

High α -linolenic acid flaxseed (*Linum usitatissimum*): some nutritional properties in humans

BY STEPHEN C. CUNNANE*, SUJATA GANGULI, CHANTALE MENARD,
ANDREA C. LIEDE, MAZEN J. HAMADEH, ZHEN-YU CHEN,
THOMAS M. S. WOLEVER AND DAVID J. A. JENKINS

Department of Nutritional Sciences, Faculty of Medicine, University of Toronto and the Clinical Risk Factor Modification Center, St Michael's Hospital, Toronto, Canada

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Although high α -linolenic acid flaxseed (*Linum usitatissimum*) is one of the richest dietary sources of α -linolenic acid and is also a good source of soluble fibre mucilage, it is relatively unstudied in human nutrition. Healthy female volunteers consumed 50 g ground, raw flaxseed/d for 4 weeks which provided 12–13% of energy intake (24–25 g/100 g total fat). Flaxseed raised α -linolenic acid and long-chain n -3 fatty acids in both plasma and erythrocyte lipids, as well as raising urinary thiocyanate excretion 2.2-fold. Flaxseed also lowered serum total cholesterol by 9% and low-density-lipoprotein-cholesterol by 18%. Changes in plasma α -linolenic acid were equivalent when 12 g α -linolenic acid/d was provided as raw flaxseed flour (50 g/d) or flaxseed oil (20 g/d) suggesting high bioavailability of α -linolenic acid from ground flaxseed. Test meals containing 50 g carbohydrate from flaxseed or 25 g flaxseed mucilage each significantly decreased postprandial blood glucose responses by 27%. Malondialdehyde levels in muffins containing 15 g flaxseed oil or flour/kg were similar to those in wheat-flour muffins. Cyanogenic glycosides (linamarin, linustatin, neolinustatin) were highest in extracted flaxseed mucilage but were not detected in baked muffins containing 150 g flaxseed/kg. We conclude that up to 50 g high- α -linolenic acid flaxseed/d is palatable, safe and may be nutritionally beneficial in humans by raising n -3 fatty acids in plasma and erythrocytes and by decreasing postprandial glucose responses.

Flaxseed: α -Linolenic acid: Dietary fibre: Humans

Flaxseed (*Linum usitatissimum*) grown in Western Canada traditionally has a content of α -linolenic acid (18:3 n -3) > 50 g/100 g total fatty acids. Flaxseed oil and purified 18:3 n -3 have been used in human studies primarily to treat overt symptoms of n -3 fatty acid deficiency, e.g. neurological and visual disturbances, scaly and haemorrhagic dermatitis, folliculitis of the scalp and growth retardation (Holman *et al.* 1982; Bjerve *et al.* 1987*a, b*, 1989). Effects of cold-pressed flaxseed oil on serum lipids, fatty acid composition and platelet function in humans have also been reported (Owren *et al.* 1964; Carlson & Walldius, 1975; Beitz *et al.* 1981; Holman *et al.* 1982; Mest *et al.* 1983; Sanders & Roshanai, 1983; Singer *et al.* 1984, 1986; Adam *et al.* 1986; Bjerve *et al.* 1987*a, b*). In the majority of these studies, large amounts of flaxseed oil (10–60 g/d) have been given, but often for 2 weeks or less (Dyerberg *et al.* 1980; Singer *et al.* 1984, 1986). 18:3 n -3 levels in serum and platelet phospholipids (PL) have generally been shown to rise with flaxseed oil supplementation. However, in only one report of healthy adults were eicosapentaenoic acid (20:5 n -3), docosapentaenoic acid (22:5 n -3), or docosahexaenoic acid (22:6 n -3) increased

* Department of Nutritional Sciences, University of Toronto, Toronto, Canada, M5S 1A8.

in serum lipids after dietary supplementation with flaxseed oil (Adam *et al.* 1986). Despite the apparently inefficient conversion of 18:3 n -3 to 20:5 n -3 and 22:6 n -3 in humans, 18:3 n -3 in flaxseed oil inhibits platelet aggregation *in vivo* (Owren *et al.* 1964; Dyerberg *et al.* 1980; Renaud & Nordoy, 1983; Budowski *et al.* 1984).

Although flaxseed may have nutritionally useful properties, its high oil content (350–400 g/kg flaxseed) and polyunsaturation (720 g/kg oil) could give rise to lipid peroxidation products such as malondialdehyde (MDA) which would be undesirable in either baked products or as a result of consuming raw flaxseed. In addition, the content of cyanogenic glycosides (linustatin, neolinustatin and linamarin) may increase blood levels of thiocyanate if raw flaxseed were consumed but would be expected to be eliminated in baked products containing flaxseed.

