# Supplementing lactating women with flaxseed oil does not increase docosahexaenoic acid in their milk[1–3]

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**ABSTRACT**

**Background:** Flaxseed oil is a rich source of 18:3n−3 (α-linolenic acid, or ALA), which is ultimately converted to 22:6n−3 (docosahexaenoic acid, or DHA), a fatty acid important for the development of the infant brain and retina.

**Objective:** The objective of this study was to determine the effect of flaxseed oil supplementation on the breast-milk, plasma, and erythrocyte contents of DHA and other n−3 fatty acids in lactating women.

**Design:** Seven women took 20 g flaxseed oil (10.7 g ALA) daily for 4 wk. Breast-milk and blood samples were collected weekly before, during, and after supplementation and were analyzed for fatty acid composition.

**Results:** Breast milk, plasma, and erythrocyte ALA increased significantly over time ($P < 0.001$) and after 2 and 4 wk of supplementation ($P < 0.05$). Over time, 20:5n−3 (eicosapentaenoic acid, or EPA) increased significantly in breast milk ($P = 0.004$) and in plasma ($P < 0.001$). In addition, plasma EPA increased significantly ($P < 0.05$) after 2 and 4 wk of supplementation. There were significant increases over time in breast-milk 22:5n−3 (docosapentaenoic acid, or DPA) ($P < 0.02$), plasma DPA ($P < 0.001$), and erythrocyte DPA ($P < 0.01$). No significant changes were observed in breast-milk, plasma, or erythrocyte DHA contents after flaxseed oil supplementation.

**Conclusions:** Dietary flaxseed oil increased the breast-milk, plasma, and erythrocyte contents of the n−3 fatty acids ALA, EPA, and DPA but had no effect on breast-milk, plasma, or erythrocyte DHA contents. *Am J Clin Nutr* 2003;77:226–33.

**KEY WORDS** Fatty acids, breast milk, human milk, α-linolenic acid, ALA, docosahexaenoic acid, DHA, eicosapentaenoic acid, EPA, docosapentaenoic acid, DPA, long-chain polyunsaturated fatty acids, arachidonic acid, flaxseed oil

## INTRODUCTION

Fifty percent of the energy in human milk is supplied by fat, which is necessary to provide energy for the rapid growth of the newborn infant. Fat also supplies n−3 and n−6 essential fatty acids needed to complete the development of the brain, retina, and other organs including the skin (1–4). The fatty acids of human milk are derived from 3 sources: mobilization of endogenous stores of fatty acids, de novo synthesis of fatty acids by the liver or breast tissue, and the diet (3, 5–9). The n−3 fatty acid, 22:6n−3 (docosahexaenoic acid, or DHA) and the n−6 fatty acid, 20:4n−6 (arachidonic acid, or AA) are stored in adipose tissue and can be secreted into breast milk after mobilization. Dietary sources can supply DHA and AA directly, or DHA and AA can be synthesized from their precursors, which are α-linolenic acid (ALA) and linoleic acid, respectively.

The fatty acid composition of human milk reflects the type of dietary fat consumed by the mother, in both the short term and the long term (1, 10–13, and WE Connor and LF Hatcher, unpublished observations, 1987). The n−3 fatty acids are of particular interest because of their role in the development of the infant's brain and retina (1–3). Harris et al (10) reported dose-dependent increases in breast-milk DHA in women taking fish oil supplements for 1–4 wk. Helland et al (12) reported that 14 d of supplementation with cod liver oil increased breast-milk DHA and 20:5n−3 (eicosapentaenoic acid, or EPA). Francois et al (11) studied the effects of 6 dietary fats, including menhaden oil and herring oil, on breast-milk fatty acids for up to 6 d after ingestion of a single, fat-rich meal. Some fatty acids increased in breast milk within 6 h after the meal, probably as a result of transfer from circulating chylomicrons. These fatty acids peaked in breast milk between 10 and 24 h and remained significantly elevated for up to 3 d.

The effects of 10 different fat supplements on the fatty acid composition of human milk were measured (WE Connor and LF Hatcher, unpublished observations, 1987). After daily supplementation with 40 g flaxseed oil (a good source of the n−3 fatty acid, ALA) for 10 d in 3 lactating women, breast-milk ALA increased significantly, as expected. However, DHA did not increase in breast milk.

Fish oil is a rich source of DHA, and lactating women who eat fish on a regular basis have much higher quantities of DHA in breast milk than do mothers who do not eat much fish (14–16). However, some lactating women are vegetarians, and others do not like fish or have limited access to fish. Flaxseed oil is rich in ALA, the precursor fatty acid in the synthetic steps that ultimately result in DHA. Studies have shown that human adults convert very

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**TABLE 1**
Fatty acid composition of flaxseed oil[1]

| Fatty acid | Content of flaxseed oil |
|---|---|
| | *% by wt of total fatty acids* |
| 12:0 | 0.0 |
| 14:0 | 0.1 |
| 16:0 | 5.4 |
| 18:0 | 3.6 |
| 20:0 | 0.0 |
| ΣSFAs[2] | 9.1 |
| 18:1n−9 | 0.0 |
| 20:1n−9 | 20.5 |
| 22:1n−11 | 0.0 |
| ΣMUFAs[3] | 20.6 |
| 18:2n−6 | 15.2 |
| 20:3n−6 | 0.1 |
| 20:4n−6 | 0.1 |
| Σn−6[4] | 15.8 |
| 18:3n−3 | 53.6 |
| 18:4n−3 | 0.02 |
| 20:4n−3 | 0.02 |
| 20:5n−3 | 0.0 |
| 22:5n−3 | 0.0 |
| 22:6n−3 | 0.0 |
| Σn−3[5] | 53.7 |

[1] $\bar{x}$.
[2] Total saturated fatty acids (SFAs), calculated by adding 8:0, 10:0, 12:0, 13:0, 14:0, 15:0, 16:0, 17:0, 18:0, 20:0, 21:0, 22:0, and 24:0.
[3] Total monounsaturated fatty acids (MUFAs), calculated by adding 14:1n−5, 16:1n−7, *trans* 18:1n−9, 18:1n−9 plus 18:1n−7, 20:1n−9, 22:1n−11, 22:1n−9, and 24:1n−9.
[4] Total n−6 fatty acids, calculated by adding 18:2n−6, 18:3n−6, 20:2n−6, 20:3n−6, 20:4n−6, 22:2n−6, 22:3n−6, 22:4n−6, and 22:5n−6.
[5] Total n−3 fatty acids, calculated by adding 18:3n−3, 18:4n−3, 20:4n−3, 20:5n−3, 22:5n−3, and 22:6n−3.

little ALA to EPA or DHA (17–19); this is also the case with laying hens (20). Our hypothesis was that supplementation of longer duration with ALA would increase the synthesis and amount of DHA in breast milk because infants require this fatty acid for brain and retinal development, and perhaps there is some signaling that enhances DHA concentrations.

## SUBJECTS AND METHODS

### Subjects

Nine healthy lactating women aged 28–39 y enrolled in this study. The subjects were recruited between 2 and 11 mo postpartum through the Oregon Health and Science University campus newsletter, e-mail announcements, word of mouth, and direct contact with study investigators. The women remained in the study for 10 wk, which included a 2-wk washout period at baseline (for subjects to stabilize their dietary intake of ALA and other n−3 fatty acids), a 4-wk flaxseed oil supplementation period, and a 4-wk postsupplementation period. One participant stopped taking the supplements immediately because of side effects, and another subject never collected the baseline breast-milk samples; therefore, 7 women completed the study. Informed consent was obtained from each subject. The study was approved by the Oregon Health and Science University Institutional Review Board, Committee on Human Research.

