Maternal diet high in fat reduces docosahexaenoic acid in liver lipids of newborn and sucking rat pups

K. Ghebremeskel[1], D. Bitsanis[1], E. Koukkou[2], C. Lowy[2], L. Poston[3] and M. A. Crawford[1]*

[1]*Institute of Brain Chemistry and Human Nutrition, The University of North London, N7 8DB, UK*
[2]*Department of Endocrinology and Diabetes, United Dental and Medical Schools, St Thomas's Hospital, London SE1 7EH, UK*
[3]*Department of Obstetrics and Gynaecology, Fetal Health Research Group, United Medical and Dental Schools, St Thomas's Hospital, London SE1 7EH, UK*

The effect of a maternal diet high in fat, similar to Western foods, and of diabetes on liver essential fatty acid composition of the mother and the newborn and sucking pups was investigated. Female Sprague-Dawley rats were fed on either a low-fat (42 g/kg) or a high-fat (329 g/kg) diet for 10 d before mating, throughout pregnancy and post-partum. On the first day of pregnancy, diabetes was induced by intravenous administration of streptozotocin in half the animals from the two diet groups. Half the pups were killed at birth, and the remaining pups and mothers at days 15 and 16 respectively. At birth, there was a significant reduction in the proportions of docosahexaenoic acid (DHA) in the liver phosphoglycerols and neutral lipids of the pups of both high-fat control and diabetic mothers compared with those of low-fat control and diabetic mothers. Diabetes decreased arachidonic (AA) and linoleic acid values in both the low- and high-fat groups at birth. The sucking pups of both the high-fat control and diabetic mothers exhibited a significant reduction in DHA and a concomitant compensatory increase in AA and a lowering in DHA–AA balance. In the mothers, the high-fat diet significantly increased the proportions of DHA in ethanolamine phosphoglycerols but had no observable effect in choline phosphoglycerols and neutral lipids. In the fetus the DHA level (g/100 g total fatty acids) was disproportionately reduced by the maternal high-fat diet. The adverse effect of the high-fat diet on the level of DHA (g/100 g total fatty acids) was greater in the neonate (and by implication the fetus) than in the sucking pups or mothers. It is concluded that a distortion of the biochemistry is induced in the offspring through a maternal high-fat diet, without genetic predisposition.

Dietary fat: Maternal diet: Fetal liver fatty acids: Docosahexaenoic acid: Diabetes mellitus

The high intake of saturated fatty acids in Western diets has been linked to endemic coronary vascular disease since the first substantive evidence from the Seven Countries Study in the 1950s (Keys, 1980, 1997). Moreover, studies spanning half a century demonstrate the athero- and thrombogenicity of saturated fatty acids and *trans*-isomers (British Nutrition Foundation, 1992).

It has been suggested that heart disease of adults is a paediatric problem. Studies on infants and children have described lesions in the coronary arteries at the earliest of ages (Osborne, 1967; Pesonen *et al.* 1990). African children are born with similar blood cholesterol levels to those of European children living in Africa, but the cardiovascular risk factors of high blood pressure and blood cholesterol are already raised in European children by the ages of 6-8 years

(Louw *et al.* 1969; Crawford *et al.* 1978; Lauer *et al.* 1978). The long-term prospective follow-up studies in Bogalusa, LA, USA, have shown that raised blood pressure and cholesterol track from early childhood to late teens (Newman *et al.* 1986; O'Neil *et al.* 1997). Epidemiological evidence has recently supported such ideas, suggesting that maternal, fetal and neonatal nutrition may programme the individual, setting the risk for CHD, stroke and non-insulin-dependent diabetes in adulthood (Barker, 1997).

The polyunsaturated fatty acids, arachidonic (AA) and docosahexaenoic (DHA), are vital structural lipid components in biomembranes. AA is a major constituent of the endothelial cell membranes (Crawford *et al.* 1997). It is the precursor for the vasodilator and endothelial protective prostacyclin, the vasoconstrictor and thrombogenic

---

thromboxane A_2, and second messengers involved in inflammation and regulation of cellular function (Lands, 1991).

DHA is a major component of the photoreceptor (Anderson & Maude, 1972; Anderson et al. 1992) and synaptosomes (Suzuki et al. 1997), and has a crucial function in signal transduction and amplification. It is cardio-protective (Burr et al. 1989; Christine et al. 1998), anti-arryhthmic (Xiao et al. 1997) and down-regulates thromboxane and inflammatory mediators (Lands, 1991). There is evidence which suggests that pre- and postnatal deficits of AA and DHA may partly contribute to the vascular and neuro-developmental complications of prematurity (Crawford et al. 1998). The developing fetus and neonate are dependent on the maternal supply of AA and DHA (Crawford et al. 1976; Ruyle et al. 1990). During the prenatal period AA and DHA are obtained from the maternal circulation through placental enrichment (Crawford et al. 1976; Crawford, 1999). This selective transfer is consistent with the finding which shows that the incorporation of the preformed AA and DHA is ten times greater than when they are biosynthesized from their respective parent compounds linoleic (LA) and α-linolenic acids (Sinclair, 1975). Placental enrichment would be reduced or denied if the mothers have impaired essential fatty acid metabolism, placental dysfunction, or the babies are born prematurely. Breast-milk provides AA, DHA and the parent essential fatty acids LA and α-linolenic to the neonate.

Dietary fatty acids are known to affect the biosynthesis and incorporation of AA and DHA into cell membranes (Holman, 1970; Gali & Socini, 1983). AA and DHA are synthesized from their parent fatty acids LA and α-linolenic acid respectively by a process of desaturation and chain elongation. The first Δ-6 desaturase (EC 1.14.99.25) in the rate-limiting step (Sprecher, 1986). In experimental diabetes the activity of the Δ-6 desaturase is impaired, reducing the proportion of LA converted to AA (Brenner et al. 1981). Reduced levels of AA and DHA have been reported in diabetic subjects (Freyburger et al. 1989; Arisaka et al. 1991). Diabetes is also associated with vascular and neurological complications (Caprio et al. 1997; Fulesdi et al. 1997) resulting from impairment of membrane integrity.

A study was set up to test the possibility that a maternal high-fat diet, alone and or in combination with diabetes, alters the composition of liver essential fatty acids of mothers and their offspring. The investigation was particularly concerned with AA and DHA because of their crucial role in the structure and function of vascular and neural membranes.

Materials and methods

Animals and diets

Female Sprague Dawley rats (aged 12–14 weeks) were fed on either a low-fat diet (42 g/kg) or a high-fat diet (329 g/kg) for 10 d before mating, throughout pregnancy and for 16 d post-partum. Lard was used for the formulation of the high-fat diet. To offset the dilution effect of the lard, the levels of protein and essential micronutrients in the high-fat diet were adjusted. The composition of fatty acids and other essential nutrients of the two experimental diets are shown in Tables 1 and 2.