In a series of experiments we have, therefore, evaluated the potential nutritional value of flaxseed as a source of n -3 fatty acids and soluble fibre (60–80 g/kg mucilage; Mazza & Biliaderis, 1989) in the human diet. We have also addressed the potential toxicological aspects of human flaxseed consumption by monitoring MDA and thiocyanate levels.

MATERIALS AND METHODS

Four separate experiments investigating several nutritional aspects of flaxseed in humans were conducted: (1) effects on plasma fatty acids and lipids, and on thiocyanate excretion, (2) relative changes in blood fatty acids after consuming flaxseed flour *v.* flaxseed oil containing an equivalent amount of 18:3 n -3, (3) effects of flaxseed and flaxseed mucilage on blood glucose responses, and (4) effects of baking flaxseed into muffins on content of MDA and cyanogenic glycosides. The flaxseed variety used throughout was Linott (1987) from Melfort, Saskatchewan. The study was approved by the Review Committee on the Use of Human Subjects, University of Toronto.

1. Serum lipids, plasma fatty acids and thiocyanate excretion

Volunteers recruited were healthy, unmedicated, non-smoking female undergraduate students (n 9; 24 (SD 3) years; body-mass index 21.5 (SD 0.7). They were divided into two groups consuming either a supplement of flaxseed flour (n 5) or bread baked with 250 g/kg flaxseed flour (n 4) providing both groups with 50 g flaxseed/d for 4 weeks. The flaxseed-flour supplement was consumed in breakfast cereal, soup, juice or yogurt. The flaxseed bread was substituted for the regular bread in the diet. For the group receiving the flaxseed flour, the 2 weeks after the supplementation period was a washout period; for those receiving the flaxseed bread, a control bread was provided during the 2 weeks after the flaxseed bread. During the 4th week of the flaxseed supplementation and during the 2nd week of the wash-out/control period, 24 h urine samples were collected for measurement of thiocyanate excretion. Diet records (3 d) were kept during each week of the study and total daily energy intake and energy from 50 g flaxseed/d were evaluated as described previously (Jenkins *et al.* 1989).

Two 7 ml venous blood samples were obtained from an anticubital forearm vein before supplementation (week 0), at the end of each of the 4 weeks of supplementation, and 2 weeks after (wash-out/control) the flaxseed supplementation. One was a serum sample for analysis of lipids (no anticoagulant) while the other was for analysis of plasma and erythrocyte fatty acids (EDTA-anticoagulated). Plasma samples were separated from the erythrocytes and 1.0 ml dissolved in chloroform containing 0.2 g butylated hydroxytoluene (antioxidant; Sigma Chemical Co., St Louis, MO, USA)/l and stored at -20° . Erythrocytes were washed three times in saline (9 g NaCl/l) and a 0.5 g portion resuspended in distilled water to lyse the cells. The diluted-lysed erythrocytes were

dissolved in 5.0 ml methanol containing 0.3 g butylated hydroxytoluene/l and stored at 4° until analysed.

2. *Flaxseed flour v. flaxseed oil*

To evaluate indirectly the bioavailability of 18:3n-3 from ground flaxseed *v.* flaxseed oil, changes in plasma 18:3n-3 were compared in a cross-over study in which 12 g 18:3n-3/d was consumed as flaxseed oil capsules (20 g/d; Omega Nutrition, Vancouver, BC, Canada) or flaxseed flour (50 g/d). Participants (five females; 20 (SD 5) years) consumed the flaxseed oil capsules or flaxseed flour for 4 weeks in randomized order with a 2 week wash-out in between. The flaxseed capsules were taken three times daily and the flaxseed flour was added to breakfast cereal, soup or yogurt. Venous blood samples were collected at 2-week intervals and plasma frozen for fatty acid analyses.

3. *Blood glucose responses to flaxseed and flaxseed mucilage*

The potential of soluble fibre mucilage in flaxseed to reduce postprandial changes in blood glucose was also examined. A standardized method has been developed in which the glycaemic response to a test carbohydrate (50 g) can be compared with 50 g of a standard carbohydrate (Wolever, 1991). After an overnight fast, six healthy volunteers (five male, one female; age 30 (SD 4) years) consumed, in random order, a test meal containing 50 g carbohydrate as bread made from white flour or flaxseed flour. Fingerprick blood samples were obtained at 0 min (pre-meal), 15, 30, 45 and 60 min after starting to consume the meal (0–5 min) and 100 µl portions of whole blood analysed for glucose.