### Dietary assessment

To monitor dietary consistency, maternal diets were assessed 3 times: at baseline, after 4 wk of flaxseed oil supplementation, and after the 4-wk postsupplementation period. Subjects were advised not to change their current dietary intake of foods and oils containing ALA and other n−3 fatty acids. An eating habits questionnaire, the Diet Habit Survey (21), was used to assess dietary intakes of cholesterol, saturated fat, total fat, carbohydrate, and fish; it was scored by a trained dietitian. This questionnaire was developed by the nutrition staff in the Section of Clinical Nutrition and Lipid Metabolism, Department of Medicine at Oregon Health and Science University. For each subject, the scores obtained included the Cholesterol-Saturated Fat Index, Carbohydrate Score, Fish Score, and Total Score.

### Flaxseed oil supplementation

During the 4-wk supplementation period, subjects took 20 g flaxseed oil daily (Spectrum Essentials Veg-Omega 3 Cold Pressed Organic Flax Oil; Spectrum Naturals Inc, Petaluma, CA). The supplements provided a total of 10.7 g ALA/d. Subjects were instructed to take the supplements 3 times/d (total of 20 capsules/d; each capsule contained 1 g flaxseed oil). The fatty acid composition of the flaxseed oil used in this study is reported in **Table 1**. As indicated, flaxseed oil is rich in ALA (53.6% of fatty acids). Another polyunsaturated fatty acid of interest was linoleic acid (15.2% of the total fatty acids in flaxseed oil). The flaxseed oil contained no EPA, 22:5n−3 (docosapentaenoic acid, or DPA), or DHA. The fat in the supplements was 69.5% polyunsaturated fatty acids, 9.1% saturated fatty acids, and 20.6% monounsaturated fatty acids.

### Breast-milk collection and analysis

A total of 10 breast-milk samples were collected from each subject at the following time points: 1 sample at study entry, 1 sample after a 2-wk washout period (the baseline sample), 4 samples at weekly intervals during the 4-wk supplementation period, and 4 samples at weekly intervals during the 4-wk postsupplementation period. During the supplementation period, subjects were instructed to collect their milk samples in the morning, before taking the supplements.

Subjects were instructed to collect mid-feeding milk samples by nursing until the first breast was partially emptied, switching to the second breast, and then expressing the milk from the partially emptied first breast into a 5-cc plastic vial, either manually or by using a breast pump. Immediately after collection, the milk samples were placed upright in the subject's freezer (to prevent leakage and contamination). The samples were stored frozen until the next study appointment, when they were transported on ice to the Clinical Research Center. There, the samples were immediately placed in a freezer (−40 °F) until subsequent analysis.

For analysis, the milk samples were thawed and shaken vigorously. The fatty acids of breast milk and the flaxseed oil were saponified in alcoholic KOH and extracted into hexane. Fatty acid methyl esters were prepared with 12% $BF_3$ in methanol. They were analyzed by gas-liquid chromatography, as described by Anderson et al (20) and Anderson (22), on a Perkin-Elmer instrument (model Sigma 3B; Perkin-Elmer, Norwalk, CT) equipped with a hydrogen flame ionization detector and a 30-m SP-2330 fused silica capillary column with a 0.25-mm internal diameter and a 0.2-μm film thickness (Supelco, Bellefonte, PA).

### Plasma and erythrocyte fatty acids

For analyzing the plasma and erythrocyte fatty acid profiles, 10 mL blood was drawn into tubes containing EDTA on 5 occasions: at the initial visit, after a 2-wk washout period (baseline sample), after 2 and 4 wk of supplementation with flaxseed oil, and 4 wk after the supplementation ended.

Plasma and erythrocytes were separated immediately by centrifugation at 1000 × *g* for 10 min at 20 °C. The erythrocytes were washed twice with saline and the lipids of the erythrocytes were extracted with chloroform and isopropanol by using the procedure of Rose and Oklander (23); the use of isopropanol in place of methanol avoids extracting the heme pigment. Aliquots of both erythrocytes and plasma were saponified with 6% ethanolic KOH for 1 h at 37 °C. Fatty acids were extracted with hexane, acidified to remove the sterols, and extracted with hexane again. Lipids were liberated directly by incubation with ethanolic KOH. Methyl esters of the fatty acids were prepared by heating in 14% boron trifluoride-methanol ($BF_3$/MeOH) for 10 min at 100 °C in tightly sealed tubes with polytetrafluoroethylene-coated screw caps (24). All solvent evaporation was carried out under a gentle stream of nitrogen to reduce lipid peroxidation.

Fatty acid methyl esters were analyzed with gas-liquid chromatography on an instrument equipped with a hydrogen flame ionization detector (Perkin-Elmer model Sigma 3B) and a 30-m SP-2330 fused silica capillary column (Supelco, Bellefonte, PA). The temperatures of the column, detector, and injection ports were 195 °C, 250 °C, and 250 °C, respectively. Helium was used as the carrier gas; the inlet pressure was 80 psi. The split ratio was 1:170. The retention time and area of each peak were measured with an HP-3390 integrator (Hewlett-Packard, Palo Alto, CA); a computer (HP85; Hewlett Packard) was used to identify and quantify each individual fatty acid by percentage. A mixture of fatty acid standards was run daily.

### Statistical methods

The data are reported as means ± SDs. Statistical significance was defined as $P < 0.05$. One-way repeated-measures analysis of variance was used to test for significant differences between baseline values and values at other time points throughout the study for breast-milk, plasma, and erythrocyte fatty acids. The Bonferroni *t* test and Dunnett's method were then used to determine which time points were significantly different from baseline (25). If the normality test failed, the data were log transformed and used for the analysis. Paired *t* tests (25) were performed to compare initial and final scores on the Diet Habit Survey. Because the Carbohydrate Score data failed the normality test, a Mann-Whitney rank-sum test was used to analyze these data. SIGMASTAT for WINDOWS, version 2.0 (Jandel Scientific, San Rafael, CA) was used for all statistical computations.

## RESULTS

### Subjects' diets

The diets of the subjects remained constant during the study. There were no significant differences in their Cholesterol-Saturated Fat Index, Carbohydrate Score, Fish Score, or Total Score between the beginning of the study and the end of the study. The macronutrient composition of their diets remained stable, as did their fish intake. Subjects consumed 2–3 servings of fish/mo on average; the fish consumed consisted primarily of white fish (eg, tuna, halibut, and snapper). The subjects consumed ≈28% of their energy as fat, 9% as saturated fat, and 58% as carbohydrate. Subjects consumed < 250 mg cholesterol/d and 2600 mg Na/d on average.

### Changes in breast-milk fatty acid composition during flaxseed oil supplementation

At baseline, the total fatty acid composition of the milk samples (n = 7) was 40.0% saturated fatty acids, 40.5% monounsaturated fatty acids, and 16.8% polyunsaturated fatty acids (**Table 2**). The polyunsaturated fatty acids consisted of 13.3 ± 3.0% linoleic acid, 1.0 ± 0.3% ALA, 0.1 ± 0.0% EPA, 0.1 ± 0.2% DPA, and 0.2 ± 0.1% DHA.

During supplementation with flaxseed oil, significant changes were observed in the fatty acid composition of the milk (Table 2). Breast-milk ALA increased significantly over time, from 1.0% of fatty acids at baseline to 6.8% of fatty acids after 1 wk of flaxseed oil supplementation. ALA remained elevated at the 2-wk and 4-wk time points. After 4 wk of supplementation, ALA peaked at 7.7% of fatty acids; it then returned to near baseline values (1.9% of fatty acids) as early as 1 wk after subjects discontinued supplementation. As expected, breast-milk monounsaturated fatty acids decreased significantly, from 40.5% of total fatty acids at baseline to 34.0% after 4 wk of supplementation.

