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Eicosapentaenoic and docosapentaenoic acids are the principal products of α-linolenic acid metabolism in young men*

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The capacity for conversion of α-linolenic acid (ALNA) to *n*-3 long-chain polyunsaturated fatty acids was investigated in young men. Emulsified [*U*-^{13}C]ALNA was administered orally with a mixed meal to six subjects consuming their habitual diet. Approximately 33 % of administered [^{13}C]ALNA was recovered as $^{13}CO_2$ on breath over the first 24 h. [^{13}C]ALNA was mobilised from enterocytes primarily as chylomicron triacylglycerol (TAG), while [^{13}C]ALNA incorporation into plasma phosphatidylcholine (PC) occurred later, probably by the liver. The time scale of conversion of [^{13}C]ALNA to eicosapentaenoic acid (EPA) and docosapentaenoic acid (DPA) suggested that the liver was the principal site of ALNA desaturation and elongation, although there was some indication of EPA and DPA synthesis by enterocytes. [^{13}C]EPA and [^{13}C]DPA concentrations were greater in plasma PC than TAG, and were present in the circulation for up to 7 and 14 d, respectively. There was no apparent ^{13}C enrichment of docosahexaenoic acid (DHA) in plasma PC, TAG or non-esterified fatty acids at any time point measured up to 21 d. This pattern of ^{13}C *n*-3 fatty acid labelling suggests inhibition or restriction of DHA synthesis downstream of DPA. [^{13}C]ALNA, [^{13}C]EPA and [^{13}C]DPA were incorporated into erythrocyte PC, but not phosphatidylethanolamine, suggesting uptake of intact plasma PC molecules from lipoproteins into erythrocyte membranes. Since the capacity of adult males to convert ALNA to DHA was either very low or absent, uptake of pre-formed DHA from the diet may be critical for maintaining adequate membrane DHA concentrations in these individuals.

α-Linolenic acid: Polyunsaturated fatty acids: Metabolism

The concentration of *n*-3 long-chain polyunsaturated fatty acids (LCPUFA), principally eicosapentaenoic acid (20 : 5*n*-3; EPA) and docosahexaenoic acid (22 : 6*n*-3; DHA), in cell membranes is an important factor in determining cell and tissue function. Western populations generally consume diets in which the principal *n*-3 essential fatty acid is α-linolenic acid (18 : 3*n*-3; ALNA), while the dietary intakes of EPA and DHA are substantially lower (Ministry of Agriculture, Fisheries and Food, 1997). Thus ability to convert ALNA to *n*-3 LCPUFA may be an important mechanism for maintaining adequate EPA and DHA concentrations in cell membranes and thus optimal tissue function. However, the extent to which human adults satisfy their metabolic demands for DHA through either dietary intake or these synthetic processes remains unclear.

Conversion of ALNA to EPA involves sequential Δ6-desaturation, chain elongation and Δ5- desaturation. EPA is, in turn, converted to docosapentaenoic acid (22 : 5*n*-3; DPA). DHA is synthesised from DPA by further chain elongation, Δ6-desaturation and limited peroxisomal β-oxidation. The peroxisomal β-oxidation step may also act as a locus of metabolic control in addition to the initial rate-limiting Δ6-desaturation reaction (Sprecher, 2000).

This pathway has been demonstrated to be active in rats (Voss *et al.* 1991; Cunnane *et al.* 1995*b*; Luthria *et al.* 1996), non-human primates (Sheaff *et al.* 1996; Su *et al.* 1999*a*,*b*) and human neonates *in vivo* (Carnielli *et al.* 1996; Salem

Abbreviations: ALNA, α-linolenic acid; AUC, area under the curve; CRF, chylomicron-enriched fraction; 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 acids; PC, phosphatidylcholine; PE, phosphatidylethanolamine; TAG, triacylglycerol.

* Part of this work has been presented in abstract form (Burdge GC, Jones AE, Wright P, Ware L & Wootton SA (2001) α-Linolenic acid metabolism in adult men: evidence for synthesis of eicosapentaenoic and docosapentaenoic acids, but not docosahexaenoic acid. *Proceedings of the Nutrition Society* **60**, 22A).

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et al. 1996; Sauerwald *et al.* 1997). The few studies that describe the metabolism of stable isotope-labelled ALNA in human adults show relatively low apparent conversions of ALNA to EPA and DPA, and limited DHA synthesis (Emken *et al.* 1994; Salem *et al.* 1999; Vermunt *et al.* 1999, 2000; Pawlosky *et al.* 2001). These studies suggest that conversion of ALNA to longer-chain metabolites is constrained at least at the level of EPA synthesis (Pawlosky *et al.* 2001). Thus it is unclear whether such limited metabolic capacity for ALNA inter-conversion is able to meet the demands of adults for EPA, DPA and DHA. However, while these studies provide some insight into the metabolic handling of ALNA, the use of differing chemical forms of labelled fatty acid (particularly methyl and ethyl esters), concurrent administration with other tracers and differing vehicles make it difficult to apply the results to free-living individuals consuming diets with mixed macronutrient content.

In the present study we have characterised the fate of [*U*-^{13}C]ALNA administered in the context of a mixed meal, designed such that the lipid component reflected the *n*-3 fatty acid content of the UK diet (Ministry of Agriculture, Fisheries and Food, 1997), to men consuming their habitual omnivorous diet. Our results describe incorporation of ^{13}C into *n*-3 LCPUFA in plasma, incorporation of ^{13}C-labelled fatty acids into erythrocyte membrane phospholipids and the extent of partitioning of [^{13}C]ALNA towards β-oxidation.

Materials and methods

Materials

Solvents were obtained from Fisher Chemicals Ltd. (Loughborough, Leicestershire, UK). Lipid standards and all other reagents, with noted exceptions, were from Sigma (Poole, Dorset, UK). [*U*-^{13}C]ALNA was obtained from Martek Biosciences Corporation (Columbia, MD, USA). GC–MS analysis showed that this preparation contained 97·1 % ALNA, the remainder being short- and medium-chain fatty acids. ^{13}C enrichment was >98 atoms %.

