Effect of reduced dietary protein intake on hepatic and plasma essential fatty acid concentrations in the adult female rat: effect of pregnancy and consequences for accumulation of arachidonic and docosahexaenoic acids in fetal liver and brain*

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During pregnancy, the accumulation of long-chain polyunsaturated fatty acids (LCPUFA) in fetal tissues places a substantial demand upon maternal lipid metabolism. As lipid metabolism is intimately linked to aspects of protein metabolism, a reduced protein intake in pregnancy may impair activities of enzymes and transport proteins responsible for supplying LCPUFA to the fetus, thereby compromising fetal development. We have investigated the effect of reduced protein intake on LCPUFA status in the non-pregnant rat and in the pregnant rat, and in fetus at day 20 of gestation. Female rats (*n* 5 per group) were either mated and fed the control diet (180 g protein/kg) or low-protein diet (90 g protein/kg, LPD) diet throughout pregnancy, or fed the control diet or LPD for 20 d (non-pregnant animals). The fatty acid compositions of maternal liver and plasma, and fetal liver and brain were determined by GC. Feeding the LPD did not lead to any gross changes either in adult or fetal growth, or in total lipid concentrations in adult rat liver. However, the LPD was associated specifically with lower liver (42·6 %) and plasma (19·4 %) phosphatidylcholine (PC), and plasma triacylglycerol (28·6 %) docosahexaenoic acid (DHA) concentrations in pregnant rats and reduced fetal brain PC- (26·1 %) and phosphatidylethanolamine- (25·6 %) DHA concentrations. Together, these results show that variations in maternal dietary protein consumption alter DHA status in pregnancy and modify DHA accumulation into the fetal brain. The present results suggest that lower maternal protein intakes reduce delivery of DHA from the mother to the fetus, which may impair development and function of the fetal brain.

Low-protein diet: Pregnancy: Docosahexaenoic acid: Brain: Rat

Long-chain polyunsaturated fatty acids (LCPUFA) are an integral part of all cell membranes and critical for normal cellular function. Thus, there is an absolute requirement for normal fetal growth (Koletzco & Braun, 1991; Leaf *et al.* 1992) and a special requirement for the development of specific tissues, such as the brain and retina (Leat *et al.* 1986; Neuringer *et al.* 1988; Innis, 1991; Salem & Niebylski, 1995). Inadequate incorporation of docosahexaenoic acid (DHA) into the membrane phospholipids of neural tissue can lead to persistent deficits in function in animal models (Connor *et al.* 1990; Riesbick *et al.* 1990, 1994; Neuringer *et al.* 1984; Leat *et al.* 1986) and in

preterm human infants (Uauy *et al.* 1990). The timing of any limitation of DHA availability for brain development may be critical, as DHA is accumulated over a period corresponding principally to the major phase of neuritogenesis. Any deficit in DHA assimilation during this period may not be repaired by subsequent supplementation (Connor *et al.* 1990).

The precursors linoleic acid (LA) and α-linolenic acid (ALNA) are typically the predominant LCPUFA in the diets of both man and laboratory animals. Longer-chain LCPUFA, such as DHA or arachidonic acid (AA), are formed in the liver through metabolic conversion: desaturation

and chain elongation. To a substantial degree, the fetus is dependent upon the mother for a supply of pre-formed DHA and AA, because the capacity of the fetal liver for desaturation and chain-elongation is not mature in early gestation in rats (Li *et al.* 2000), pigs (Chambaz *et al.* 1985) or human subjects (Sanders & Rana, 1987). Although desaturation and elongation of LA and/or ALNA has been demonstrated in the near-term fetuses of both non-human primate (Su *et al.* 1999*a,b*) and human subjects (Demmelmair *et al.* 1995; Carnielli *et al.* 1996; Salem *et al.* 1996; Sauerwald *et al.* 1997), this capacity appears to be limited and its contribution compared with the mother for meeting fetal demands for LCPUFA has not been defined. The ability of the mother to satisfy fetal demands for AA and DHA will depend upon her capacity for inter-conversion of LA and ALNA, for mobil-isation of LCPUFA, principally from the liver, and for transfer within the maternal circulation to the placenta and fetus. Any reduction in the activities of enzymes and transporter proteins responsible for these processes would potentially limit the ability of the mother to supply adequate LCPUFA to the fetus, which would in turn have important consequences for fetal development and subsequent function in later life.