The EPA content of breast milk increased significantly over time with flaxseed oil supplementation, from 0.08% at baseline to 0.14% after 1 wk, 0.13% after 2 wk, and 0.11% after 4 wk of supplementation. However, none of these time points were significantly different from baseline; only the trend was significant. Also, breast-milk DPA increased significantly over time during flaxseed oil supplementation, from 0.19% at baseline to 0.20% after 2 wk of supplementation. The value then decreased to 0.17% after 4 wk of supplementation. As with EPA, none of these time points were significantly different from baseline; only the trend was significant. Breast-milk DHA content did not increase over time or at any time points. It remained constant at ≈0.1–0.2% throughout the supplementation and postsupplementation periods.

At baseline, breast-milk linoleic acid accounted for 13.3% of total fatty acids. Although linoleic acid makes up 15.2% of the fatty acids in flaxseed oil, breast-milk linoleic acid did not change significantly during flaxseed oil supplementation. The ratio of n−6 to n−3 fatty acids in breast milk also decreased significantly, from 9.5 at baseline to 1.9 after 4 wk of supplementation. The breast-milk content of *trans* fatty acids did not change significantly during supplementation with flaxseed oil, remaining at ≈3% of total fatty acids.

### Changes in plasma fatty acid composition during flaxseed oil supplementation

During supplementation with flaxseed oil, there were significant changes from baseline in the fatty acid composition of maternal plasma (**Table 3**). Plasma ALA increased significantly over time and after both 2 wk and 4 wk of supplementation, from 0.6% at baseline to 2.8% at 2 wk and 3.4% at 4 wk. ALA returned to near baseline values (0.7% of total fatty acids) by 4 wk postsupplementation.

Plasma EPA increased significantly over time, from 0.7% at baseline to 1.5% after 2 wk and 1.6% after 4 wk of supplementation; it returned to 0.7% at 4 wk postsupplementation. Plasma DPA increased significantly over time, from 0.5% at baseline to 0.8% after both 2 wk and 4 wk of supplementation; it returned to 0.6% by 4 wk postsupplementation. Plasma DHA remained fairly

**TABLE 2**
Breast-milk fatty acid composition at baseline, after 2 and 4 wk of flaxseed oil supplementation, and at 4 wk postsupplementation[1]

| Fatty acid | Baseline ($n$ = 7) | After 2 wk of supplementation ($n$ = 7) | After 4 wk of supplementation ($n$ = 4) | 4 wk postsupplementation ($n$ = 5) | $P$ |
|---|---|---|---|---|---|
| | | | *% by wt of total fatty acids* | | |
| 8:0 | 0.1 ± 0.0 | 0.1 ± 0.0 | 0.1 ± 0.1 | 0.1 ± 0.1 | NS |
| 10:0 | 1.1 ± 0.3 | 1.1 ± 0.2 | 1.2 ± 0.2 | 1.0 ± 0.5 | NS |
| 12:0 | 4.9 ± 1.3[a] | 5.7 ± 1.6[b] | 5.8 ± 0.9[b] | 5.0 ± 1.5[a] | 0.04 |
| 14:0 | 6.2 ± 1.8 | 6.4 ± 2.4 | 6.7 ± 1.3 | 6.8 ± 2.0 | NS |
| 16:0 | 19.8 ± 1.3 | 16.5 ± 2.3 | 17.8 ± 2.5 | 18.9 ± 1.6 | NS |
| 18:0 | 7.2 ± 1.2 | 6.0 ± 1.3 | 6.9 ± 0.7 | 7.1 ± 1.5 | NS |
| 20:0 | 0.06 ± 0.04 | 0.13 ± 0.06 | 0.10 ± 0.03 | 0.11 ± 0.03 | 0.04 |
| ΣSFAs[2] | 40.0 ± 4.8 | 36.6 ± 5.9 | 39.5 ± 3.2 | 40.2 ± 6.4 | NS |
| 18:1n−9 | 35.0 ± 3.3[a] | 31.3 ± 3.4[a] | 27.9 ± 1.7[b] | 33.1 ± 3.9[a] | 0.01 |
| *trans* 18:1n−9 | 2.6 ± 1.1 | 2.2 ± 0.9 | 3.1 ± 0.7 | 2.6 ± 1.1 | NS |
| 20:1n−9 | 0.7 ± 0.2 | 0.5 ± 0.1 | 0.5 ± 0.1 | 0.6 ± 0.1 | NS |
| 22:1n−11 | 0.1 ± 0.0 | 0.1 ± 0.0 | 0.1 ± 0.0 | 0.1 ± 0.0 | NS |
| ΣMUFAs[3] | 40.5 ± 3.1[a] | 36.7 ± 3.5[a] | 34.0 ± 2.3[b] | 39.1 ± 3.7[a] | 0.03 |
| 18:2n−6 | 13.3 ± 3.0 | 15.5 ± 2.8 | 15.0 ± 1.2 | 15.1 ± 4.9 | NS |
| 20:3n−6 | 0.3 ± 0.1 | 0.2 ± 0.1 | 0.2 ± 0.1 | 0.2 ± 0.1 | NS |
| 20:4n−6 | 0.4 ± 0.1 | 0.5 ± 0.1 | 0.5 ± 0.1 | 0.4 ± 0.1 | NS |
| Σn−6[4] | 14.6 ± 3.0 | 16.9 ± 2.9 | 16.2 ± 1.2 | 16.4 ± 4.9 | NS |
| 18:3n−3 | 1.0 ± 0.3[a] | 7.3 ± 1.5[b] | 7.7 ± 1.0[b] | 1.4 ± 0.5[a] | <0.001 |
| 20:5n−3 | 0.08 ± 0.03 | 0.13 ± 0.03 | 0.11 ± 0.01 | 0.07 ± 0.02 | 0.004 |
| 22:5n−3 | 0.19 ± 0.05 | 0.20 ± 0.04 | 0.17 ± 0.02 | 0.13 ± 0.02 | <0.02 |
| 22:6n−3 | 0.2 ± 0.1 | 0.2 ± 0.1 | 0.1 ± 0.1 | 0.1 ± 0.0 | NS |
| Σn−3[5] | 1.6 ± 0.3[a] | 7.9 ± 1.5[b] | 8.2 ± 1.0[b] | 1.8 ± 0.4[a] | <0.001 |
| n−6:n−3 | 9.5 ± 1.4[a] | 2.2 ± 0.5[b] | 1.9 ± 0.2[b] | 9.1 ± 0.8[a] | <0.001 |
| ΣPUFAs[6] | 16.8 ± 3.3[a] | 25.2 ± 3.7[b] | 25.0 ± 2.0[b] | 17.4 ± 5.6[a] | <0.001 |

[1] $\bar{x}$ ± SD. Values in the same row with different superscript letters are significantly different, $P < 0.05$.
[2] Total saturated fatty acids (SFAs) include 8:0, 10:0, 12:0, 13:0, 14:0, 15:0, 16:0, 17:0, 18:0, 20:0, 21:0, 22:0, and 24:0.
[3] Total monounsaturated fatty acids (MUFAs) include 14:1n−5, 16:1n−7, *trans* 18:1n−9, 18:1n−9 plus 18:1n−7, 20:1n−9, 22:1n−11, 22:1n−9, and 24:1n−9.
[4] Total n−6 fatty acids, calculated by adding 18:2n−6, 18:3n−6, 20:2n−6, 20:3n−6, 20:4n−6, 22:2n−6, 22:3n−6, 22:4n−6, and 22:5n−6.
[5] Total n−3 fatty acids, calculated by adding 18:3n−3, 18:4 n−3, 20:4n−3, 20:5n−3, 22:5n−3, and 22:6n−3.
[6] Total PUFAs include total n−6, total n−3, and other PUFAs (16:2n−4, 16:4n−1, 20:3n−9, and 22:3n−9).

constant at ≈1.2–1.3% of total fatty acids throughout the supplementation and postsupplementation periods. The plasma ratio of n−6 to n−3 fatty acids decreased significantly from 13.0 at baseline to 6.8 after 4 wk of supplementation.

Plasma total monounsaturated fatty acids decreased significantly from 24.5% of total fatty acids at baseline to 21.1% of total fatty acids after 4 wk of supplementation. Plasma *trans* fatty acids remained fairly constant, at ≈1% of total fatty acids, after supplementation with flaxseed oil.

