*British Journal of Nutrition* (2003), **90**, 345–352
DOI: 10.1079/BJN2003909
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https://doi.org/10.1079/BJN2003909 Published online by Cambridge University Press

# Effect of reduced maternal protein intake in pregnancy in the rat on the fatty acid composition of brain, liver, plasma, heart and lung phospholipids of the offspring after weaning

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*(Received 19 December 2002 – Revised 19 March 2003 – Accepted 8 April 2003)*

Reduced protein intake during pregnancy decreased maternal hepatic and plasma docosahexaenoic acid concentrations and impaired docosahexaenoic acid accumulation into fetal brain in the rat. The present study investigated whether restriction of maternal protein intake during pregnancy in the rat alters membrane phospholipid fatty acid composition in the offspring after weaning. Female rats (six per group) were mated and fed diets containing either 180 or 90 g protein/kg throughout pregnancy. Mothers were transferred to standard chow after delivery and the litters reduced to eight pups. Weaning was at 28 d and pups were killed 5 to 6 d later. Tissue weights or membrane total phosphatidylcholine (PC) and phosphatidylethanolamine (PE) concentrations in the offspring did not differ between dietary groups. There were significant differences between the 180 and 90 g/kg groups in liver, brain, lung and heart fatty acid composition that differed between tissues and phospholipid classes. For example, docosahexaenoic and arachidonic acid concentrations were 23 and 10 % lower respectively in hepatic PC, but not PE, in the 90 g/kg group. In brain, docosahexaenoic acid concentration was 17 % lower in PC, but not PE, while arachidonic acid content was 21 % greater in PE but unchanged in PC. The greatest differences were in unsaturated fatty acids, which suggests alterations to desaturase activities and/or the specificity of phospholipid biosynthesis. These results suggest that restricted maternal protein intake during pregnancy results in persistent alterations to membrane fatty acid content.

**Low-protein diet: Polyunsaturated fatty acids: Rats: Phospholipids**

Long-chain polyunsaturated fatty acids (PUFA) are important structural components of cell membrane phospholipids and are critical for normal cell function (Salem & Niebylski, 1995; Calder, 2001). There is an absolute requirement for PUFA to support normal growth and development (Koletzko & Braun, 1991; Leaf *et al.* 1992). For example, lower concentrations of *n*-3 PUFA docosahexaenoic acid (22 : 6*n*-3) in the central nervous system are associated with a significant deficit in function (Neuringer *et al.* 1984, 1986, 1988; Leat *et al.* 1986; Bourre *et al.* 1989; Connor *et al.* 1990; Lin *et al.* 1990; Reisbick *et al.* 1990, 1994; Innis, 1991; Salem & Niebylski, 1995; Pawlosky *et al.* 1997). Arachidonic acid (20 : 4*n*-6) and docosahexaenoic acid are relatively minor components of the diets of both man and laboratory animals compared with the precursor essential fatty acids linoleic acid (18 : 2*n*-6) and α-linolenic acid (18 : 3*n*-3). Thus maintenance of appropriate concentrations of arachidonic and docosahexaenoic acids within cell membranes is dependent, at least in part, on capacity for desaturation and chain elongation of linoleic acid and α-linolenic acid.

A pathway for conversion of linoleic and α-linolenic acids to their respective long-chain metabolites has been described (Sprecher, 2000). This involves the sequential activities of Δ6-desaturase, elongases, and Δ5-desaturase to form 20-carbon PUFA. Synthesis of docosahexaenoic acid involves further addition of four carbons, desaturation at the Δ6 position and limited peroxisomal β-oxidation. Alterations to the capacity to convert linoleic and α-linolenic acids to longer-chain PUFA may alter the concentrations of arachidonic and docosahexaenoic acids, respectively, within membranes, and so alter cell function.

Dietary protein intake has long been known to alter Δ6- and Δ5-desaturase activities in rats and so potentially alter the availability of PUFA for incorporation into membrane phospholipids. Reduced dietary protein intake up regulates hepatic Δ6-desaturase activity in the non-pregnant female rat (Peluffo & Brenner, 1974), but is associated with lower hepatic Δ6-, Δ5- and Δ9-desaturase activities in pregnant animals (De Thomas *et al.* 1983). This suggests that maternal protein restriction during pregnancy may reduce capacity for fatty acid desaturation

**Abbreviations:** FAME, fatty acid methyl esters; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PUFA, polyunsaturated fatty acids.