Subjects

Subjects were healthy, non-smoking males (*n* 6), median age of 36 (27–40) years and median BMI of 24·7 (21·5–28·9) kg/m^2. None of the subjects consumed fish oil dietary supplements or regularly ate fish. Routine biochemical analysis showed fasting plasma total triacylglycerol (TAG) (0·97 (SE 0·47) mmol/l), total cholesterol (4·6 (SE 0·79) mmol/l) and HDL-cholesterol (1·56 (SE 0·57) mmol/l) to be within normal ranges (Department of Chemical Pathology, Southampton General Hospital, Southampton, UK). None of the subjects had raised concentrations of plasma enzyme markers of hepatic dysfunction. Ethical approval was granted by the Joint Ethics Committee of Southampton and South West Hampshire, and volunteers gave written consent.

Administration of [U-^{13}C]α-linolenic acid, and blood and breath sampling

Subjects consumed their habitual diet, but excluded ^{13}C-enriched foods for 3 d before the study. On the day preceding the study, the subjects consumed only three standardised meals (total energy 11·2 MJ/d; macronutrient distribution protein 12 %, carbohydrate 45 %, fat 43 % total energy) and then fasted overnight for 12 h. On the day of the study, breath samples were collected for measurement of baseline $^{13}CO_2$ excretion (Jones *et al.* 1999) and total body CO_2 excretion determined using a GEM indirect calorimeter (PDZ-Europa, Crewe, Cheshire, UK). A plastic cannula was inserted into a forearm vein and a fasting baseline blood sample (10 ml) collected using lithium heparin as anticoagulant. [*U*-^{13}C]ALNA (700 mg, approximately 10 mg/kg) free fatty acid was emulsified with double cream (22 g), casein (12 g), beet sugar (4·5 g) and glucose (9 g) and Nesquik milkshake powder (Nestle, Vevey, Switzerland; 10 g) according to a modification of Jones *et al.* (1999). The final fat composition of the emulsion was adjusted with safflower and cod-liver oils to provide a combined test meal and emulsion with a fatty acid composition modelled on the UK dietary *n*-3 fatty acid intake (Table 1). The emulsion containing ^{13}C-labelled ALNA was consumed as a drink (160 ml) at approximately 8.00 hours, with a standard breakfast (Table 1).

Blood samples (10 ml) were collected at 2 h intervals for 24 h after ingestion of [*U*-^{13}C]ALNA, then at 48 and 72 h, 1, 2, and 3 weeks. Breath samples were collected and whole body CO_2 excretion measured by indirect calorimetry at 2 h intervals for the first 12 h of the study and then at 24 h. Subjects were fed standardised meals at 6 and 12 h (Table 1) and then returned to their habitual diet for the remainder of the study period.

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

Fatty acid	Mass in test meal and emulsion		% Total energy/meal*
	Mg	%	
12 : 0	1393	3·1	1·25
14 : 0	4562	10·1	4·10
16 : 0	11992	26·5	10·78
18 : 0	5550	12·3	4·99
16 : 1*n*-7	789	1·7	0·71
18 : 1*n*-9	12263	27·1	11·02
20 : 1	22	0·1	0·02
22 : 1	14	0·03	0·01
18 : 2*n*-6	7596	16·8	6·83
ALNA	1003	2·2†	0·9
20 : 3*n*-6	3	0·01	>0·01
20 : 4*n*-6	0		>0·01
EPA	56	0·1	0·05
DHA	46	0·1	0·04
n-3 : *n*-6	0·15		
Total fatty acids (g)	45·3		

ALNA, α-linolenic acid, EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

* Total energy intake was 3995 kJ (protein 10·2 %, carbohydrate 49·1 % and fat 40·7 %). Total energy intake for the first 24 h after ingestion of the label was 11·4 MJ (protein 11 %, carbohydrate 45 % and fat 45 %), divided approximately equally between three meals.

† Including [*U*-^{13}C]ALNA.

Estimation of ^{13}C fatty acid oxidation

Enrichment of $^{13}CO_2$ on breath was measured at each time point by continuous flow isotope ratio mass spectrometry using an automated breath carbon analyser interface (GSL; PDZ-Europa) and a 20/20 Stable Isotope Analyser (PDZ Europa) (Jones *et al.* 1999). Values for $^{13}CO_2$ excretion on breath, expressed as δ per mil (‰), were used with whole body CO_2 excretion to estimate the proportion of the administered dose of labelled fatty acid that was excreted on breath over 24 h (Watkins *et al.* 1982; Jones *et al.* 1999).

Blood lipid analyses

Blood samples were separated into plasma and erythrocytes by centrifugation at 1125 ***g*** for 15 min at 4°C. Plasma was mixed with protease inhibitors (1·7 μg phenylmethylsulfonylfluoride, 7 μg aprotinin, 37·2 μg ethylenediaminetatraacetic acid/ml plasma) and stored at −20°C. A chylomicron-enriched fraction (CRF; Svedberg flotation units >400) was prepared from plasma (3 ml) by ultracentrifugation (Redgrave *et al.* 1975). Plasma (1 ml) or the CRF were extracted with chloroform–methanol (2:1, v/v) (Folch *et al.* 1957) containing butyrated hydroxytoluene (50 μg/ml) using triheptadecanoin, diheptadecanoyl phosphatidylcholine (PC) and heneicosanoic acid as internal recovery standards. TAG, PC and non-esterified fatty acids (NEFA) were isolated by solid phase extraction on 100 mg aminopropyl silica cartridges (BondElute; Varian Ltd., Surrey, UK) and *trans*-esterified to form fatty acid methyl esters (FAME) (Burdge *et al.* 2000).

Erythrocytes were washed twice with 0·9 % (w/v) saline and resuspended in the original blood volume. Washed erythrocytes (2 ml) were extracted with chloroform–methanol (2:1, v/v) (Folch *et al.* 1957) containing butyrated hydroxytoluene using diheptadecanoyl PC and diheptadecanoyl PE internal recovery standards. PC and PE were isolated from total lipid extracts by solid phase extraction (Caesar *et al.* 1988) and FAME prepared.

Measurement of ^{13}C enrichment in plasma fatty acids

^{13}C enrichment in individual fatty acids was determined by GC-combustion-isotope ratio MS (GC-C-IRMS). FAME were resolved on a 50 m × 0·25 μm × 0·32 mm BPX-70 fused silica capillary column (SGE Europe Ltd., Milton Keynes, UK) using an HP6890 GC (Hewlett Packard, Wokingham, Berkshire, UK) with an Orchid interface (PDZ-Europa), combusted to CO_2 by heating to 830°C in the presence of PtCuO and the $^{13}CO_2$:$^{12}CO_2$ ratio was determined by a 20/20 Stable Isotope Analyser (PDZ Europa) using tricosanoic acid methyl ester as isotopic reference standard (δ −31·99 ‰) (Stolinski *et al.* 1997). Baseline resolution was achieved for all *n*-3 FAME, including complete separation of DPA and DHA from 24 : 0 and 24 : 1.

