	0·51	0·109	0·001, 0·218
Endpoint	1·37	0·42	1·25	0·45		
18 : 2*n*-6 (LA)‡	*n* 74		*n* 81			
Baseline					0·015	−0·001, 0·031
Median	16·31		15·36			
IQR	14·61, 17·42		14·4, 16·87			
Endpoint						
Median	18·01		17·18			
IQR	16·41, 18·98		16·1, 18·48			
20 : 2*n*-6 (Eicosadienoic)‡	*n* 58		*n* 55			
Baseline					0·020	−0·013, 0·053
Median	0·40		0·37			
IQR	0·37, 0·45		0·31, 0·45			
Endpoint						
Median	0·39		0·37*			
IQR	0·34, 0·44		0·31, 0·44			
20 : 3*n*-6 (DGLA)§	*n* 74		*n* 81			
Baseline						−0·012, 0·095
Median	2·38		2·24		0·042	
IQR	1·92, 2·92		1·84, 2·65			
Endpoint						
Median	2·42		2·23			
IQR	1·87, 2·90		1·75, 2·66			
20 : 4*n*-6 (AA)	*n* 74		*n* 81			
Baseline	7·47	1·90	7·73	1·78	0·154	−0·341, 0·649
Endpoint	8·31	1·63	8·35	2·19		
22:4*n*-6 (Adrenic)	*n* 54		*n* 54			
Baseline	0·47	0·14	0·46	0·15	−0·021	−0·064, 0·021
Endpoint	0·46	0·13	0·48	0·14		
22 : 5*n*-6 (*n*-6 DPA)	*n* 59		*n* 58			
Baseline	0·49	0·16	0·52	0·19	0·003	−0·064, 0·057
Endpoint	0·49	0·21	0·49	0·20		
total *n*-6 LCPUFA	*n* 74		*n* 81			
Baseline	9·87	2·29	10·00	2·07	0·259	−0·290, 0·808
Endpoint	10·72	1·98	10·61	2·48		
20 : 5*n*-3 (EPA)	*n* 21		*n* 21			
Baseline	0·20	0·07	0·18	0·06	0·030	−0·041, 0·101
Endpoint	0·22	0·11	0·24	0·11		
22 : 5*n*-3 (*n*-3 DPA)‡	*n* 52		*n* 50			
Baseline					0·021	−0·030, 0·072
Median	0·35		0·36			
IQR	0·24, 0·40		0·29, 0·44			
Endpoint						
Median	0·38		0·42			
IQR	0·28, 0·45		0·31, 0·52			
22 : 6*n*-3 (DHA)	*n* 73		*n* 80			
Baseline	1·67	0·61	1·59	0·55	0·073	−0·066, 0·212
Endpoint	1·72	0·53	1·63	0·59		
DGLA:LA ratio	*n* 65		*n* 61			
Baseline	0·16	0·05	0·15	0·04	0·001	−0·002, 0·003
Endpoint	0·02	0·01	0·021	0·01		

Table 3. *Continued*

Zn (µmol/l) and fatty acids (% of total fatty acids)	Zn + filter		Filter		Intervention effect	
	Mean	SD	Mean	SD	β	95 % CI
AA:DGLA ratio	*n* 74		*n* 81			
Baseline	3·22	0·87	3·52	0·88	−0·151	−0·472, 0·170
Endpoint	3·61	0·96	3·86	1·18*		
AA:LA ratio	*n* 74		*n* 81			
Baseline	0·48	0·13	0·50	0·12	−0·007	−0·043, 0·029
Endpoint	0·47	0·11	0·49	0·14		
n-6 DPA:DHA ratio	*n* 59		*n* 58			
Baseline	0·28	0·10	0·30	0·10	−0·006	−0·036, 0·024
Endpoint	0·28	0·11	0·28	0·11		