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in the mother when presented with increased demands and so restrict supply of PUFA to the offspring. This is supported by the observation that feeding rats a diet containing 90 g protein/kg during gestation impaired their ability to increase liver and plasma phosphatidylcholine (PC) docosahexaenoic acid concentration, although no effect was found in non-pregnant animals (Burdge *et al.* 2002). This was associated with an approximate 25 % decrease in fetal brain docosahexaenoic acid concentration. In addition, feeding a diet containing 80 g protein/kg to rats during pregnancy and lactation resulted in lower hepatic Δ5-desaturase activity in the offspring at 3 months of age and was associated with a selective decrease in hepatic docosahexaenoic acid concentration (Ozanne *et al.* 1998). One possible interpretation of these data is that maternal protein intake during pregnancy programs lower capacity for PUFA synthesis in the offspring.

It is unclear whether the adverse effects of feeding a lower maternal protein during pregnancy alone on membrane phospholipid composition in the fetus (Burdge *et al.* 2002) persist in the free-living offspring. In the present study a well-established rat model was used (Langley & Jackson, 1994) to determine the effect of feeding a reduced-protein diet during pregnancy on the phospholipid fatty acid compositions of tissues in the offspring after weaning. The fatty acid compositions of the major phospholipid classes, PC and phosphatidylethanolamine (PE) will be reported separately as they are distributed differentially between membrane leaflets and they are derived from different diacylglycerol substrate pools (Burdge *et al.* 1994).

## Materials and methods

### *Materials*

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

### *Animal procedures*

Animal procedures were essentially as described previously (Langley & Jackson, 1994; Burdge *et al.* 2002). The design of the diet was identical to Langley & Jackson (1994) based upon the American Institute of Nutrition AIN-93G purified diet (Reeves *et al.* 1993). Although the linoleic acid:α-linolenic acid value was high (Table 1) the concentrations of individual *n*-3 PUFA in liver and brain in the present study were comparable with those reported by Su *et al.* (1996) of a linoleic acid:α-linolenic acid ratio of 9:1. No markers of *n*-3 deficiency were found. Briefly, virgin female Wistar rats (six per group) were mated and then fed *ad libitum* isoenergetic diets containing either 180 or 90 g casein/kg (Table 1) throughout pregnancy (Langley & Jackson, 1994). At delivery, litters were reduced to eight pups and the mothers fed standard chow during lactation. Pups were weaned at 28 d after birth and killed 5 to 6 d later by $CO_2$ asphyxiation. Blood was collected by cardiac puncture using lithium heparin as anti-coagulant, separated into plasma and cells by centrifugation at 1125 ***g*** for 10 min, and the plasma stored at −80°C. Tissues were frozen in liquid $N_2$ and stored at −80°C. Results are presented for tissues from eight pups per dietary group. Tissues were analysed from equal numbers of males and females chosen at random and distributed between all six pregnancies per diet (no more than two pups were studied per pregnancy).

**Table 1.** Composition of experimental diets (g/kg)

| | Dietary group | |
|---|---|---|
| | 180 g protein/kg | 90 g protein/kg |
| Casein | 180 | 90 |
| Maize starch | 425 | 482 |
| 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 |
| Total energy (MJ/kg) | 20·2 | 19·9 |

*The fatty acid composition of the maize oil was (g/kg): palmitic acid 116, stearic acid 16, oleic acid 309, linoleic acid 549 and α-linolenic acid 7. Palmitoleic, dihomo γ-linolenic, arachidonic, eicosapentaenoic, docosapentaenoic and docosahexaenoic acids were not detected.