Pregnancy is associated with hyperlipidaemia, which is thought to facilitate the transfer of lipid from the mother to the fetus. In addition, there is a specific increase in the DHA content of plasma phosphatidylcholine (PC) and/or triacylglycerol (TAG) in several species (Neuringer *et al.* 1984; Chen *et al.* 1992; Burdge *et al.* 1994; Burdge & Postle, 1994; Postle *et al.* 1995; Otto *et al.* 1997). There is progressive DHA enrichment from maternal liver to fetal liver (biomagnification) (Crawford *et al.* 1976), sug-gestive of a substantial drive within the mother to deliver DHA. These adaptations have the appearance of facilitat-ing the supply of DHA to the fetus from the mother, as for the human subject and guinea-pig the timing of the increase in DHA concentration in plasma coincides with the principle period of DHA accumulation into fetal brain (Clandinin *et al.* 1980*a,b*; Burdge & Postle, 1995*a*). It seems likely that these adaptations in DHA status in the mother are specific and enable the demands of develop-ing fetal brain to be met. By contrast, for the pregnant rat, the increase in plasma PC-DHA concentration preceded the principle phase of DHA assimilation into developing brain in the neonatal period (Sinclair & Crawford, 1972), per-haps to increase DHA availability in anticipation of the needs for lactation. In pregnant women, rats and guinea-pigs increased plasma PC-DHA concentration was due to a selective increase in plasma 1-palmitoyl, 2-docosahexaenoyl PC (Burdge *et al.* 1994; Burdge & Postle, 1994; Postle *et al.* 1995). In the rat, this was due to increased DHA concentration in maternal hepatic diacylglycerol pools used as substrates for PC synthesis, greater cytidine 5′-diphosphate: choline 1,2-diacylglycerol cholinephosphotransferase activity and increased PC synthesis *de novo* (Burdge *et al.* 1994).

There are important interactions between lipid metab-olism and protein metabolism. The consumption of a diet with reduced protein content impairs the synthesis of pro-teins involved in both LCPUFA synthesis and mobilisation of lipid from the liver (Jackson *et al.* 2001). Dietary protein

intake has been shown to regulate Δ6-desaturation in hepa-tic microsomes from non-pregnant adult female rats (Peluffo & Brenner, 1974). In the pregnant rat, feeding a diet containing 50 g protein/kg reduced maternal Δ6-de-saturase activity, the rate-limiting step in LCPUFA syn-thesis (Sprecher *et al.* 1999), at day 20 of gestation compared with controls fed 250 g protein/kg (De Thomas *et al.* 1983). This was associated with lower hepatic phospholipid DHA concentration in the pups at weaning, which was reversed by cross-fostering the pups at birth to mothers consuming a control diet (250 g protein/kg). Reduced hepatic Δ5-desaturase activity at 3 months of age has also been reported in the offspring of mothers fed 80 g protein/kg diet during pregnancy (Ozanne *et al.* 1998). Marin *et al.* (1995) found a progressive reduction in brain weight and phospholipid content of the pups when pregnant rats were fed diets containing 250, 150, 100 or 50 g protein/kg. Thus, variations in maternal protein intake may both modify LCPUFA supply to the fetus and programme the essential fatty acid metabolism in the off-spring, possibly by altering capacity for synthesis of hepatic Δ6- and Δ5-desaturases.

None of the preceding studies reported the effects of feeding a low-protein diet (LPD) on maternal LCPUFA status during pregnancy or upon accumulation of LCPUFA into fetal tissues. A reduction (50 %) in dietary protein intake in pregnancy in the rat is associated with marked alterations to the physiology of the offspring (Langley & Jackson, 1994; Langley-Evans *et al.* 1999). One mechanism by which this dietary modification may act is by reducing maternal capacity for synthesis of enzymes and proteins involved in lipid transfer to the fetus. In the present study, we have fed non-pregnant and pregnant female rats a diet with a reduced protein content (50 %) to test the hypothesis that feeding a LPD in preg-nancy decreases maternal LCPUFA status leading to lower levels of these fatty acids in fetal tissues at term. We report total lipid and LCPUFA concentrations in liver and plasma from both non-pregnant rats and in pregnant adult rats, and fetal liver and brain at day 20 of gestation.

Materials and methods

Materials

Solvents were purchased from Fisher Chemicals Limited (Loughborough, Leics., UK). Lipid standards and other regents were from Sigma (Poole, Dorset, UK). Solid-phase extraction cartridges were from Varian (Walton-on-Thames, Surrey, UK).