Measurement of ^{13}C enrichment of FAME at natural abundance showed greater variability and increased δ values when the mass of sample injected into the GC column was below 160 ng. Since *n*-3 fatty acids were relatively minor components of plasma lipids, we were concerned that the lack of carbon mass may lead to an artefactual increase in ^{13}C enrichment. Methyl esters of these fatty acids at natural ^{13}C abundance (ALNA δ = −29·54; EPA δ = −26·22; DHA δ = −29·12 ‰) were added before GC-C-IRMS analysis such that the mass of fatty acid injected onto the column was above 160 ng (approximately 200 ng). Addition of these FAME increased the precision of measurement of ^{13}C enrichment from a CV of 38·0 % (unspiked) to a CV of 6·3 % (spiked) for repeated analysis (*n* 10) of ALNA in a single plasma specimen. ^{13}C abundance greater than two standard deviations above the mean natural abundance for the study group was considered to represent enrichment (ALNA −23·01‰, EPA −24·76‰, DPA −23·44‰ and DHA −24·68‰). FAME were identified by retention times relative to standards and confirmed by electron-impact ionisation GC–MS.

Concentrations of individual *n*-3 fatty acids were calculated by integration of baseline-corrected peak areas on chromatograms derived from the total ion current generated by the GC-C-IRMS. For extraction of 1 ml plasma or erythrocytes:

$$\begin{aligned}&\text{Total concentration target fatty acid/ml}\\&\quad=(\text{peak area of target fatty acid/}\\&\qquad\text{peak area 23 : 0 methyl ester standard})\\&\qquad\times\text{mass of 23 : 0 added.}\end{aligned}\tag{A}$$

$$\begin{aligned}&\text{Fractional recovery}\\&\quad=(\text{peak area 23 : 0/peak area internal standard}).\end{aligned}\tag{B}$$

The same mass of internal standard and 23 : 0 methyl ester were added to each specimen. Fractional ^{13}C enrichment of individual *n*-3 fatty acids was determined by interpolation against a [*U*-^{13}C]ALNA fatty acid methyl ester calibration curve (C). The concentration of ^{13}C-labelled fatty acids was calculated as:

$$\begin{aligned}&\text{Concentration of }^{13}\text{C target fatty acid/ml}\\&\quad=((\text{A})\times(\text{C}))\times(\text{B}).\end{aligned}$$

Statistical analysis

Data are presented as mean and standard error concentrations. Statistical analysis of both peak ^{13}C fatty acid concentrations and integrated areas under the curve (AUC) was carried out using Student's paired *t* test.

Results

Handling of [U-^{13}C]α-linolenic acid in plasma

The excursions of labelled *n*-3 fatty acids in plasma total TAG, NEFA and PC are summarised in Figs. 1, 2 and 3, respectively. [^{13}C]ALNA was detected in plasma TAG by 2 h and reached maximum concentration between 4 to

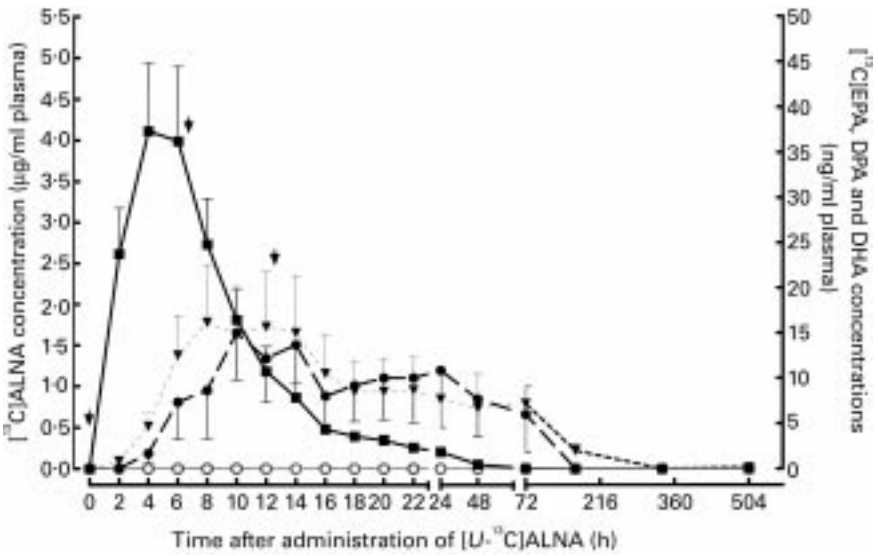

Fig. 1. Time course of ^{13}C-labelled *n*-3 fatty acid concentrations in plasma triacylglycerol following administration of [*U*-^{13}C]α-linolenic acid (ALNA). Blood samples were collected at 2 h intervals after administration of labelled fatty acid up to 24 h, then at 48 and 72 h, and 1, 2, and 3 weeks (represented as approximately 168, 336 and 504 h). [^{13}C]ALNA (■) concentration is indicated on the left *y* axis; eicosapentaenoic acid (EPA, ●), docosapentaenoic acid (DPA, ▼) and docosahexaenoic acid (DHA, ○) on the right *y* axis. Mean values are shown, with standard errors of the mean represented by vertical bars. ↓, Meal times.

6 h (Fig. 1). Peak [^{13}C]ALNA concentration occurred later in plasma NEFA (6 h) and total PC (6 to 10 h) (Figs. 2 and 3) and at lower concentrations (10- and 20-fold, respectively) compared with plasma total TAG. Calculation of the AUC for the changes in the concentration of labelled ALNA in plasma lipids showed that [^{13}C]ALNA excursion was significantly ($P<0{\cdot}0001$) greater in TAG than in NEFA (9-fold) and PC (18-fold) (Table 2).