PZn, plasma Zn; OA, oleic acid; LA, linoleic acid; GLA, γ-linolenic acid; DGLA, dihomo-γ-linolenic acid; AA, arachidonic acid; ALA, α-linolenic acid; LCPUFA, long-chain PUFA.
* Values changed significantly from baseline to endpoint ($P < 0.05$).
† Intervention effects were estimated by one-way ANCOVA, adjusted for respective baseline value, sex, age and BMI. Fatty acids with low sample sizes (<21 in Zn + filter and 21 in filter group), due to peak area counts below 0·1 % of the total chromatogram were not included in analysis.
‡ Data were log transformed to perform ANCOVA.
§ Data were squared to perform ANCOVA.

lower *n*-6 LCPUFA in the blood/tissue of Zn-deficient animals, indicating a disruption in desaturation and/or elongation of FA[8,15,18,19,49]. A novel finding of this study is that Zn fortification prevented the reduction of nervonic acid, a MUFA. This suggests that greater availability of circulating Zn not only increases desaturase activity, but may increase activity of the elongase enzymes in the *n*-9 synthesis pathway. Nervonic acid is produced from oleic acid, which occurs naturally in various animal and vegetable fats and oils, after undergoing three consecutive chain elongation steps catalysed by elongases. FA elongases, which function with FA desaturases to generate many of the long-chain MUFA- and PUFA, are likely affected by Zn status[50] as an inhibition in the terminal desaturase reaction has been described in Zn deficiency[49]. This finding may be physiologically relevant as nervonic acid is required for the biosynthesis of sphingomyelin in the human brain[51].

Overall, the proposed molecular mechanisms by which Zn affects the activity of desaturase and/or elongase enzymes were supported by results from this study. Even with a low dosage of Zn and a small intervention effect[33], Zn fortification had a detectable effect on the FA composition of Zn-deficient children.

The strengths of this study include the randomized and controlled study design, the direct supervision of each child during water consumption to assess compliance, the provision of highly bioavailable Zn which doubled the amount of absorbable Zn intake from the usual diet[4], the strict procedures during collection and processing to avoid Zn contamination of plasma samples[4,33] and the extensive characterisation of FA profiles in a relatively large number of children. However, this study had limitations. This study was a secondary analysis of a larger study that was not designed or powered to assess changes in FA profiles[33], this may have introduced bias into our analysis. Furthermore, although PZn concentrations in the Zn + filter group were significantly higher than in the filter group during the intervention, PZn concentrations did not increase in the Zn + filter group. This was attributed to a seasonal increase in infections occurring towards the end of the intervention which cause PZn depression[33], thus reducing the availability of circulating Zn. This may have reduced our ability to differentiate group

differences in FA profile during the intervention. It is possible that a more efficacious Zn fortification intervention (or Zn supplementation with higher doses) might have resulted in a more pronounced group differences in the FA profile. Also, although baseline FA composition was similar in the two groups suggesting no large differences in dietary fat intake, potential group differences in dietary fat intake during the intervention could have biased our results, as dietary fat intake is correlated with plasma FA composition[52]. finally, our study was limited by the use of plasma for the measurement of FA status[53]. The use of erythrocyte membranes or adipose tissue would have been more sensitive to reflect long-term changes in LCPUFA than plasma, as the latter is used as a short term FA biomarker[54–56].

In conclusion, our findings suggest PZn concentrations are positively associated with the activity of FA desaturases (particularly Δ-6-desaturase), and/or elongases in children. Furthermore, Zn fortification for 20 weeks prevented reduction of nervonic acid in plasma total phospholipids, especially in children who were Zn deficient. This was unexpected as previous studies have reported that Zn deficiency mainly affects *n*-6 FA composition. We provide evidence for the interaction between PZn and plasma total phospholipid FA composition in humans and our findings support the value of the LA:DGLA or DGLA:LA ratio as a potential biomarker of Zn status. Future research should investigate the impact of changes in Zn status on these ratios, and on nervonic acid concentrations, preferably through controlled Zn supplementation studies in Zn-deficient children and adults.

Supplementary material

For supplementary material/s referred to in this article, please visit https://doi.org/10.1017/S000711451700366X

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