Early Growth Restriction, Membrane Phospholipid Fatty Acid Composition, and Insulin Sensitivity

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An animal model of protein restriction during pregnancy and lactation with subsequent dietary fatty acid manipulation was used to investigate the association between poor early growth, defective unsaturated fatty acid handling, and later disease. Both control and early growth–restricted animals fed a diet rich in saturated fatty acids showed a doubling of the plasma insulin levels as well as a reduced degree of unsaturation in liver and skeletal muscle membrane phospholipids compared with animals fed diets rich in unsaturated fatty acids. The skeletal muscle of early growth–restricted animals weaned onto a saturated fat diet had reduced proportions of 22:6n-3 and increased proportions of 18:1n-9. This reduction in 22:6n-3 is similar to that observed in Pima Indians, a population with a high prevalence of type 2 diabetes.

A NUMBER OF studies have shown a strong relationship between the fatty acid composition of skeletal muscle membrane phospholipids and insulin sensitivity.[1-3] One of the most striking correlations is between the ratio of arachidonic acid (20:4n-6) to cis-8,11,14-eicosatrienoic acid (20:3n-6) and insulin sensitivity.[1,3] This correlation has been interpreted as indicating a link between the activity of 5-desaturase (which is responsible for the interconversion of these fatty acids) and insulin sensitivity. In addition, Pima Indians, who have the highest prevalence of type 2 diabetes in the world, have been shown to have lower proportions of 22:6n-3 (the longest and most polyunsaturated fatty acid) in their skeletal muscle phospholipids than the Caucasian Australian population.[3] Therefore, the inability to incorporate, or perhaps generate, certain fatty acids has been suggested to predispose individuals to insulin resistance and diabetes.

It is unknown whether the associations between membrane phospholipid fatty acid composition and whole-body insulin sensitivity reflect cause or effect. A number of factors need to be clarified to resolve this issue. One of these issues is whether the 5-desaturase enzyme per se is an important regulator of insulin sensitivity. It has been suggested previously that an increased degree of membrane unsaturation is associated positively with insulin action.[1,3] It is unknown whether the mechanistic basis of this relationship is through an alteration in the physical properties of the lipid environment, which may, for example, affect insulin receptor signaling, or through specific fatty acids acting as intracellular messengers regulating genes involved in insulin sensitivity. The possibility that fatty acids or their metabolites could act as messengers controlling insulin sensitivity is strengthened by the fact that 15d-$^{12,14}PGJ_2$ (which is an arachidonic acid [20:4n-6] metabolite) is a ligand of the peroxisome proliferator-activated receptor (PPAR),[4] which is also postulated to be the receptor of the new antidiabetic drugs, the thiazolidinediones.[4,5]

Indexes of poor fetal and infant growth have been associated with the development of insulin resistance and type 2 diabetes later in life.[6,7] Protein restriction during pregnancy and lactation in the rat results in early growth restriction of the offspring.[8] These offspring have been shown previously to have defective unsaturated fatty acid metabolism (including reduced 5-desaturase activity) and possibly an inability to incorporate certain fatty acids into membranes.[9] However, in these studies the adult diet used (LAD1) contained arachidonic acid (20:4n-6), the product of 5-desaturase enzyme. It was speculated that these early growth–restricted animals would be more susceptible to insulin resistance if they were fed diets that lacked 20:4n-6 and other long-chain polyunsaturated fatty acids. The present study was designed to test this possibility.

In the present study, offspring of mothers who were fed a control or low-protein diet during pregnancy and lactation were weaned onto 1 of 3 different diets that varied specifically in their fatty acid composition. These adult diets were all low in fat content (10% of calories as fat); thus the aim was to study the quality of fat rather than the quantity. As descibed above, the LAD1 diet contained 20:4n-6 and was the only one to do so. It also contained other polyunsaturated fatty acids such as 18:2n-6, 18:3n-3, and 22:5n-3. Compared with the LAD1 diet, the EFA diet had higher levels of 18:2n-6, lower levels of 18:3n-3, and no 22:5n-3. Because n-6 and n-3 fatty acids compete for the same desaturase and elongase enzymes,[10-14] membranes of the EFA groups would be expected to have increased n-6 but decreased n-3 fatty acids compared with LAD1. Finally, we used a diet called SAT that was rich in saturated fatty acids and had only minute levels of 18:2n-6. Such a diet would be expected to reduce the proportions of the n-6 and n-3 series of polyunsaturated fatty acids in membrane phospholipids, whereas those of n-9 should increase.