[^{13}C]ALNA concentration was maximal in CRF TAG at 4 h, but at a lower magnitude (2·6 (SE 0·6) μg/ml, $P=0{\cdot}005$) compared with total plasma TAG. CRF TAG [^{13}C]ALNA level decreased to approximately half maximum concentration by 6 h, but had not returned to baseline by 10 h. [^{13}C]ALNA concentration in CRF PC was maximal at 4 h (3·3 (SE 3·1) ng/ml) which was significantly lower compared with total plasma PC (73-fold,

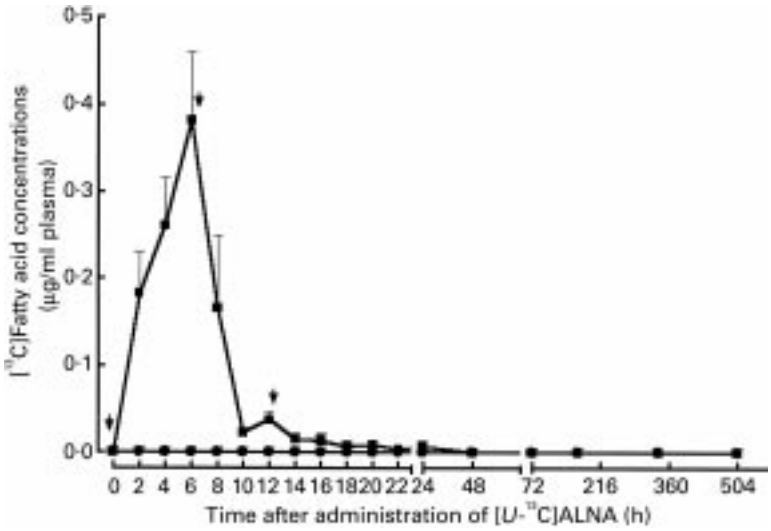

Fig. 2. Time course of ^{13}C-labelled *n*-3 non-esterified fatty acids following administration of [*U*-^{13}C]α-linolenic acid (ALNA). Blood samples were collected at 2 h intervals after administration of labelled fatty acid up to 24 h, then at 48 and 72 h, and 1, 2, and 3 weeks (represented as approximately 168, 336 and 504 h). (■), [^{13}C]ALNA; (●), eicosapentaenoic acid (EPA); (▼), docosapentaenoic acid (DPA); (○),docosahexaenoic acid (DHA) concentrations. Mean values are shown, with standard errors of the mean represented by vertical bars. ↓, Meal times.

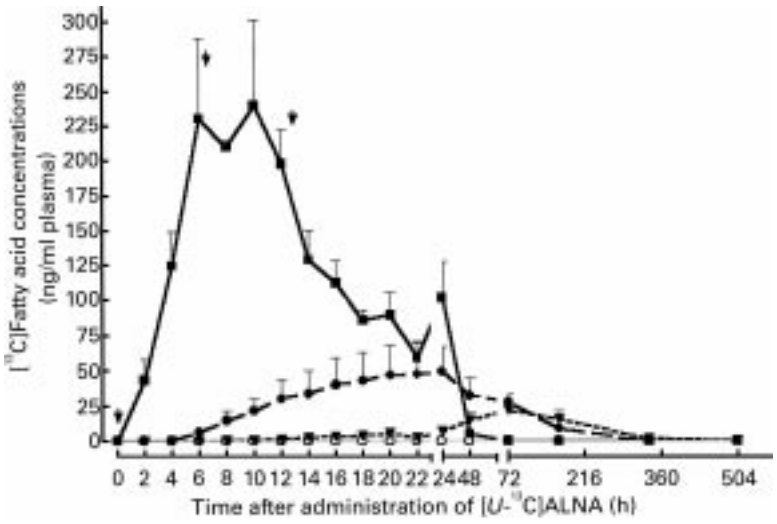

Fig. 3. Time course of ^{13}C-labelled *n*-3 fatty acid concentrations in plasma phosphatidylcholine following administration of [*U*-^{13}C]α-linolenic acid (ALNA). Blood samples were collected at 2 h intervals after administration of labelled fatty acid up to 24 h, then at 48 and 72 h, and 1, 2, and 3 weeks (represented as approximately 168, 336 and 504 h). (■), [^{13}C]ALNA; (●), eicosapentaenoic acid (EPA); (▼), docosapentaenoic acid (DPA); (○),docosahexaenoic acid (DHA) concentrations. Mean values are shown, with standard errors of the mean represented by vertical bars. ↓, Meal times.

$P<0{\cdot}0001$) (Fig. 3) and CRF TAG (788-fold, $P<0{\cdot}0001$). Although the concentration of CRF PC ALNA was substantially lower than CRF TAG ALNA, the time course of [^{13}C]ALNA was similar in these two pools.

Handling of ^{13}C-labelled long-chain n-*3 long-chain polyunsaturated fatty acids in plasma*

Incorporation of ^{13}C into long-chain *n*-3 metabolites of ALNA (EPA, DPA and DHA) was used to indicate the capacity for elongation and desaturation of ALNA. Maximum concentrations of ^{13}C-labelled EPA and DPA in plasma total TAG occurred after peak ^{13}C enrichment of ALNA (Fig. 1). There was no significant ^{13}C enrichment of DHA above natural abundance at any of the time points measured over 21 d (Fig. 1). The time course and magnitude of [^{13}C]EPA and [^{13}C]DPA excursions in plasma total TAG were similar (Fig. 1). However, peak ^{13}C enrichments in EPA and DPA were both approximately 273-fold lower than maximum ^{13}C incorporation into ALNA ($P<0{\cdot}0001$). Calculation of AUC for ^{13}C-labelled n-3 LCPUFA over 21 d in plasma TAG (Table 2) showed that the relative excursions in plasma total TAG for each fatty acid over 21 d were: ALNA 94·9 %; EPA 2·4 %; DPA 2·4 %; DHA 0 %.

There was no significant ^{13}C enrichment above natural abundance in EPA, DPA and DHA plasma NEFA.