Table 1. Composition (g/kg) of some nutrients of the low- and high-fat diets

Nutrients	Low-fat diet	High-fat diet
Total lipids	42·8	329
Crude protein (N×6·25)	223	180
Carbohydrate	557	385
Gross energy (kJ/kg)	1526	2277
Arginine	15·6	12·1
Cystine	3·6	2·6
Histidine	5·8	3·5
Methionine	4·7	3·5
Lysine	14·1	11·1
Isoleucine	10·5	8·3
Leucine	17·8	12·5
Threonine	9·2	6·4
Tryptophan	2·8	2·1
Valine	11·6	8·2
Choline (mg/kg)	1882	1317
Cyanocobalamin (μg/kg)	28·2	19·7
Folic acid (mg/kg)	2·7	1·9
Pyridoxine (mg/kg)	19·3	13·5
Thiamin (mg/kg)	25·7	19·1
Riboflavin (mg/kg)	10·9	7·6

On days 1–2 of pregnancy, diabetes was induced in half the animals by caudal injection of streptozotocin (30 mg/kg; Upjohn Co., Kalamazoo, MI, USA). Diabetes was confirmed by the demonstration of glycosuria (using glucostix®; Boehringer Mannheim, Lewes, East Sussex, UK) 48 h after injection. The severity of diabetes was monitored for glucose and ketones every 2 d during pregnancy using glucostix® and ketostix® (Boehringer Mannheim) respectively, and in order to partially control the diabetes, insulin was given in the form of an implant (half tablet; release rate 2 U/24 h per implant for >40 d; Limplant, Mollegard,

Table 2. Fatty acid composition (g/100 g total fatty acids) of the low- and high-fat diets

Fatty acids	Low-fat diet	High-fat diet
14:0	0·56	1·52
16:0	15·92	24·62
18:0	2·46	13·63
20:0	0·17	0·20
22:0	0·65	–
24:0	0·11	–
Total saturates	19·87	39·97
16:1n-9	0·71	2·27
18:1n-9	18·55	38·33
20:1n-9	0·90	0·84
22:1n-9	0·11	–
Total monoenes	20·27	41·11
18:2n-6	51·60	15·48
20:2n-6	0·06	0·34
20:3n-6	–	0·03
20:4n-6	0·12	0·14
Total n-6	51·78	15·99
18:3n-3	5·73	1·66
22:5n-3	0·15	0·13
22:6n-3	0·09	0·06
Total n-3	5·97	1·85
18:2n-6/18:3n-3	9·0	9·3
n-6:n-3	8·67	8·64

Table 3. The effects of low-fat and high-fat diets on the weights (g) of the control mothers or streptozotocin-induced diabetic mothers during gestation and lactation, and of the sucking pups†

(Mean values and standard deviations for numbers of rats shown)

| | Maternal | | | | | | | | | Offspring (day 15) | | |
| | Day 1 gestation | | | Day 21 gestation | | | Day 16 post-partum | | | | | |
Diet groups	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n
Low-fat: Control	281·8	28·7	10	386·4	19·9	10	311·5	36·2	10	39·6	3·6	10
Diabetic	256·7	28·6	9	340·8	26·3	9	282·8	26·7	9	35·2	3·6	9
High-fat: Control	270·7	35·7	10	333·0	35·4	10	275·0	47·4	10	37·8	6·9	8
Diabetic	262·8	26·2	8	305·6***	27·6	8	268·3	19·3	8	27·9***	7·3	4

Mean values were significantly different from those for the low-fat control group: ***$P < 0.001$.

† For details of diets and procedures, see pp. 396–397 and Tables 1 and 2.

Denmark) which was inserted subcutaneously when ketonuria was first detected.

The pregnant rats which entered the study were: control rats on low-fat diet (n 15), diabetic rats on low-fat diet (n 10), control rats on high-fat diet, (n 24) and diabetic rats on high-fat diet (n 12).

The rats were weighed every 2 d until 20–21 d of pregnancy and their food and water intakes monitored regularly. Details of the diet and growth characteristics are given in Tables 1–3. At delivery, a proportion of each litter were killed, in order that five to six pups were left with the mother in all four groups. The remaining pups and mothers were killed at days 15 and 16 respectively and their liver lipids were extracted and analysed. Vascular function was assessed separately and is reported elsewhere (Koukkou et al. 1998).

Fatty acid analysis

Total lipid was extracted by the method of Folch et al. (1957) by homogenizing the samples in chloroform–methanol (2 : 1, v/v; containing 0·1 g butylated hydroxytoluene/l as an antioxidant) under N_2. Lipid classes were separated by thin-layer chromatography on silica gel plates. Chloroform–methanol–water (60 : 30 : 4, by vol.; containing 0·1 g butylated hydroxytoluene/l, and light petroleum (b.p. 40–60°)–diethyl ether–formic acid–methanol (85 : 15 : 2·5 : 1, by vol.; with butylated hydroxytoluene) were used as developing solvents for the separation of phospholipids and neutral-lipid fractions respectively. Bands were detected by spraying with a methanolic solution of 2,7-dichloroflurescein (0·1 g/l) and identified by the use of standards.

A modification of the method previously reported from our laboratory (Ghebremeskel et al. 1995) was used to determine the fatty acid composition of the liver lipid classes. Fatty acid methyl esters were prepared by heating the lipid fractions with 5 ml acetyl chloride in methanol (150 ml/l) in a sealed vial at 70° for 3 h under N_2. Fatty acid methyl esters were separated using GLC (HRGC MEGA 2 Series; Fisons Instruments, Milan, Italy) fitted with a BP 20 capillary column (25 m × 0·32 mm i.d., 0·25 μm film; SGE (UK) Ltd, Milton Keynes, Bucks., UK). H_2 was used as a carrier gas, and the injector, oven and detector temperatures were 235, 210 and 260° respectively. The fatty acid methyl esters were identified by comparison of retention times with authentic standards and interpretation of equivalent chain-length values. Peak areas were quantified using a computer chromatography data system (EZChrom Chromatography Data System; Scientific Software Inc., San Ramon, CA, USA).

Data analyses

Data are expressed as means and standard deviations. Parametric and non-parametric statistical methods were used to test for any difference in the levels of the fatty acids among the groups. A statistical package, SPSS for Windows (release 7; SPSS UK Ltd, Woking, Surrey, UK), was used to analyse the data.

Results

Mean weights of the mothers during gestation and lactation, and the sucking pups are given in Table 3. There was no significant difference in weight gain during gestation between the low-fat control, low-fat diabetic and high-fat control mothers. Similarly, the weights of the sucking pups from the three groups of mothers were comparable. However, the high-fat diabetic rats at 21 d gestation and their sucking pups had significantly lower body weight ($P < 0.001$) compared with the low-fat control mothers and the sucking offspring of the low-fat controls respectively.

