

## Conversion of $\alpha$ -linolenic acid to eicosapentaenoic, docosapentaenoic and docosahexaenoic acids in young women

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The extent to which women of reproductive age are able to convert the *n*-3 fatty acid  $\alpha$ -linolenic acid (ALNA) to eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA) was investigated *in vivo* by measuring the concentrations of labelled fatty acids in plasma for 21 d following the ingestion of [ $U$ - $^{13}$ C]ALNA (700 mg). [ $^{13}$ C]ALNA excursion was greatest in cholesteryl ester (CE) (224 (SEM 70)  $\mu$ mol/l over 21 d) compared with triacylglycerol (9-fold), non-esterified fatty acids (37-fold) and phosphatidylcholine (PC, 7-fold). EPA excursion was similar in both PC (42 (SEM 8)  $\mu$ mol/l) and CE (42 (SEM 9)  $\mu$ mol/l) over 21 d. In contrast both [ $^{13}$ C]DPA and [ $^{13}$ C]DHA were detected predominately in PC (18 (SEM 4) and 27 (SEM 7)  $\mu$ mol/l over 21 d, respectively). Estimated net fractional ALNA inter-conversion was EPA 21%, DPA 6% and DHA 9%. Approximately 22% of administered [ $^{13}$ C]ALNA was recovered as  $^{13}$ CO<sub>2</sub> on breath over the first 24 h of the study. These results suggest differential partitioning of ALNA, EPA and DHA between plasma lipid classes, which may facilitate targeting of individual *n*-3 fatty acids to specific tissues. Comparison with previous studies suggests that women may possess a greater capacity for ALNA conversion than men. Such metabolic capacity may be important for meeting the demands of the fetus and neonate for DHA during pregnancy and lactation. Differences in DHA status between women both in the non-pregnant state and in pregnancy may reflect variations in metabolic capacity for DHA synthesis.

### Women: $\alpha$ -Linolenic acid: Stable isotopes: Docosahexaenoic acid: Plasma

*n*-3 Long-chain polyunsaturated fatty acids (LCPUFA), principally docosahexaenoic acid (22:6*n*-3; DHA), are important structural components of cell membranes. Maintenance of appropriate levels of *n*-3 LCPUFA in membrane phospholipids is important for ensuring cell function. Alterations to the eicosapentaenoic acid (20:5*n*-3; EPA) and DHA content of membrane phospholipids relative to other fatty acids, in particular those of the *n*-6 series, may modify cell function (Innis, 1991; Calder, 1999). However,  $\alpha$ -linolenic acid (18:3*n*-3; ALNA), the putative precursor of EPA and DHA, is the principal dietary *n*-3 fatty acid in individuals consuming a Western diet (about 1.5 g/d). Since estimated intakes of ALNA are 25-fold and 15-fold greater than EPA and DHA, respectively (Ministry of Agriculture, Fisheries and Food, 1997), the metabolic capacity of an individual for ALNA conversion and for mobilisation of its longer-chain metabolites within the body may be important for maintaining membrane EPA and DHA levels.

A pathway for conversion of ALNA to EPA and DHA

has been identified in rats (Voss *et al.* 1991; reviewed in Sprecher, 2000). Briefly, ALNA is converted to EPA by the sequential activities of  $\Delta$ 6 and  $\Delta$ 5 desaturases and by elongation of the carbon chain. Docosapentaenoic acid (22:5*n*-3; DPA) is formed by addition of C<sub>2</sub> to EPA, which is converted subsequently to 24:5*n*-3 and 24:6*n*-3 by further chain elongation and  $\Delta$ 6 desaturation. DHA is synthesised from 24:6*n*-3 by peroxisomal  $\beta$ -oxidation, which shortens the carbon chain by C<sub>2</sub>. This pathway has also been demonstrated in the pig (Li *et al.* 2000) and baboons (Su *et al.* 1999*a,b*). While  $\Delta$ 6-desaturase appears to be the rate-limiting step for this pathway, its overall regulation is unclear and may contain several loci of metabolic control including translocation of 24:6*n*-3 and DHA between the endoplasmic reticulum and peroxisomes (Sprecher, 2000). It has been assumed that hepatic capacity for ALNA conversion and mobilisation from the liver by VLDL represents the major source of newly synthesised EPA and DHA for supply to the periphery. However, since some non-hepatic tissues, in particular brain and

**Abbreviations:** ALNA,  $\alpha$ -linolenic acid; AUC, area under the curve; CE, cholesteryl ester; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; FAME, fatty acid methyl esters; GC–C-IRMS, gas chromatography–combustion-isotope ratio mass spectrometry; LCPUFA, long-chain polyunsaturated fatty acid; NEFA, non-esterified fatty acid; PC, phosphatidylcholine; TAG, triacylglycerol.

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skeletal muscle, express both  $\Delta 6$  and  $\Delta 5$  desaturases (Cho *et al.* 1999a,b) it is possible that ALNA conversion within peripheral tissues may also be important for maintaining membrane EPA and DHA concentrations.

In adult men the extent of conversion of ALNA to EPA and DHA has been studied by administration of either  $^2\text{H}$ - or  $^{13}\text{C}$ -labelled ALNA. Estimates for fractional conversion of labelled ALNA derived from excursions of individual metabolites in plasma show about 8% was present as EPA, but apparent synthesis of DHA ranged between 0% (Burdge *et al.* 2001) to 4% (Emken *et al.* 1994). Despite methodological differences between studies, the overall capacity for EPA synthesis in men appears to be limited and conversion to DHA at best constrained severely (Emken *et al.* 1994; Salem *et al.* 1999; Vermunt *et al.* 2000; Burdge *et al.* 2001). This is consistent with the findings of studies in which increased dietary ALNA intake was not associated with an increase in plasma and/or cell membrane DHA concentration (reviewed by Gerster, 1998). These observations suggest that men may be particularly dependent upon intake of pre-formed DHA for maintenance of membrane DHA concentration.