### *Fatty acid analysis*

Fatty acid methyl esters (FAME) were prepared from phospholipids extracted from tissues (approximately 100 mg) and plasma (0·5 ml) as described previously (Burdge *et al.* 2000). Briefly, tissues were homogenised in 0·15 M-NaCl (0·8 ml). Dipentadecanoyl phosphatidylcholine and diheptadecanoyl phosphatidylethanolamine were used as internal standards. Total lipids were extracted with chloroform–methanol (2:1, v/v) (Folch *et al.* 1957). PC and PE were isolated by solid phase extraction using 100 mg aminopropyl silica cartridges (Burdge *et al.* 2000). FAME were prepared by incubation with methanol containing 2 % (v/v) sulfuric acid at 50°C for 18 h (Burdge *et al.* 2000). FAME were analysed using a Hewlett Packard 6890 gas chromatograph (Hewlett Packard, Wokingham, Berks., UK) equipped with a BPX-70 fused silica capillary column (30 m × 0·25 μm × 0·25 mm) equipped with flame ionisation detection. Fatty acid concentrations were calculated from their relative peak areas compared with the internal standard. Total phospholipid concentrations were estimated from the sum of the concentrations of individual FAME corrected for mol of fatty acid per mol of phospholipid.

### *Statistical analysis*

Statistical analysis of the tissue weight, and fatty acid and phospholipid concentrations of tissues from the offspring of mothers fed either the 180 or 90 g protein/kg diets during pregnancy was by unpaired Student's *t* test. Statistically significant difference was assumed for values of $P < 0{\cdot}05$.

https://doi.org/10.1079/BJN2003909 Published online by Cambridge University Press

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## Results

There were no significant effects of maternal protein restriction during pregnancy on the body weight or the weight of brain, liver, heart or lung of their offspring post-weaning (Table 2). In addition, there were no significant differences between the 180 and 90 g/kg groups in the total concentrations of PC and PE in any of the tissues measured (Tables 3–6). There were no differences in any of the measurements between male and female pups.

### *Effect of reduced maternal dietary protein on the saturated fatty acid content of membrane phospholipids in the offspring*

Tissue and plasma PC and PE fatty acid compositions are summarised in Tables 3 to 6. Reducing the maternal protein intake during pregnancy produced differential effects on saturated fatty acid content between tissues and between PC and PE within a tissue. There were no significant differences in the saturated fatty acid content of lung and heart phospholipids (Tables 5 and 6). The lignoceric acid (24 : 0) content of both liver and plasma PC was greater (66·7 and 50·0 %, respectively) in the 90 g/kg group compared with the 180 g/kg group (Table 3). In contrast, maternal protein intake during pregnancy did not alter the concentrations of saturated fatty acids in hepatic PE. The palmitic acid (16 : 0) content of brain PC was greater and there was a trend for stearic acid (18 : 0) to be less in the 90 g/kg group (Table 4), while the lignoceric acid level was not altered. However, in brain PE, lignoceric acid concentration was 18·1 % greater ($P<0·05$) in the 90 g/kg group, while palmitic and stearic acid contents were not changed (Table 4). Myristic acid (14 : 0) was only detected in heart and lung, and was not altered by maternal protein intake during pregnancy (Tables 5 and 6).

### *Effect of reduced maternal dietary protein on the monounsaturated fatty acid content of membrane phospholipids in the offspring*

Reduced maternal dietary protein during pregnancy altered the monounsaturated fatty acid content of liver, plasma, brain, heart and lung (Tables 3–6). Palmitoleic acid (16 : 1*n*-7) concentration was increased in liver (57·1 %) and plasma (50·0 %) PC, but not PE, in the 90 g/kg group (Table 3). There was no difference between the 180 and 90 g/kg groups in the palmitoleic acid concentration of brain PC and PE, while oleic acid (18 : 1*n*-9) concentration was lower in both PC (6·2 %) and PE (26·7 %) in the 90 g/kg group (Table 4). In addition, nervonic acid (24 : 1*n*-9) was lower in brain PC (50·0 %) in the 90 g/kg group, but was unchanged in PE. There was a small but significant increase (11·8 %) in the palmitoleic acid content of lung PC, but not PE, in the 90 g/kg group, while the concentrations of oleic and nervonic acids were unchanged in both phospholipid classes (Table 5). Palmitoleic and oleic acid concentrations were greater (10 and 66·7 %, respectively) and nervonic acid lower (21·1 %) in heart PC in the 90 g/kg group, while the monounsaturated fatty acid composition of heart PE was unchanged (Table 6).