Flaxseed mucilage was obtained from whole flaxseed by soaking the flaxseed in boiling water (with constant stirring) followed by cooling to room temperature for 30 min. The mucilage dissolved in the water within 30 min and was filtered from the flaxseed through a screen. The dissolved mucilage was shell-frozen, freeze-dried over 72 h, and reconstituted in warm water (25 g in 400 ml). The effect of 50 g glucose *v.* 50 g glucose mixed with 25 g mucilage was then compared over 120 min.

4. *MDA and cyanogenic glycosides in flaxseed muffins*

Although effective and palatable in providing a source of dietary flaxseed, the flaxseed bread used in the first experiment was found to be cumbersome and limited dietary flexibility. Accordingly a flaxseed muffin mix was developed which was evaluated with respect to MDA and cyanogenic glycoside content. The flaxseed muffin mix contained the following ingredients (g) per twenty-four muffins with each muffin containing 25 g ground flaxseed: white flour 960, milk 658, flaxseed flour 600, eggs 300, honey 300, baking powder 96, maize oil 30, salt 17. In the control muffins flaxseed flour was replaced by white flour. The muffins were baked at 230° for 15–18 min.

Analytical procedures

Plasma total cholesterol (tCH), high-density-lipoprotein-cholesterol (HDL-CH), and triacylglycerols (TG) were measured by automated Lipid Research Clinic-standardized procedures (CDC, Atlanta, GA, USA) and low-density-lipoprotein-cholesterol (LDL-CH) calculated as described previously (Jenkins *et al.* 1989). Serum phospholipid (PL) was quantified by the phosphorus assay of Bartlett as modified by Christie (Cunnane, 1988).

All organic solvents used in the extraction and chromatographic separation of flaxseed, plasma and erythrocyte lipids and fatty acids were glass-redistilled before use (Fisher Scientific, Toronto, Canada). Total lipids were extracted into chloroform–methanol (2:1, *v:v*; 10 vol.) after partitioning of the organic phase with 2 vol. saline (9 g NaCl/l). The organic phase was dried under N₂ gas and the PL and TG of plasma and flaxseed or the

phosphatidylcholine and phosphatidylethanolamine of erythrocytes separated by thin-layer chromatography (Cunnane, 1988). Fatty acids in these lipid classes were transmethylated under N_2 at 90° for 30 min using boron trifluoride in methanol (140 g/l; Sigma). The proportional composition of the resulting fatty acid methyl esters was determined by gas-liquid chromatography (Hewlett-Packard 5890A) using a capillary column (Durabond 23, 30 m, $0.25 \mu\text{m}$ I.D.; J&W Scientific, Folsom, CA, USA) with automated sample delivery and injection.

Whole capillary blood glucose was measured as previously described (Jenkins *et al.* 1989). Thiocyanate in urine and flaxseed muffins and cyanogenic glycosides in flaxseed muffins were determined by high-performance liquid chromatography as previously reported (Schilcher & Wilkens-Sauter, 1986). MDA in flaxseed products was measured spectrophotometrically (Wade & van Rij, 1989).

Statistics

Within individual experiments, statistical comparisons were done using two-way ANOVA or Student's paired *t* test, or both, as appropriate.

RESULTS

Fatty acid profile of flaxseed lipids

The flaxseed used contained 415 g TG/kg and 11 g PL/kg. The fatty acid profile of the lipid extract (g/100 g) was polyunsaturated fatty acids 72–73 with linoleic acid (18:2 n -6) 15.0 and 18:3 n -3 58.2 (Table 1).

1. *Flaxseed or flaxseed bread supplementation*

Consumption of raw, ground flaxseed or bread made with flaxseed flour did not significantly influence body weight throughout the study (54 (SD 8) *v.* 55 (SD 8) kg while on flax). The mean total energy intake was not significantly influenced by flaxseed intake (7.69 (SD 2.84) *v.* 8.28 (SD 3.09) MJ (1840 (SD 680) *v.* 1980 (SD 740) kcal)/d on flaxseed). Mean total fat intake was also not significantly affected by flaxseed intake (75 (SD 31) *v.* 77 (SD 43) g/d on flaxseed). During the 4-week supplementation period, flaxseed provided 12–13% of the total energy or 24–25% of the total fat intake/d. The influence of flaxseed on total *n*-3 fatty acid intake was not determined because of the paucity of *n*-3 fatty acid values for many common foods.