The extent to which women are able to convert ALNA to EPA, DPA and DHA is not known. As the capacity for essential fatty acid desaturation and chain-elongation appears to be limited in the human fetus (Chambaz *et al.* 1985), the fetus is dependent upon the effective supply of pre-formed EPA and DHA from the maternal circulation, either from the maternal diet, tissue reserves or synthesis from ALNA. The metabolic demands for DHA by the fetal-placental unit represent a substantial burden for *n*-3 fatty acids upon the mother above that incurred in maintaining her own tissue *n*-3 fatty acid levels in the non-pregnant state, exceeding 860 mg/week in late gestation. This is based upon the sum of estimated accumulation of DHA into the developing brain, liver and adipose tissue (Clandinin *et al.* 1981), but is likely to be a marked under-representation of the true demands, as it does not include the remaining fetal tissues or placenta. In the absence of a selective increase in dietary DHA intake, such increased demands can only be met by mobilisation of fatty acid stores accumulated before pregnancy and/or the up regulation of ALNA conversion.

Therefore, there is a need to determine whether young women, in the non-pregnant state, are different from men in the way in which they handle *n*-3 PUFA and, in particular, their capacity to form EPA and DHA from dietary ALNA. In the present study we have characterised the metabolic fate of [ $U$ - $^{13}\text{C}$ ]ALNA in young women, in terms of the extent of incorporation into *n*-3 LCPUFA in plasma lipids and partitioning towards  $\beta$ -oxidation.

## Materials and methods

### Materials

[ $U$ - $^{13}\text{C}$ ]ALNA (>98 atom%) was purchased from Martek Biosciences Corporation (Columbia, ML, USA). This preparation contained 97.1% ALNA, the remainder being short- and medium-chain fatty acids. Solvents were from Fisher Chemicals Limited (Loughborough, Leics., UK).

Fatty acid standards and all other reagents were obtained from Sigma (Poole, Dorset, UK). BondElut solid phase extraction cartridges were from Varian Limited (Walton-on-Thames, Surrey, UK).

### Subjects

Subjects ( $n$  6) were healthy women aged  $28 \pm 4$  years with BMI  $22.4 \pm 2.8$  kg/m<sup>2</sup>. None of the subjects consumed fish oil dietary supplements or regularly ate oily fish. Routine biochemical analyses of fasting blood lipid concentrations were within ranges regarded as normal (Department of Chemical Pathology, Southampton University Hospitals Trust, Southampton, UK) and none of the subjects had raised concentrations of enzyme markers of hepatic dysfunction. Three subjects regularly used a combined oral contraceptive (30–35  $\mu\text{g}$  17  $\alpha$ -ethinyloestradiol/d) while the others did not take synthetic oestrogens. Ethical approval was granted by the Joint Ethics Committee of Southampton and South West Hampshire and subjects gave written consent.

### Administration of [ $U$ - $^{13}\text{C}$ ] $\alpha$ -linolenic acid and specimen collection

All subjects were studied on the tenth day after the start of their menstrual period. The protocol for administration of [ $^{13}\text{C}$ ]ALNA was essentially the same as Burdge *et al.* (2001). Briefly, on the day preceding the start of the study subjects consumed only three standardised meals (total energy 11.2 MJ/d) and then fasted overnight for 12 h. On the study day, whole body  $\text{CO}_2$  excretion was measured by using a GEM indirect calorimeter (PDZ-Europa, Crewe, Ches., UK) and a sample of breath was collected at baseline. A blood sample was collected from a forearm vein by venesection using lithium heparin as an anti-coagulant. Subjects then consumed [ $U$ - $^{13}\text{C}$ ]ALNA (700 mg) free fatty acid emulsified with double cream (22 g), casein (12 g), beet sugar (4.5 g), glucose (9 g) and Nesquik milkshake powder (Nestlé, Vevey, Switzerland; 10 g). The lipid composition of the test meal was adjusted with sunflower-seed and fish oils so that the fatty acid composition of the combined emulsion and test meal reflected the estimated *n*-3 fatty content of the typical UK diet (Ministry of Agriculture, Fisheries and Food, 1997). The fatty acid composition of the combined test meal and emulsion was confirmed by GC (Table 1). Labelled ALNA was consumed as a drink (150 ml) and accompanied by a standard test breakfast. The total macronutrient and energy contents of the emulsion and test meal are summarised in Table 1. Subjects consumed two further standardised meals at 6 and 12 h after ingestion of labelled ALNA and then resumed their habitual diet for the remainder of the study period. Breath samples were collected at 2 h intervals for 12 h and then at 24 h. Venous blood specimens were collected by venesection at 24, 48, and 72 h and at 1, 2 and 3 weeks.

### Preparation of fatty acid methyl esters from plasma lipids

Blood samples were separated into plasma and erythrocytes

**Table 1.** Fatty acid composition of emulsion and test meal determined by gas chromatographic analysis of total lipid extracts\*

Fatty acid	Typical mass in test meal and emulsion (mg)
12:0	1393
14:0	4562
16:0	11992
18:0	5550
16:1 <i>n</i> -7	789
18:1 <i>n</i> -9	12263
20:1	22
22:1	14
18:2 <i>n</i> -6	7596
α-Linolenic acid	1003†
20:3 <i>n</i> -6	3
20:4 <i>n</i> -6	0
Eicosapentaenoic acid	56
Docosahexaenoic acid	46

\* Total energy intake was 3995 kJ (protein 10.2%, carbohydrate 49.1% and fat 40.7%). Total mass of fatty acids was 45.3 g.