### Changes in erythrocyte fatty acid composition during flaxseed oil supplementation

After supplementation with flaxseed oil, similar changes were observed in the fatty acid composition of maternal erythrocytes (**Table 4**). Erythrocyte ALA increased significantly over time and was significantly increased after both 2 and 4 wk of supplementation; the values were 0.1% at baseline, 0.6% at 2 wk, and 0.7% at 4 wk. Erythrocyte EPA increased significantly over time, from 0.6% at baseline to 1.0% after 2 wk and 0.7% after 4 wk of supplementation; it returned to 0.8% at 4 wk postsupplementation. Erythrocyte DPA increased significantly over time, from 2.4% at baseline to 2.7% after both 2 wk and 4 wk of supplementation. Erythrocyte DHA did not change significantly, remaining at ≈3.5% throughout the supplementation and postsupplementation periods.

Erythrocyte linoleic acid did not change significantly over time or at any time points. Erythrocyte total monounsaturated fatty acids decreased from 18.6% at baseline to 18.0% after 4 wk of supplementation; this decrease was not significant. The erythrocyte ratio of n−6 to n−3 fatty acids decreased from 5.1 at baseline to 4.1 after 4 wk of supplementation. Erythrocyte *trans* fatty acids did not change significantly after supplementation with flaxseed oil, remaining at ≈1% of total fatty acids.

## DISCUSSION

In this study of 7 lactating women, ALA, EPA, and DPA increased in breast milk, plasma, and erythrocytes after flaxseed oil supplementation. These results were different from what we observed in 7 nonlactating control subjects supplemented with flaxseed oil (26). In that previous study, subjects ranged in age from 24 to 54 y and included 3 men and 4 women; all the women were premenopausal. The subjects received 15 g flaxseed oil (11 g ALA) daily for 12 wk, which did not increase EPA, DPA, DHA, or total n−3 fatty acids in plasma or erythrocytes. However, in the present study of lactating women, we measured significant increases in milk and plasma EPA and milk, plasma, and erythrocyte DPA. This finding supports our hypothesis that lactating women synthesized some of the longer-chain n−3 fatty acids, whereas nonlactating control subjects did not.

**TABLE 3**
Maternal plasma fatty acid composition at baseline, after 2 and 4 wk of flaxseed oil supplementation, and at 4 wk postsupplementation[1]

| Fatty acid | Baseline ($n$ = 8) | After 2 wk of supplementation ($n$ = 8) | After 4 wk of supplementation ($n$ = 6) | 4 wk postsupplementation ($n$ = 6) | $P$ |
|---|---|---|---|---|---|
| | | | *% by wt of total fatty acids* | | |
| 12:0 | 0.1 ± 0.1 | 0.2 ± 0.2 | 0.2 ± 0.3 | 0.1 ± 0.1 | NS |
| 14:0 | 0.9 ± 0.4 | 1.4 ± 0.7 | 1.3 ± 0.9 | 1.1 ± 0.5 | NS |
| 16:0 | 19.3 ± 1.1 | 19.0 ± 1.7 | 18.8 ± 0.9 | 19.6 ± 1.7 | NS |
| 18:0 | 7.4 ± 0.8 | 7.5 ± 0.6 | 8.0 ± 0.7 | 7.2 ± 0.5 | NS |
| 20:0 | 0.1 ± 0.2 | 0.1 ± 0.2 | 0.1 ± 0.2 | 0.0 ± 0.0 | NS |
| ΣSFAs[2] | 28.7 ± 1.3 | 29.4 ± 2.7 | 29.3 ± 1.7 | 28.9 ± 2.1 | NS |
| 18:1n−9 | 20.4 ± 2.7 | 17.6 ± 2.7 | 17.5 ± 1.2 | 20.3 ± 2.9 | 0.004 |
| *trans* 18:1n−9 | 1.0 ± 0.3 | 1.0 ± 0.2 | 1.0 ± 0.2 | 0.9 ± 0.3 | NS |
| 20:1n−9 | 0.3 ± 0.1 | 0.3 ± 0.1 | 0.3 ± 0.1 | 0.3 ± 0.1 | NS |
| 22:1n−11 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | NS |
| ΣMUFAs[3] | 24.5 ± 2.9 | 21.3 ± 2.2 | 21.1 ± 1.7 | 25.4 ± 6.0 | 0.02 |
| 18:2n−6 | 30.9 ± 2.6 | 31.9 ± 3.5 | 32.1 ± 2.2 | 31.2 ± 4.6 | NS |
| 20:3n−6 | 1.6 ± 0.3 | 1.1 ± 0.2 | 1.2 ± 0.2 | 1.4 ± 0.2 | 0.001 |
| 20:4n−6 | 7.5 ± 1.8 | 6.5 ± 1.4 | 6.2 ± 1.4 | 7.3 ± 1.3 | 0.001 |
| Σn−6[4] | 41.3 ± 3.7 | 40.6 ± 4.1 | 41.0 ± 2.5 | 38.7 ± 11.3 | NS |
| 18:3n−3 | 0.6 ± 0.2[a] | 2.8 ± 0.9[b] | 3.4 ± 1.2[b] | 0.7 ± 0.1[a] | <0.001 |
| 20:5n−3 | 0.7 ± 0.2[a] | 1.5 ± 0.3[b] | 1.6 ± 0.4[b] | 0.7 ± 0.1[a] | <0.001 |
| 22:5n−3 | 0.5 ± 0.1[a] | 0.8 ± 0.1[b] | 0.8 ± 0.1[b] | 0.6 ± 0.1[a] | <0.001 |
| 22:6n−3 | 1.3 ± 0.4 | 1.3 ± 0.4 | 1.2 ± 0.3 | 1.3 ± 0.3 | NS |
| Σn−3[5] | 3.3 ± 0.5[a] | 6.5 ± 1.0[b] | 7.1 ± 1.4[b] | 3.3 ± 0.4[a] | 0.001 |
| n−6:n−3 | 13.0 ± 2.8[a] | 6.4 ± 1.3[b] | 6.8 ± 1.7[b] | 12.6 ± 2.8[a] | <0.001 |
| ΣPUFAs[6] | 45.2 ± 3.4 | 47.7 ± 3.9 | 48.2 ± 2.2 | 45.3 ± 5.3 | NS |

[1] $\bar{x}$ ± SD. Values in the same row with different superscript letters are significantly different, $P < 0.05$.
[2] Total saturated fatty acids (SFAs) include 8:0, 10:0, 12:0, 13:0, 14:0, 15:0, 16:0, 17:0, 18:0, 20:0, 21:0, 22:0, and 24:0.
[3] Total monounsaturated fatty acids (MUFAs) include 14:1n−5, 16:1n−7, *trans* 18:1n−9, 18:1n−9 plus 18:1n−7, 20:1n−9, 22:1n−11, 22:1n−9, and 24:1n−9.
[4] Total n−6 fatty acids, calculated by adding 18:2n−6, 18:3n−6, 20:2n−6, 20:3n−6, 20:4n−6, 22:2n−6, 22:3n−6, 22:4n−6, and 22:5n−6.
[5] Total n−3 fatty acids, calculated by adding 18:3n−3, 18:4 n−3, 20:4n−3, 20:5n−3, 22:5n−3, and 22:6n−3.
[6] Total polyunsaturated fatty acids (PUFAs) include total n−6, total n−3, and other PUFAs (16:2n−4, 16:4n−1, 20:3n−9, and 22:3n−9).

Contrary to our hypothesis, DHA did not increase in breast milk, plasma, or erythrocytes. There are several possible reasons for the lack of increase in breast-milk DHA concentrations after ALA consumption.