Blood glucose concentrations of the newborn and sucking pups, and of the mothers on day 16 post-partum are presented in Table 4. The glucose concentrations of the diabetic rats on both diets were significantly higher than those of the control rats on the same diet. ($P < 0.01$).

Fatty acids

The high-fat maternal diet had a differential effect on the liver fatty acid composition of the mothers, neonates and sucking pups. Moreover, the different lipid classes were either affected differently, or not to the same extent. Surprisingly, some of the effects were not consistent with the fatty acid composition of the high-fat diet. The composition of certain major fatty acids of liver choline and ethanolamine phosphoglycerols (CPG and EPG respectively) of the rats and their pups are shown in Table 5.

K. Ghebremeskel *et al.*

Table 4. The effects of low-fat and high-fat diets on blood glucose concentrations (mmol/l) of the newborn and sucking pups, and the control and streptozotocin-induced diabetic mothers at day 16 post-partum†

(Mean values and standard deviations for numbers of rats shown)

Diet group	Newborn			Sucking			Mother		
	Mean	SD	n	Mean	SD	n	Mean	SD	n
Low-fat: Control	4·7	0·8	9	16·0	4·0	10	11·8	2·4	10
Diabetic	5·1	1·5	10	13·6	4·0	8	26·4**	7·7	9
High-fat: Control	4·6	3·3	10	14·6	2·3	8	12·8	4·1	10
Diabetic	8·3	9·5	5	12·7	5·2	4	26·3**	8·7	8

Mean values were significantly different from those for the low- and high-fat control groups: **$P < 0.01$.
† For details of diets and procedures, see pp. 396–397 and Tables 1 and 2.

Table 5. The effects of low-fat and high-fat diets on fatty acids of liver choline (CPG) and ethanolamine (EPG) phosphoglycerols for the control and streptozotocin-induced diabetic mothers and their pups*

(Mean values and standard deviations for numbers of rats shown in parentheses)

Diet group...	Fatty acid	Lipid fraction	Low-fat				High-fat			
			Control		Diabetic		Control		Diabetic	
			Mean	SD	Mean	SD	Mean	SD	Mean	SD
Newborn pups			(10)		(9)		(15)		(6)	
	16:0	CPG	29·5	1·6	27·8	1·8	27·3	3·7	26·5	1·8
		EPG	21·1	1·5	18·1	2·1	17·1	4·3	16·9	1·6
	18:0	CPG	17·4	0·7	19·2	1·6	17·6	2·8	19·4	1·0
		EPG	22·5	1·1	24·5	1·8	23·1	2·6	23·6	1·8
	18:1	CPG	6·80	0·6	6·57	0·3	11·8	3·3	13·0	3·4
		EPG	2·90	0·6	2·78	0·4	4·62	1·6	5·10	2·2
	18:2	CPG	6·94	0·7	8·19	1·7	9·21	4·3	8·57	2·6
		EPG	1·75	0·4	2·28	0·4	3·63	2·5	3·29	2·1
	20:4n-6	CPG	21·7	1·1	20·3	1·3	17·9	4·7	16·5	3·8
		EPG	16·8	0·7	18·2	1·4	22·2	3·1	20·9	3·2
	22:6n-3	CPG	11·3	1·1	11·2	1·4	8·35	1·9	8·56	2·6
		EPG	25·4	2·5	23·7	1·9	16·7	4·5	17·6	6·5
Sucking pups			(16)		(8)		(7)		(4)	
	16:0	CPG	25·3	1·6	28·4	1·6	25·2	1·5	27·4	2·6
		EPG	20·5	1·3	17·1	1·7	14·2	1·0	15·7	1·9
	18:0	CPG	20·4	1·4	18·8	0·7	22·8	1·3	20·5	2·7
		EPG	23·9	1·4	21·9	0·8	25·8	1·2	24·3	1·9
	18:1	CPG	3·50	0·4	3·60	0·6	5·30	0·6	5·60	0·9
		EPG	1·80	0·5	3·40	0·3	4·00	0·5	4·20	0·4
	18:2	CPG	11·6	1·3	12·4	1·2	7·90	0·7	7·10	0·8
		EPG	3·90	2·8	11·8	0·8	10·4	0·9	9·80	0·7
	20:4n-6	CPG	19·9	1·7	16·9	1·3	23·9	1·2	21·9	2·6
		EPG	18·3	1·6	15·8	1·1	20·6	1·1	19·4	2·2
	22:6n-3	CPG	13·3	1·2	13·5	0·8	10·2	0·3	12·4	1·8
		EPG	23·6	1·6	21·3	0·8	17·7	1·0	18·8	1·8
Mothers			(15)		(10)		(24)		(12)	
	16:0	CPG	18·6	2·3	17·5	3·6	18·1	4·5	19·0	3·7
		EPG	14·8	1·2	14·1	2·2	12·7	1·6	14·1	2·5
	18:0	CPG	27·9	3·4	28·6	3·1	28·9	7·1	29·5	2·0
		EPG	22·0	1·7	23·5	1·8	27·2	1·6	27·0	1·6
	18:1	CPG	7·34	1·81	6·50	2·7	5·63	0·94	5·71	1·1
		EPG	5·52	0·99	5·44	1·0	3·77	0·42	4·35	0·51
	18:2	CPG	15·7	2·0	16·7	2·8	11·0	1·7	10·6	2·1
		EPG	18·4	1·7	19·6	2·3	12·8	1·8	14·1	1·9
	20:4n-6	CPG	15·3	2·5	15·5	4·3	22·1	3·9	21·2	3·6
		EPG	16·1	1·6	14·4	2·5	18·8	4·0	16·4	3·5
	22:6n-3	CPG	7·20	0·92	7·61	2·89	8·96	3·2	8·99	2·8
		EPG	13·8	1·5	13·4	3·3	16·5	3·1	17·0	2·8

* For details of diets and procedures, see pp. 396–397 and Tables 1 and 2.

Saturated fatty acids

The high-fat maternal diet either significantly reduced ($P < 0.01$), or did not change ($P > 0.05$), the proportions of palmitate (16:0) in liver phospholipids (CPG and EPG; Table 5), triacylglycerols (TG) and free fatty acids (FFA; values not shown) of the control and diabetic rats and their offspring. This finding was unexpected, since the level of palmitate in the high- and low-fat diets was 24·6 and 15·9 g/100 g total fatty acids respectively (Table 2). In contrast, the proportions of stearate (18:0) either increased significantly ($P < 0.001$) or remained unchanged ($P > 0.005$) in the high-fat, control, and diabetic mothers and their pups. The increase in stearate was more striking in the sucking pups.

Oleate

There was a significant increase of oleate (18:1) in the CPG, EPG, TG and FFA of the newborn ($P = 0.0001$) and sucking ($P = 0.001$) pups of the high-fat control and diabetic rats. In the mothers, despite their high-oleate diet (38·3 g/100 g total fatty acids), the level of the fatty acid decreased in the phospholipids (CPG and EPG; $P = 0.004$) and remained unchanged in the TG. However, it increased significantly in the FFA ($P = 0.0001$).