Animal procedures

Virgin female Wistar rats were assigned (*n* 5 per dietary group) to a diet containing either 180 (control) or 90 (LPD) g protein/kg (Langley & Jackson, 1994) and main-tained on these diets for 20 d. The nutrient compositions of these diets are summarised in Table 1. A second group of rats were mated and maintained on the control or LPD diets (*n* 5 per dietary group) until day 20 of ges-tation. All procedures were carried out in accordance

Table 1. Composition (g/kg) of the control and low-protein diets*

Diet...	Control	LPD
Casein	180	90
Maize starch	425	485
Sucrose	213	243
Choline	2	2
Methionine	5	5
Vitamins	5	5
Minerals	20	20
Cellulose	50	50
Magnesium sulfate	0·5	0·5
Maize oil†	100	100

LPD, low protein diet.
* The diets were isoenergetic (control 20·2 MJ/kg, LPD 19·9 MJ/kg).
† The maize oil contained (g/kg diet): palmitic acid 11·6, stearic acid 1·6, oleic acid 30·9, linoleic acid 54·9, α-linolenic acid 0·7.

with Home Office regulations. Adult rats were sacrificed by CO_2 asphyxiation. Blood was collected from adults by cardiac puncture using lithium heparin as anticoagulant and plasma isolated by centrifugation at $1125\,\textit{g}$ for 15 min at 4°C. Maternal liver, and fetal liver and brain were excised rapidly and frozen immediately in liquid N_2. Plasma and tissues were stored at −20°C.

Isolation of lipids and analysis of fatty acid compositions

Liver, brain and plasma total lipid extracts were prepared by a modification of Folch *et al.* (1957) as described by Burdge *et al.* (2000). Briefly, tissues were homogenised in 0·8 ml 0·15 M-NaCl. Dipentadecanoyl PC, diheptadecanoyl phosphatidylethanolamine (PE), heneicosanoic acid and triheptadecanoin were added to the tissue homogenates or plasma (1·0 ml) as recovery standards as appropriate. Total lipids were isolated by extraction with chloroform–methanol (2: 1, v/v) containing butyrated hydroxytoluene (50 μg/ml), the organic phase collected and dried under N_2 at 40°C (Burdge *et al.* 2000). TAG, PC, PE and non-esterified fatty acids (NEFA) were isolated by solid-phase extraction on aminopropylsilica cartridges (100 mg) and fatty acid methyl esters prepared using methanol containing H_2SO_4 (20 ml/l) (Burdge *et al.* 2000).

Fatty acid methyl esters were resolved using a Hewlett Packard 5890 GC (Hewlett Packard, Wokingham, Berks., UK) equipped with a 50 m × 0·25 μm × 0·32 mm BPX-70 fused silica capillary column (SGE Europe Limited, Milton Keynes, Bucks., UK) and GC–flame ionisation detection. Data were acquired and processed using Chemstation software (Hewlett Packard). Peaks were identified routinely by retention times relative to authentic standards. These identities were confirmed in representative specimens by comparison of electron impact ionisation spectra generated by GC–MS under identical chromatographic conditions to the GC–flame ionisation detection analysis with those produced by authentic standards (results not shown). Fatty acid concentrations were determined relative to the internal standard. Total lipid concentrations were calculated from summation of individual fatty acid concentrations, corrected for mol fatty acid/mol TAG, PC or PE.

Data are presented as mean values and standard deviations. Statistical analysis of the results from adult animals was by ANOVA using Bonferroni's correction for *post-hoc* analysis of multiple comparisons. Analysis of data from fetuses was carried out using Students unpaired *t* test.

Results

Adult and fetal growth and tissue weights

There was no significant weight gain in the non-pregnant animals over the 20 d of the study, while the pregnant rats showed equivalent weight gains in both dietary groups (control 35·0 %, LPD 31·5 %) (Table 2). There were no statistically significant differences between dietary groups in starting or final weight. Adult liver weights did not differ significantly between groups. However, for both the control and LPD rats liver weights were greater ($P < 0.01$) in the pregnant animals compared with non-pregnant animals (control 41·1 %, LPD 40·9 %). There were no significant differences between dietary groups in placental or fetal carcass, liver or brain weights (Table 2).

Effect of pregnancy and reduced protein intake on total lipid concentrations in adult liver and plasma

There were no significant differences between dietary groups in liver and plasma total lipid concentrations in non-pregnant rats (Table 3). In addition, there was no effect of pregnancy upon liver PC, PE and TAG concentrations or in plasma PC and NEFA levels (Table 3). Plasma TAG was increased significantly in both the control (3·2-fold) and LPD (3·4-fold) groups relative to the non-pregnant animals, but there was no significant difference between groups in plasma TAG concentration in pregnancy at day 20 (Table 3).