By these measures, it should be possible to determine whether the membrane long-chain fatty acid composition is a major determinant of insulin sensitivity and whether any such effect interacted with any effect of early growth restriction.

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MATERIALS AND METHODS

Materials

Analytical grade biochemicals and solvents were obtained from Sigma-Aldrich Company Ltd and BDH Biochemicals (both Dorset, England). [1-^{14}C]8,11,14-eicosatrienoic acid (47 mCi/mmol) was obtained from Dupont Ltd (Hertfordshire, England). [1-^{14}C]Arachidonic acid (56 mCi/mmol) was obtained from Amersham Life Science Ltd (Buckinghamshire, England). Microcuvettes for the measurement of blood glucose were purchased from Hemocue Ltd (Sheffield, England).

Synthetic Rat Diets

Five synthetic rat diets were used during the course of the study. The 8% and 20% protein diets given to pregnant and lactating female rats and the adult diets EFA and SAT were purchased from Hope Farm (Woerden, The Netherlands). Their nutrient and fatty acid compositions are shown in Tables 1 and 2, respectively. The LAD1 adult diet was purchased from Special Diet Services (Essex, England); Tables 1 and 2 show its nutrient and fatty acid composition, respectively.

Animals

Virgin female Sprague-Dawley rats (Charles River Ltd, Kent, England) were mated when they weighed 240 to 255 g. After mating (when a vaginal plug was expelled), mothers were fed either a 20% (wt/wt) protein diet (n = 29; control) or an isocaloric 8% (wt/wt) protein diet (n = 27; low protein). Rats were maintained on these diets throughout the gestational and suckling periods and were allowed to feed ad libitum.

Offspring were born at approximately day 21 of pregnancy, and the litter size was recorded. On day 2 after birth, litter size was standardized to 8 (2 female and 6 male). For simplicity, the male offspring of mothers fed a control or low-protein diet during pregnancy and lactation are referred to as control and low protein, respectively. When the male offspring reached 28 days of age, they were weaned onto 1 of 3 different diets (LAD1, EFA, SAT) whose nutrient and fatty acid compositions are described above. The rats were maintained on their respective diets and allowed to eat ad libitum throughout the study. At 3 months of age (ie, after 8 weeks of eating their respective diets), animals were starved overnight (18 hours) before procedures were begun.

Blood Sampling and Plasma Lipid/Hormone Assays

Blood was collected from the tail vein for the measurement of plasma insulin, total cholesterol, non-esterified fatty acids (NEFAs), and triacylglycerols. Blood glucose was measured using a Hemocue glucose analyzer. Insulin was measured by radioimmunoassay (RIA) using a Linco rat insulin kit (Biogenesis Ltd, Dorset, England). Plasma triacylglycerols[15] and total cholesterol[16] were measured using kits purchased from Sigma-Aldrich Company Ltd. NEFA concentrations[17,18] were measured using a kit purchased from Boehringer Mannheim (East Sussex, England).

Tissue Collection and Liver Microsomal Preparation

Liver and muscle (tibilalis anterior) tissues were removed and weighed immediately after the animals were killed by cervical dislocation in accordance with the 1986 British Home Office Animals Act. Tibialis anterior muscle (0.4 g) was frozen immediately in liquid nitrogen and stored at −70°C for further analysis. Livers were rinsed with ice-cold homogenization buffer (0.25 mol/L sucrose, 1 mmol/L EDTA, 0.1 mmol/L KH_2PO_4, pH 7.2), minced finely on an ice-cold petri dish and homogenized with buffer (5 volumes). Liver microsomes were prepared by differential centrifugation as previously described.[9] The protein concentration of liver microsomes was measured by a modification of the method of Lowry et al (1951)[19] (bicinchoninic acid kit for protein determination; Sigma-Aldrich Company Ltd).