Linoleate

The level of LA (18:2) decreased in the CPG and EPG ($P = 0.0001$; Table 5) and increased in the TG and FFA ($P = 0.0001$) of the high-fat control and diabetic mothers. However, in their newborn and sucking pups, LA increased in the EPG ($P < 0.05$) and decreased in the TG and FFA ($P < 0.05$). In the CPG, LA did not change in the neonate, but decreased significantly in the sucking pups ($P < 0.001$).

Long-chain polyunsaturated fatty acids

Interesting findings regarding the effects of the high-fat maternal diet on the metabolism of the highly-unsaturated n-3 and n-6 fatty acids emerged by comparing the liver composition of the newborn, sucking pups and the mothers. It was evident that the high-fat diet primarily and disproportionately reduced the levels of DHA in the newborn pups. In the sucking pups and mothers, however, the main effect of the high-fat diet was to reduce the proportions of the intermediate metabolic products, dihomo-γ-linolenic (20:3n-6), eicosapentaenoic acid (20:5n-3; EPA) and docosapentaenoic (22:5n-3) acids, particularly in the phospholipids.

Docosahexaenoic acid

The newborn pups of the high-fat control and diabetic rats had lower proportions of DHA in liver CPG ($P = 0.021$), EPG ($P < 0.05$; Fig 1(a)), TG ($P < 0.0005$; Fig. 1(b)) and FFA ($P < 0.05$) compared with those of the pups of the low-fat control and diabetic groups. Similarly, the sucking pups of the high-fat control rats had significantly lower levels of DHA in liver EPG and TG ($P < 0.0005$), CPG ($P < 0.0001$), and FFA ($P < 0.001$) than the pups of the control rats on the
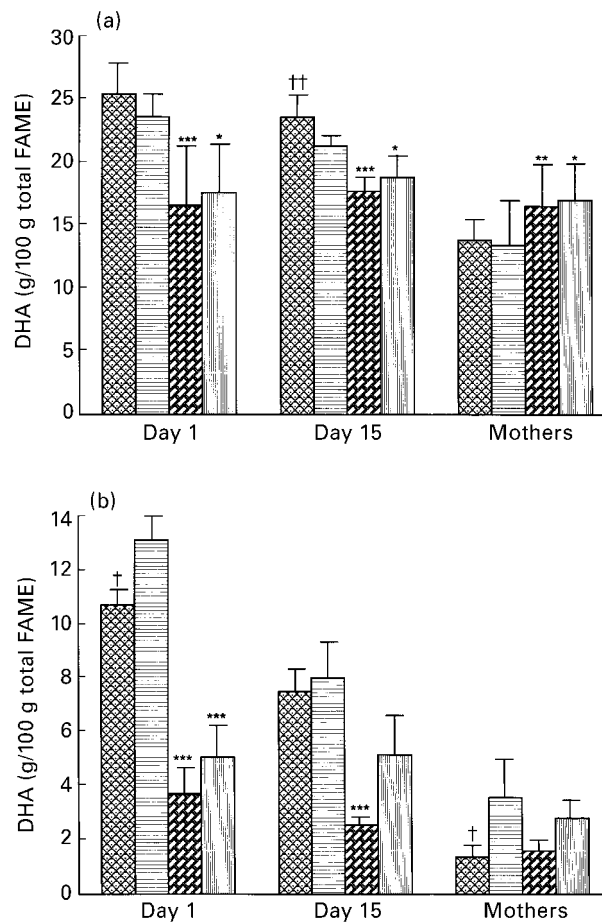
Fig. 1. Docosahexaenoic acid (DHA) in liver ethanolamine phosphoglycerols (a) and triacylglycerols (b) of control and streptozotocin-induced diabetic rats fed on low-fat and high-fat diets and their newborn (day 1) and sucking pups (day 15). (▦), Control low-fat; (▤), diabetic low-fat; (▨), control high-fat; (▥), diabetic high-fat. Values are means and standard deviations represented by vertical bars. Mean values were significantly different from those for the corresponding low-fat group: *$P < 0.05$, **$P < 0.001$, ***$P < 0.0005$. Mean values were significantly different from those of the corresponding low-fat diabetic group: †$P < 0.05$, ††$P < 0.001$. For details of diets and procedures, see pp. 396–397 and Tables 1 and 2. FAME, fatty acid methyl esters.

low-fat diet (Fig. 1 (a and b)). There was also a significant difference in the proportions of DHA in liver EPG (Fig. 1(a)) and FFA ($P < 0.05$) between the sucking pups of diabetic rats on the low- and high-fat diets. In contrast to the newborn and sucking pups, the high-fat control and diabetic mothers had higher levels of DHA in liver EPG ($P < 0.05$) compared with the corresponding control and diabetic rats on the low-fat diet (Fig. 1(a)). DHA in CPG, TG and FFA was not different in the four group of mothers.

Eicosapentaenoic acid

EPA in liver phospholipids (CPG and EPG) was not significantly different between the pups of the four groups of mothers at birth. However, the pups of the high-fat control and diabetic rats had lower proportions of EPA in

liver TG and FFA ($P = 0.0001$) than the pups of the control and diabetic rats on the low-fat diet (Fig. 2(b and c)). The high-fat control and diabetic rats and their sucking pups had significantly lower levels of EPA ($P < 0.0001$) in liver CPG (Fig. 2(a)), TG (Fig. 2(b)), FFA (Fig. 2(c)) and EPG

compared with the corresponding control and diabetic rats on the low-fat diet and their sucking pups.

Arachidonic acid

The newborn pups of the high-fat control and diabetic rats had lower AA ($20:4n$-6) in CPG ($P < 0.05$) but higher AA in EPG compared with the pups of the control ($P = 0.0001$) and diabetic ($P = 0.04$) rats on the low-fat diet respectively (Fig. 3(a and b)). In contrast, the high-fat control and diabetic rats and their sucking offspring had higher levels of AA both in the CPG and EPG ($P < 0.01$) in comparison with the corresponding rats on the low-fat diet and their pups (Fig. 3(a and b)).

Docosapentaenoic acid

The control rats on the high-fat diet and their newborn and sucking pups had significantly higher levels of docosapentaenoic acid in EPG compared with the corresponding control rats on the low-fat diet and their newborn and sucking offspring ($P = 0.0001$; Fig. 3(c)). Similarly, the diabetic rats on the high-fat diet and their pups had higher proportions of docosapentaenoic acid in EPG compared with their diabetic counterparts and their offspring ($P < 0.05$; Fig. 3(c)).

