Content of phospholipids in human diets studied by the duplicate-portion technique

BY BJÖRN ÅKESSON

Department of Clinical Chemistry and Dalby Community Health Research Centre, University of Lund, Lund, Sweden

(*Received 13 May 1981 – Accepted 23 October 1981*)

1. The phospholipid intake by eight healthy women was studied by the duplicate-portion technique, i.e. chemical analyses of duplicates of the food consumed. Their intake of phospholipids was 1·5–2·5 mmol lipid-phosphorus/d, which corresponds to 0·023–0·059 mmol lipid-P/g dietary fat and 0·24–0·45 mmol lipid-P/MJ respectively.

2. Choline phosphoglyceride constituted 480–700 mmol/mol and ethanolamine phosphoglyceride 170–240 mmol/mol total lipid-P. Most of the choline in nature occurs as choline phospholipids, the intake of which ranged between 0·91–1·85 mmol/d or 0·15–0·33 mmol/MJ.

3. Palmitate, stearate, oleate, and linoleate were the four major fatty acids both in choline phosphoglyceride and ethanolamine phosphoglyceride. Significant amounts of highly unsaturated, C_{20} and C_{22} fatty acids were also observed. It was calculated that 13–33 mg/g total dietary fatty acids was consumed as phospholipids. Together with bile phospholipids, dietary phospholipids and their degradation products may be important in the digestion and absorption processes.

The main dietary lipid is triacylglycerol but the quantity and composition of less-abundant acyl lipids, such as phospholipids, are not much studied. Most available information concerns individual foodstuffs whereas information on the polar lipid composition in human diets, mixed and prepared for consumption is almost completely lacking. Together with bile phospholipids dietary phospholipids may have important functions in the intestinal lumen (Borgström, 1976) and may influence, for example, triacylglycerol release from the intestine (O'Doherty *et al.* 1973), and the absorption of cholesterol (Rodgers & O'Connor, 1975). Bile and dietary phospholipids may be metabolized in the same way (Arnesjö *et al.* 1969) although a difference has been proposed (Boucrot & Clement, 1971). The consumption of phospholipids is also relevant for the provision of dietary choline, most of which is lipid-bound.

The intake of phospholipids by healthy free-living subjects has been studied. This study was part of a project designed to assess dietary intake by chemical analysis of so-called duplicate portions (Borgström *et al.* 1975; Borgström *et al.* 1979). This methodological approach is especially advantageous for components which are not listed in customary food composition tables, such as polar lipids.

EXPERIMENTAL

The subjects studied were eight healthy women, aged 34–69 years, who formed part of a reference group in a study on dietary intake in relation to blood pressure. Other aspects of their dietary intake are reported elsewhere (Thulin *et al.* 1980). The nutrient intake agreed well with the pattern normally encountered in Sweden. Each subject collected six daily portions as described previously (Thulin *et al.* 1980). The portions were homogenized as described previously (Borgström *et al.* 1975) and 2–5 g of the homogenate was extracted for 1 h with 5 vol. chloroform–methanol (1:2, v/v). The mixture was filtered and the extraction was repeated with 3 vol. chloroform–methanol (1:1, v/v). Enough water to give a value for water–methanol of 1:1 was added to the combined extracts and the chloroform