^{13}C incorporation into plasma total PC *n*-3 fatty acids was, again, only detected in ALNA, EPA and DPA (Fig. 3). Since the range of ^{13}C enrichments of plasma PC DHA were not significantly different from baseline there appeared to be no ^{13}C enrichment in plasma PC DHA over 21 d. Maximal incorporation of ^{13}C into plasma PC total EPA and DPA occurred at later time points and at lower concentrations (EPA 5-fold, $P<0{\cdot}001$; DPA 11-fold, $P<0{\cdot}0005$) compared with plasma total PC ALNA (Fig. 3). This is consistent with a precursor–product relationship within the fatty acid substrate pool destined for incorporation into plasma total PC. Calculation of AUC for ^{13}C-labelled *n*-3 PUFA over

Table 2. Excursions of ^{13}C-labelled *n*-3 fatty acids in individual plasma lipid classes (Mean values and standard errors of the mean)

	AUC (μg/ml over 21 d)							
	ALNA		EPA		DPA		DHA	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
TAG	41·6	5·8	1·0	0·3	1·0	0·3	ND	
NEFA	2·3	0·4	ND		ND		ND	
PC	4·5	0·6	4·9	1·5	4·3	1·3	ND	

AUC, area under the curve; ALNA, α-linolenic acid; EPA, eicocapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid; TAG, triacylglycerol; ND, not detected; NEFA, non-esterified fatty acids; PC, phosphatidylcholine.

21 d showed similar excursions of labelled ALNA, EPA and DPA (Table 2). Thus the fractional distribution of ^{13}C-labelled *n*-3 fatty acids in plasma total PC over 21 d was ALNA 32·9 %, EPA 35·7 %, DPA 31·4 % and DHA 0 %.

The fractional distribution from AUC of plasma total *n*-3 fatty acids, derived from summation of AUC calculations of TAG, NEFA and PC excursions, over the 21 d study period for individual ^{13}C-labelled fatty acids were: ALNA 84·0 %; EPA 7·9 %; DPA 8·1 %; DHA 0 %.

In CRF TAG maximum ^{13}C enrichment was detected in both EPA (1·8 (SE 1·0) ng/ml) and DPA (1·7 (se 0·9) ng/ml) at 6 h. After 10 h, [^{13}C]DPA concentration had decreased by half, while there was no significant change in ^{13}C enrichment of EPA between 6 and 10 h. Comparison of maximal [^{13}C]EPA and [^{13}C]DPA concentrations showed that the levels of these fatty acids were significantly lower in CRF TAG (10-fold) compared with total plasma TAG ($P<0·0001$). There was no significant ^{13}C enrichment in DHA in CRF TAG, and no enrichment of EPA, DPA and DHA in CRF PC.

Incorporation of ^{13}C-labelled n-*3 fatty acids into erythrocyte membrane phospholipids*

Erythrocyte membrane phospholipids, in particular PE, have been used frequently as markers of cellular uptake of *n*-3 LCPUFA. The patterns of ^{13}C enrichment of erythrocyte PC *n*-3 fatty acids are summarised in Fig. 4. Maximum ^{13}C incorporation into EPA and DPA were significantly lower (7·6-fold, $P<0·005$; 2·9-fold, $P<0·01$, respectively) than into [^{13}C]ALNA (Fig. 4). There was no significant ^{13}C enrichment of erythrocyte PC DHA (Fig. 4) or PE *n*-3 fatty acids (data not shown).

Excretion of $^{13}CO_2$ on breath

Excretion of $^{13}CO_2$ on breath followed a similar pattern for all subjects. ^{13}C enrichment of breath CO_2 was detected by 2 h after administration of [*U*-^{13}C]ALNA and reached maximum levels (3·25 (SE 0·41) % administered dose/h) between 6 and 8 h and decreased to approximately half maximum levels by 12 h (1·22 (SE 0·15) % administered dose/h). Calculation of the AUC over the first 24 h showed that 33·2 (SE 9·1) % of the administered dose (equivalent to 232 mg) was recovered as $^{13}CO_2$ on the breath.

Discussion

The results of the present study show that the metabolism of [*U*-^{13}C]ALNA presented in the context of a mixed macronutrient meal with a comparable *n*-3 fatty acid content to the typical UK diet was characterised by differential incorporation into plasma lipid pools and significant partitioning towards β-oxidation. Furthermore, the principal products of desaturation and elongation of ALNA were EPA and DPA. There was no evidence for increased ^{13}C enrichment in DHA.

Approximately one-third of administered [^{13}C]ALNA was used as an energy source during the first 24 h of the study. This may represent an underestimate as a proportion of the $^{13}CO_2$ released by fatty acid oxidation may be retained in bicarbonate pools (Irving *et al.* 1983). These results are comparable to that reported by Vermunt *et al.* (2000) for labelled ALNA over a shorter observation period (15·7 to 20·4 % of administered [*U*-^{13}C]ALNA over 12 h). The recovery of label on breath was also comparable to our previous measurements of [1-^{13}C]palmitic acid oxidation in young men (23·4 to 31·7 % administered dose) (Bennoson *et al.* 1999). This suggests no selective

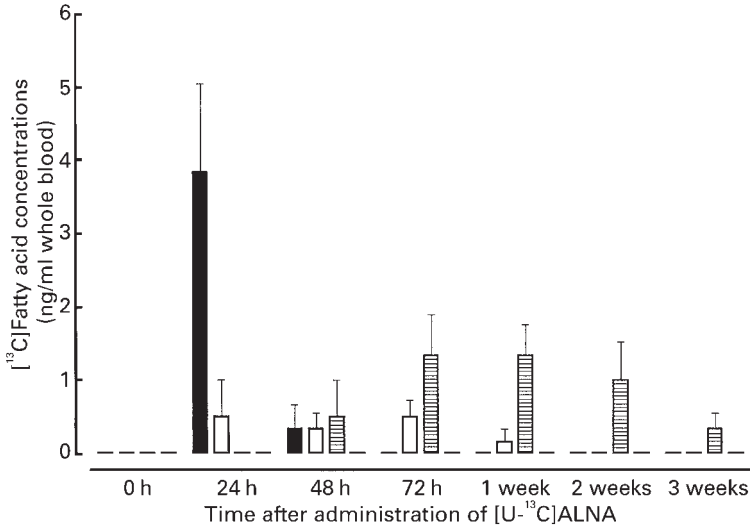

Fig. 4. Concentrations of ^{13}C-labelled α-linolenic acid (ALNA, ■), eicosapentaenoic acid (EPA, □), docosapentaenoic acid (DPA, ▤) and docosahexaenoic acid (DHA, ▬) in erythrocyte phosphatidylcholine. Results are mean ^{13}C fatty acid concentrations/ml whole blood, with standard errors of the mean represented by vertical bars.

sparing or use of ALNA as an energy source. The present data are in marked contrast to [^{14}C]ALNA catabolism in rats which showed extensive (>60 % administered dose) and preferential partitioning towards oxidation compared with other fatty acids (Leyton *et al.* 1987). This suggests that the extent to which ALNA is used as an energy source may be driven by the precise metabolic requirements or dietary intake of the organism. Thus extrapolation of such animal data to man must be considered with caution.