Arachidonic acid : linoleic acid

There was a significant difference in AA : LA ratio in CPG between the newborn ($P < 0.05$) and sucking ($P = 0.007$) pups of the control rats on the low-fat diet and those of the corresponding diabetic rats on the same diet (Fig. 4(a)). Similarly, the sucking pups of the two group of rats on the low-fat diet had a different AA : LA value in CPG ($P < 0.01$). The sucking pups of the high-fat control and diabetic rats had higher values for AA : LA in CPG compared with the corresponding pups of the low-fat control ($P = 0.0001$) and diabetic rats ($P = 0.01$) (Fig. 4(a)).

Discussion

The study has produced unexpected findings which may have a wider significance in relation to the understanding of the impact of an unfavourable uterine and postnatal nutritional environment in early development.

Although there was a slight time lag between birth and tissue sampling, the liver fatty acids of the newborn pups
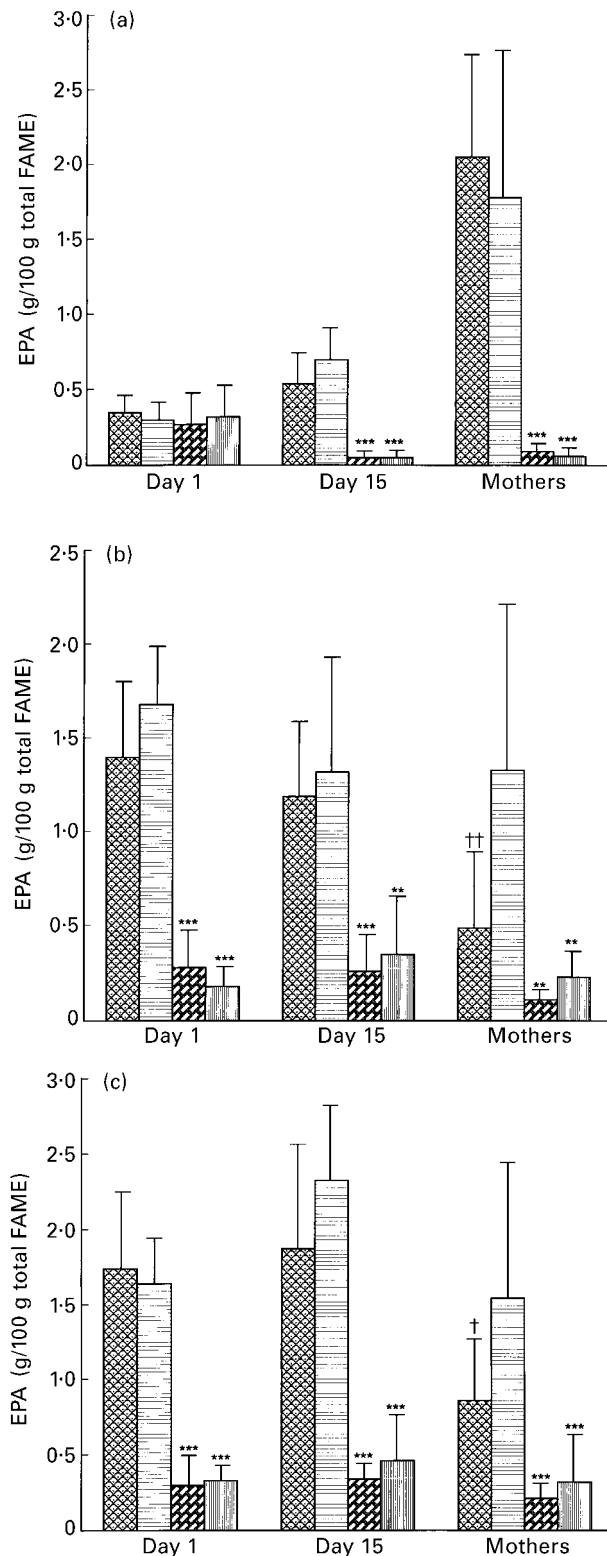
Fig. 2. Eicosapentaenoic acid (EPA) in liver choline phosphoglycerols (a), triacylglycerols (b) and free fatty acids (c) of control and streptozotocin-induced diabetic rats fed on low-fat and high-fat diets and their newborn (day 1) and sucking pups (day 15). (▨), Control low-fat; (▤), diabetic low-fat; (▧), control high-fat; (▥), diabetic high-fat. Values are means and standard deviations represented by vertical bars. Mean values were significantly different from those for the corresponding low-fat group: **$P < 0.005$, ***$P < 0.0001$. Mean values were significantly different from those of the corresponding low-fat diabetic group: †$P < 0.05$, ††$P = 0.004$. For details of diets and procedures, see pp. 396–397 and Tables 1 and 2. FAME, fatty acid methyl esters.

relate to the intrauterine accumulation. Consequently, the data for the newborn pups will also be referred as fetal data.

Our discussion will concentrate on AA and DHA because of their vital role in membrane structure and function and the metabolic impairment in diabetes. However, the significant reduction in palmitate (EPG, TG and FFA) and

oleate (CPG and EPG) in the high-fat control mothers when there was a substantial increase in the dietary composition of the two fatty acids is noteworthy, and merits further investigation.

Both diabetes and the high-fat diet appeared to impair the metabolism of AA and DHA in the pups (newborn and sucking) and mothers. The effect of diabetes on the fatty acids was more striking in the low-fat diet rats and pups than in the corresponding high-fat group. In the high-fat diabetic group the independent influence of diabetes appears to have been masked by the greater effect of the high-fat diet.
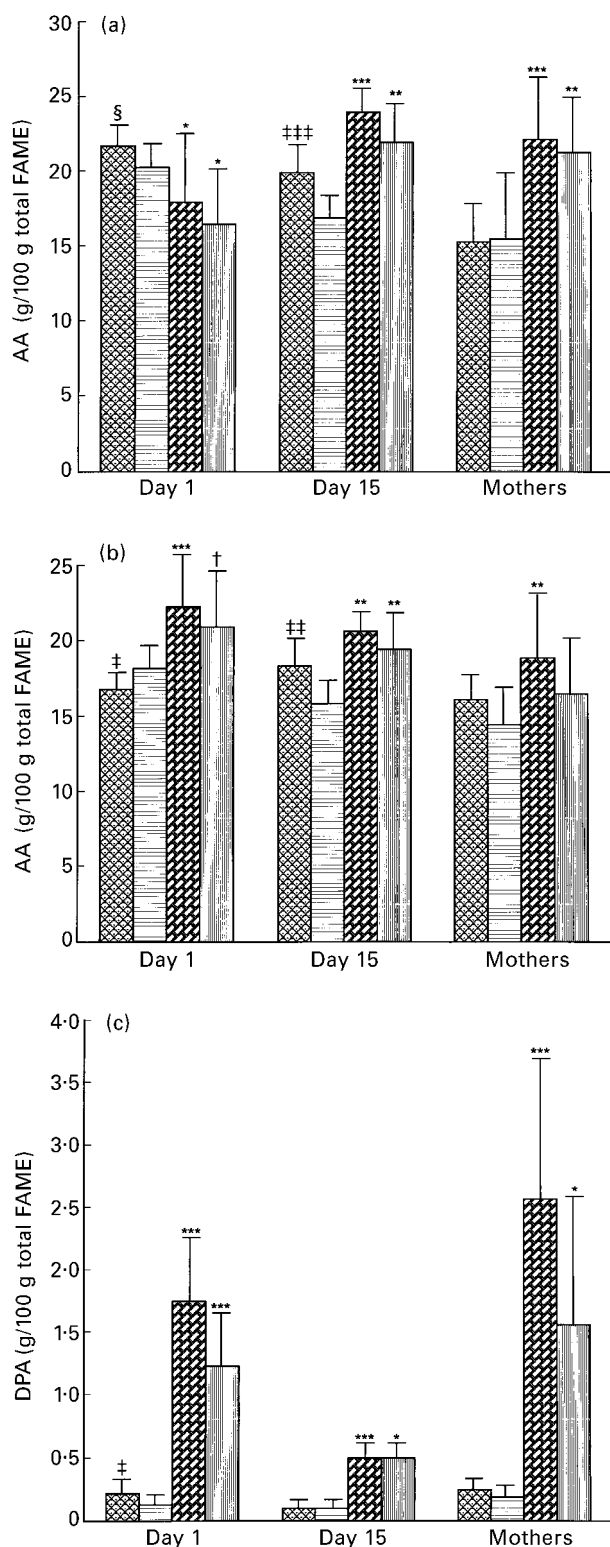
Diabetes