**Table 2.** Tissue wet weights (g) from pups (eight per group) of mothers fed either 180 or 90 g protein/kg diet during pregnancy*

(Mean values and standard deviations)

| | 180 g/kg | | 90 g/kg | |
|---|---|---|---|---|
| Diet... | Mean | SD | Mean | SD |
| Body weight (g) | 77·9 | 3·6 | 74·1 | 6·8 |
| Lung (pair) | 0·8 | 0·2 | 0·7 | 0·1 |
| Heart | 0·4 | 0·1 | 0·5 | 0·1 |
| Liver | 3·8 | 0·7 | 3·4 | 0·3 |
| Brain | 1·5 | 0·1 | 1·4 | 0·1 |

* For details of diets and procedures, see Table 1 and p. 346.

### *Effect of reduced maternal dietary protein on the polyunsaturated fatty acid content of membrane phospholipids in the offspring*

There was no effect of reduced maternal protein intake on the *n*-6 or *n*-3 PUFA content of lung, and heart PC and PE in their offspring (Table 5–6). The effects of lower maternal protein intake during pregnancy on the concentrations of *n*-6 PUFA in tissue phospholipids from their offspring were as follows. Linoleic acid (18 : 2*n*-6) was lower in liver PC (16·9 %) and PE (19·7 %), and in plasma PC in the 90 g/kg group (Table 3). Linoleic acid was also reduced in brain PC (22·2 %), but not PE (Table 4). In contrast, while dihomo γ-linolenic acid (20 : 3*n*-9) and arachidonic acid (20 : 4*n*-6) concentrations were unchanged in brain PC, dihomo γ-linolenic acid was 28·5 % lower and arachidonic acid 21·1 % greater in brain PE in the 90 g/kg group (Table 4).

For the *n*-3 series, eicosapentaenoic acid (20 : 5*n*-3), when present, and docosapentaenoic acid (22 : 5*n*-3) concentrations were not altered significantly in liver PC and PE, plasma PC or brain PC and PE (Tables 3 and 4). In contrast, docosahexaenoic acid concentration was lower in liver PC (23·0 %), but not PE, and also reduced in plasma PC (16·8 %) in the 90 g/kg group (Table 3). Docosahexaenoic acid concentration was also lower in brain PC (16·9 %), but not PE, in the 90 g/kg group (Table 4).

## Discussion

The results of the present study show that there was no significant effect of a moderate restriction in maternal protein intake during pregnancy on the growth of specific tissues in the offspring after weaning, or on total membrane phospholipid concentrations. However, there were significant differences between the 180 and 90 g protein/kg groups in phospholipid fatty acid compositions that differed between tissues and between phospholipid classes.

Feeding the 90 g protein/kg diet to pregnant rats did not alter the growth of heart, lung, liver and brain. This is consistent with our previous data showing that this dietary regimen did not alter fetal growth (Burdge *et al.* 2002). However, some (Langley-Evans *et al.* 1996; Langley-Evans & Nwagwu, 1998), but not all (Langley & Jackson, 1994),

**Table 3.** Phospholipid fatty acid composition of rat liver and plasma phospholipids (g/kg) from pups (eight per group) of mothers fed either 180 or 90 g protein/kg diet during pregnancy†

(Mean values and standard deviations)

| | Liver | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Phosphatidylcholine | | | | Phosphatidylethanolamine | | | | Plasma phosphatidylcholine | | | |
| | 180 g/kg | | 90 g/kg | | 180 g/kg | | 90 g/kg | | 180 g/kg | | 90 g/kg | |
| Diet... | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| Saturated fatty acids | | | | | | | | | | | | |
| Palmitic | 238 | 28 | 248 | 25 | 190 | 10 | 197 | 14 | 248 | 24 | 249 | 10 |
| Stearic | 165 | 89 | 205 | 8 | 238 | 9 | 236 | 4 | 209 | 19 | 199 | 10 |
| Lignoceric | 12 | 5 | 20* | 6 | 21 | 9 | 32 | 9 | 8 | 3 | 13* | 4 |
| Monounsaturated fatty acids | | | | | | | | | | | | |
| Palmitoleic | 7 | 4 | 11* | 2 | 3 | 1 | 4 | 2 | 4 | 3 | 6* | 1 |
| Oleic | 39 | 14 | 49 | 6 | 29 | 6 | 29 | 4 | 50 | 7 | 57 | 4 |
| Nervonic | 4 | 2 | 4 | 1 | ND | | ND | | 6 | 1 | 7 | 1 |
| *n*-6 Polyunsaturated fatty acids | | | | | | | | | | | | |
| Linoleic | 130 | 26 | 108* | 9 | 71 | 11 | 57* | 8 | 235 | 25 | 209* | 15 |
| γ-Linolenic | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 3 | 6 | 4 | 9 |
| DGLA | 9 | 7 | 10 | 2 | 5 | 1 | 7 | 1 | 5 | 3 | 9 | 1 |
| Arachidonic | 271 | 31 | 245* | 14 | 256 | 14 | 271 | 9 | 166 | 63 | 190 | 19 |
| *n*-3 Polyunsaturated fatty acids | | | | | | | | | | | | |
| α-Linolenic | 3 | 1 | 4 | 1 | 1 | 1 | 1 | 1 | ND | | ND | |
| EPA | 2 | 1 | 2 | 1 | 3 | 1 | 2 | 1 | 2 | 2 | 1 | 3 |
| DPA | 15 | 4 | 15 | 2 | 24 | 4 | 26 | 3 | 10 | 1 | 10 | 2 |
| DHA | 104 | 23 | 80* | 10 | 159 | 21 | 137 | 17 | 54 | 7 | 45* | 6 |
| Total (μmol/g) | 9·3 | 3·5 | 9·9 | 1·8 | 4·1 | 0·7 | 3·5 | 0·8 | | | | |
| Total (mmol/l) | | | | | | | | | 1·0 | 0·2 | 1·0 | 0·1 |