Analysis of ^{13}C-labelled fatty acid concentrations in plasma TAG, PC and NEFA showed distinct patterns for the time taken to reach peak concentration and decrease to baseline levels, and differences in the relative proportions of the products of elongation and desaturation. Incorporation of labelled ALNA into plasma lipids was substantially greater in TAG compared with either NEFA or PC. Labelled ALNA concentration was also greater in CRF TAG compared with CRF PC. This is consistent with ingested ALNA being exported from the enterocyte primarily within chylomicron TAG. Although labelled ALNA in CRF TAG was substantially lower than the maximum concentration by 10 h, labelled ALNA persisted in total plasma TAG for up to 72 h. This suggests the possibility of labelled ALNA incorporation into other lipoproteins, in particular VLDL, by the liver. While the time course of labelled ALNA in CRF PC was similar to CRF TAG, maximum labelled ALNA concentration in total plasma PC occurred later (6 to 10 h) and at a higher concentration compared with CRF PC. This suggests that incorporation of labelled ALNA into plasma PC was primarily the result of hepatic PC biosynthesis and mobilisation from the liver by VLDL.

Plasma NEFA concentration represents the net contributions of the release of fatty acids from adipose tissue by the action of hormone-sensitive lipase, incomplete entrapment of fatty acids released from lipoproteins by lipoprotein lipase activity and uptake into tissues (Sniderman *et al.* 1998). At early time points (about 2 h), appearance of labelled ALNA in the plasma NEFA pool is likely to reflect primarily fatty acid released directly into the circulation from chylomicrons by lipoprotein lipase activity, while at later time points (4–6 h) the presence of [^{13}C]ALNA in the NEFA fraction is likely to reflect mainly mobilisation from adipose tissue. The almost complete clearance of labelled ALNA from plasma by 10 h suggests that plasma NEFA are a relatively short-term source of ALNA for supply to tissues compared with either plasma PC and TAG.

Labelled *n*-3 LCPUFA with chain lengths greater than C_{18} were detected in plasma TAG and PC. In both TAG and PC, the principal products of labelled ALNA desaturation and elongation were EPA and DPA. Labelled EPA and labelled DPA appeared in both total plasma and CRF TAG by 2 h. These data suggest that desaturation and elongation of ALNA may occur within the enterocyte, consistent with reports of desaturase activities in microsomes prepared from human intestinal epithelium (Garg *et al.* 1992). This is supported by the observation that maximum concentration of labelled EPA and labelled DPA in plasma total TAG occurred later than in CRF TAG. However, it is possible that the presence of labelled EPA and DPA in CRF TAG may reflect contamination of the CRF preparation with VLDL. Labelled DPA concentration increased in total plasma TAG before labelled EPA, while labelled EPA preceded labelled DPA in CRF TAG. The relative change in the concentrations of labelled EPA and DPA in CRF TAG are consistent with sequential synthesis of these metabolites, while the excursions of [^{13}C]EPA and [^{13}C]DPA in total plasma TAG do not follow this model. One possible explanation is that incorporation of [^{13}C]*n*-3 LCPUFA into CRF TAG results from enterocyte metabolism, while labelled EPA and labelled DPA content of total TAG is the net product of both enterocyte and hepatic synthesis which may have masked the precursor–product relationship between these metabolites. The excursion of labelled ALNA into CRF TAG and PC was comparable to that described by Emken *et al.* (1994) but prolonged compared with [1-^{13}C]palmitic acid (Bennoson *et al.* 1999). This suggests mobilisation of *n*-3 essential fatty acids from the enterocyte may be regulated independently from saturated fatty acids.

The results of the present study are in broad agreement with previous reports in human adults which show limited conversion of ALNA to EPA and marginal DHA synthesis (Emken *et al.* 1994; Salem *et al.* 1999; Vermunt *et al.* 1999, 2000; Pawlosky *et al.* 2001). We have detected consistently ^{13}C incorporation into ALNA, EPA and DPA, but failed to detect ^{13}C-labelling of DHA, while others have reported synthesis of all four metabolites. It is possible that spiking the samples with DHA at natural abundance may have diluted the level of ^{13}C enrichment to produce a false negative result. However, we have measured elevated ^{13}C enrichment in the other three metabolites at levels similar to those reported for DHA (Vermunt *et al.* 2000). Secondly, the dose of [U-^{13}C]ALNA administered in the present study was nearly sixteen times greater than that used by Vermunt *et al.* (1999, 2000) and so would be expected to produce even higher concentrations of labelled DHA in plasma if conversion occurred at equivalent rates. If the addition of DHA at natural ^{13}C abundance to increase the mass of fatty acid by about 25 % did dilute the level of enrichment so that it was not distinguishable from background, then the level of enrichment in the unspiked sample would have been so low that the extent of DHA synthesis is likely to be of negligible biological significance. Since only plasma fatty acids were studied these results do not exclude the possibility that DHA synthesis did occur within tissues, but was not was released into plasma. Alternatively, it is possible that the results of studies which suggest a greater extent of ALNA interconversion than reported here may reflect the use of labelled ALNA presented in a chemical form not found in the diet and/or in a non-physiological meal vehicle (Emken *et al.* 1994; Vermunt *et al.* 1999, 2000; Pawlosky *et al.* 2001). In contrast, in the present study [^{13}C]ALNA was presented both in a chemical present in the diet in a mixed macronutrient meal. However, the precise effect of such factors upon the extent of ALNA inter-conversion remains to be determined.

Previous studies have demonstrated inhibition of ALNA desaturation and elongation by increasing dietary EPA and/or DHA intakes (Emken *et al.* 1999; Vermunt *et al.* 2000).

The subjects in the present study did not consume diets enriched in fish or take fish oil supplements and so product inhibition of the ALNA conversion pathway seems unlikely to explain the apparent absence of DHA synthesis. The proportion of ^{13}C-labelled metabolites accounted for by ALNA (84 %) and EPA (7·9 %) in the present study was similar to that reported previously (Emken *et al.* 1994). However, the excursion of [^{13}C]DPA was about twice that reported by these authors, which suggests inhibition of conversion of DPA to DHA. This is consistent with the proposal that the β-oxidation step in DHA synthesis may represent a point of metabolic control (Sprecher, 2000). Since these subjects were essentially well-nourished it is possible that their demands for DHA were met adequately by their diet and thus there was no physiological drive for DHA synthesis leading to down regulation of conversion of DPA to DHA.