One possible reason is that there is very limited conversion of ALA to DHA in human adults (11, 17–19). Pawlosky et al (27) recently quantified the inefficiency of the conversion of ALA to DHA. Only 0.2% of the plasma ALA was available for synthesis of EPA. Of this, 63% was available for synthesis of DPA, of which 37% was accessible for production of DHA. Thus, <0.05% of the plasma ALA was available for synthesis of DHA. Even so, long-term vegetarians with virtually no dietary DHA intake have DHA in their plasma phospholipids (18). In studies of vegans who ate no foods of animal origin (including fish), DHA was still found in erythrocyte and breast-milk fatty acids (16, 28). These data suggest that conversion of ALA to DHA must occur in humans. Likewise, infant monkeys fed prenatal and postnatal diets containing only ALA from soy oil had DHA in the blood, brain, retina, and other organs, indicating that synthesis of DHA from ALA did indeed occur in the monkeys as well (29). Similar results were found in human infants fed formulas containing ALA but no DHA; DHA was found in their blood, although the concentration was lower than that in infants fed human milk, which contains DHA as well as ALA (30). The failure of conversion of ALA to DHA in the present study may have resulted from limited conversion in combination with a relatively short duration of supplementation (4 wk).

A second possible reason for the lack of increase in breast-milk DHA is that DHA may not have increased because of competitive enzyme inhibition. The $\Delta^6$ enzyme is required for 2 steps of the DHA synthesis pathway (**Figure 1**). The first step is the conversion of 18:3n−3 (ALA) to 18:4n−3. The second step is the conversion of 24:5n−3 to 24:6n−3, with subsequent retroconversion to 22:6n−3. An excess of the substrate ALA (from the flaxseed oil) might provide for synthesis of some fatty acids (eg, EPA) in the initial phases of the synthetic pathway to DHA, but the excess of substrate might have suppressed the final $\Delta^6$-desaturase step needed to produce 24:6n−3 from 24:5n−3 later in the pathway.

A third possible reason for a lack of increase in breast-milk DHA is that high dietary DHA intakes can suppress the conversion of ALA to DHA, but in the present study, this was unlikely because the subjects did not appear to have high DHA intakes. The average amount of DHA in the milk of these American women was much lower (≈0.2% of total fatty acids) than the amounts found in the milk of women throughout the world, especially in China, where the concentrations are 4 or 5 times those found in the United States. These high concentrations have been correlated with the consumption of large amounts of fish (14). Even in Cuba, breast-milk concentrations of DHA are double those reported in the milk of US women (0.4% of total fatty acids versus 0.2%, respectively). Cuban lactating women generally consume 1 serving of fish each day, in comparison with our study subjects, who consumed 2–3 servings of fish/mo; this probably

**TABLE 4**
Maternal erythrocyte fatty acid composition at baseline, after 2 and 4 wk of flaxseed oil supplementation, and at 4 wk postsupplementation[1]

| Fatty acid | Baseline ($n$ = 8) | After 2 wk of supplementation ($n$ = 8) | After 4 wk of supplementation ($n$ = 6) | 4 wk postsupplementation ($n$ = 6) | $P$ |
|---|---|---|---|---|---|
| | | | *% by wt of total fatty acids* | | |
| 12:0 | 0.3 ± 0.1 | 0.1 ± 0.1 | 0.2 ± 0.1 | 0.2 ± 0.2 | NS |
| 14:0 | 0.4 ± 0.2 | 0.5 ± 0.1 | 0.4 ± 0.2 | 0.4 ± 0.1 | NS |
| 16:0 | 19.6 ± 1.0 | 19.1 ± 0.8 | 19.2 ± 1.3 | 19.8 ± 1.2 | NS |
| 18:0 | 15.9 ± 0.7 | 15.9 ± 0.8 | 16.2 ± 1.2 | 17.2 ± 1.6 | NS |
| 20:0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.0 ± 0.0 | 0.1 ± 0.0 | NS |
| ΣSFAs[2] | 39.3 ± 1.1 | 38.5 ± 1.0 | 39.4 ± 1.3 | 39.4 ± 1.3 | NS |
| 18:1n−9 | 15.8 ± 1.1 | 15.4 ± 1.0 | 14.9 ± 1.0 | 15.7 ± 1.2 | NS |
| *trans* 18:1n−9 | 1.0 ± 0.2 | 1.1 ± 0.3 | 1.3 ± 0.2 | 1.1 ± 0.1 | NS |
| 20:1n−9 | 0.4 ± 0.1 | 0.4 ± 0.1 | 0.4 ± 0.1 | 0.4 ± 0.0 | NS |
| 22:1n−11 | 0.0 ± 0.0 | 0.1 ± 0.1 | 0.0 ± 0.1 | 0.0 ± 0.1 | NS |
| ΣMUFAs[3] | 18.6 ± 0.9 | 18.0 ± 1.4 | 18.0 ± 1.0 | 18.6 ± 1.0 | NS |
| 18:2n−6 | 11.6 ± 1.2 | 11.6 ± 0.7 | 11.7 ± 0.8 | 11.6 ± 0.8 | NS |
| 20:3n−6 | 1.7 ± 0.4 | 1.5 ± 0.3 | 1.4 ± 0.4 | 1.6 ± 0.1 | 0.001 |
| 20:4n−6 | 14.8 ± 1.1 | 14.9 ± 1.0 | 14.0 ± 1.4 | 14.0 ± 1.4 | 0.04 |
| Σn−6[4] | 32.7 ± 1.8 | 32.7 ± 1.1 | 31.6 ± 2.2 | 32.6 ± 1.1 | NS |
| 18:3n−3 | 0.1 ± 0.0[a] | 0.6 ± 0.1[b] | 0.7 ± 0.2[b] | 0.2 ± 0.1[a] | <0.001 |
| 20:5n−3 | 0.6 ± 0.2[a] | 1.0 ± 0.2[b] | 0.7 ± 0.2[a] | 0.8 ± 0.2[a] | <0.001 |
| 22:5n−3 | 2.4 ± 0.4 | 2.7 ± 0.4 | 2.7 ± 0.3 | 2.8 ± 0.4 | <0.001 |
| 22:6n−3 | 3.5 ± 0.9 | 3.7 ± 1.1 | 3.3 ± 0.9 | 3.3 ± 1.1 | NS |
| Σn−3[5] | 6.7 ± 1.3[a] | 8.1 ± 1.4[b] | 7.9 ± 1.2[b] | 7.1 ± 1.5[a] | <0.001 |
| n−6:n−3 | 5.1 ± 1.1[a] | 4.2 ± 0.8[b] | 4.1 ± 0.8[b] | 4.7 ± 1.0[a] | <0.001 |
| ΣPUFAs[6] | 40.3 ± 1.6 | 41.8 ± 1.2 | 40.5 ± 1.7 | 40.5 ± 1.5 | NS |

[1] $\bar{x}$ ± SD. Values in the same row with different superscript letters are significantly different, $P < 0.05$.
[2] Total saturated fatty acids (SFAs) include 8:0, 10:0, 12:0, 13:0, 14:0, 15:0, 16:0, 17:0, 18:0, 20:0, 21:0, 22:0, and 24:0.
[3] Total monounsaturated fatty acids (MUFAs) include 14:1n−5, 16:1n−7, *trans* 18:1n−9, 18:1n−9 plus 18:1n−7, 20:1n−9, 22:1n−11, 22:1n−9, and 24:1n−9.
[4] Total n−6 fatty acids, calculated by adding 18:2n−6, 18:3n−6, 20:2n−6, 20:3n−6, 20:4n−6, 22:2n−6, 22:3n−6, 22:4n−6, and 22:5n−6.
[5] Total n−3 fatty acids, calculated by adding 18:3n−3, 18:4 n−3, 20:4n−3, 20:5n−3, 22:5n−3, and 22:6n−3.
[6] Total polyunsaturated fatty acids (PUFAs) include total n−6, total n−3, and other PUFAs (16:2n−4, 16:4n−1, 20:3n−9, and 22:3n−9).