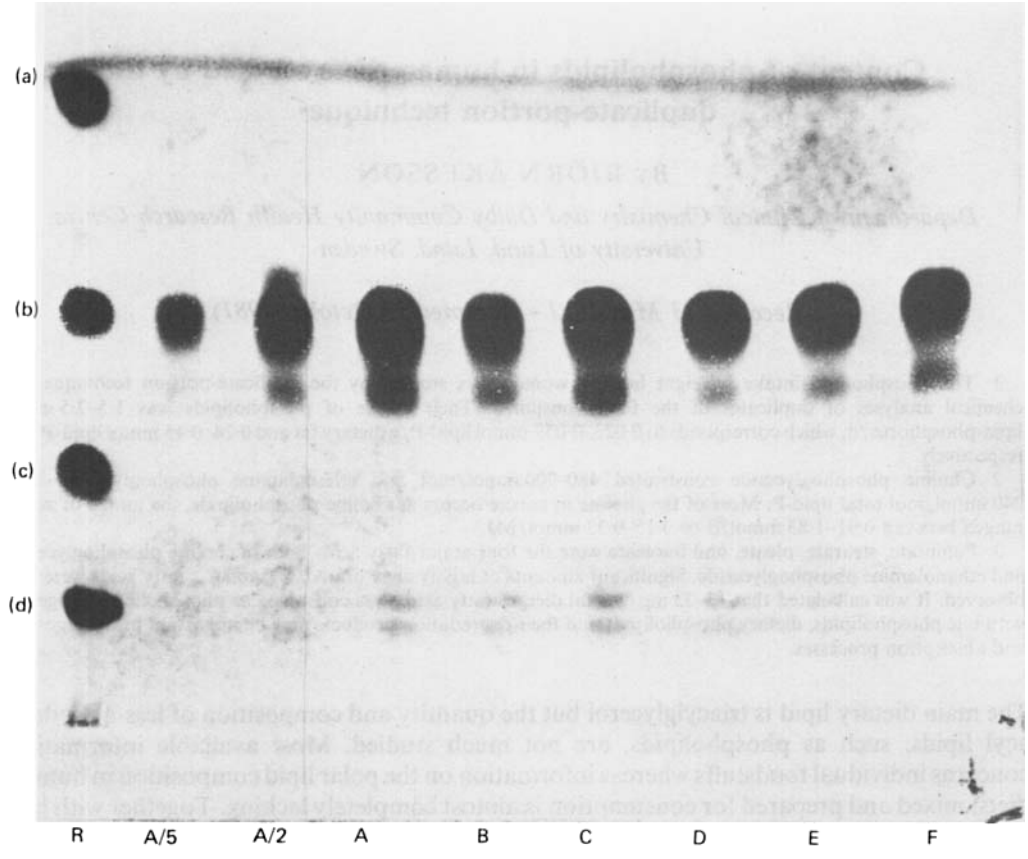

Fig. 1. Thin-layer chromatography of non-polar dietary lipids isolated from six daily portions from subject 6. The reference substances to the left (R) are (*a*) cholesterol oleate, (*b*) triolein, (*c*) oleic acid and (*d*) cholesterol. Between the reference and the six samples one fifth and one half the amount of the A sample was applied. The developing solvent was diethyl ether–petroleum ether–acetic acid (30:70:1, v/v). Charring with 0·6% $K_2Cr_2O_7$ in 55% H_2SO_4.

layer was recovered after phase separation. After drying and weighing, total lipids were dissolved in chloroform and separated into a neutral lipid fraction and a polar fraction by column chromatography on silicic acid (< 200 mesh; Mallinckrodt). The first eluent was chloroform (20 ml/g silicic acid) and the second eluent chloroform-methanol (1:1, v/v) plus methanol (each 7–10 ml/g silicic acid). The phosphorus content in the polar fraction was determined according to Chen *et al.* (1956). Phospholipids were separated by thin-layer chromatography on silica gel H with chloroform–methanol–acetic acid–water (65:25:4:4, v/v) as the developing solvent. The phospholipid fractions were either eluted with chloroform–methanol–acetic acid–water (50:39:1:10, v/v) (Åkesson *et al.* 1970) or directly measured by P determination (Rouser *et al.* 1966). Some of the isolated fractions were further purified by thin-layer chromatography with chloroform–methanol–concentrated ammonia (65:35:8, v/v) as the developing solvent. Fatty acid analysis by gas–liquid chromatography of methyl esters was performed as described previously (Åkesson *et al.* 1970). The components were identified by comparing the retention times with those of authentic standards.