Effect of pregnancy and reduced protein intake on adult rat liver and plasma long-chain polyunsaturated fatty acid concentrations

Pregnancy was associated with decreased concentrations of hepatic PC-dihomo-γ-linolenic acid (DGLA) (control 41·4 %, LPD 35·2 %), AA (control 33·0 %, LPD 33·4 %) and DPA (control 50·0 %, LPD 48·5 %) (Table 4). There was no significant difference in LA concentration between non-pregnant and pregnant animals. In contrast, hepatic PC-DHA concentration was significantly greater in pregnant rats fed the control diet (39·2 %). However, there was no significant difference in liver PC-DHA concentration between non-pregnant and pregnant animals fed the LPD (Table 4). Thus, feeding a reduced-protein diet resulted in significantly ($P < 0.005$) lower maternal hepatic DHA concentration in pregnancy (42·6 %) compared with animals fed the control diet.

Pregnancy was associated with decreased hepatic PE-LCPUFA concentrations in animals in both dietary groups (Table 4), while liver TAG-*n*-3 and -*n*-6 fatty acid contents were not affected significantly by pregnancy.

Table 2. Whole-body and tissue weights of adult and fetal rats§

(Mean values and standard deviations)

	Dietary group			
	Control		LPD	
	Mean	SD	Mean	SD
Adult non-pregnant (*n* 5 per group)				
Whole-body weight at start (g)	262	18	265	11
Whole-body weight at 20 d (g)	272	18	282	14
Liver weight (g)	9·5	0·9	8·8	1·5
Adult pregnant (*n* 5 per group)				
Whole-body weight at start of pregnancy (g)	260	16	254	14
Whole-body weight at 20 d (g)	351*‡	32	334*‡	28
Liver weight (g)	12·7	1·0	12·4	2·0
Placenta (*n* 20 per group)‖				
Weight (g)	0·53	0·13	0·54	0·11
Fetus (*n* 20 per group)‖				
Carcass weight (g)	3·44	0·4	3·46	0·59
Liver weight (g)	0·26	0·07	0·27	0·09
Brain weight (g)	0·14	0·01	0·14	0·02

LPD, low-protein diet.

Mean values were significantly different from those of non-pregnant animals within the same dietary group: *$P<0·01$.

Mean values were significantly different from those at the start of pregnancy within the same dietary group: ‡$P<0·0005$.

§ For details of diets and procedures, see Table 1 and pp. 380–381.

‖ Four fetuses or placentas were selected at random from each pregnancy.

DPA was not detected in liver TAG in either non-pregnant or pregnant rats.

Plasma PC-LA, -DGLA, -AA and -DPA concentrations were not altered significantly by either pregnancy or by feeding diets with differing protein contents (Table 5).

Table 3. Adult rat liver and plasma total lipid concentrations‡

(Mean values and standard deviations for five rats per group)

	Dietary group			
	Control		LPD	
	Mean	SD	Mean	SD
Liver				
Non-pregnant				
PC (μmol/g)	15·7	3·2	13·7	0·7
PE (μmol/g)	4·9	1·1	4·7	0·1
TAG (μmol/g)	3·6	0·7	3·0	1·6
Pregnant				
PC (μmol/g)	16·5	1·0	14·6	0·5
PE (μmol/g)	4·0	0·5	3·5	0·5
TAG (μmol/g)	3·2	0·7	4·1	0·6
Plasma				
Non-pregnant				
PC (mmol/l)	0·8	0·2	0·9	0·1
NEFA (mmol/l)	0·8	0·1	1·1	0·1
TAG (mmol/l)	0·9	0·2	0·9	0·3
Pregnant				
PC (mmol/l)	1·0	0·2	1·4	0·3
NEFA (mmol/l)	1·2	0·1	1·4	0·1
TAG (mmol/l)	2·9****	0·3	3·1****	0·7

LPD, low-protein diet; PC, phosphatidylcholine; PE, phosphatidylethanolamine; TAG, triacylglycerol; NEFA, non-esterified fatty acid.

Mean values were significantly different from those of non-pregnant animals within the same dietary group: ****$P<0·0001$.

‡ For details of diets and procedures, see Table 1 and pp. 380–381.