† Mass includes 700 mg [ $U\text{-}^{13}\text{C}$ ] α-linolenic acid.

by centrifugation at 1125 *g* for 15 min at 4°C. Plasma was collected and mixed with protease inhibitors (phenylmethylsulfonylfluoride 1.7 μg; aprotinin 7 μg; ethylenediamine tetra-acetic acid 32.2 μg/ml plasma) and stored at -20°C. Total lipids were prepared from plasma (1 ml) by extraction with chloroform-methanol (2:1, v/v) (Folch *et al.* 1957) containing butyrate hydroxytoluene (50 μg/ml). Triheptadecanoin, diheptadecanoyl phosphatidylcholine (PC), heneicosanoate and cholesteryl behenate were added as internal standards. Triacylglycerol (TAG), PC, non-esterified fatty acid (NEFA) and cholesteryl esters (CE) were isolated from total lipid extracts by solid phase extraction using aminopropylsilica cartridges (100 mg) and trans-esterified as described (Burdge *et al.* 2000). Purified lipids were dissolved in toluene and converted to their corresponding fatty acid methyl esters (FAME) by addition of methanol containing 2% (v/v) H<sub>2</sub>SO<sub>4</sub> and incubation at 50°C for 18 h. The reaction mixture was neutralised with 0.25 M KHCO<sub>3</sub> and 0.5 M K<sub>2</sub>CO<sub>3</sub> and the FAME extracted with hexane. Samples were dried under N<sub>2</sub> before analysis of isotopic enrichment (Burdge *et al.* 2000).

#### Analysis of <sup>13</sup>C enrichment in plasma fatty acids

<sup>13</sup>C enrichment of *n*-3 fatty acids was determined by GC-combustion-isotope ratio mass spectrometry (GC-C-IRMS). FAME were resolved on a 50 m × 0.25 μm × 0.32 mm BPX-70 fused silica capillary column (SGE Europe Limited, Milton Keynes, UK) using an HP6890 GC (Hewlett Packard, Wokingham, Berks., UK). FAME were converted to CO<sub>2</sub> by heating to 860°C in the presence of PtCuO using an Orchid IRMS interface (PDZ-Europa) and <sup>13</sup>CO<sub>2</sub>:<sup>12</sup>CO<sub>2</sub> was determined by a 20/20 Stable Isotope Analyser (PDZ-Europa). Tricosanoic acid methyl ester was used as isotopic enrichment standard (1.135 atom%). ALNA, EPA, DPA and DHA were all resolved to baseline including complete separation of DPA from 24:0 and 24:1. FAME were identified by

their retention times relative to standards. Concentrations of total fatty acids were calculated by integration of baseline-corrected peak areas on chromatograms generated from the total ion current by the GC-C-IRMS. For 1 ml plasma:

(A) target fatty acid concentration (mol/l) = (peak area target fatty acid ÷ peak area internal standard) × internal standard (mol) × 1000;

(B) fractional enrichment determined by interpolation using a calibration curve of fractional enrichment plotted against atom % enrichment;

(C) concentration of <sup>13</sup>C-labelled fatty acid = (A) × (B).

Repeated analysis of the same specimen showed that the CV for measurement of the concentration of <sup>13</sup>C-labelled fatty acids in individual plasma lipid classes was consistently less than 4%. This technique was able to resolve <sup>13</sup>C enrichment at 0.002% total fatty acid mass.

#### Estimation of [<sup>13</sup>C]fatty acid oxidation

<sup>13</sup>C enrichment of CO<sub>2</sub> excreted on breath was determined by continuous flow isotope ratio MS using an automated breath carbon analyser interface (GSL; PDZ-Europa) and a 20/20 Stable Isotope Analyser (PDZ-Europa) (Jones *et al.* 1999). Fractional oxidation of labelled fatty acids over 24 h was calculated from whole body CO<sub>2</sub> excretion and <sup>13</sup>CO<sub>2</sub> enrichment on breath (Watkins *et al.* 1982). No attempt was made to adjust values for retention of <sup>13</sup>CO<sub>2</sub> within bicarbonate pools.

#### Statistical analysis

Data are presented as mean and standard error of the mean (SEM) concentrations in plasma. Statistical comparisons both of fatty acid concentrations and areas under the curve (AUC) were carried out by ANOVA using Bonferroni's *post hoc* correction for multiple comparisons between groups, with the exception of DPA, which was only present in two plasma lipid classes and so was analysed using Student's paired *t* test.

## Results

#### Concentrations of *n*-3 fatty acids in plasma lipids

Concentrations of total ALNA, EPA, DPA and DHA are summarised in Table 2. Analysis of the fatty acid composition of plasma lipids from fasted subjects at baseline showed *n*-3 LCPUFA had distinct distributions between classes. The greatest ALNA concentration was in the CE fraction, which was significantly greater than in PC (3-fold), TAG (5-fold) and NEFA (26-fold) fractions. EPA was present in similar concentration in both plasma PC and CE, both of which were significantly greater than that seen in TAG (15-fold) and NEFA (79-fold) fractions (Table 2). DPA was not detected in plasma NEFA and CE, but was present in PC at a concentration 8-fold greater than in TAG. Plasma PC DHA concentration was significantly greater than in CE (2-fold), TAG (17-fold) and NEFA (41-fold).

**Table 2.** Concentrations of total *n*-3 long-chain polyunsaturated fatty acids in plasma lipids at baseline for six fasted women (Mean values and standard errors of the mean)

	Concentration ( $\mu\text{mol/l}$ )							
	TAG		NEFA		PC		CE	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
ALNA	27.2 <sup>a†,b*,c‡</sup>	6.7	4.8 <sup>d†,e‡</sup>	0.7	40.0 <sup>f‡</sup>	7.7	124.9	29.5
EPA	3.5 <sup>a*,b†,c‡</sup>	0.6	0.7 <sup>d†,e‡</sup>	0.1	51.4	9.1	55.2	31.9
DPA	5.1 <sup>b†</sup>	0.9	ND		38.7	7.7	ND	
DHA	10.4 <sup>a*,b†,c‡</sup>	2.8	4.4 <sup>d†,e‡</sup>	0.3	181.2 <sup>f*</sup>	19.2	77.8	21.4