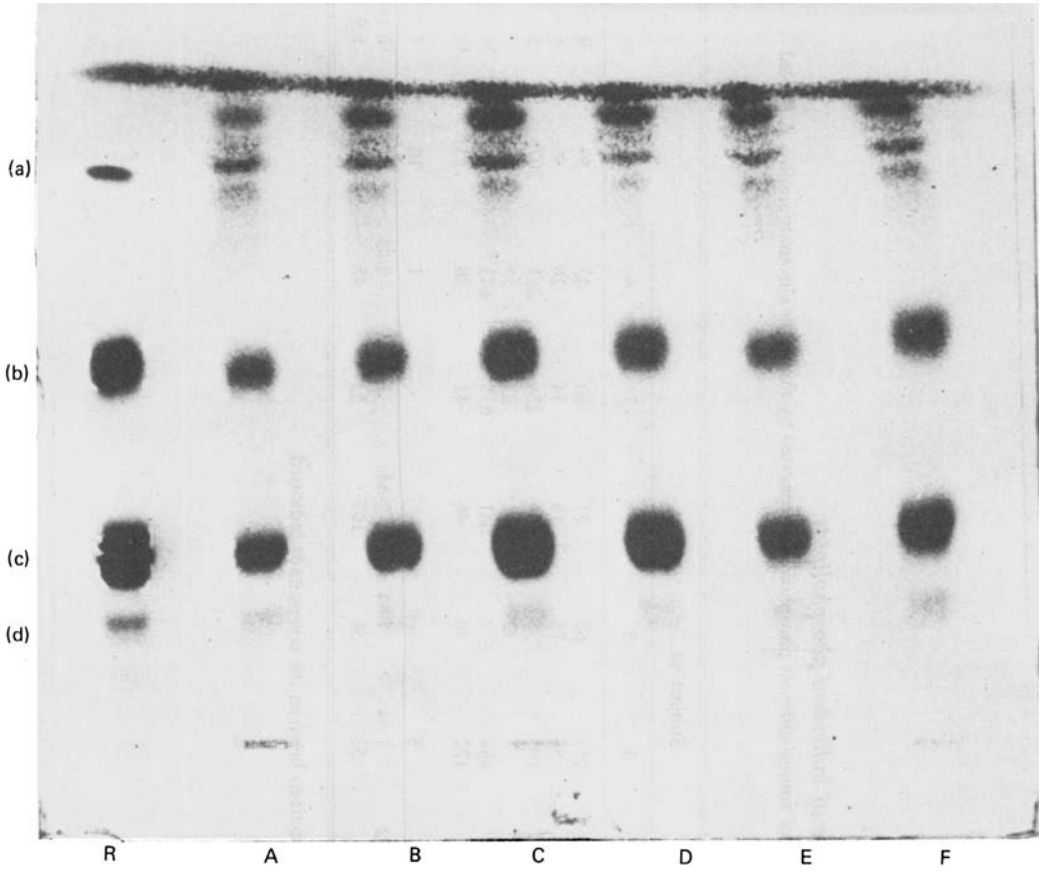

Fig. 2. Thin-layer chromatography of polar dietary lipids isolated from six daily portions from subject 6. The reference to the left (R) is rat liver phospholipid: (*a*) cardiolipin, (*b*) ethanolamine phosphoglyceride, (*c*) choline phosphoglyceride and (*d*) sphingomyelin. The developing solvent was chloroform–methanol–acetic acid–water (65:25:4:4, v/v). Charring with 0·6% $K_2Cr_2O_7$ in 55% H_2SO_4.

RESULTS

Lipid classes in the diet

Total dietary lipid was separated into a non-polar and a polar fraction by column chromatography, and the fractions were analysed by thin-layer chromatography. The non-polar fraction contained mainly triacylglycerol with smaller amounts of compounds with the chromatograhic mobilities of diacylglycerol, sterols and unesterified fatty acid.

The intake of phospholipids was 1·5–2·5 mmol/d, determined from the content of lipid-P in the polar fraction (Table 1). The intake in relation to fat and energy was 0·023–0·059 mmol lipid-P/g fat and 0·24–0·45 mmol lipid-P/MJ respectively. The energy intake was 3·7–10·1 MJ/d and fat intake 38–107 g/d. For one subject, the daily variation in phospholipid intake was investigated (Table 2). Total phospholipid intake varied approximately twofold. It could be calculated that 13–33 mg/g total dietary fatty acids were consumed as phospholipids. Although the contribution by phospholipids for most dietary fatty acids is small, their role may be greater for fatty acids enriched in phospholipids, such as long-chain, highly-unsaturated acids (see p. 228).