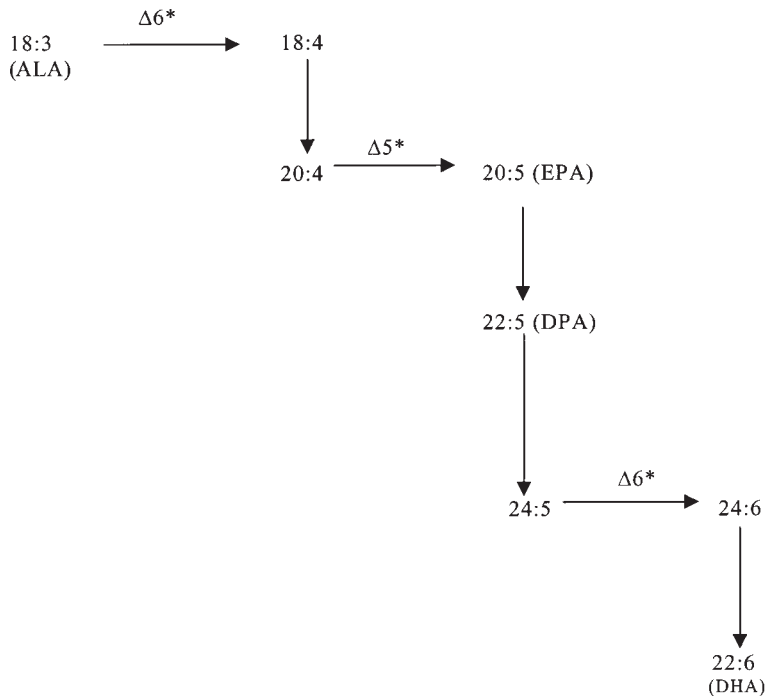


**FIGURE 1.** The conversion of α-linolenic acid (ALA) to docosahexaenoic acid (DHA) via desaturation and elongation. EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; *, desaturase enzymes.

explains the greater DHA concentrations found in the milk of Cuban mothers (31).

Also of interest is the fact that ALA is known to be catabolized rapidly (32). Our data support the rapid catabolism of ALA (Table 2). One week after the flaxseed oil supplementation was discontinued, ALA concentrations in the blood and breast milk had declined to baseline concentrations. In milk, ALA decreased from 7.7% to < 2.0% of total fatty acids. However, the decline in breast-milk DPA concentrations was much slower, with baseline values not reached until 3 wk postsupplementation. For EPA, baseline values were also reached at 3 wk postsupplementation. Similar findings were noted for plasma and erythrocyte ALA, DPA, and EPA, although the declines of their concentrations were much less rapid in the erythrocytes than in the plasma.

Although there were no significant changes in the *trans* fatty acid content of the breast milk, plasma, or erythrocytes, we reported these data because of the potential adverse effects of dietary *trans* fatty acids. These potential adverse effects include reduced serum HDL concentrations (33, 34) and impaired biosynthesis of long-chain polyunsaturated fatty acids (33, 35).

In conclusion, 4 wk of supplementation with flaxseed oil was not an effective way to increase DHA concentrations in maternal plasma, erythrocytes, or breast milk. Thus, flaxseed oil supplementation would not be an adequate method of increasing the availability of DHA for the developing infant. Increasing the maternal intake of DHA from fish or fish oil would still be the most effective way to increase the DHA in breast milk and provide this long-chain polyunsaturated fatty acid that is so critical for infant development (36).

We are grateful to the volunteers for their commitment to the study. We also thank Pam Smith of Oregon Health and Science University for her assistance in drawing the participants' blood and Greg Anderson for his statistics expertise.

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## Paleolithic diet, sweet potato eaters, and potential renal acid load

Dear Sir:

In a recent article in the Journal, Sebastian et al (1) provided a detailed analysis of the probable effect of ancestral preagricultural diets on systemic acid load (net endogenous acid production, or NEAP) and compared this with the average acid load of contemporary diets. The NEAP was calculated for retrojected preagricultural diets for which compositions were suggested by Eaton and Konner (2). Current food tables served to estimate the respective nutrient content. Final computation was based on an existing calculation model (3, 4) that was modified to more accurately estimate those food-dependent acid loads that lead to endogenous production (and renal excretion) of sulfate and organic acids (OAs).

This modified approach, which considers individual dietary sulfur-containing amino acids (instead of average protein content) and dietary determinants of OA production, offers an important improvement to existing estimation models for net acid excretion. However, we do not fully agree with Sebastian et al, who argue that food-dependent endogenous OA production can be sufficiently predicted (with specific formulas) from the same nutrients (sodium, potassium, calcium, magnesium, chlorine, and phosphorus) that are needed to estimate the major food-dependent component (apart from sulfur-containing amino acids) of NEAP or of potential renal acid load (PRAL).

It is highly probable that the renal excretion of different OAs is dependent on diet. Aromatic organic acids are a dietary component, not mentioned by Sebastian et al, that may have a particularly strong effect. For example, phenolic and benzoic acids, which are found in considerable amounts especially in fruit (5, 6), are metabolically inactivated (detoxified) and excreted (mainly via the kidney) as acids, largely in the form of hippuric acid.

Interestingly, in the highlands of New Guinea, some Papuan tribes consume a low-protein vegetarian diet consisting predominantly of sweet potatoes. These sweet potato eaters excrete extremely high amounts of hippuric acid (31 mmol/d on average compared with 4 mmol/d in European control subjects), which adds substantially to their basal (not primarily food-dependent) urinary OA excretion (7). Basal OA excretion can be estimated from average anthropometric data as follows (3, 4):

$$\text{Basal OA excretion} = \text{body surface area } (\text{m}^2) \times 41 \text{ mEq} \times \text{d}^{-1} \times (1.73 \text{ m}^2)^{-1} \quad (1)$$

As a result, ≈36 mEq/d is yielded for sweet potato eaters [young adult males weighing 53 kg, 1.55 cm tall, and with a body surface area of 1.5 $m^2$; (7)], which together with their hippuric acid output amounts to 67 mEq total OA excretion/d.

The reported data (7) on average daily food intake and 24-h urinary excretion of sodium (7 mmol/d), chloride (4 mmol/d), potassium (180 mmol/d), and total nitrogen (2.6 g/d) allowed us to estimate the NEAP and PRAL of the sweet potato eaters. Urinary excretion rates not given in the original article (7) were calculated (3, 4) from the corresponding daily intakes of magnesium (443 mg/d), calcium (728 mg/d), and phosphorus (936 mg/d). They were obtained from the reported food consumption by using food tables (8) and yielded values of 12, 9, and 34 mEq/d, respectively. Urinary sulfate output (11 mEq/d) was estimated from protein degradation, ie, from total nitrogen excretion (*see* above), corresponding to an absorbed amount of 16.3 g protein/d. The nutrient-dependent PRAL (sulfate + phosphate + chloride − sodium − potassium − magnesium − calcium) was then calculated as −159 mEq/d. Because the NEAP corresponds to PRAL + OA, an average overall endogenous acid production of −92 mEq/d was finally yielded. This NEAP, directly calculated for "modern" stone age farmers by using measured (ie, hard) data for the intake and renal excretion of nutrients, is nearly identical to the average NEAP (−88 mEq/d) found by Sebastian et al for 159 retrojected preagricultural diets. However, the protein intake of the sweet potato eaters was very low (22 g protein/d, as estimated from urinary nitrogen output under the assumption of 75% net absorption), whereas protein intakes of ≈200 g are assumed for most ancestral diets (1, 9).