Plasma fatty acid profiles at entry (week 0) or during the 4 weeks of flaxseed supplementation did not differ significantly according to whether the participants consumed flaxseed flour or flaxseed bread. Hence the values for all nine participants have been combined. 18:3 n -3 increased 2.3-fold in serum PL and 3.2-fold in plasma TG after 4 weeks of flaxseed supplementation ($P < 0.01$), and returned to week 0 values after the 2-week wash-out/control period (Fig. 1). 20:5 n -3 also increased in plasma PL but did not change in serum TG during flaxseed supplementation. 22:5 n -3 (values not shown) and 22:6 n -3 did not change significantly in either serum TG or PL during flaxseed supplementation (Fig. 1). None of the *n*-3 fatty acids were significantly altered in erythrocyte phosphatidylethanolamine during flaxseed supplementation. However, in erythrocyte phosphatidylcholine, 22:6 n -3 increased by 80% after 4 weeks flaxseed supplementation and remained elevated during the wash-out/control period (Fig. 2).

Saturated, monounsaturated and *n*-6 fatty acids did not change significantly in plasma TG or PL over the course of the flaxseed study but, in erythrocyte phosphatidylcholine, C₂₀ and C₂₂ *n*-6 fatty acids were increased significantly after 3–4 weeks of flaxseed supplementation and remained elevated during the wash-out/control period (Table 2).

Table 1. Fatty acid composition of triacylglycerols and phospholipids of high α -linolenic acid flaxseed (*Linum usitatissimum* var. *Linott*)
(Mean values and standard deviations for four determinations)

	Triacylglycerols		Phospholipids	
	Mean	SD	Mean	SD
Total (g/kg seed)	415	10	10.5	0.2
16:0				
g/kg seed	20	2	1.2	0.1
g/100 g fatty acids	4.8	0.3	11.4	1.9
18:0				
g/kg seed	10	1	0.4	0.1
g/100 g fatty acids	2.4	0.2	3.1	0.6
18:1n-9				
g/kg seed	78	5	0.5	0.1
g/100 g fatty acids	19.2	1.0	23.3	2.7
18:2n-6				
g/kg seed	60	2	4.2	0.3
g/100 g fatty acids	15.0	0.6	35.1	6.5
18:3n-3				
g/kg seed	242	9	3.5	0.2
g/100 g fatty acids	58.2	1.4	27.0	2.5

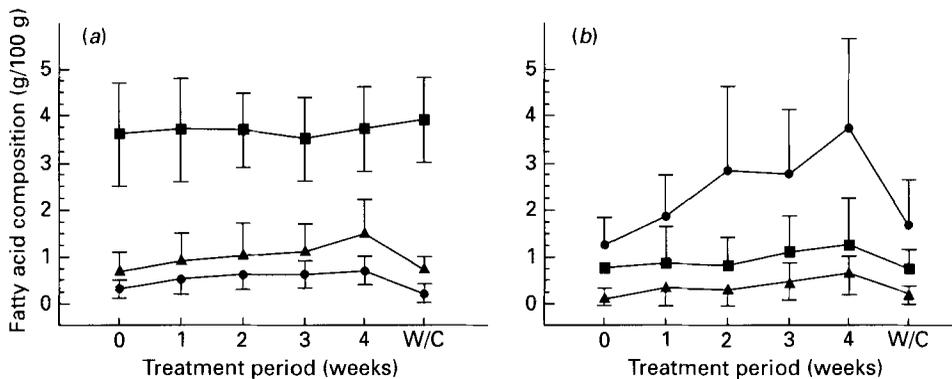


Fig. 1. Fatty acid compositions (g/100 g total fatty acids) of plasma lipids before (week 0), during (weeks 1–4) and after (washout/control; W/C) flaxseed (*Linum usitatissimum*) supplementation at 50 g/d in human subjects. (a) Plasma phospholipids: α -linolenic acid, (●—●); eicosapentaenoic acid, (▲—▲); docosahexaenoic acid, (■—■); (b) plasma triacylglycerols: α -linolenic acid, (●—●); eicosapentaenoic acid, (▲—▲); docosahexaenoic acid, (■—■). Values are means and standard deviations, represented by vertical bars, for nine subjects. For detail of procedures, see pp. 444–446.

Serum tCH decreased 9% and LDL-CH decreased 18% after 4 weeks consumption of flaxseed ($P < 0.01$) with no significant difference between the groups consuming raw flaxseed v. flaxseed bread. Serum HDL-CH and TG levels remained unaffected by flaxseed consumption (Fig. 3).