ND, not detected; DGLA, dihomo γ-linolenic acid; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid.
Mean values were significantly different between pups from mothers fed the 180 and 90 g protein/kg diets within the same phospholipid class: $^{*}P<0{\cdot}05$.
† For details of diets and procedures, see Table 1 and p. 346.

**Table 4.** Phospholipid fatty acid composition (g/kg) of rat brain phospholipids from pups (eight per group) of mothers fed either 180 or 90 g protein/kg diet during pregnancy†

(Mean values and standard deviations)

| | Phosphatidylcholine | | | | Phosphatidylethanolamine | | | |
|---|---|---|---|---|---|---|---|---|
| | 180 g/kg | | 90 g/kg | | 180 g/kg | | 90 g/kg | |
| Diet... | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| Saturated fatty acids | | | | | | | | |
| Palmitic | 399 | 37 | 436* | 23 | 124 | 19 | 126 | 27 |
| Stearic | 183 | 11 | 173 | 5 | 242 | 31 | 249 | 6 |
| Lignoceric | 21 | 2 | 21 | 4 | 55 | 10 | 65* | 5 |
| Monounsaturated fatty acids | | | | | | | | |
| Palmitoleic | 4 | 1 | 5 | 1 | 3 | 1 | 2 | 1 |
| Oleic | 194 | 13 | 182* | 6 | 165 | 43 | 121* | 10 |
| Nervonic | 12 | 7 | 6* | 2 | 1 | 2 | 1 | 1 |
| *n*-6 Polyunsaturated fatty acids | | | | | | | | |
| Linoleic | 9 | 2 | 7* | 1 | 5 | 2 | 3 | 1 |
| DGLA | 3 | 1 | 3 | 6 | 7 | 2 | 5* | 1 |
| Arachidonic | 85 | 12 | 95 | 9 | 165 | 26 | 200** | 10 |
| *n*-3 Polyunsaturated fatty acids | | | | | | | | |
| α-Linolenic | 4 | 4 | 3 | 2 | 4 | 2 | 3 | 1 |
| DPA | 1 | 1 | 1 | 1 | 2 | 3 | 2 | 3 |
| DHA | 83 | 14 | 69* | 9 | 226 | 30 | 224 | 16 |
| Total (μmol/g) | 11·4 | 2·2 | 10·1 | 0·6 | 6·0 | 0·6 | 6·0 | 0·5 |

DGLA, dihomo γ-linolenic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid.
Mean values were significantly different between pups from mothers fed the 180 and 90 g protein/kg diets within the same phospholipid class: $^{*}P<0{\cdot}05$, $^{**}P<0{\cdot}01$.
† For details of diets and procedures, see Table 1 and p. 346.