Plasma PC-DHA concentration was significantly greater in pregnant animals (control 80·0 %, LPD 32·8 %). However, plasma PC-DHA concentration in pregnant rats was significantly lower in those fed the LPD (19·4 %) compared with controls (Table 5). There were no effects of either pregnancy or diet upon plasma NEFA-LCPUFA composition or plasma TAG-DGLA or -DPA concentrations (Table 5). DGLA and DPA were not detected in plasma NEFA. Plasma TAG-LA (control 2·6-fold, LPD 3·2-fold), AA (control 2·2-fold, LPD 1·6-fold) and DHA (control 2·3-fold, LPD 3·3-fold) concentrations were significantly greater in pregnant animals compared with non-pregnant rats (Table 5). There were no significant effects of dietary protein intake on plasma TAG-LA or -AA levels. However, plasma TAG-DHA concentration in pregnancy was significantly lower (28·6 %) in the mothers fed the LPD compared with controls (Table 5).

Effect of reduced maternal protein intake in pregnancy on fetal liver and brain lipid compositions

Fetal liver and brain total lipid concentrations are summarised in Table 6. There was no effect of maternal dietary protein intake on fetal liver PC, PE or TAG concentration. There was also no significant difference in the LCPUFA content of liver PC or PE between fetuses of mothers fed control or LPD diets. However, LA concentration was greater (2·0-fold) in liver TAG of fetuses from mothers fed the LPD compared with controls. Brain PE concentration was significantly ($P<0·01$) less (17·5 %) in fetuses from mothers fed the LPD compared with controls, although there was no difference in brain PC concentration between dietary groups (Table 7). Analysis of the LCPUFA

Table 4. Adult liver phospholipid and triacylglycerol (TAG) long-chain polyunsaturated fatty acid (LCPUFA) compositions‡

(Mean values and standard deviations for five rats per group)

	Dietary group							
	Control				LPD			
	Non-pregnant		Pregnant		Non-pregnant		Pregnant	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
PC (nmol/g)								
LA	1759	438	1822	157	1508	93	1494	140
DGLA	94	20	55**	8	88	22	57***	4
AA	4908	109	3288***	292	4278	298	2850**	283
DPA	66	16	33***	10	66	11	34***	8
DHA	1241	236	1727***	180	1058	149	991	195
PE (nmol/g)								
LA	335	111	223**	63	270	105	170	32
DGLA	20	3	11**	2	23	3	11**	1
AA	1471	438	817***	34	1214	426	721***	73
DPA	50	16	25***	5	51	8	20***	6
DHA	703	187	410***	306	542	187	306***	86
TAG (nmol/g)								
LA	583	132	761	99	601	167	868	102
DGLA	12	7	10	3	6****	2	15	5
AA	57	14	39	14	41	11	27	16
DHA	14	3	14	6	15	2	16	6

LPD, low-protein diet; PC, phosphatidylcholine; LA, linoleic acid; DGLA, dihomo-γ-linolenic acid; AA, arachidonic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid; TAG, triacylglycerol.

Mean values were significantly different from those of non-pregnant animals within the same dietary group: ** $P < 0.01$, *** $P < 0.005$.

Mean value was significantly different from that of the control-diet group for either non-pregnant or pregnant animals: **** $P < 0.0001$.

‡ For details of diets and procedures, see Table 1 and pp. 380–381.

Table 5. Adult plasma phosphatidylcholine (PC), non-esterified fatty acids (NEFA) and triacylglycerol (TAG) long-chain polyunsaturated fatty acid (LCPUFA) compositions‡

(Mean values and standard deviations for five rats per group)

	Dietary group							
	Control				LPD			
	Non-pregnant		Pregnant		Non-pregnant		Pregnant	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
PC (μmol/l)								
LA	166	39	181	36	184	35	246	38
DGLA	5	1	3	1	6	2	5	1
AA	211	23	149	40	214	31	197	38
DPA	3	1	2	1	3	1	2	1
DHA	40	5	72***	7	39	5	58**†	6
NEFA (μmol/l)								
LA	27	8	39	3	34	1	41	8
AA	6	1	6	2	6	1	11	5
DHA	1	0	2	1	1	1	2	1
TAG (μmol/l)								
LA	463	186	1218***	192	365	117	1162***	315
DGLA	5	3	7	2	3	1	8	1
AA	31	8	67***	15	34	6	56**	9
DPA	2	1	2	2	1	1	2	2
DHA	6	2	14***	3	3	2	10***††	1

LPD, low-protein diet; LA, linoleic aicd; DGLA, dihomo-γ-linolenic acid; AA, arachidonic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid; NEFA, non-esterified fatty acid; TAG, triacylglycerol; PC, phosphatidylcholine.