These present data are consistent with the results of studies which showed that increasing ALNA intake was associated with increased EPA and/or DPA concentrations in plasma and/or membrane phospholipids (Chan *et al.* 1993; Freese *et al.* 1994; Allman *et al.* 1995; Cunnane *et al.* 1995*a*; Li *et al.* 1999). However, there was no significant change in DHA concentration in any of these trials. However, increased plasma DHA concentration following increased ALNA intakes has been reported in some studies (Beitz *et al.* 1981; Valsta *et al.* 1996; Ezaki *et al.* 1999). Together these data are consistent with the view that DHA synthesis from ALNA is severely constrained (Gerster, 1998). The few studies which report increased DHA levels following ALNA supplementation suggest that the extent to which ALNA is converted to DHA may differ between groups of individuals.

Erythrocytes have been used in a number of studies as an indicator of uptake of dietary fatty acids, in particular essential fatty acids, into cells. The present data show that the pattern of incorporation of labelled *n*-3 fatty acids into erythrocyte PC reflected the excursions of these metabolites in plasma PC. Direct transfer of intact PC molecules from lipoproteins to erythrocyte membranes has been reported previously in human subjects *in vivo* (Galli *et al.* 1992). Thus one possible explanation for the pattern of ^{13}C-labelling of erythrocyte membrane phospholipids is uptake of intact PC molecules from lipoproteins.

The principal conclusion of the present study is that these adult men possessed the capacity to synthesise EPA and DPA from ALNA to an extent comparable to that seen by others. However, DHA synthesis, if it occurred, was insufficient to increase enrichment above background to levels that could be detected using this methodology. These results, taken together with the findings of other isotopic studies and dietary intervention trials, would suggest that the initial conversion of ALNA to EPA and DPA occurs at the levels of dietary ALNA intake typically consumed by adult men, but that the conversion to DHA is severely restricted. The nutritional demands for DHA in healthy adults are likely to be modest as they reflect principally the need to supply DHA to support turnover and resynthesis of cell membranes. In these well-nourished men, metabolic demands for DHA may have been satisfied by existing pools of DHA within the body or by dietary supply of DHA so that further synthesis has been down regulated to the point where the rates of conversion were so low as to be of questionable biological importance. In other individuals with different metabolic demands for DHA and under different experimental conditions, these demands may not be met, necessitating an increase in DHA synthesis to rates greater than that observed here. If for any reason, the ability to increase DHA synthesis was constrained to levels seen in the present study, these demands could only be met by an increased intake and assimilation of pre-formed DHA from the diet.

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References

Allman MA, Pena MM & Peng D (1995) Supplementation with flaxseed oil versus sunflower oil in healthy young men when consuming a low fat diet: effects on platelet composition and function. *European Journal of Clinical Nutrition* **49**, 169–178.

Beitz J, Mest HJ & Forster W (1981) Influence of linseed oil diet on the pattern of serum phospholipids in man. *Acta Biologica Medica Germany* **40**, K31–K35.

Bennoson J, Humayun MA, Jones AE, Hounslow A & Wootton SA (1999) Within-individual variability in the gastrointestinal handling and metabolism of [1,1,1-^{13}C]tripalmitin. *Proceedings of the Nutrition Society* **58**, 25A.

Burdge GC, Wright P, Jones AE & Wootton SA (2000) A method for separation of phosphatidylcholine, triacylglycerol, non-esterified fatty acids and cholesterol esters from plasma by solid phase extraction. *British Journal of Nutrition* **84**, 781–787.

Caesar PA, Wilson SJ, Normand ICS & Postle AD (1988) A comparison of the specificity of phosphatidylcholine synthesis by human fetal lung maintained in either organ or organotypic culture. *Biochemical Journal* **253**, 451–457.

Carnielli CP, Wattimena JDL, Luijendijk IHT, Boerlage A, Dagenhart HJ & Sauer PJJ (1996) The very-low-birth-weight premature infant is capable of synthesizing arachidonic and docosahexaenoic acid from linolenic and linolenic acid. *Pediatric Research* **40**, 169–174.

Chan JK, McDonald BE, Gerrard JM, Bruce VM, Weaver BJ & Holub BJ (1993) Effect of dietary α-linolenic acid and its ratio to linoleic acid on platelet and plasma fatty acids and thrombogenesis. *Lipids* **28**, 811–817.

Cunnane SC, Hamadeh MJ, Leide AC, Thompson LU, Wolver TMS & Jenkins DJA (1995*a*) Nutritional attributes of tradition flaxseed in healthy young adults. *American Journal of Clinical Nutrition* **61**, 62–68.

Cunnane SC, Ryan MA, Craig KS, Brookes S, Koletzko B, Demmelmair H, Singer J & Kyle DJ (1995*b*) Synthesis of linoleate and (-linolenate by chain elongation in the rat. *Lipids* **30**, 781–783.

Emken EA, Adolf RO, Duval SM & Nelson GJ (1999) Effect of dietary docosahexaenoic acid on desaturation and uptake in vivo of isotope-labeled oleic, linoleic and linolenic acids by male subjects. *Lipids* **34**, 785–798.

Emken EA, Adolf RO & Gully RM (1994) Dietary linoleic acid influences desaturation and acylation of deuterium-labeled linoleic and linolenic acids in young adult males. *Biochimica Biophysica Acta* **1213**, 277–288.

Ezaki O, Takahashi M, Shigematsu T, Shimamura K, Kimura J, Ezaki H & Gotoh T (1999) Long-term effects of dietary alpha-linolenic acid from perilla oil on serum fatty acid composition and on the risk factors of coronary heart disease in Japanese elderly subjects. *Journal of Nutritional Science and Vitaminology (Tokyo)* **45**, 759–772.

Folch JL, Lees M & Sloane-Stanley GH (1957) A simple method for the isolation and purification of total lipides from animal tissues. *Journal of Biological Chemistry* **226**, 497–509.

Freese R, Mutanen M, Valsta LM & Salminen I (1994) Comparison of the effects of two diets rich in monounsaturated fatty acids differing in their linoleic/α-linolenic acid ratio on platelet aggregation. *Thrombosis and Haemostasis* **71**, 73–77.