Diabetes reduced the conversion of LA to AA in the low-fat mothers, and even more significantly in the offspring (newborn and sucking). Brenner (1981) has shown that diabetes reduces the activity of Δ-6 desaturase in the rat. This enzyme catalyses the rate-limiting first-step reaction in the conversion of LA to AA and α-linolenic acid to DHA. Also, it catalyses the final desaturation step in the synthesis of DHA (Sprecher, 1992). The low AA:LA in the mothers suggests the impairment of the activity of Δ-6 desaturase.

The marked reduction in AA:LA, particularly in the offspring of the low-fat rats, is unexpected as it was the mothers who were diabetic. The smaller reduction in AA:LA in the mothers is possibly due to the fact that membrane phosphoglycerol fatty acids in adults have a long turnover time and are carefully protected (Anderson et al. 1992). The fetus and sucking pups are growing rapidly and forming new membranes. Consequently, metabolic effects which are difficult to detect in the mother may well be expected to have a more profound impact on her offspring. We are not aware of published data which show that a metabolic disturbance in the mother exerts a greater reduction in membrane AA in the fetus than in the mother. Our finding questions the concept that the fetus is a 'perfect parasite' unaffected by maternal conditions (Dally, 1998).

High-fat diet

The data show that levels of DHA, AA and their intermediate metabolites were altered in the control and diabetic rats and their offspring by the high-fat diet. The effect of the high-fat diet on membrane AA and DHA was again more pronounced in the fetus than in the sucking pups and the mothers. Since the growing fetus is reliant on direct placental transfer of AA and DHA from the maternal circulation (Crawford et al. 1976; Ruyle et al. 1990; Al et al. 1995;

Fig. 3. Arachidonic acid (AA) in liver choline (a) and ethanolamine (b) phosphoglycerols and docosapentaenoic acid (DPA) in liver ethanolamine phosphoglycerols (c) of control and streptozotocin-induced diabetic rats fed on low-fat and high-fat diets and their newborn (day 1) and sucking pups (day 15). (▦), Control low-fat; (▤), diabetic low-fat; (▨), control high-fat; (▥), diabetic high-fat. Values are means and standard deviations represented by vertical bars. Mean values were significantly different from those for the corresponding low-fat group: *$P<0.05$, **$P<0.005$, ***$P<0.0001$, †$P=0.04$. Mean values were significantly different from those for the corresponding low-fat diabetic group: ‡$P<0.05$, ‡‡$P<0.001$, ‡‡‡$P=0.0001$, §$P=0.031$. For details of diets and procedures, see pp. 396–397 and Tables 1 and 2. FAME, fatty acid methyl esters.
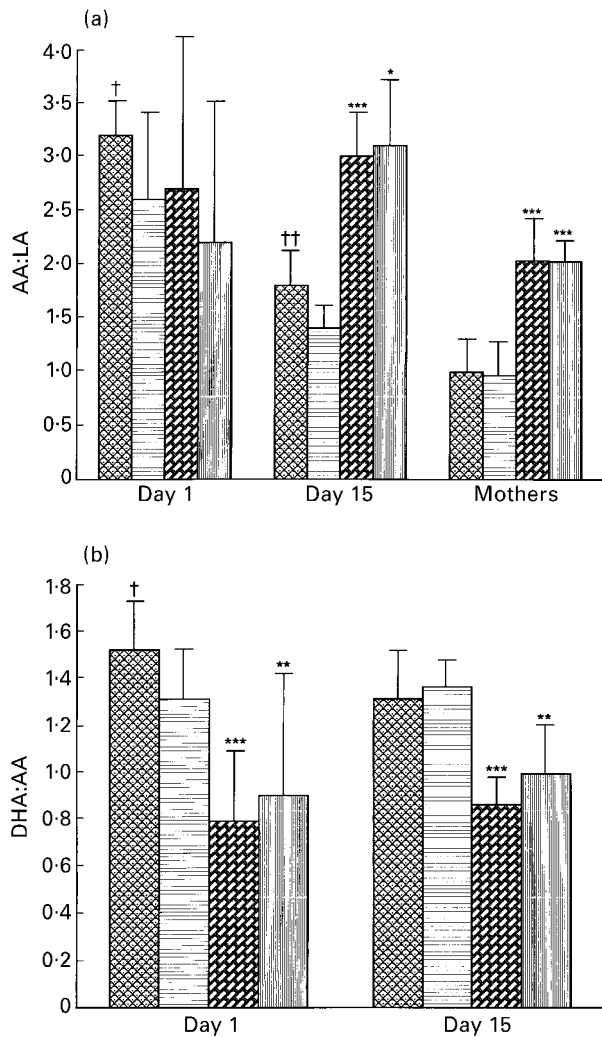
Fig. 4. (a) Arachidonic acid (AA): linoleic acid (LA) in liver choline phosphoglycerols and (b) docosahexaenoic acid (DHA): AA in liver ethanolamine phosphoglycerols of rats fed on low-fat and high-fat diets and their newborn (day 1) and sucking pups (day 15). (▦), Control low-fat; (▤), diabetic low-fat; (▨), control high-fat; (▥), diabetic high-fat. Values are means and standard deviations represented by vertical bars. Mean values were significantly different from those for the corresponding low-fat group: $*P = 0.01$, $**P = 0.005$, $***P = 0.001$. Mean values were significantly different from those for the corresponding low-fat diabetic group: $† P < 0.05$, $†† P < 0.007$.