Table 1. *Intake of individual phospholipids*

(The values represent the relative distribution of phosphorus mmol/mol among different phospholipids separated by thin-layer chromatography. A pooled sample from six daily portions was analyzed for each person)

Lipid fraction	Subject no. 1	2	3	4	5	6	7	8	Mean	SE
Solvent front	84	38	38	113	62	31	60	43	59	9
A*	63	20	49	55	28	16	34	26	36	6
Ethanolamine phosphoglyceride	193	183	174	195	236	234	226	211	207	8
B*	22	23	3	11	22	11	23	25	18	3
Choline phosphoglyceride	528	704	676	484	622	661	615	673	620	27
Sphingomyelin + lysophosphatidylcholine	79	30	59	122	30	46	41	20	53	11
Origin	31	2	—	5	—	—	—	1	10	7
Total lipid-P (mmol/d)	2·35	1·62	2·52	1·54	1·84	2·44	1·49	2·05	1·98	0·15
Total fat (g/d)	92	38	43	53	51	107	53	45	60·3	8·9

A, B, are intermediate fractions between the compounds indicated.

Table 2. *Phospholipids in individual daily portions from subject no. 6*

Day...	a	b	c	d	e	f
Total lipid-phosphorus (mmol/d)	1·4	1·8	3·2	2·6	1·5	2·0
Lipid-P: mmol/g total fat	0·012	0·024	0·024	0·027	0·014	0·019
Lipid-P: mmol/MJ energy	0·14	0·22	0·29	0·31	0·15	0·19

The polar fraction was analyzed by thin-layer chromatography. Spots migrating as ethanolamine phosphoglyceride and choline phosphoglyceride were the major components but several unidentified spots were seen, especially close to the solvent front. Quantitative P analysis showed that the choline phosphoglyceride fraction constituted 480–700 mmol/mol total phospholipids and the ethanolamine phosphoglyceride fraction 170–240 mmol/mol total phospholipids (Table 1). The intake of choline phospholipids, calculated from total lipid-P and the sum of the relative amounts of choline phosphoglyceride, sphingomyelin and lysophosphatidylcholine, ranged between 0·91 and 1·85 mmol/d or 0·15 and 0·33 mmol/MJ.

Fatty acid composition

The fatty acid composition of total polar lipids in the diet was similar for different persons (Table 3). Only the proportions of oleic acid and linoleic acid varied reciprocally. The fatty acid composition of dietary choline phosphoglyceride, as expected, resembled that of total polar lipids in several respects (Table 4). The proportions of palmitate and oleate were somewhat higher and that of linoleate lower than in total polar lipids. The ethanolamine phosphoglyceride fraction contained a higher proportion of linoleate than choline phosphoglyceride, and also a larger amount of total fatty acids than expected from the content of P. This fraction and the choline phosphoglyceride fraction (pooled sample from eight subjects) was therefore further purified by thin-layer chromatography. The purified choline phosphoglyceride contained 890 mg/g of the fatty acids in the starting fraction, the rest probably partly representing inositol phosphoglyceride. The purified dietary ethanolamine phosphoglyceride contained 530 mg/g fatty acids in the starting fraction. The rest was mainly recovered in a compound migrating as digalactosyl diacylglycerol. The fatty acid

Table 3. *Fatty acid composition (mg/g) in total polar lipids consumed by eight subjects*
(Each sample represents six pooled daily portions)

	Subject no.									
Fatty acid	1	2	3	4	5	6	7	8	Mean	SE
16:0	202	242	244	215	198	229	222	251	225	7
16:1	5	9	7	4	3	5	5	7	6	1
18:0	120	158	121	80	112	120	97	112	115	8
18:1	120	240	232	170	147	206	186	238	192	16
18:2	357	230	287	359	363	345	363	273	322	18
18:3+20:1	40	13	27	41	59	23	40	30	34	5
20:4	53	62	34	21	45	38	31	34	40	5
20:5	20	11	5	27	21	2	10	7	13	3
22:6	72	30	38	79	47	28	41	43	47	7

Table 4. *Fatty acid composition (mg/g) of individual dietary polar lipids*
(Mean values with their standard errors for eight subjects)

Fatty acid	Choline phosphoglyceride fraction		Ethanolamine phosphoglyceride fraction		Choline phospho-glyceride*	Ethanolamine phospho-glyceride*	Diglycosyl diacyl-glycerol*
	Mean	SE	Mean	SE			
16:0	277	7	165	7	290	145	135
16:1	12	1	10	1	17	2	
18:0	120	8	140	19	101	202	22
18:1	238	19	157	13	256	179	71
18:2	210	11	378	30	197	205	681
18:3+20:1	24	5	44	6	20	35	71
20:4	35	4	54	9	33	109	
20:5	16	5	8	1	16	16	
22:5	9	2	6	1	6	9	
22:6	57	10	44	3	51	86	