Mean values were significantly different from those of non-pregnant animals within the same dietary group: ** $P < 0.01$, *** $P < 0.0001$.

Mean values were significantly different from those of the control-diet group for either non-pregnant or pregnant animals: † $P < 0.05$, †† $P < 0.01$.

‡ For details of diets and procedures, see Table 1 and pp. 380–381.

Table 6. Fetal liver phosphatidylcholine (PC), phosphatidylethanolamine (PE) and triacylglycerol (TAG) long-chain polyunsaturated fatty acid (LCPUFA) compositions†

(Mean values and standard deviations for five rats per group)

	Dietary group			
	Control		LPD	
	Mean	SD	Mean	SD
PC (nmol/g)				
LA	645	148	642	107
DGLA	72	12	67	13
AA	720	120	642	107
DPA	17	6	18	5
DHA	243	62	241	97
Total FA‡	5900	1800	5600	1300
PE (nmol/g)				
LA	141	30	145	23
DGLA	20	3	20	3
AA	545	87	581	74
DPA	14	3	18	5
DHA	219	46	248	48
Total FA‡	2200	300	2300	300
TAG (nmol/g)				
LA	57	27	115**	40
AA	17	6	17	7
DHA	15	5	12	5
Total FA‡	700	300	800	300

LPD, low-protein diet; PC, phosphatidylcholine; LA, linoleic aicd; DGLA, dihomo-γ-linolenic acid; AA, arachidonic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid; TAG, triacylglycerol.
Mean value was significantly different from that of the control-diet group: **$P < 0.01$.
† For details of diets and procedures, see Table 1 and pp. 380–381.
‡ Total FA represents the sum of all FA within the lipid fraction, including saturated and monounsaturated species.

Table 7. Fetal brain phosphatidylcholine (PC), and phosphatidylethanolamine (PE), long-chain polyunsaturated fatty acid (LCPUFA) compositions†

(Mean values and standard deviations for five rats per group)

	Dietary group			
	Control		LPD	
	Mean	SD	Mean	SD
PC (nmol/g)				
LA	69	12	64	15
DGLA	22	6	19	5
AA	633	97	613	111
DHA	314	61	232**	46
Total FA‡	7500	600	7400	700
PE (nmol/g)				
LA	49	28	59	24
AA	704	83	730	68
DHA	558	87	415**	69
Total FA‡	4000	900	3300**	300

LPD, low-protein diet; PC, phosphatidylcholine; LA, linoleic aicd; DGLA, dihomo-γ-linolenic acid; AA, arachidonic acid; DHA, docosahexaenoic acid; FA, fatty acid; PE, phosphatidylethanolamine.
Mean values were significantly different from those of the control-diet group: **$P < 0.01$.
† For details of diets and procedures, see Table 1 and pp. 380–381.
‡ Total FA represents the sum of all fatty acids within the lipid fraction, including saturated and monounsaturated species.

composition of fetal brain PC and PE showed that there was no significant difference in the concentrations of *n*-6 fatty acids between dietary groups (Table 7). In contrast, DHA concentration was significantly ($P < 0.01$) lower in both brain PC (26·1%) and PE (25·6%) in fetuses from mother fed the LPD. Only 17·5% of the reduction in brain PE-DHA could be accounted for by the decrease in brain total PE, indicating that there was a specific effect of LPD on DHA levels independent of total PE concentration.

Discussion

The results of the present study demonstrate that following the consumption of a diet which provided 90 g protein/kg for 20 d, there were no gross differences in the non-pregnant or pregnant animals, compared with the diet providing 180 g protein/kg. Nevertheless, our present results show that reducing maternal dietary protein intake in pregnancy resulted in a lower concentration of DHA in maternal liver and plasma and impaired accumulation of DHA into fetal brain phospholipids. ALNA was the sole source of *n*-3 fatty acids in the diet of these rats and therefore the results provide clear evidence that feeding the LPD compromised one or more of the metabolic–physiological steps involved in the formation and/or supply of DHA from mother to fetus. This is a novel observation, and since accumulation of DHA into the developing central nervous system results in long-term impairment of neurological function (Connor *et al.* 1990; Riesbick *et al.* 1990, 1994; Innis, 1991), it raises the possibility that such deficits represent an insult to the development and subsequent function of fetal brain.