Hornstra et al. 1995; Crawford, 1998), and there was an increase in maternal phosphoglycerol DHA, the finding suggests an impairment in the transfer mechanism by the high-fat diet.

In the sucking pups and mothers, in contrast to the fetus, there was a greater influence on the intermediate metabolites, dihomo-γ-linolenic acid (20 : 3n-6) and EPA, in the phospholipids and TG. Both the sucking pups and mothers synthesize DHA and AA, whereas the fetus receives preformed AA and DHA via the placenta. Hence, the reduction in the intermediate metabolites indicates that the synthesis process was adversely influenced by the high-fat diet.

The reduction in DHA in all cell-membrane and neutral-lipid fractions by the high-fat diet was confirmed by the rise in AA and docosapentaenoic acid. The increase in docosa-pentaenoic acid, which was particularly striking in the EPG lipid fraction of all high-fat groups, is a classical response to the reduction in the proportions of DHA (Holman et al. 1991). An increase in membrane long-chain n-6 fatty acids, AA, docosatetraenoate (22 : 4n-6) and docosapentaenoic acid when DHA is either marginal or deficient has been reported in primates (Fiennes et al. 1973), rats and human subjects (Holman et al. 1982; Crawford et al. 1990). Interestingly, in previous studies of placental transfer of fatty acids, Kuhn et al. (1990) found that the placental uptake and fetal effluent of radiolabelled AA from the perfused placentas of pregnancies complicated by insulin-dependent diabetes mellitus was significantly increased. The Kuhn et al. (1990) data are consistent with the details reported in the present paper, where the AA proportions were increased in the EPG in the newborn and in both EPG and CPG in the sucking pups. It is also in agreement with our data in insulin-dependent diabetes mellitus pregnancies, where there was a specific reduction in the proportions of DHA in plasma choline phosphoglycerols in mother and fetus (Ghebremeskel et al. 1997).

The reduction in DHA and EPA in offspring induced by the high-fat maternal diet would be expected to compromise vascular integrity. The additional impact of the diabetes is difficult to detect from the data, which suggest that the high-fat diet overwhelms the metabolic effect of the diabetes. Nonetheless, the data did indicate that an additive effect would be expected on the metabolic intermediates. The impact of the high-saturated-fat diet on the expression of diabetes is suggested by companion papers (Koukkou et al. 1998), where it has been shown that the high-fat diet in these animals induced vascular dysfunction in the rat pups measured at 15 d. The relaxation dysfunction was worsened by the presence of diabetes. In the absence of the high-fat diet, the diabetes itself had little effect on vascular relaxation.

Implications to Western diets

Research requires to be done to establish if the high-saturated-fat Western diet is compromising human fetal nutritional status in the way suggested by the data presented here. Is it the reduction in cardio-protective DHA induced by the high-fat diet which gives rise to the vascular and neuropathology of diabetes in Western population? Is this one of the mechanisms underlying the fetal and neonatal genesis of cardiovascular disease endemic in populations using the high-saturated-fat Western diet? Could supplementation with the cardio-protective DHA (Burr et al. 1989; Xiao et al. 1997; Christine et al. 1998) during pregnancy and the early postnatal period from the basis of the prevention and treatment of the vascular and neurological complications of diabetes?

Conclusion

The high-fat diet had a major effect in reducing the cardio- and vascular-protective DHA, thus changing the DHA : AA value (Fig. 4(b)) quite strikingly, in a manner expected to up-regulate thrombogenic potential. In view of the interest in the early effects of nutrition on risk for cardiovascular

disease in later life, the data presented here clearly demonstrates that a high-saturated-fat diet during pregnancy changes the profile of fetal liver membrane lipids in a manner which would be expected to pose a risk to vascular development.

Acknowledgements

References

Al MDM, Houwelingen AC, Kester ADM, Hasaart THM, De-Jong AET & Hornstra G (1995) Maternal essential fatty acid patterns during normal pregnancy and relationship to neonatal essential fatty acid status. *British Journal of Nutrition* **74**, 55–68.

Anderson RE & Maude MB (1972) Lipids of ocular tissues: VII. The effects of essential fatty acid deficiency on the phospholipids of the photoreceptor membranes of rat retina. *Archives of Biochemistry and Biophysics* **151**, 270–276.

Anderson RE, O'Brien PJ, Weigand RD, Koutz CA & Stinson AM (1992) Conservation of docosahexaenoic acid in the retina. In *Advances in Experimental Medical Biology*. vol. 318, *Neurobiology of Essential Fatty Acids* pp. 285–294 [NG Bazan, MG Murphy and G Toffano, editors]. New York and London: Plenum Press.

Arisaka M, Arisaka O & Yamashiro Y (1991) Fatty acid and prostaglandin metabolism in children with diabetes mellitus. II. The effect of evening primrose oil supplementation on serum fatty acid and plasma prostaglandin levels. *Prostaglandins, Leukotrienes and Essential Fatty Acids* **43**, 197–201.

Barker DJ (1997) Maternal nutrition, fetal nutrition, and disease in later life. *Nutrition* **13**, 807–813.

Brenner RR (1981) Nutritional and hormonal factors influencing desaturation of essential fatty acids. *Progress in Lipid Research* **20**, 41–47.

British Nutrition Foundation (1992) Unsaturated fatty acids, thrombogenesis and fibrinolysis. In *Unsaturated Fatty Acids: Nutritional and Physiological Significance. Report of the Nutrition Foundation's Task Force*, pp. 92–111. London: Chapman & Hall.

Burr ML, Fehily AM, Gilbert JF, Rogers S, Holliday RM, Sweetnam PM, Elwood PC & Deadman NM (1989) Effects of changes in fat, fish, and fibre intakes on death and myocardial re-infarction trial (DART). *Lancet* **ii**, 757–761.

Caprio S, Wong S, Alberti KG & King G (1997) Cardiovascular complications of diabetes. *Diabetologia* **40**, Suppl. 3, B78–B82.

Christine M, Albert CM, Hennekens CH, O'Donnell CJ, Ajani UJ, Carey VJ, Willett WC, Ruskin JN & Manson JE (1998) Fish consumption and risk of sudden cardiac death. *Journal of the American Medical Association* **279**, 23–28.

Crawford MA (1999) The placental delivery of arachidonic and docosahexaenoic acids: implications for the lipid nutrition of the preterm infant. *American Journal of Clinical Nutrition* (In the Press).

Crawford MA, Costeloe K, Doyle W, Leighfield MJ, Lennon EA & Meadows N (1990) Potential diagnostic value of the umbilical artery as a definition of neural fatty acid status of the fetus during its growth. *Biochemical Society Transactions* **18**, 761–766.

Crawford MA, Costeloe K, Ghebremeskel K & Phylactos AC (1997) Are deficits of arachidonic and docosahexaenoic acids responsible for the neural and vascular complications of preterm babies? *American Journal of Clinical Nutrition* **66**, 1032S–1041S.