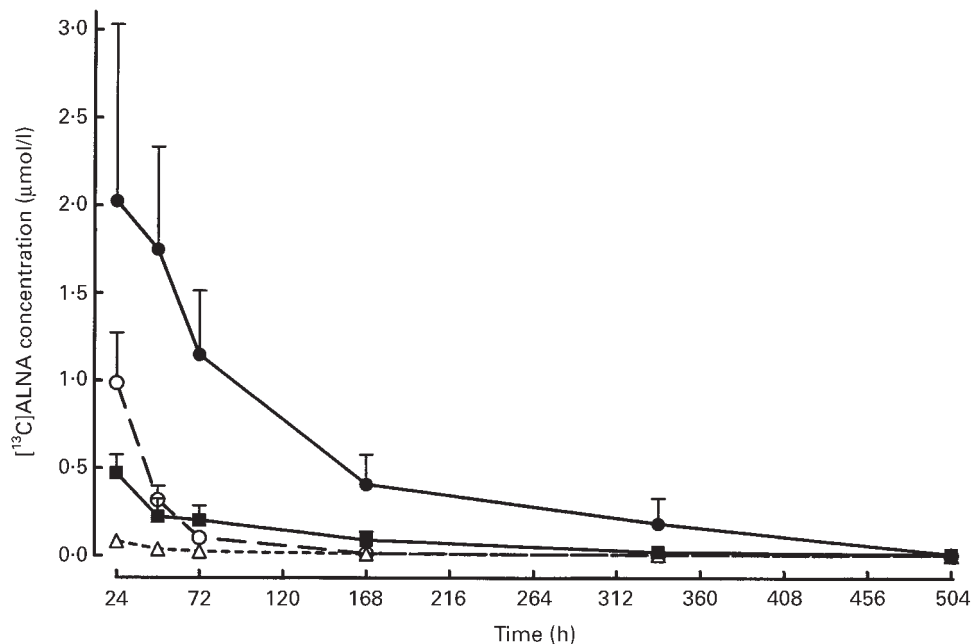
TAG, triacylglycerol; NEFA, non-esterified fatty acids; PC, phosphatidylcholine; CE, cholesteryl ester; ALNA,  $\alpha$ -linolenic acid; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid; ND, not detected.

Significant differences between lipid classes are indicated by: <sup>a</sup>TAG v. NEFA, <sup>b</sup>TAG v. PC, <sup>c</sup>TAG v. CE, <sup>d</sup>NEFA v. PC, <sup>e</sup>NEFA v. CE and <sup>f</sup>PC v. CE. Comparisons between lipid classes for ALNA, EPA and DHA were by ANOVA while comparisons between TAG and PC for DPA was by Student's unpaired *t* test. Mean values in a row were significantly different: \**P*<0.01, †*P*<0.001, ‡*P*<0.0001.

### Handling of [<sup>13</sup>C] $\alpha$ -linolenic acid in plasma

The excursions of labelled fatty acids in plasma lipids between 24 h and 21 d are summarised in Figs. 1 and 2. Labelled ALNA was detected in all four lipid classes at 24 h (Fig. 1). [<sup>13</sup>C]ALNA concentration was greatest in the plasma TAG at 24 h (0.47 (SEM 0.11)  $\mu\text{mol/l}$ ), reached approximately half-maximum values (0.22 (SEM 0.10)  $\mu\text{mol/l}$ ) by 48 h and had decreased to baseline by 21 d (Fig. 1). The time-course of [<sup>13</sup>C]ALNA in plasma PC and NEFA fractions was similar to that seen in the TAG fraction. [<sup>13</sup>C]ALNA concentration in NEFA at 24 h was 0.08 (SEM 0.02)  $\mu\text{mol/l}$ , which decreased to approximately half-maximum level by 48 h (0.04 (SEM 0.01)  $\mu\text{mol/l}$ ) and

returned to baseline by 21 d (Fig. 1). In plasma PC, labelled ALNA concentration was greatest at 24 h (0.99 (SEM 0.29)  $\mu\text{mol/l}$ ), decreasing to half-maximum levels by 48 h (0.32 (SEM 0.08)  $\mu\text{mol/l}$ ) and reached baseline enrichment by 21 d (Fig. 1). [<sup>13</sup>C]ALNA concentration in the CE fraction at 24 h was 2.0 (SEM 1.0)  $\mu\text{mol/l}$ , which decreased to approximately half-maximum concentration by 72 h (1.15 (SEM 0.37)  $\mu\text{mol/l}$ ) and returned to baseline by 21 d (Fig. 1). The relative excursions of [<sup>13</sup>C]ALNA in plasma lipids were calculated from summation of the absolute excursion of labelled ALNA in each lipid class (Table 3) and expressed as a percentage: TAG 9.2, NEFA 2.1, PC 10.5 and CE 78.2. Therefore, [<sup>13</sup>C]ALNA was associated predominately with the CE fraction over



**Fig. 1.** Excursion of [<sup>13</sup>C] $\alpha$ -linolenic acid (ALNA) in plasma lipids over 21 d. Mean values for six women are shown with standard errors of the mean represented by vertical bars. (■), Triacylglycerol; (△), non-esterified fatty acid; (○), phosphatidylcholine; (●), cholesteryl ester concentrations in plasma.

**Table 3.** Excursions of individual [<sup>13</sup>C]-labelled *n*-3 fatty acids in plasma lipids over 21 d

	AUC (μmol/l × d)							
	TAG		NEFA		PC		CE	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
ALNA	26.3 <sup>a†,c†</sup>	4.8	5.9 <sup>d†,e†</sup>	0.5	30.2 <sup>†</sup>	4.5	224.4	70.1
EPA	3.3 <sup>a†,b*,c*</sup>	0.9	0.3 <sup>d†,e†</sup>	0.1	42.2	7.8	41.5	8.9
DPA	3.2 <sup>b*</sup>	1.1	ND		17.7 <sup>a*</sup>	3.8	ND	
DHA	3.8 <sup>a*,b†</sup>	2.0	0.5 <sup>d†,e*</sup>	0.2	26.8 <sup>†</sup>	7.0	2.9	1.1

TAG, triacylglycerol; NEFA, non-esterified fatty acids; PC, phosphatidylcholine; CE, cholesteryl ester; ALNA, α-linolenic acid; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid; ND, not detected.