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**Table 5.** Phospholipid fatty acid composition (g/kg) of rat lung phospholipids from pups (eight per group) of mothers fed either 180 or 90 g protein/kg diet during pregnancy†
(Mean values and standard deviations)

| | Phosphatidylcholine | | | | Phosphatidylethanolamine | | | |
|---|---|---|---|---|---|---|---|---|
| | 180 g/kg | | 90 g/kg | | 180 g/kg | | 90 g/kg | |
| Diet... | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| Saturated fatty acids | | | | | | | | |
| Myristic | 32 | 2 | 31 | 3 | 17 | 7 | 7 | 7 |
| Palmitoleic | 497 | 1 | 500 | 32 | 213 | 25 | 224 | 39 |
| Stearic | 82 | 5 | 83 | 8 | 142 | 15 | 146 | 22 |
| Lignoceric | 24 | 6 | 23 | 4 | 81 | 11 | 89 | 15 |
| Monounsaturated fatty acids | | | | | | | | |
| Palmitoleic | 30 | 2 | 34** | 2 | 15 | 10 | 13 | 9 |
| Oleic | 111 | 5 | 118 | 7 | 138 | 17 | 135 | 10 |
| Nervonic | 11 | 1 | 10 | 2 | 23 | 4 | 23 | 5 |
| *n*-6 Polyunsaturated fatty acids | | | | | | | | |
| Linoleic | 80 | 5 | 75 | 5 | 55 | 7 | 55 | 8 |
| DGLA | 4 | 1 | 5** | 1 | 5 | 4 | 6 | 5 |
| Arachidonic | 109 | 15 | 98 | 25 | 255 | 30 | 248 | 67 |
| *n*-3 Polyunsaturated fatty acids | | | | | | | | |
| α-Linolenic | 1 | 1 | 1 | 1 | ND | | ND | |
| DPA | 7 | 2 | 7 | 2 | 25 | 7 | 26 | 5 |
| DHA | 11 | 3 | 15 | 14 | 32 | 20 | 27 | 6 |
| Total (μmol/g) | 5·1 | 1·1 | 5·5 | 1·5 | 2·2 | 0·5 | 2·1 | 0·4 |

ND, not detected; DGLA, dihomo γ-linolenic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid.
Mean values were significantly different between pups from mothers fed the 180 and 90 g protein/kg diets within the same phospholipid class: $^{**}P<0·01$.
† For details of diets and procedures, see Table 1 and p. 346.

studies have shown altered growth in this model. Such differences between the present and previous studies in outcome may reflect the number of animals studied.

Apolipoprotein B100 synthesis is down regulated by reduced protein consumption in human subjects (Jackson *et al.* 2001), which implies impaired VLDL secretion. However, since there was no change in plasma total plasma PC concentration in the 90 g protein/kg rat pups this suggests that lipoprotein secretion was not compromised. This is in accordance with previous results that showed no effect of the 90 g protein/kg diet on hepatic or plasma PC concentrations in adult non-pregnant or

**Table 6.** Phospholipid fatty acid composition (g/kg) of rat heart phospholipids from pups (eight per group) of mothers fed either 180 or 90 g protein/kg diet during pregnancy†
(Mean values and standard deviations)

| | Phosphatidylcholine | | | | Phosphatidylethanolamine | | | |
|---|---|---|---|---|---|---|---|---|
| | 180 g/kg | | 90 g/kg | | 180 g/kg | | 90 g/kg | |
| Diet... | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| Saturated fatty acids | | | | | | | | |
| Myristic | 2 | 1 | 3 | 1 | 7 | 6 | 14 | 15 |
| Palmitic | 204 | 11 | 210 | 22 | 123 | 75 | 168 | 99 |
| Stearic | 242 | 7 | 235 | 10 | 283 | 34 | 289 | 16 |
| Lignoceric | 24 | 2 | 22 | 2 | 41 | 7 | 43 | 12 |
| Monounsaturated fatty acids | | | | | | | | |
| Palmitoleic | 2 | 1 | 3* | 1 | 1 | 1 | 1 | 1 |
| Oleic | 21 | 3 | 35* | 17 | 24 | 12 | 18 | 12 |
| Nervonic | 38 | 7 | 30* | 5 | 77 | 35 | 59 | 37 |
| *n*-6 Polyunsaturated fatty acids | | | | | | | | |
| Linoleic | 144 | 21 | 156 | 20 | 86 | 32 | 74 | 16 |
| DGLA | 4 | 2 | 6 | 2 | 14 | 22 | 21 | 22 |
| Arachidonic | 245 | 14 | 228 | 12 | 221 | 60 | 205 | 63 |
| *n*-3 Polyunsaturated fatty acids | | | | | | | | |
| α-Linolenic | 1 | 1 | 1 | 1 | ND | | ND | |
| DPA | 20 | 3 | 20 | 3 | 25 | 11 | 24 | 15 |
| DHA | 54 | 9 | 49 | 8 | 97 | 46 | 84 | 53 |
| Total (μmol/g) | 7·4 | 0·6 | 7·8 | 1·8 | 3·3 | 1·2 | 2·8 | 1·6 |