Crawford MA, Costeloe K, Ghebremeskel K & Phylactos A (1998) The inadequacy of the essential fatty acid content of present preterm feeds. *European Journal of Pediatrics* **157**, Suppl. 1, S23–S27.

Crawford MA, Hassam AG & Rivers JPW (1978) Vulnerability of the artery during growth. *Post Graduate Medical Journal* **54**, 149–153.

Crawford MA, Hassam AG, Williams G & Whitehouse WL (1976) Essential fatty acids and fetal brain growth. *Lancet* **i**, 452–453.

Dally A (1998) Thalidomide: was the tragedy preventable? *Lancet* **351**, 1197–1199.

Fiennes RNT-W, Sinclair AJ & Crawford MA (1973) Essential fatty acid studies in primates: linolenic acid requirements of Capuchins. *Journal of Medical Primatology* **2**, 155–169.

Folch J, 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.

Freyburger G, Gin H, Heape A, Juguelin H, Boisseau MR & Cassagne C (1989) Phospholipid and fatty acid composition of erythrocytes in type I and type II diabetes. *Metabolism* **38**, 673–678.

Fulesdi B, Limburg M, Bereczki D, Michels RP, Neuwirth G, Legemate D, Valikovics A & Csiba L (1997) Impairment of cerebrovascular reactivity in long-term type 1 diabetes. *Diabetes* **46**, 1840–1845.

Gali C & Socini A (1983) Dietary lipids in pre- and post-natal development. In *Dietary Fats and Health*, vol. 16, pp. 278–301 [EG Perkins and WJ Visek, editors]. Chicago, IL: American Oil Chemists' Society.

Ghebremeskel K, Leighfield MJ, Leaf A, Costeloe KL & Crawford MA (1995) Fatty acid composition of plasma and red cell phospholipids of preterm babies fed on breast milk and formulae. *European Journal of Pediatrics* **154**, 46–52.

Ghebremeskel K, Thomas B, Min Y, Stacey F, Koukkou E, Lowy C, Erskine K, Crawford MA & Offley-Shore B (1997) Fatty acids in pregnant women and neonates: implications for pre and post-natal development. *Prostaglandins, Leukotrienes and Essential Fatty Acids* **57**, M26.

Hornstra G, Al M, Houwelingen AC & Foreman-van Drongelen MHP (1995) Essential fatty acids in pregnancy and early human development. *European Journal of Obstetric and Gynaecological Reproductive Biology* **61**, 57–62.

Holman RT (1970) Biochemical activities of and requirement for polyunsaturated fatty acids. *Progress in the Chemistry of Fats and Lipids* **9**, 607–687.

Holman RT, Johnson SB & Hatch TF (1982) A case of human linolenic acid deficiency involving neurological abnormalities. *American Journal of Nutrition* **35**, 617–623.

Holman RT, Johnson SB & Ogburn PL (1991) Deficiency of essential fatty acids and membrane fluidity during pregnancy and lactation. *Proceedings of the National Academy of Sciences USA* **88**, 4835–4839.

Keys A (1980) *Seven Countries: A Multivariate Analysis of Death and Coronary Heart Disease*. London: Harvard University Press.

Keys A (1997) Coronary heart disease in seven countries: 1970. *Nutrition* **13**, 250–252.

Koukkou E, Lowy C & Poston L (1998) Young offspring of normal and diabetic rats fed saturated fats in pregnancy exhibit vascular dysfunction. *Circulation* (In the Press).

Kuhn DC, Crawford MA, Stuart MJ, Botti JJ & Demers LM (1990) Alterations in transfer and lipid distribution of arachidonic acid in placentas of diabetic pregnancies. *Diabetes* **39**, 914–918.

Lands WEM (1991) Biosynthesis of prostaglandins. *Annual Review of Nutrition* **11**, 41–60

Lauer RM, Clarke WR & Rames LK (1978) Blood pressure and its

significance in childhood. *Post Graduate Medical Journal* **54**, 206–210.

Louw ME, du Plessis JP & Van den Berg AS (1969) Inter-correlation study of dietary and biochemical data from school children in the Pretoria area. *South African Medical Journal* **43**, 1516–1518.

Newman WP, Freedman DS, Voors AW, Gard PD, Srinivasan SR, Cresanta JL, Williamson GD, Webber LS & Berensen GS (1986) Relation of serum lipoprotein levels and systolic blood pressure to early atherosclerosis: the Bogalusa Heart Study. *New England Journal of Medicine* **314**, 138–144.

O'Neil CE, Nicklas TA, Myers L, Johnson CC & Berenson GS (1997) Cardiovascular risk factors and behavior lifestyles of young women: implications from findings of Bogalusa Heart Study. *American Journal of Medical Sciences* **314**, 385–395.

Osborne G (1967) *Stages in Development of Coronary Artery Disease Observed From 1,500 Young Subjects.* Paris: Editions du Centre National de la Recherché Scientifique.

Pesonen E, Noris R, Hirvonen J, Karkola K, Kuusela V, Laaksonen H, Mottonen M, Nikkari T, Raekallioet J & Viikari J (1990) Intimal thickening in the coronary arteries of infants and children as an indicator of risk factors for coronary heart disease. *European Heart Journal* **11**, Suppl. E, 53–60.

Ruyle M, Connor WE, Anderson GJ & Lowensohn RL (1990) Placental transfer of essential fatty acids in humans: venous-arterial difference for docosahexaenoic acid in fetal umbilical erythrocytes. *Proceedings of the National Academy of Sciences USA* **87**, 7902–7905.

Sinclair AJ (1975) Long chain polyunsaturated fatty acids in the mammalian brain. *Proceedings of the Nutrition Society* **34**, 287–291.

Sprecher H (1986) The metabolism of (n-3) and (n-6) fatty acids and their oxygenation by platelet cyclooxygenase and lipo-oxygenase. *Progress in Lipid Research* **25**, 19–28.

Sprecher H (1992) Interconversions between 20- and 22-carbon n-3 and n-6 fatty acids via 4-desaturase independent pathways. In *Essential Fatty Acids and Eicosanoids*, pp. 18–22 [AJ Sinclair and R Gibson, editors]. Champaign, IL: American Oil Chemists' Society.

Suzuki H, Manabe S, Wada O & Crawford MA (1997) Rapid incorporation of docosahexaenoic acid from dietary sources into brain microsomal, synaptosomal and mitochondrial membranes in adult mice. *International Journal of Vitamin and Nutrition Research* **67**, 272–278.

Xiao YF, Gomez AM, Morgan JP, Lederer WJ & Leaf A (1997) Suppression of voltage-gated L-type Ca^{2+} currents by poly-unsaturated fatty acids in adult and neonatal rat ventricular myocytes. *Proceedings of the National Academy of Sciences USA* **94**, 4182–4187.