If the protein intake of sweet potato eaters was to isoenergetically increase by only 100 g/d (with protein replacing carbohydrates), the NEAP would increase (ie, net base production would fall) to −43 mEq/d. Therefore, it appears to us that the average net base production of −88 mEq/d (ie, the absolute figure) calculated by Sebastian et al may be too high for Stone Age persons with high protein intakes. This is also confirmed if the average PRAL and NEAP are calculated from the average nutrient intakes of Stone Age persons as recently published by Eaton and Eaton (9). Using their figures on daily nutrient intakes, we estimated a negative PRAL of −39 mEq/d, leading to an NEAP of 22 mEq/d, which is markedly lower than current net acid excretion (64 mEq) in the United States (**Table 1**).

Taken together, we also conclude that the average Paleolithic diet principally led to net base production (yielding a negative PRAL), but was possibly less alkaline than suggested by Sebastian et al. One of several uncertainties in this respect is obviously the intake of those OAs not metabolically combusted but renally excreted, eg, phenolic acid, which is excreted in the form of hippuric acid. Reasons for the historical shift from negative to positive PRAL are not only the displacement of alkali-rich plant foods in the ancestral diet by cereal grains and nutrient-poor foods in the temporary diet but also the modern processing and preparation of foods, which lead to considerable losses of base-forming nutrients such as potassium and magnesium.

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**TABLE 1**
Potential renal acid load (PRAL) and net endogenous acid production (NEAP) in the Paleolithic Age and today

| | Paleolithic diet (3000 kcal/d) | | US diet today (2500 kcal/d) | |
|---|---|---|---|---|
| | Intake[1] | Urine[2] | Intake[1] | Urine[2] |
| | *g/d* | *mEq/d* | *g/d* | *mEq/d* |
| Protein/sulfate | 226.0 | 110 | 79.0 | 39 |
| Phosphorus | 3.22 | 118 | 1.51 | 55 |
| Potassium | 10.50 | −215 | 2.50 | −51 |
| Calcium | 1.62 | −20 | 0.92 | −12 |
| Magnesium | 1.22 | −32 | 0.32 | −8 |
| PRAL | | −39 | | 23 |
| Organic acids[3] | | 61 | | 41 |
| NEAP | | 22 | | 64 |

[1] Intake data from Eaton and Eaton (9), with protein intake calculated from given ratios of protein to potassium (0.84 g/mmol for Stone Age persons and 1.24 g/mmol for Americans).

[2] Potential renal excretion, calculated according to references 3 and 4 by using the following conversion factors (g intake → mEq excretion): sulfate, 0.4888; phosphorus, 36.6; potassium, 20.5; calcium, 12.5; magnesium, 26.3.

[3] Basal organic acid excretion was calculated for a subject with a body surface area of 1.73 $m^2$ (41 mEq/d). A further diet-dependent organic acid excretion (eg, hippuric acid) value, assumed to be 20 mEq/d, was added for the Stone Age persons.

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## Reply to T Remer and F Manz

Dear Sir:

In estimating net endogenous acid production (NEAP) in preagricultural humans and their hominid ancestors (1), we computed the contribution to the NEAP of the body's total organic acid production as a function of the "unmeasured anion" content of the diet, on the basis of the following considerations. Ultimately, one must determine the contribution to the NEAP of total organic acid production as that fraction of organic acids produced whose dissociated organic anions escape into the urine, leaving behind the protons once associated with them (2). Those organic anions that do not so escape are metabolized to bicarbonate, which backtitrates the dissociated protons. As total organic acid production increases, so does the spillover of organic anions into the urine. Total organic acid production increases in response to increased bicarbonate input to the body—evidently a homeostatic response to mitigate the alkalinization (3)—and as a result the excretion rate of the dissociated organic anions increases. The increase in lactic acid and ketoacid production, and the excretion of their anions, after alkali administration exemplifies that fact (3), as does the dose-dependent increase in total organic anion excretion in response to increased dietary bicarbonate precursors (4). The dose-dependent increase in citrate excretion in response to bicarbonate administration or to dietary bicarbonate precursors also exemplifies that fact (5, 6), which is contributed to by reduced reabsorption of citrate filtered at the renal glomerulus (3).

Accordingly, organic anion excretion increases in response to increases in the so-called unmeasured anion (UA) content of the diet—computed as $Na^+ + K^+ + Ca^{2+} + Mg^{2+} - Cl^- - P_i$ in mEq/d—because such UAs consist of organic anions largely metabolized to bicarbonate and thus are dietary bicarbonate precursors (4). Empirically, in adults eating a wide variety of diets, organic anion excretion correlates positively and quantifiably with the UA content of the diet (4, 6). Thus, in our study (1), we computed the contribution of organic acid production to the NEAP from the UA content of the diet by using the regression equation of Kleinman and Lemann (4).

Remer and Manz prefer to estimate organic anion excretion not from dietary UA but from body surface area (BSA) independent of diet composition. This approach ignores the physiology discussed above, in that persons with a given BSA may have markedly different organic anion excretion rates depending on the content of bicarbonate precursors in the diet. The BSA approach might identify a basal component of organic anion excretion independent of diet. However, our method accounts for any such baseline effect because the organic anion excretion intercept at zero diet UA content in the regression of the former on the latter among adult diets has a positive value, which is incorporated in the calculation for organic anion excretion (4). Moreover, because the UA content of contemporary Western diets is almost an order of magnitude lower than that in preagricultural diets (1), it has little influence on organic anion excretion relative to the diet-independent basal rate. This may explain why Remer and Manz's BSA approach works reasonably well for contemporary diets, and why their approach may be questioned for preagricultural diets.

In part for the reasons cited above, we take exception to the estimation of the positive value of NEAP for the single retrojected preagricultural diet shown in Remer and Manz's Table 1. We consider their estimation of organic acid contribution to NEAP in the table problematic, not only because it is based on an assumed BSA and thereby ignores the effect of the diet's content of bicarbonate precursors independent of BSA but also because it posits an arbitrary diet-dependent substantial production of a nonmetabolized organic acid, hippuric acid, which might result from eating large amounts of noncombustible hippuric acid precursors in their undissociated acid form. Although a few plant foods (eg, cranberries and plums) do contain substantial amounts of noncombustible organic acids, such foods would substantially alter

interpretation of the quantitative relation between organic anion excretion and dietary UA content only if they were a regular and major component of the daily diet. Estimating NEAP as we did for a large number of preagricultural diets ($n = 159$), with widely differing plant food group distribution ratios, gives a more comprehensive picture of the potential range of preagricultural diet-induced NEAPs, most of which were computed as decidedly negative values (1).

Nothwithstanding their comments, Remer and Manz accept the main conclusion of our article. They end by writing, "Taken together, we also conclude that the average Paleolithic diet principally led to net base production," although they suggest that the average NEAP we reported for 159 diets, −88 mEq/d, might slightly overestimate net base production because of the presence of noncombustible organic acids in some food items. We do not necessarily disagree but doubt that the adjustment is large averaged over 159 different diets, given the small fraction of natural food items with substantial noncombustible organic acid content present in the undissociated acid form. Further limiting the effect of any such noncombustible organic acids, some fraction of those acids in a food item exist in their dissociated organic anion form, the amount depending on the acid's $pK_a$ and the pH of the food. Because such non-bicarbonate-generating organic anions appear as UAs, they get computed both as part of the bicarbonate yield of the food and as part of its contribution to the organic anion excretion rate. Therefore, their effect on the NEAP tends to be cancelled out.

As to meat-eating sweet potato eaters, we concede that odd 2-food item combinations might yield lower estimates of net base load than our reported average for preagricultural diets. We reported several such examples in our paper, even some with net acid loads (1). It seems unlikely that ancestral hominid diets consisting predominately of such odd 2-food item combinations were habitually ingested over millions of years, and therefore it seems unlikely that they played a dominant role in conditioning the genetic makeup of humans.

We end by expressing our appreciation to Remer and Manz for their numerous contributions over many years to our knowledge of diet effects on NEAP and for their trailblazing efforts in tackling the problem of computing the NEAP from diet composition. To the extent that our findings suggest that natural selection likely has adapted human metabolic machinery and integrated organ physiology to habitual ingestion of a net base-producing diet, and not to the modern net acid-producing diet, Remer and Manz merit a share in the discovery.