Feeding the LPD did not alter significantly the weight of the non-pregnant animals or the weight gain of the pregnant rats. Neither was there a significant difference in liver weight between dietary groups in either the non-pregnant or pregnant rats. Thus a 50% reduction in protein intake did not restrict the growth of the adult animals. Furthermore, there were no significant effects of the LPD on liver or plasma total lipid concentrations This is in contrast to previous studies where a more severe protein restriction (80%) resulted in accumulation of TAG and lower PC levels in liver tissue accompanied by decreased plasma TAG concentration in non-pregnant rats (Ristic *et al.* 1985), presumably due to impaired mobilisation of TAG by VLDL. Together, these results indicate that feeding a diet with a moderately reduced protein content did not produce marked changes in lipid handling in the adult rat. In particular, there was no apparent impairment of the capacity of the liver to maintain membranes, to export lipoproteins and to sustain the increased plasma TAG concentration associated with late gestation.

There was no effect of the LPD in non-pregnant rats on the LCPUFA composition of liver PC, PE or TAG or plasma PC, TAG and NEFA. Since the fat source in both control and LPD diets did not contain AA or DHA, tissue levels of these fatty acids reflect metabolic capacity for their synthesis from LA and ALNA respectively. These results indicate that in non-pregnant females the activities of the desaturation–elongation pathways responsible for

LA and ALNA inter-conversion were adequate to meet the demand on the reduced-protein intake. From the present results, it is not possible to say whether there was any effect at all of the LPD, as the demands for AA and DHA may be relatively low in non-pregnant adults, such that any changes in capacity for LA and ALNA inter-conversion were insufficient to limit adequate availability of LCPUFA for membrane synthesis.

During pregnancy, the demands for LCPUFA are increased above those of the non-pregnant adult female, to support the development of the fetus and placenta. The rats consumed a diet lacking AA and DHA and therefore any increased demands for these fatty acids by fetal tissues would have to have been met by adaptations to maternal lipid metabolism. Pregnancy was associated with selective changes in the LCPUFA concentrations in maternal liver and plasma. In particular, liver PC from pregnant animals fed the control diet contained less DGLA, AA and DPA than non-pregnant animals. Maternal liver PE-n-6 and -n-3 LCPUFA concentrations were also lower in pregnant animals, while there was no effect of pregnancy on liver TAG-LCPUFA composition. In contrast, plasma PC, DGLA, AA and DPA levels were not significantly different at term compared with non-pregnant rats. Plasma TAG-LA, -AA and -DHA concentrations were increased in pregnancy. One possible explanation for the different patterns of pregnancy-associated changes in lipid composition is selective incorporation of individual LCPUFA species into different hepatic lipid pools destined either for incorporation into tissue or mobilisation from the liver.

DHA concentration was significantly greater in both hepatic and plasma PC, and in plasma TAG in pregnant animals fed the control diet compared with non-pregnant rats. Increased plasma PC-DHA concentration is, in part, the result of selective adaptation to maternal hepatic PC synthesis (Burdge *et al.* 1994), which may increase the availability of DHA to the placenta and/or mammary gland. In the absence of an increased dietary intake, increased availability of DHA would be achieved by mobilisation of DHA from adipose tissue, and/or up-regulation of ALNA inter-conversion, either of which would require increased activity of enzymes and transport proteins involved in these pathways. In contrast, in the pregnant rats fed the LPD there was no increase in the DHA content of hepatic PC above those found in non-pregnant animals, and there was a lower increment in both plasma PC- and TAG-DHA. This finding is consistent with the suggestion that feeding the LPD had an adverse effect and was a limitation in the mechanisms responsible for modulating the tissue and plasma levels of DHA that only became manifest when the biochemical–physiological processes were stressed by increased demands for DHA associated with pregnancy. Feeding pregnant rats a diet containing 50 g protein/kg has been shown to decrease the activity of the rate-limiting hepatic enzyme Δ6-desaturase in late gestation and during lactation (De Thomas *et al.* 1983), which may explain the reduced DHA levels in liver phospholipid in the pups at weaning in the pups (De Thomas *et al.* 1983). Both DHA and AA are products of the same pathway (Sprecher *et al.* 1999) and if, therefore,

a reduction in Δ6-desaturase activity was the primary mechanism for reduced DHA status in pregnant rats, then it would be expected that hepatic and plasma AA levels would also be lower in the LPD group. Alternatively, a specific effect on DHA, which was not seen for AA, could be explained by selective down-regulation of the Δ6-desaturase responsible for conversion of 24 : 5 n-3 to 24 : 6 n-3 and/or the peroxisomal β-oxidation step which is required for DHA, but not AA, synthesis.