ND, not detected; DGLA, dihomo γ-linolenic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid.
Mean values were significantly different between pups from mothers fed the 180 and 90 g protein/kg diets within the same phospholipid class: $^{*}P<0·05$.
† For details of diets and procedures, see Table 1 and p. 346.

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pregnant female rats (Burdge *et al.* 2002). This is in contrast to the effect of an 80 % restriction in dietary protein in adult rats, which increased triacylglycerol and decreased PC levels in the liver, and lower plasma triacylglycerol concentration, which suggested impaired VLDL secretion (Ristic *et al.* 1985).

Tissue growth and maturation require an adequate supply of appropriate fatty acids to support membrane synthesis. Such demands differ between tissues and time points in development (Burdge *et al.* 1993*a*,*b*; Burdge & Postle, 1995). The marked differences between the pattern of fatty acids consumed in the diet and the composition of cell membranes implies that differential selection and molecular transformations of dietary fatty acids are fundamental processes in meeting the demands of individual tissues. For example, since the only PUFA in the diet were linoleic and α-linolenic acids, the availability of longer-chain PUFA, such as arachidonic and docosahexaenoic acids, for incorporation into cell membranes was dependent entirely upon capacity for conversion of these precursors to longer-chain metabolites. Impairment of these processes during development may lead to altered membrane fatty acid composition (Ozanne *et al.* 1998) and modified tissue function. The present data show that reducing maternal protein intake during pregnancy was associated with differential alterations to the fatty acid composition of membrane phospholipids between tissues. One interpretation is that maternal dietary protein restriction during pregnancy resulted in long-term changes to mechanisms responsible for determining membrane phospholipid composition. Differences in docosahexaenoic acid concentration may, in part, reflect impaired docosahexaenoic acid supply from mother to fetus during gestation (Burdge *et al.* 2002). However, this cannot account for differential changes in the concentrations of other fatty acid differences between tissues and between phospholipid classes within a tissue. One possible explanation is that maternal protein intake during pregnancy persistently altered the specificity of phospholipid biosynthesis and/or supply of fatty acids destined for incorporation into specific diacylglycerol pools that are substrates for PC and PE synthesis. Alternatively, such changes to the phospholipid composition of fatty acids in the offspring may reflect the net effect of the maternal protein restriction on fatty acid supply both during pregnancy and lactation, together with the differential demands of individual tissues for specific fatty acids. Irrespective of the underlying mechanism, the present data show that reduced maternal protein intake during pregnancy may have persistent, potentially adverse, effects on the fatty acid composition and the function of tissues in offspring. This is supported by the observation that short-term *n*-3 PUFA deficiency during the perinatal period in rat pups resulted in hypertension at 63 d after birth (Weisinger *et al.* 2001).

In contrast to our earlier report that showed no effect of maternal dietary protein restriction on liver phospholipid composition at 20 d gestation (Burdge *et al.* 2002), the present data showed marked changes to the hepatic membrane fatty acid composition. However, this is consistent with the selective decrease in liver microsome docosahexaenoic acid concentration at 3 months of age (Ozanne *et al.* 1998). One possible explanation is that demands on hepatic lipid metabolism, in particular lipoprotein synthesis and secretion, are greater in the free-living rat compared with the fetus. Such physiological demands may reveal limitations in fatty acid metabolism that are not present in the immature tissue. This is consistent with the observation that reduced protein intake led to lower plasma docosahexaenoic acid concentration in pregnant rats, which represents a substantial nutritional and physiological challenge, but not in non-pregnant animals (Burdge *et al.* 2002). Since the liver is largely responsible for supply of PUFA within the body, decreased hepatic arachidonic and docosahexaenoic acid concentrations may reduce supply of these fatty acids to other tissues.