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## Metabolic effects of dietary fructose

Dear Sir:

Elliott et al (1) wrote an interesting article concerning fructose, weight gain, and the insulin resistance syndrome. In that review they concluded that an increased consumption of fructose might be one of the environmental factors contributing to the development of obesity and the accompanying abnormalities of the insulin resistance syndrome. It is true that the prevalence of obesity in the United States and worldwide is increasing, and it is important to identify the acquired causes contributing to this increase. However, several lifestyle factors other than an increased consumption of fructose are much more probable contributors to the development of obesity (eg, a high intake of fat and minimal physical activity).

Elliott et al described a few mechanisms by which the consumption of dietary fructose might influence glucose metabolism and insulin resistance. However, they did not address the prolonged effects of dietary fructose on glucose metabolism, which are worthy of review. Type 2 diabetes (or, adult-onset diabetes) is one of the biggest health problems in the Western world, and it is suggested to be a consequence of increased energy intake and decreased physical activity. In persons with type 2 diabetes, the need for insulin is greater than that able to be produced by the pancreas. Therefore, the foods that produce a lower secretion of insulin (ie, foods that have a low glycemic index) are known to be beneficial for glucose metabolism. Koivisto and Yki-Jarvinen (2) studied the effects of dietary fructose (20% of calories as carbohydrate calories; 45–65 g/d for 4 wk) on insulin concentration and glycated hemoglobin in 10 patients with type 2 diabetes. In that study, subjects were fed—in a double-blind, randomized crossover design—a crystalline fructose or isocaloric complex carbohydrate (control) diet evenly as 4 meals or snacks per day while hospitalized. The mean diurnal blood glucose concentration decreased during both diets, but serum insulin concentration remained unchanged. Glycated hemoglobin, measured to determine glucose balance long term, improved only during the fructose diet (9.0% compared with 8.0%; $P < 0.02$) (2). In that study, insulin sensitivity also increased, by 34% ($P < 0.05$), during the fructose diet but remained unchanged during the control diet.

Even more long-term effects of a fructose diet on glycemic control were studied by Osei et al (3). They performed an outpatient study in 18 patients with type 2 diabetes who consumed either 60 g crystalline fructose/d ($n = 9$) or their usual meals ($n = 9$; control group) for 12 wk. Osei et al reported that both serum glucose and glycated hemoglobin concentrations progressively decreased in the group treated with fructose but had a tendency to increase in the control group during the study. The authors concluded that a slight improvement in glycemic control and alterations in the apoprotein composition that favor a decreased risk of coronary

artery disease may occur with an increased consumption of fructose (3). The conclusion by Elliott et al that dietary fructose has only detrimental metabolic and endocrine effects is somewhat misleading. However, Elliott et al do suggest that much more research is needed to fully understand the metabolic effects of dietary fructose, particularly in humans.

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## Reply to TJ Vasankari

Dear Sir:

We agree with Vasankari that dietary fat and inactivity are likely environmental factors contributing to the marked worldwide increase in the prevalence of obesity, and this was noted in our review on the metabolic effects of fructose (1). However, we believe that an increase in fructose consumption also deserves attention as a potential third factor contributing to the escalation of obesity. Because we are not aware of quantitative data regarding the relative contributions of the 3 factors, we consider it premature to conclude that dietary fat and reduced physical activity are "much more probable contributors" to the obesity epidemic. As we and Bray (2) have pointed out, fructose consumption has increased concurrently with the obesity epidemic. Furthermore, because fructose—similarly to dietary fat—does not stimulate insulin secretion, leads to decreased leptin production, and does not suppress the orexigenic gastric hormone ghrelin (3), the lack of effects of long-term fructose consumption on these endocrine systems involved in the regulation of energy homeostasis could lead to increased energy intake, weight gain, and obesity. We therefore regard increased fructose consumption to be a likely contributor to the increased prevalence of obesity in the past 2–3 decades.

Vasankari discusses the inclusion of fructose in the diets of patients with diabetes because of its low glycemic index. In our review we briefly addressed this topic and also cited evidence that the consumption of small catalytic amounts of fructose increases hepatic glucose uptake and reduces glucose excursions after treatment with oral glucose in subjects with type 2 diabetes (1). Thus, we agree that modest amounts of dietary fructose may be beneficial in the dietary management of diabetes. Although we intentionally did not provide an in-depth discussion of or cite additional literature on studies of fructose consumption in diabetes, we believe that certain issues should be considered before high-fructose diets are recommended for diabetic patients. First, when large amounts of fructose are rapidly consumed, a sufficient amount of fructose may escape hepatic uptake to significantly elevate systemic circulating fructose concentrations. Protein fructosylation could contribute to diabetic complications (4), particularly because fructose is a major product of the polyol-sorbitol pathway and because tissue fructose accumulation has been implicated in diabetic neuropathy and other complications of diabetes. It was reported that consumption of a high-fructose diet increases both the formation of cataracts and of oxidative byproducts in the kidneys of streptozotocin-diabetic rats (5). In nondiabetic rats, fructose consumption for 1 y led to increased glycation (fructosamine and glycated hemoglobin) and markers of lipid peroxidation and aging when compared with animals that consumed glucose (6). The effect of dietary fructose on glycation and oxidation-related products deserves further investigation, and it is important to determine whether increased glycation and oxidation occur in humans consuming high amounts of dietary fructose.

In addition, although not all studies have shown an adverse effect of fructose consumption on lipids, several studies (7–9), including our own (3), showed that—compared with glucose—fructose increases postprandial triacylglycerol. Although this effect is likely to be highly dependent on the amount of fructose consumed, evidence exists that fructose-induced hypertriglyceridemia is magnified in subjects with insulin resistance (10) or hypertriglyceridemia (6, 11). Therefore, caution should be exercised when recommending diets high in fructose to patients with the metabolic syndrome or type 2 diabetes. Furthermore, in preliminary studies, we found that overweight women with normal fasting triacylglycerol concentrations who consumed 25% of energy from fructose-sweetened beverages for 10 wk had markedly increased postprandial triacylglycerol concentrations (compared with a baseline diet high in complex carbohydrate) and significantly elevated concentrations of atherogenic apolipoprotein B (12). In contrast, postprandial triacylglycerol and apolipoprotein B concentrations did not increase in subjects who consumed 25% of energy as glucose, which induced larger postprandial circulating glucose and insulin excursions than did the consumption of complex carbohydrate or fructose. Thus, the consumption of glucose-sweetened beverages—which have a high glycemic index—does not result in adverse changes in the postprandial lipid profile.

In summary, the effects of fructose on postprandial triacylglycerol and apolipoprotein B concentrations suggest that long-term consumption of high amounts of fructose could contribute to the risk of cardiovascular disease. As indicated by some studies, this effect is likely to be exacerbated in subjects with preexisting metabolic disease. Finally, as discussed above, the lack of effects on endocrine systems involved in body weight regulation suggests that the long-term consumption of diets high in fructose may lead to weight gain, obesity, and the development of type 2 diabetes. Clearly, much additional research is needed to more fully understand the metabolic effects of high-fructose diets, particularly in subjects at risk of metabolic diseases, ie, obese, insulin-resistant, or hyperlipidemic persons. Limiting fructose consumption may be a desirable objective in the management of obesity and hyperlipidemia in individual persons and in the prevention of weight gain and its metabolic consequences at the population level.

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## Erratum

Francois CA, Connor SL, Bolewicz LC, Connor WE. Supplementing lactating women with flaxseed oil does not increase docosahexaenoic acid in their milk. Am J Clin Nutr 2003;77:226–33.

In Table 1 on page 227, the values for fatty acids 18:1n−9 and 20:1n−9 were interchanged. The value for 18:1n−9 should read 20.5% by wt of total fatty acids, and the value for 20:1n−9 should read 0.0% by wt of total fatty acids.