Significant differences between lipid classes are indicated by: <sup>a</sup>TAG v. NEFA, <sup>b</sup>TAG v. PC, <sup>c</sup>TAG v. CE, <sup>d</sup>NEFA v. PC, <sup>e</sup>NEFA v. CE and <sup>†</sup>PC v. CE. Comparisons between lipid classes for ALNA, EPA and DHA were by ANOVA while comparison between TAG and PC for DPA was by Student's unpaired *t* test.

Mean values in a row were significantly different: \**P*<0.01, †*P*<0.001.

the 3-week period following ingestion of the labelled test meal.

*Handling of labelled eicosapentaenoic acid, docosapentaenoic acid and docosahexaenoic acid in plasma*

The time-courses of the excursions of labelled EPA, DPA and DHA are summarised in Fig. 2. In plasma TAG, [<sup>13</sup>C]EPA concentration was greatest at 24 h (36 (SEM 8) nmol/l) and decreased to baseline by 14 d (Fig. 2 (a)). Labelled DPA followed a similar time-course to EPA, with maximum concentration at 24 h (26 (SEM 7) nmol/l) decreasing to baseline by 12 d. Peak [<sup>13</sup>C]DHA concentration was at 48 h (12 (SEM 6) nmol/l), which returned to baseline by 21 d. The relative excursions of labelled *n*-3 fatty acids in plasma TAG were: ALNA 71.9%; EPA 9.0%; DPA 8.7%; DHA 10.4% (Table 3).

Incorporations of both [<sup>13</sup>C]EPA and [<sup>13</sup>C]DHA were detected in plasma NEFA (Fig. 2). However, there was no detectable incorporation of [<sup>13</sup>C]DPA above baseline <sup>13</sup>C abundance in the NEFA fraction. Maximum concentrations of labelled fatty acids were: EPA 2.2 (SEM 0.8) nmol/l at 48 h; DHA 1.0 (SEM 0.7) nmol/l at 72 h. The relative excursions of [<sup>13</sup>C]fatty acids in plasma NEFA were: ALNA 88.0%; EPA 4.5%; DPA 0%; DHA 7.5% (Table 3).

[<sup>13</sup>C]EPA concentration was greatest in plasma PC at 24 h (418 (SEM 85) nmol/l), which decreased to baseline over the subsequent 20 d (Fig. 2). Peak [<sup>13</sup>C]DPA concentration in plasma PC was at 72 h (88 (SEM 24) nmol/l), which returned to baseline enrichment by 21 d. Maximum [<sup>13</sup>C]DHA concentration in plasma PC occurred later than for labelled EPA and DPA at 7 d (79 (SEM 23) nmol/l), and remained significantly (*P*<0.01) elevated above baseline <sup>13</sup>C abundance at 21 d (31 (SEM 9) nmol/l). The relative excursions of <sup>13</sup>C-labelled *n*-3 fatty acids in plasma PC were: ALNA 25.8%; EPA 36.1%; DPA 15.2%; DHA 22.9% (Table 3).

Peak [<sup>13</sup>C]EPA concentration in plasma CE was at 72 h (213 (SEM 56) nmol/l), which decreased to baseline by 21 d (Fig. 2 (d)). There was no detectable incorporation of [<sup>13</sup>C]DPA into plasma CE above baseline <sup>13</sup>C abundance

over 21 d. <sup>13</sup>C enrichment of DHA in plasma CE was marginal with a peak concentration of 4 (SEM 2) nmol/l at 48 h. The relative excursions of labelled ALNA metabolites in plasma CE were: ALNA 83.5%; EPA 15.4%; DPA 0%; DHA 1.1%.

Calculation of the AUC for each of the labelled *n*-3 fatty acids in plasma over 21 d showed a differential distribution between lipid classes (Table 3). [<sup>13</sup>C]ALNA was carried predominately as plasma CE (≥7-fold compared with the other plasma lipid classes). The excursion of labelled EPA was similar in plasma PC and CE, both of which were substantially greater (>13-fold) than in TAG or NEFA. Both [<sup>13</sup>C]DPA and [<sup>13</sup>C]DHA were carried principally in plasma PC (both >5-fold compared with other lipid classes) (Table 3).