Feeding the 90 g protein/kg diet to pregnant rats specifically reduced the concentration of docosahexaenoic acid in fetal brain PC and PE at 20 d gestation (Burdge *et al.* 2002). However, in post-weaning pups docosahexaenoic acid concentration was lower only in the PC fraction. There were also differential changes between PC and PE in the concentrations of palmitic acid, monounsaturated fatty acids and arachidonic acid. In part, this may reflect differences in brain maturation since accumulation of PUFA and structural development occurs principally in the postnatal period (Kishimoto *et al.* 1965; Sinclair & Crawford, 1972). The greater brain PC palmitic acid and lower oleic and nervonic acid concentrations may indicate a greater proportion of *sn*-1 palmitic acid PC molecular species relative to *sn*-1 oleic and nervonic acid species, which is found in immature brain (Burdge & Postle, 1995). Since a deficit in docosahexaenoic acid assimilation is associated with reduced neurological function (Neuringer *et al.* 1984, 1986; Leat *et al.* 1986; Bourre *et al.* 1989; Connor *et al.* 1990; Reisbick *et al.* 1990, 1994; Pawlosky *et al.* 1997), one possible implication is that lower maternal protein intake during pregnancy may result in impaired neurological development. If so, impaired PUFA metabolism due to restricted protein intake either by the mother or neonate may contribute to lower docosahexaenoic acid status in malnourished human infants (Smit *et al.* 1997, 1999). Restriction of the effects of the low-protein diet to the PC fraction suggests that the impaired accumulation of docosahexaenoic acid during fetal development may have been partly offset during lactation. Feeding fish oil to neonatal monkeys deprived of *n*-3 fatty acids during gestation reversed the deficit in docosahexaenoic acid accumulation into retina, but did not impaired retinal function (Connor & Neuringer, 1988). It is possible that although the deficit in docosahexaenoic acid was reduced in the post-weaning pups in the 90 g protein/kg group, any deficit in neurological function associated with impaired accumulation during gestation may not have been completely reversed.

In tissues where there were differences between the 180 and 90 g protein/kg groups in membrane composition, there were differential effects on PC and PE fatty acid contents. Since PC and PE are derived, at least in the liver, from separate diacylglycerol pools (Burdge *et al.* 1994) and are substrates for different acyl-remodelling mechanisms (Tijburg *et al.* 1991; Burdge *et al.* 1993*a*, 1994) specific processes may differ in their susceptibility to restricted

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amino acid supply during development. Although the precise functional consequences of such differential effects on membrane composition are not known, the net effect may be to provide a less favourable environment for the activities of membrane-associated proteins. Phospholipid biosynthesis requires adequate supply of the appropriate fatty acids for incorporation into diacylglycerol substrate pools.

One possible mechanism to account for the changes to unsaturated fatty acid concentrations is alterations to desaturase activities. Reduced protein intake has been associated with increased Δ6-desaturase activity in non-pregnant adult rats (Peluffo & Brenner, 1974), but decreased Δ9, Δ6- and Δ5-desaturase activities in pregnant animals (De Thomas *et al.* 1983) and lower Δ5-desaturase activity in 3-month-old pups (Ozanne *et al.* 1998). While the capacity for fatty acid desaturation has not been determined in the present study, the restriction of the effects of the restricted maternal protein intake largely to unsaturated fatty acids is consistent with altered desaturase activities. In addition, impaired desaturase activity may explain why there was no increase in docosapentaenoic acid (22 : 5*n*-6) in tissues in which docosahexaenoic acid concentration was decreased.

The results of the present study suggest that maternal protein intake during pregnancy may alter the fatty acid composition of cell membranes; further there is evidence for differential effects for individual tissues and phospholipid pools. The precise mechanisms by which this occurs and the processes that make specific tissues or lipid pools more or less vulnerable remain to be determined. However, such differential changes to membrane phospholipid composition suggest the possibility of tissue-specific alterations to the actions of developmental and/or functional regulators, for example, by modifications to the activities of membrane proteins by altering membrane fluidity (Mitchell *et al.* 1992; Litman & Mitchell, 1996) and/or phospholipase-mediated signalling pathways (Heung & Postel, 1995; Sanchez-Pinera *et al.* 1999). If so, this may contribute to the adverse effects of poor maternal diet during pregnancy on tissue function and development in the offspring.

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