Galli C, Sirtori CR, Mosconi C, Medini L, Giafranceschi G, Vaccarino V & Scolastico C (1992) Prolonged retention of doubly labelled phosphatidylcholine in human plasma and erythrocytes after oral administration. *Lipids* **27**, 105–1012.

Garg ML, Keelan M, Thomson ABR & Clandinin MT (1992) Desaturation of linoleic acid in the small bowel is increased by short-term fasting and by dietary content of linoleic acid. *Biochimica Biophysica Acta* **1126**, 17–25.

Gerster H (1998) Can adults convert α-linolenic acid (18:3n-3) to eicosapentaenoic acid (20:5n-3) and docosahexaenoic acid (22:5n-3)? *International Journal of Vitamin and Nutrition Research* **68**, 159–173.

Irving CS, Wong WW, Schulman RJ, Smith EO & Klein PD (1983) [^{13}C]Bicarbonate kinetics in humans: intra- vs. inter-individual variations. *American Journal of Physiology* **245**, R190–R202.

Jones AE, Stolinski M, Smith RD, Murphy JL & Wootton SA (1999) Effect of fatty acid chain length and saturation on the gastrointestinal handling and metabolic disposal of dietary fatty acids in women. *British Journal of Nutrition* **81**, 37–43.

Leyton J, Drury PJ & Crawford MA (1987) Differential oxidation of saturated and unsaturated fatty acids *in vivo* in the rat. *British Journal of Nutrition* **57**, 383–393.

Li D, Sinclair A, Wilson A, Nakkote S, Kelly F, Abedin L, Mann N & Turner A (1999) Effect of dietary α-linolenic acid on thrombotic risk factors in vegetarian men. *American Journal of Clinical Nutrition* **69**, 872–882.

Luthria DL, Mohammed S & Sprecher H (1996) Regulation of the biosynthesis of 4,7,10,13,16,19-docosahexaenoic acid. *Journal of Biological Chemistry* **271**, 16020–16025.

Ministry of Agriculture, Fisheries and Food (1997) *Dietary Intake of Iodine and Fatty Acids*. Food Information Surveillance Sheet 127. London: Ministry of Agriculture, Fisheries and Food.

Pawlosky RJ, Hibbeln JR, Novotny JA & Salem N (2001) Physiological compartmental analysis of α-linolenic acid metabolism in adult humans. *Journal of Lipid Research* **42**, 1257–1265.

Redgrave TG, Roberts DCK & West CE (1975) Separation of plasma lipoproteins by density gradient ultracentrifugation. *Analytical Biochemistry* **65**, 42–49.

Salem N, Powlosky R, Wegher B & Hibbeln J (1999) In vivo conversion of linoleic acid to arachidonic acid in human adults. *Prostaglandins, Leukotrienes and Essential Fatty Acids* **60**, 407–410.

Salem N, Wegher B, Mena P & Uauy R (1996) Arachidonic and docosahexaenoic acids are biosynthesized from their 18-carbon precursors in human infants. *Proceedings of the National Academy of Sciences USA* **93**, 49–54.

Sauerwald TU, Hachey DL, Jensen CL, Chen H, Anderson RE & Heird WC (1997) Intermediates in endogenous synthesis of C22:6ω3 and C20:4ω6 by term and preterm infants. *Pediatric Research* **41**, 183–187.

Sheaff RC, Zhang Q, Goodman KJ, Giussani DA, Nathanielsz PW & Brenna JT (1996) Linoleate, α-linolenate and docosahexaenoate recycling into saturated and monounsaturated fatty acids is a major pathway in pregnant or lactating adults and fetal or infant Rhesus monkeys. *Journal of Lipid Research* **37**, 2675–2686.

Sniderman AD, Cianflone K, Arner P, Summers LKM & Frayn KN (1998) The adipocyte, fatty acid trapping and atherogenesis. *Atheriosclerosis, Thrombosis and Vascular Biology* **18**, 147–151.

Sprecher H (2000) Metabolism of highly unsaturated n-3 and n-6 fatty acids. *Biochimica et Biophysica Acta* **1486**, 219–231.

Stolinski M, Murphy JL, Jones AE, Jackson AA & Wootton SA (1997) Stable-isotope method for determining the gastrointestinal handling of [1-^{13}C]palmitic acid. *Lipids* **32**, 337–340.

Su HM, Bernardo L, Mirmiran M, Ma XH, Corso TN, Nathanielsz PW & Brenna JT (1999*a*) Bioequivalence of dietary alpha-linolenic and docosahexaenoic acids as sources of docosahexaenoate accretion in brain and associated organs of neonatal baboons. *Pediatric Research* **45**, 87–93.

Su HM, Bernardo L, Mirmiran M, Ma XH, Nathanielsz PW & Brenna JT (1999*b*) Dietary 18:3n-3 and 22:6n-3 as sources of 22:6n-3 accretion in neonatal baboon brain and associated organs. *Lipids* **34**, S347–S350.

Valsta LM, Salminen I, Aro A & Mutanen M (1996) Alpha-linolenic acid in rapeseed oil partly compensates for the effect of fish restriction on plasma long chain n-3 fatty acids. *European Journal of Clinical Nutrition* **50**, 229–235.

Vermunt SHF, Mensink RP, Simonis AMG & Hornstra G (1999) Effects of age and dietary n-3 fatty acids on the metabolism of [^{13}C]-α-linolenic acid. *Lipids* **34**, S127 abstr.

Vermunt SHF, Mensink RP, Simonis AMG & Hornstra G (2000) Effects of dietary (-linolenic acid on the conversion and oxidation of [^{13}C]-α-linolenic acid. *Lipids* **35**, 137–142.

Voss A, Reinhart M, Sankarappa S & Sprecher H (1991) The metabolism of 7,10,13,16,19-docosapentaenoic acid to 4,7,10,13,16,19-docosahexaenoic acid in rat liver is independent of a 4-desaturase. *Journal of Biological Chemistry* **266**, 19995–20000.

Watkins JB, Klein PD, Schoeller DA, Kirschner BS, Park R & Perman JA (1982) Diagnosis and differentiation of fat malabsorption in children using [^{13}C]-labelled lipids: trioctanoin, triolein and palmitic acid breath tests. *Gastroenterology* **82**, 911–917.