Urinary thiocyanate excretion was itself not significantly affected by previous consumption of flaxseed for 4 weeks (47 (SD 35) v. 106 (SD 75) μ mol/l on flaxseed) but was

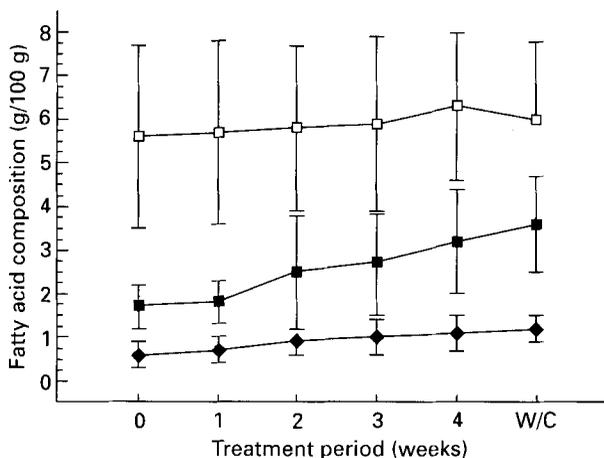


Fig. 2. *n*-3 fatty acid composition (g/100 g total fatty acids) of erythrocyte lipids before (week 0), during (weeks 1–4) and after (washout/control; W/C) flaxseed (*Linum usitatissimum*) supplementation at 50 g/d in human subjects. Docosahexaenoic acid in erythrocyte phosphatidylcholine, (■—■); docosapentaenoic acid (*n*-3) in erythrocyte phosphatidylcholine (◆—◆); docosahexaenoic acid in erythrocyte phosphatidylethanolamine, (□—□). Values are means and standard deviations, represented by vertical bars, for nine subjects. For details of procedures, see pp. 444–446.

Table 2. *n*-6 fatty acids (g/100 g total fatty acids) in erythrocyte phosphatidylcholine before (week 0), during (weeks 1–4) and after (washout/control) 4 weeks of flaxseed (*Linum usitatissimum*) supplementation (50 g/d) in human subjects†
(Mean values and standard deviations for nine subjects)

Fatty acid...	18:2 <i>n</i> -6		20:4 <i>n</i> -6		22:4 <i>n</i> -6		22:5 <i>n</i> -6	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Pre-flax								
Week 0	19.3	2.9	7.3	1.8	0.6	0.3	0.1	0.1
Flax								
Week 1	19.2	2.8	7.5	2.6	0.6	0.4	0.2	0.2
Week 2	17.9	2.6	8.7	2.4	0.9	0.4	0.3	0.3
Week 3	18.5	3.2	9.3	2.6*	0.9	0.6	0.3	0.3
Week 4	17.5	3.0	9.9	2.4*	1.1	0.5*	0.4	0.3*
Post-flax (washout/control)	17.5	2.5	11.0	2.2*	1.1	0.7*	0.5	0.3*

Mean values were significantly different from those at week 0 (two-way ANOVA and paired Student's *t* test): * $P < 0.01$.

† For details of subjects and procedures, see pp. 444–446.

affected in comparison with creatinine excretion (11 (SD 4) v. 33 (SD 29) $\mu\text{mol}/\text{mmol}$ on flaxseed; $P < 0.01$).

2. Flaxseed flour v. flaxseed oil

Consumption of 12 g 18:3*n*-3 fatty acid/d for 4 weeks as either 20 g flaxseed oil/d or 50 g flaxseed flour/d raised 18:3*n*-3 in plasma TG to a similar extent; the increase in 18:3*n*-3 in plasma PL was significant only after the flaxseed flour (week 2 only; $P < 0.01$). Total *n*-3 fatty acid products (20:5*n*-3, 22:5*n*-3, 22:6*n*-3) were raised significantly in plasma TG after the flaxseed flour (week 2 only) and after flaxseed oil (week 4 only; Fig. 4).