* Pooled sample representing eight subjects.

composition of this compound deviated distinctly from that of the phospholipids, since it contained 680 mg linoleic acid/g (Table 4). In comparison to choline phosphoglyceride, purified ethanolamine phosphoglyceride had a higher stearate:palmitate value and also contained larger proportions of highly unsaturated fatty acids. Since these acids are very minor components in dietary triacylglycerol, their main location in the diet may be the phospholipids. It could be calculated that the mean consumption of C 20:4 from ethanolamine phosphoglyceride was 0·09 mmol/d and from choline phosphoglyceride 0·08 mmol/d. The corresponding intake of C 22:6 was 0·07 and 0·13 mmol/d respectively. A nutritional role for such fatty acids has been suggested in brain development (Crawford *et al.* 1973) and also in prostaglandin synthesis (Dyerberg *et al.* 1978). *Trans*-isomers of octadecenoate constitute approximately 50 mmol/mol dietary octadecenoate (Åkesson *et al.* 1981), but the proportion of such isomers in dietary phospholipids has not been studied.

DISCUSSION

In the intestinal lumen dietary phospholipids are mixed with biliary phospholipids. The secretion rate of biliary phospholipids, mainly phosphatidylcholine, is in the range 9·2–23·1 mmol/d (Northfield & Hoffmann, 1975) or 4·4–5·0 g/d (5·7–6·5 mmol/d, Shioda *et al.* 1968). Since the diets studied by the present authors contained 1·5–2·5 mmol phospholipid/d it can be concluded that the bile is the dominating origin of lumen phospholipid under normal conditions. Phosphatidylcholine from both sources is probably metabolized in the same way (Nilsson, 1968; Arnesjö *et al.* 1969) although a heterogeneity has been suggested (Boucrot & Clement, 1971). A proposed enterohepatic circulation of biliary phosphatidylcholine has not been verified (Larsson & Nilsson, 1978).

Since phosphatidylcholine is the dominating source of choline in the diet, the present results also give information on choline intake. Choline is essential for several animals but the requirement in humans is not known (Lucas & Ridout, 1967; Griffith & Dyer, 1968). For infant formulas 7 mg choline/100 kcal (0·16 mmol/MJ) is recommended (Committee on Nutrition, American Academy of Pediatrics, 1976), and the present intake of choline phospholipids corresponded to 0·15–0·33 mmol/MJ.

Previous estimates of choline intake showed in three human rations as mean daily values

7 mmol phospholipid and 3·5 mmol choline, corresponding to 0·078 mmol lipid-P/g fat and 0·039 mmol choline/g fat (Ridout *et al.* 1952). An experimental diet for young men contained 150 mg choline/d (1·4 mmol; Connor Johnsson *et al.* 1945). Borglin (1947) calculated that an average Swedish diet would contain 500 mg choline/3450 kcal (4·8 mmol/d or 0·33 mmol choline/MJ). These values and those of Mudd & Poole (1975) are in the same range as those reported here.

Foodstuffs rich in phospholipids include egg yolk, meat, fish, dairy products and several grains (Lucas & Ridout, 1967), and one estimation indicated that half of the dietary choline originated from eggs (Borglin, 1947). In phosphatidylcholine from eggs (Noble & Moore, 1965) and also from milk (Jensen, 1973) oleate dominates over linoleate which is contrary to the situation for dietary phosphatidylcholine (Table 4). This finding indicated that other phosphatidylcholines rich in linoleate, such as soya-bean phosphatidylcholine, occur in the diet in addition to egg phosphatidylcholine.

Several aspects of dietary phospholipids in human nutrition require further elucidation. Detailed knowledge of the intake of different dietary lipids by persons on various regimens is one prerequisite. The duplicate-portion technique permits such analyses on free-living individuals and such dietary information can be related to the health status of the consumer. Previously, the intake of fatty acids and sterols was studied by the same technique (Borgström *et al.* 1975; Borgström *et al.* 1979). The present study further exemplifies the usefulness of this approach.

Ms Brigitta Mårtensson and Ms Elizabeth Nilsson gave excellent technical assistance. This study was supported by grants from the Swedish Medical Research Council (project 3968), A. Påhlsson's Foundation and the Swedish Nutrition Foundation.

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Printed in Great Britain