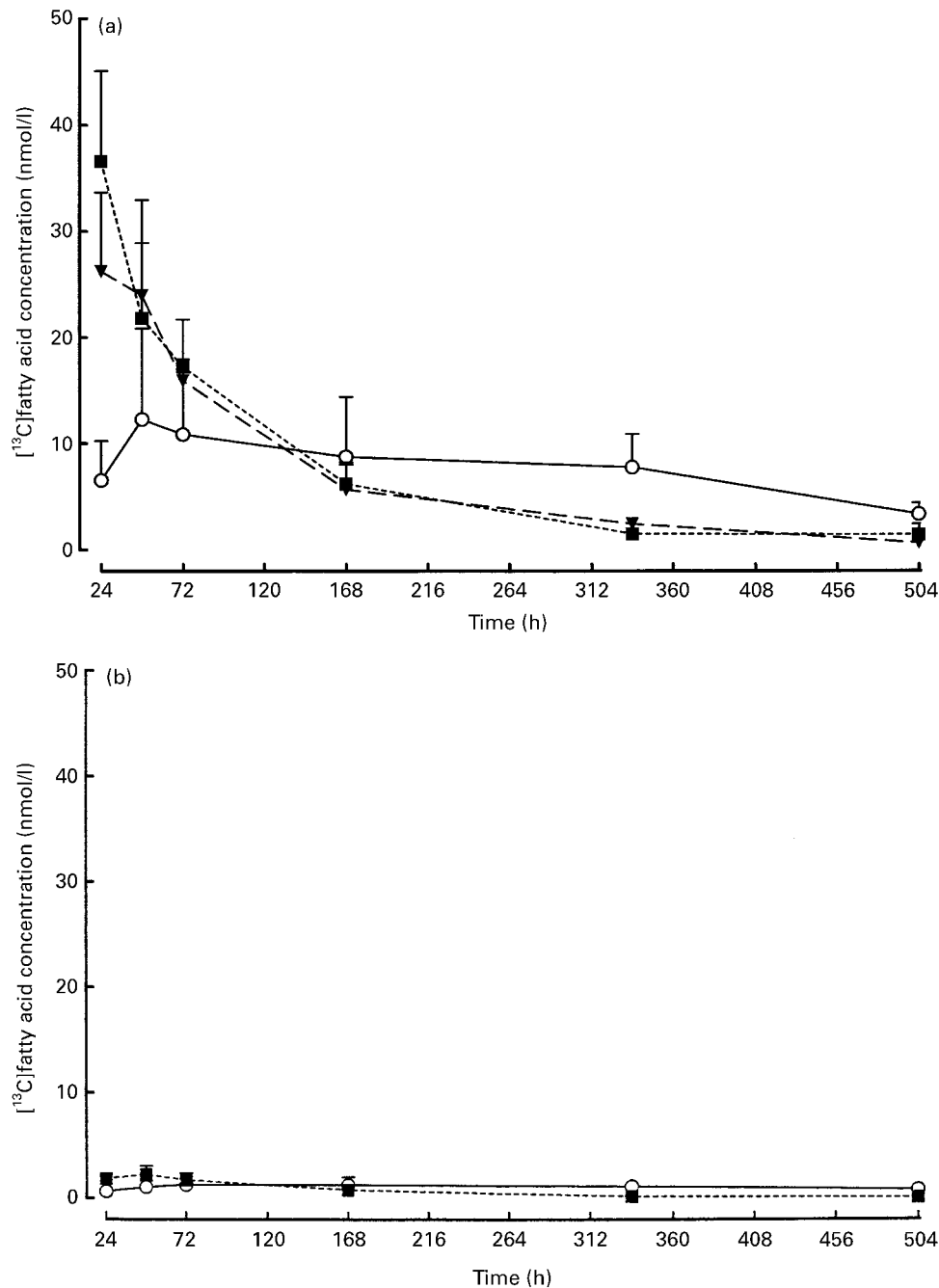
The excursion of labelled *n*-3 fatty acids in total plasma lipids has been used previously to estimate the apparent fractional conversion of ALNA to longer-chain fatty acids (Emken *et al.* 1994; Burdge *et al.* 2001). Summation of the excursion of each fatty acid in all four plasma lipids expressed as a proportion of the total excursion of [<sup>13</sup>C]*n*-3 fatty acids showed that the fractional distributions of labelled fatty acids were: ALNA 63.7 (SEM 4.0)%; EPA 21.1 (SEM 2.1)%; DPA 5.9 (SEM 1.4)%; DHA 9.2 (SEM 2.9)%. The fractional excursion of [<sup>13</sup>C]DHA in plasma PC tended to be greater in the women using the contraceptive pill (individual values 40.3, 30.0, 29.0 μmol/l × d) compared with the non-pill users (individual values 22.8, 3.0, 13.4 μmol/l × d).

*Recovery of labelled fatty acid as <sup>13</sup>CO<sub>2</sub> on breath*

Recovery of [<sup>13</sup>C]ALNA on breath derived from integration of the AUC of the excursion of labelled CO<sub>2</sub> on breath over 24 h and whole body CO<sub>2</sub> excretion indicated that 22.2 (SEM 1.5) % of the administered dose of labelled ALNA was recovered as <sup>13</sup>CO<sub>2</sub> over the first 24 h of the study. There was no detectable enrichment in breath <sup>13</sup>CO<sub>2</sub> above baseline abundance after 24 h.

**Discussion**

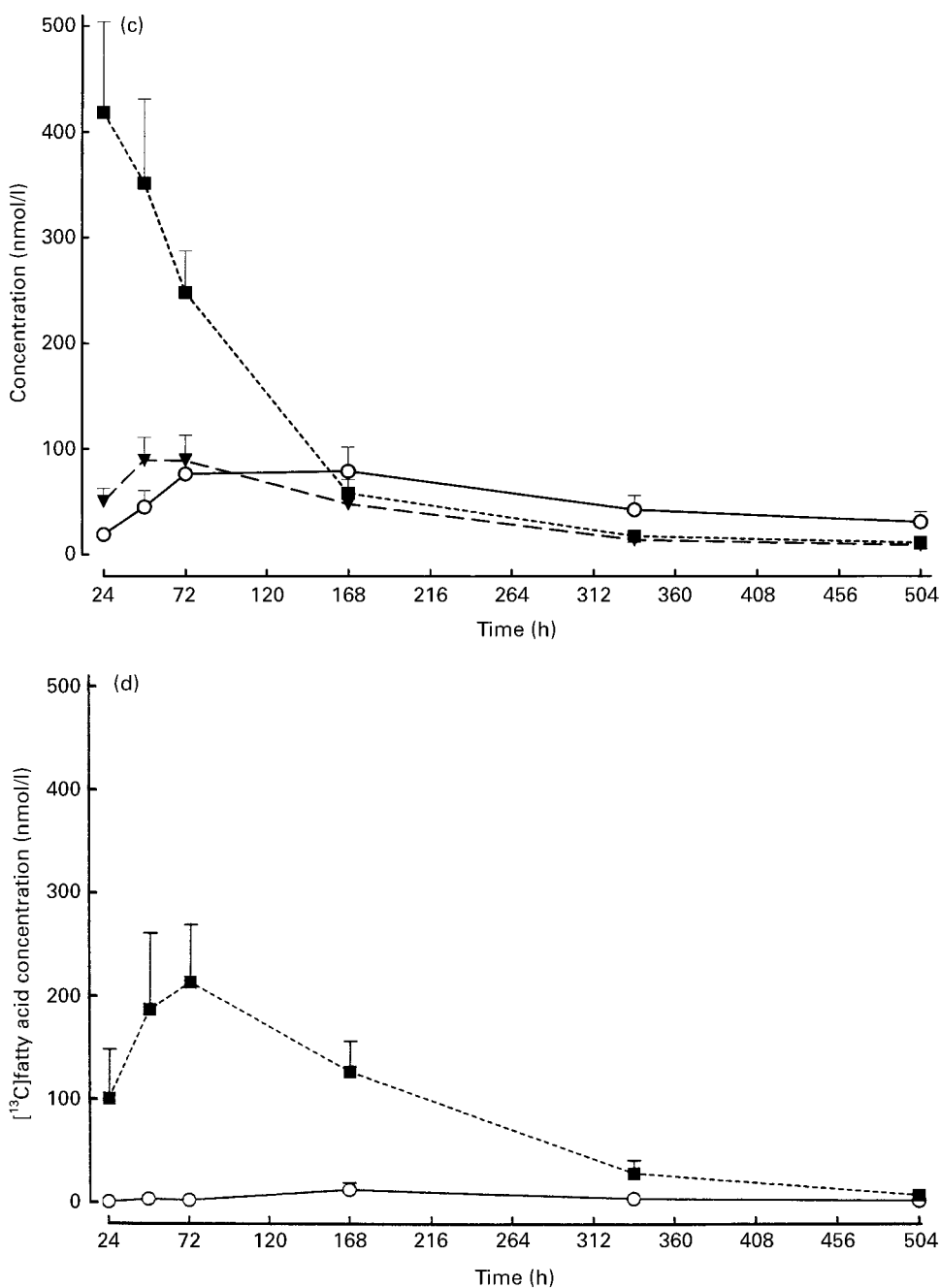
The present study is the first that specifically characterises