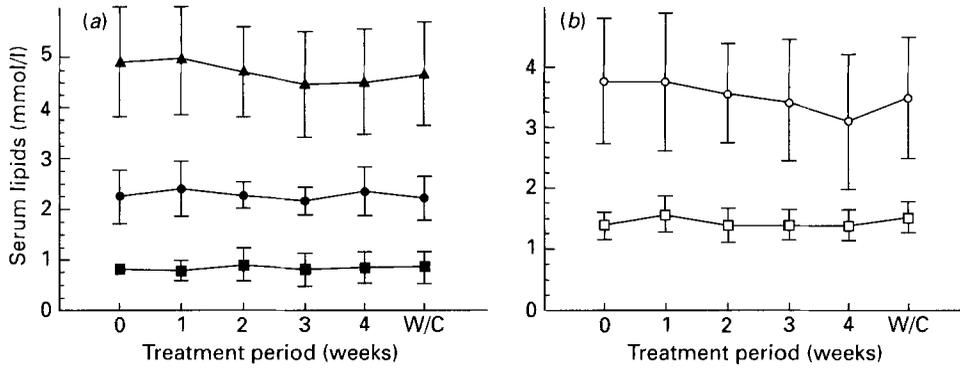


Fig. 3. Serum lipids (mmol/l) before (week 0), during (weeks 1–4) and after (washout/control; W/C) flaxseed (*Linum usitatissimum*) supplementation at 50 g/d in human subjects. (a) Total cholesterol, (▲—▲); total phospholipid, (●—●); total triacylglycerol, (■—■); (b) low-density-lipoprotein-cholesterol (○—○); high-density-lipoprotein-cholesterol (□—□). Values are means and standard deviations, represented by vertical bars, for nine subjects. For details of procedures, see pp. 444–446.

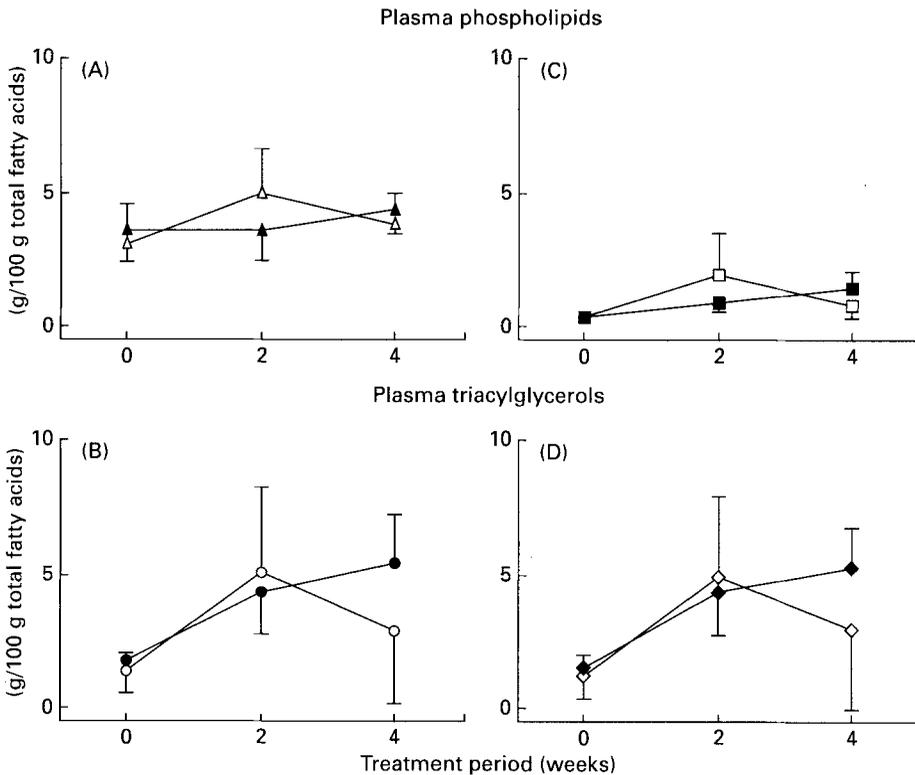


Fig. 4. Changes in total *n*-3 fatty acids (A,B) and α -linolenic acid (C,D) in plasma triacylglycerols and phospholipids after consuming 12 g α -linolenic acid/d as either 50 g flaxseed (*Linum usitatissimum*) flour/d (△, □, ○, ◇) or 20 g flaxseed oil capsules/d (▲, ■, ●, ◆) in human subjects. Values are means and standard deviations, represented by vertical bars, for four determinations per time-point. For details of procedures, see pp. 444–446.