There were no effects of the LPD during pregnancy on placental or fetal carcass weight, fetal liver weight, total phospholipid and TAG, nor LCPUFA concentrations. These results differ from those found in other studies that showed decreased levels of DHA and/or AA in rat liver phospholipids, measured at weaning or 3 months of age (De Thomas *et al.* 1983; Ozanne *et al.* 1998). These differences may be explained by the different ages at which the studies were carried out, or may be related to a more severe reduction in protein intake (De Thomas *et al.* 1983), producing more severe effects on lipid metabolism (Marin *et al.* 1995).

There was no significant effect of reduced maternal protein intake on fetal brain growth. In contrast, fetuses from mothers fed the LPD showed decreased brain PE concentration and a specific reduction in PC- and PE-DHA contents compared with controls. Marin *et al.* (1995) showed that brain weight at weaning was reduced in pups from mothers fed a LPD compared with controls. Since brain growth in the rat occurs principally during the period between birth and weaning (Dobbing & Sands, 1979), the absence of any such effect on growth in the current study may reflect the age at which the animals were studied. The selective reduction in brain total PE concentration is consistent with the lower total lipid-P reported previously (Marin *et al.* 1995). The significance of a proportional increase in brain PC : PE may be indicative of altered brain structure and function, and this will need to be investigated further. In guinea-pigs ingesting ethanol during pregnancy, the PC : PE value in fetal brain at term was increased and was associated with altered motor function (Burdge & Postle, 1995*b*).

The substantial accumulation of DHA into the phospholipids in the developing fetal and neonatal brain and retina is an aspect of functional maturation. Deficits in brain and retinal DHA assimilation are associated with impaired neurological function (Neuringer *et al.* 1984; Neuringer *et al.* 1988; Connor *et al.* 1990; Innis, 1991). Fetuses of mothers fed the LPD had significantly ($P<0.01$) lower brain PC- and PE-DHA concentrations, which raises the real possibility that this intervention might have been associated with impaired maturation or neurological dysfunction. The magnitude of the difference between dietary groups in fetal brain PE-DHA concentration (about 25 %) was greater than reported for rat pups at weaning from mothers fed an ALNA- and DHA-deficient diet during pregnancy (14 %; Leat *et al.* 1986). One possible explanation is that the pregnant rat fed an n-3 fatty acid-deficient diet is able to meet the demands of the fetus for DHA in part by mobilisation of fatty acid stores. In contrast, the apparent constraints on n-3 fatty acid handling in pregnancy associated with the LPD suggest that the ability of

the mother to adapt her essential fatty acid metabolism during gestation may be a greater determinant of DHA accretion in fetal brain than dietary *n*-3 LCPUFA intake. However, as the majority (93 %) of DHA accumulation and neuritogenesis in rat brain occurs during the sucking period (Kishimoto *et al.* 1965; Sinclair & Crawford, 1972), any limitation in DHA assimilation during late fetal life may be offset in part by DHA provision during lactation. While fetal brain-phospholipid composition and DHA content were altered significantly by feeding the mother the LPD, there was no effect on fetal liver-lipid composition. This suggests that the developing fetal brain is more sensitive than liver to impaired maternal DHA. Langley-Evans *et al.* (1996) showed that the effect of altered maternal protein intake on the programming of blood pressure in the rat could be modified by the type of oil used in the feed. Whether the adverse impact of the LPD diet on maternal and fetal DHA status can be modified in a similar manner remains to be determined.

The results of the present study show that in rats fed a diet lacking DHA, reducing protein intake in pregnancy decreased both maternal DHA status and accumulation of DHA into fetal brain. Whether variation in maternal protein intake is an important determinant of DHA supply from mother to fetus in pregnant women consuming both ALNA and DHA remains to be determined. The pregnancy-associated increase in plasma phospholipids-DHA concentration (Postle *et al.* 1995; Otto *et al.* 1997) suggests that the capacity to up-regulate LCPUFA synthesis and mobilisation may be a primary factor in determining DHA supply to the fetus and neonate. If so, it will be important to identify the extent to which the observed variation between women in the increment of plasma phospholipid DHA during pregnancy (about 25 %) (Postle *et al.* 1995; Otto *et al.* 1997) can be accounted for by differences in dietary protein intake. It remains to be established whether such variations in maternal DHA status are associated with differences in neurological function in the offspring.

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