the metabolism of ALNA in young women. These findings show differential partitioning of individual metabolites between plasma lipid classes, significant conversion to EPA, DPA and DHA and marked conversion to  $\text{CO}_2$  by  $\beta$ -oxidation. These data indicate that plasma CE may act as a long-term source of ALNA within the circulation while EPA, DPA and DHA were associated primarily with PC. In comparison with previous studies, it also appears that the metabolic capacity for conversion of ALNA to DHA may be substantially greater in women than in men.

In studies conducted in young men, we have shown that  $^{13}\text{C}$ ALNA appears as TAG in the circulation during the

early postprandial period (up to 10 h), which represents the mobilisation of ingested labelled fatty acid from the enterocyte (Burdge *et al.* 2001). Labelled ALNA in TAG almost completely returns to baseline within 24 h, so any  $^{13}\text{C}$ ALNA that remains in the TAG fraction after the first 24 h most probably represents hepatic mobilisation of labelled PUFA as VLDL TAG. The results of the present study focus on the subsequent partitioning of ALNA and *n*-3 LCPUFA metabolites in plasma after the initial postprandial period. In young women, the excursion of labelled ALNA in plasma TAG and PC followed a similar time-course to men (Burdge *et al.* 2001). However,  $^{13}\text{C}$ ALNA was present in plasma CE for up to 21 d,



**Fig. 2.** Excursion of (■),  $[^{13}\text{C}]$ eicosapentaenoic acid; (▼),  $[^{13}\text{C}]$ docosapentaenoic acid; (○),  $[^{13}\text{C}]$ docosahexaenoic acid in plasma lipids over 21 d. Mean values for six women are shown with standard errors of the mean represented by vertical bars. (a), Triacylglycerol; (b), non-esterified fatty acid; (c), phosphatidylcholine; (d), cholesteryl ester  $[^{13}\text{C}]$ fatty acid concentrations.

with half-maximum concentration at about 7 d (Fig. 1). Whether this is specific to women is not clear as analysis of  $[^{13}\text{C}]$ ALNA incorporation into plasma CE has not been previously reported in men. This suggests that  $[^{13}\text{C}]$ ALNA incorporation into CE by the liver and mobilisation on VLDL and/or CE synthesis from PC by the action of lecithin-cholesterol acyl transferase may serve as a mechanism for delivery of ALNA to peripheral tissues. One potential consequence of this observation is that tissues with active desaturation and elongation pathways

may be able to maintain membrane EPA, DPA and DHA levels independent of pre-formed metabolites from the liver by taking up ALNA from CE in the circulation. This is supported by the observation that brain, lung and skeletal muscle have substantial expressions of both  $\Delta 6$  and  $\Delta 5$  desaturases (Cho *et al.* 1999a,b). Conversely, tissues with low expressions of these enzymes, such as kidney and pancreas (Cho *et al.* 1999a,b), may be dependent upon the supply of pre-formed EPA, DPA and DHA either from the diet or from hepatic ALNA conversion.

Conversion of [ $^{13}\text{C}$ ]ALNA to EPA, DPA and DHA was detected in all six individuals. EPA was carried to a similar extent in both plasma CE and PC, while the highest concentrations of labelled DPA and DHA were in the PC fraction. These reflect largely the distribution of total  $n$ -3 fatty acids between plasma lipid classes. Such differential distribution suggests that incorporation of newly synthesised LCPUFA into plasma lipids is a primary determinant of the  $n$ -3 fatty acid composition of these lipid classes. Alternatively, the differential distribution of both total and labelled fatty acids between plasma lipid classes may reflect the net product of the specificity of hepatic lipid synthesis, and selective turnover and removal from the circulation. It may be that both processes contribute to the net  $n$ -3 content of plasma TAG, CE and PC. The high concentration of both total and labelled EPA and DHA in plasma PC suggests that PC may play an important role in supplying these fatty acids pre-formed to peripheral tissues.