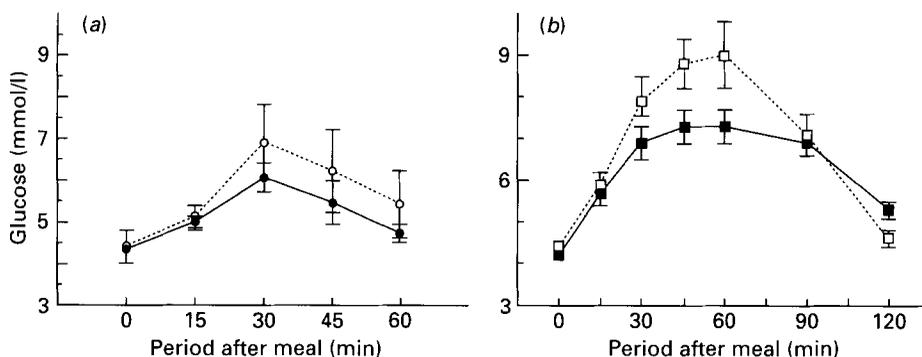


Fig. 5. Blood glucose responses to a breakfast meal consisting of 50 g carbohydrate as: (a) white bread (○—○) compared with flaxseed (*Linum usitatissimum*) bread (●—●). Values are means and standard deviations, represented by vertical bars, for six subjects. (b) Meal consisting of 50 g glucose in 400 ml water (□---□) compared with 50 g glucose plus 25 g flaxseed mucilage in 400 ml water (■—■). Values are means and standard deviations, represented by vertical bars, for four subjects. For details of procedures, see pp. 444–446.

3. Blood glucose responses

The incremental area under the blood glucose curve obtained within 60 min of consuming the flaxseed bread test meal was 28% lower than under the standard white bread test meal (Fig. 5; $P < 0.01$). The incremental area under the blood glucose curve during the flaxseed mucilage/glucose test was 27% lower than under the glucose test alone (Fig. 5).

4. MDA and cyanogenic glycosides

MDA levels were similar in raw or baked muffins made with whole flaxseeds, flaxseed flour, flaxseed oil or in muffins made with wheat flour containing no flaxseed flour or oil (mean of all groups 22 (SD 3) $\mu\text{g/g}$). Storage of the flaxseed flour at -20° for up to 2 years did not significantly affect the muffin MDA levels compared with freshly ground flaxseed (values not shown). Freeze-dried flaxseed mucilage contained 2–4 μg cyanogenic glycosides (linamarin, linustatin and neolinustatin)/g. Only linustatin and neolinustatin were detected in whole flaxseed (2.6 and 3.5 $\mu\text{g/g}$ respectively) while none of these three cyanogenic glycosides were detected in flaxseed oil or in muffins made with flaxseed flour, suggesting they were destroyed at cooking temperatures.

DISCUSSION

Our results indicate that flaxseed consumed as either raw flour or incorporated into bread reduced serum cholesterol while raising 18:3n-3 and desaturated/elongated n-3 fatty acids in serum and erythrocyte lipids. The amount of flaxseed which had these effects was 50 g/d consumed over 4 weeks. Based on the similar changes in 18:3n-3 and longer chain n-3 fatty acids in serum after either flaxseed flour or flaxseed oil (both supplying 12 g/d 18:3n-3), the bioavailability of 18:3n-3 from flaxseed seems as high as from flaxseed oil (Fig. 3). Although energy-dense (about 1.17 MJ (280 kcal)/50 g), flaxseed supplementation did not alter body weight or total dietary fat intake over a 4-week period. Thus, the flaxseed supplement seems to have displaced other sources of energy rather than adding to total fat and energy intake.

Since the intake of 18:3n-3 while consuming flaxseed was about 10-fold the usual dietary 18:3n-3 intake, the change in plasma 18:3n-3 is an indirect measure of compliance with either flaxseed or flaxseed oil capsule consumption. In comparison with the flaxseed oil values, plasma 18:3n-3 levels while consuming flaxseed decreased between weeks 2 and 4 and had wider variance suggesting a possible lack of compliance in some individuals.

Previous studies have described no increase in long-chain *n*-3 fatty acids after feeding as much as 60 ml flaxseed oil/d (20–25 g 18:3*n*-3/d) for 2 weeks but an increase in both *n*-3 and *n*-6 fatty acids in erythrocytes when much lower amounts of 18:3*n*-3 are consumed for longer periods (Holman *et al.* 1982; Bjerve 1987*a, b*). Our results after 2 weeks of flaxseed supplementation (10–12 g/d) are in agreement with the studies of Singer *et al.* (1984, 1986), e.g. little or no change in longer-chain *n*-3 fatty acids, but show that after 4 weeks of flaxseed supplementation, longer-chain *n*-3 fatty acids are raised in plasma and erythrocyte lipids (Figs. 1–2). Singer *et al.* (1984, 1986) showed that although *n*-3 fatty acids were unchanged after flaxseed oil supplementation (both hyperlipidaemic and normolipidaemic subjects), serum lipids were decreased in cases of primary hyperlipidaemia. Hence, an increase in 20:5*n*-3 and 22:6*n*-3 after flaxseed oil supplementation may not be necessary to achieve a hypolipidaemic effect. Conversely, a lack of change of 20:5*n*-3 or 22:6*n*-3 after giving 18:3*n*-3 is not necessarily due to lower desaturation/elongation in hyperlipidaemia but may be due to substrate inhibition of the desaturases by the high dose of 18:3*n*-3 (Poisson & Cunnane, 1991). In view of our present findings as well as those of Bjerve *et al.* (1987*a, b*, 1989), it appears that two factors have an important influence on synthesis of longer-chain *n*-3 fatty acids from 18:3*n*-3 present in flaxseed oil: (1) the feeding period needs to be at least 4 weeks, and (2) it is possible that substrate inhibition of the Δ 6 and Δ 5 desaturases may occur if the supplement of 18:3*n*-3 is too high (above 12–15 g/d).

As reported in the studies of Bjerve *et al.* (1987*a, b*, 1989), we have also seen an increase in long-chain *n*-6 fatty acids in erythrocyte phosphatidylcholine after flaxseed supplementation (Fig. 2). Although these observations contradict those of most animal studies in which fatty acids of liver and serum lipids are most commonly evaluated (Mohrhauer & Holman, 1963; Budowski & Crawford, 1985; Garg *et al.* 1989; Cunnane *et al.* 1990), we are not aware of studies besides those of Bjerve *et al.* (1987*a, b*) which have reported changes in human erythrocyte fatty acids. Hence, the increase in long-chain *n*-6 fatty acids after flaxseed or pure 18:3*n*-3 supplementation may be specific to the erythrocyte.

Flaxseed was hypocholesterolaemic in this group of normocholesterolaemic female subjects, with the effect being mainly on LDL-CH since HDL-CH and TG were unchanged after the 4-week flaxseed supplementation period. It is not clear from the present study whether the 18:3*n*-3 or mucilage in the flaxseed or, indeed, an effect of dietary flaxseed on reducing intake of saturated fat would have been responsible for lowering serum tCH. Since long-chain *n*-3 fatty acids do not usually lower serum tCH, but 18:3*n*-3 has been reported to do so (Bjerve *et al.* 1989), further investigation of the mechanism and clinical significance of this observation is necessary.

Flaxseed was hypoglycaemic in two test meal situations, one involving flaxseed flour incorporated into a test bread compared with a standard white bread, and the other involving flaxseed mucilage combined with glucose compared with glucose alone (Fig. 5). In both cases the lowering of the post-meal change in blood glucose was about 27%. Hence, the increase in blood glucose caused by glucose-rich cereals or snack products could be potentially reduced if these products also contained flaxseed meal (oil removed) or flaxseed mucilage. This could increase the range of foods available to diabetic patients. Flaxseed mucilage might also be hypolipidaemic as has been observed with other soluble fibres (Jenkins *et al.* 1985, 1987).

Two possible concerns about the safety of flaxseed have been addressed in preliminary form in the present study. First, the presence of cyanogenic glycosides in the flaxseed and mucilage was reduced below detectable limits after baking. Thus, although the cyanogenic glycosides could be consumed intact in products containing raw flaxseed, our results indicate that cooking the ground flaxseed (hot cereals or baked products) seems to eliminate them. A related question is whether thiocyanate is produced in increased

quantities after flaxseed consumption. From our present findings, there seems to be little significant effect of 50 g flaxseed/d on thiocyanate excretion, although it was increased relative to creatinine excretion. Considering that 50 g/d is a relatively high amount of flaxseed, the flaxseed-induced increase in urinary thiocyanate (relative to creatinine) would be less on a lower flaxseed intake. Second, MDA levels were similar in baked muffins or raw muffin dough regardless of whether they contained only wheat flour or wheat flour mixed with 150 g flaxseed flour, flaxseed oil or whole flaxseeds/kg. Thus, normal cooking time and temperature does not significantly increase MDA levels in flaxseed. We, therefore, advocate the muffin as an appropriate vehicle to consume up to 50 g/d of flaxseed. From the point of view of safety, acceptability and efficacy, flaxseed appears to be a grain with useful potential in human nutrition. We conclude that further studies of the nutritional impact of flaxseed are warranted.

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