The extent of ALNA conversion to DHA in these young women was greater than reported in previous studies in men, although direct comparisons are difficult due to differences between studies in the chemical form of labelled ALNA, the meal context in which it was administered and the size of dose given. The relative excursions of labelled fatty acids in plasma have been used previously to estimate fractional conversion of ALNA to longer-chain metabolites (Emken *et al.* 1994; Burdge *et al.* 2001). The limitation of this approach is that it probably reflects an underestimate of hepatic conversion due to partitioning of metabolites into storage pools and towards  $\beta$ -oxidation, and differential incorporation into and turnover within plasma lipids. However, in the absence of techniques for measuring hepatic ALNA conversion directly the results of such calculations are useful for comparative purposes within and between studies. In the present study the fractional excursions of labelled fatty acids in total plasma lipids was EPA 21%, and DHA 9.2%. Previous estimates of ALNA inter-conversion in young men showed lower fractional excursions of both EPA and DHA (EPA 8%, and DHA 4% (Emken *et al.* 1994); EPA 7.9%, DHA 0% (Burdge *et al.* 2001)). These data suggest greater synthesis of both EPA and DHA in women compared with men. The relative excursions of EPA and DHA in plasma PC in young women were approximately 36 and 22%, respectively. Thus while the fractional excursion of EPA was similar to that which we reported previously in young men over 21 d (36%) (Burdge *et al.* 2001), the relative AUC for DHA was substantially greater in women than in men (0%). This is consistent with the observation that the total fractional excursions of EPA, DPA and DHA in plasma PC were greater in young women (74%) than reported in men (59.6%) (Emken *et al.* 1994). Other reports of the metabolism of labelled ALNA in adults have not described the handling of longer-chain metabolites in individual plasma lipid classes. Direct comparisons between the present study and those reported by Salem *et al.* (1999) and Vermunt *et al.* (2000) are also difficult as the gender composition of the subject group reported by the former was not disclosed and the volunteers in the latter study were an undefined mixture of men and

women. However, both studies reported lower peak concentrations of [ $^{13}\text{C}$ ]EPA (approximately 150 and 123 nmol/l, respectively, re-calculated from published data) and [ $^{13}\text{C}$ ]DHA (4 and 5 nmol/l, respectively) than presented here for PC alone. Together these observations indicate that EPA and DHA synthesis was greater in women in the present study in both absolute as well as relative amounts.

Unfortunately, as there appear to be no direct comparisons of plasma  $n$ -3 fatty acid concentrations between men and women, the extent to which these apparent gender differences in ALNA conversion are reflected in the circulating concentrations of these fatty acids is not clear.

The major increase in conversion of [ $^{13}\text{C}$ ]ALNA to longer-chain metabolites was in DHA synthesis. The similarity in the excursion of [ $^{13}\text{C}$ ]EPA accompanied by a lower fractional excursion of labelled DPA in young women (15%) compared with men (34%) (Burdge *et al.* 2001) together with substantially greater fractional AUC for [ $^{13}\text{C}$ ]DHA in women (23%) than in men (0%) suggests up regulation of the desaturation and elongation pathway down-stream of EPA synthesis resulting in increased conversion of DPA to DHA. While it is possible that conversion of 24:5 $n$ -3 to 24:6 $n$ -3 by  $\Delta$ 6-desaturase activity may have been up regulated, these observations are also consistent with increased flux through the peroxisomal  $\beta$ -oxidation step, which has been proposed as a locus of control of this pathway (Sprecher, 2000).

The greater conversion of ALNA to DHA in pill users compared with non-pill users, and previous studies in both women (Silfverstolpe *et al.* 1981; Ottosson *et al.* 1984) and rats (Eden *et al.* 1987), suggests that one possible explanation for the greater apparent synthesis of DHA in women compared with men may be up regulation of the desaturation and elongation pathway by oestrogen. If true, the capacity to regulate ALNA conversion by the action of sex hormones may contribute to the physiological increase in maternal plasma DHA concentration in pregnancy (Neuringer *et al.* 1984; Burdge *et al.* 1994; Burdge & Postle, 1994; Postle *et al.* 1995; Otto *et al.* 1997).

Estimates of [ $^{13}\text{C}$ ]ALNA fractional oxidation showed that 22% of administered fatty acid was recovered as  $^{13}\text{CO}_2$  over 24 h. However, trapping of  $^{13}\text{CO}_2$  in bicarbonate pools within the body may have resulted in a 30% underestimate of actual extent of [ $^{13}\text{C}$ ]ALNA oxidation (Irving *et al.* 1983) and so up to 29% of labelled fatty acid may have been used as an energy source over the 'first 24 h of the study. Fractional oxidation of [ $^{13}\text{C}$ ]ALNA in women was about 27% lower than we have observed previously in young men (32% administered dose over 24 h uncorrected for trapping in bicarbonate pools; GC Burdge, AE Jones and SA Wootton, unpublished results). Although this may suggest selective sparing of ALNA from  $\beta$ -oxidation in women, [ $^{13}\text{C}$ ]palmitate oxidation has also been shown to be lower in women compared with men (Jones *et al.* 1998, 1999) and at a comparable level to that reported here for [ $^{13}\text{C}$ ]ALNA, possibly reflecting, in part, lower rates of energy metabolism in women. Thus, as in men (Burdge *et al.* 2001), there does not appear to be any selective sparing or use of ALNA as an



energy source. In addition, women tend to use carbohydrate as the preferred metabolic fuel in both the fasted (Toth *et al.* 1998) and postprandial state (Jones *et al.* 1998). Thus although lower ALNA oxidation in women may not reflect differential selection of fatty acid species for use as metabolic fuels, the net result may be to increase availability of ALNA for supply to the liver either for further inter-conversion and mobilisation as PC or for export without modification as CE by VLDL. Both processes would potentially increase availability of *n*-3 fatty acids to peripheral tissues.

Together these data indicate that hepatic capacity for conversion of ALNA to DHA and the subsequent mobilisation of both ALNA and longer-chain *n*-3 fatty acids from the liver are important determinants of the supply of these fatty acids to peripheral tissues in women. One implication of these observations is that EPA and DHA status in these women may depend substantially upon the metabolic capacity for ALNA conversion. Thus variations in metabolic capacity for ALNA desaturation and elongation, which may be due in part to differences in oestrogen exposure rather than diet alone, may be an important source of differing EPA and DHA status between individuals. Such regulatory influences may account in part for the 25% variation in maternal DHA status observed between pregnant women (Postle *et al.* 1995). Whether such variation in metabolic capacity for ALNA conversion and/or the magnitude and mobilisation of body DHA stores leads to differences in tissue function in women or in fetal development remain to be established.

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