5-Desaturase Assay and Tissue Membrane Phospholipid Fatty Acid Composition

The activity of 5-desaturase activity was determined as described previously,[20] by measuring the conversion of [1-^{14}C]cis-8,11,14-eicosatrienoic acid to [1-^{14}C]arachidonic acid. Incubations were performed at 37°C for 20 minutes using 0.5 mg of microsomal protein and 150 nmol of [1-^{14}C]cis-8,11,14-eicosatrienoic acid (1 Ci) in 1 mL incubation volume. The incubation mixture consisted of 0.5 mmol/L nicotinamide, 1.5 mmol/L glutathione, 1.0 mmol/L NADH, 0.25 mmol/L CoA, 5 mmol/L $MgCl_2$, 5 mmol/L adenosine triphosphate, and 1 mg bovine serum albumin in homogenization buffer. The reaction was terminated by the addition of 1 mL of 10 % (wt/vol) KOH in methanol. Lipids were then saponified and acidified with 6 mol/L HCl. The fatty acids were extracted with hexane, methylated with 14% (wt/wt) BF_3/methanol, and dissolved in chloroform. Samples were then spotted onto 10% silver nitrate–impregnated silica gel thin-layer chromatography plates (BDH Biochemicals, Poole, Dorset, England) with known standards, and the plates were developed in toluene:acetonitrile (97.5:2.5) as described previously.[21] Radiolabeled fatty acid methyl esters were identified by autoradiography and quantified by liquid scintillation counting.

The fatty acid components of liver microsomes and muscle phospholipids were extracted and derivatized as described previously.[9]

Statistical Analyses

Results were compared using 2-way analysis of variance (ANOVA) or Student *t* test when there were only 2 groups. In the ANOVA, effects associated with the maternal diet and the adult diet were used as independent variables. Post hoc comparisons were performed using the Sheffe test. If the data were not normally distributed, they were log-transformed before analyses to allow the appropriate use of parametric statistical analysis. A P value of $<.05$ was considered statistically significant. Correlations were assessed using linear regression and are shown with the Pearson correlation coefficient.

Table 1. Nutrient Composition of Experimental Diets

	Maternal Diet		Adult Diet		
	8% Protein	20% Protein	LAD1	EFA	SAT
Protein	8.1 (8.8)	19.8 (21.3)	21.3 (22.7)	19.6 (19.7)	19.6 (19.7)
Fat	4.4 (10.8)	4.5 (10.9)	3.3 (7.9)	5.2 (11.8)	5.2 (11.8)
Carbohydrate	70.3 (76.5)	59.3 (63.7)	58.3 (62.1)	62.7 (63.2)	62.7 (63.2)
Gross energy (kcal/kg)	3,674.0	3,722.7	3,755.9	3,970.1	3,970.1

NOTE. Values are expressed in g/100 g of diet. The caloric percentage of each nutrient is in parentheses.

Table 2. Fatty Acid Composition of Experimental Diets

	Maternal Diet		Adult Diet		
	8% Protein	20% Protein	LAD1	EFA	SAT
Saturated					
C8:0-C12:0	—	—	0.05	—	2.96
C14:0 myristic acid	—	—	0.13	—	0.92
C16:0 palmitic acid	0.45	0.45	0.25	0.55	0.47
C18:0 stearic acid	—	—	0.02	0.10	0.47
Monounsaturated					
C14:1 myristoleic acid	—	—	0.04	—	—
C16:1 n-7 palmitoleic acid	—	—	0.27	—	—
C18:1 n-9 oleic acid	0.89	0.89	0.86	1.28	0.05
Polyunsaturated					
C18:2 n-6 linoleic acid	2.20	2.20	0.71	3.00	0.10
C18:3 n-3 α-linolenic acid	0.33	0.33	0.08	0.05	—
C20:4 n-6 arachidonic acid	—	—	0.23	—	—
C22:5 n-3 clupanodonic acid	—	—	0.06	—	—

NOTE. Values are expressed in g/100 g of diet. Missing data indicate that the fatty acid was not detectable.

RESULTS

Body Measurements

There were no differences in litter size between the mothers fed the control and low-protein diets during pregnancy: control, 13.9 (SEM 0.4) pups per litter (n = 29); low protein, 14.3 (SEM 0.4) pups per litter (n = 27). Mean pup weights on day 2 after birth and at weaning (age 28 days) were significantly reduced in the mothers fed the low-protein diet compared with controls (Table 3). At age 3 months, after weaning on a normal-protein diet, the low-protein animals still had lower body weights than their corresponding controls, irrespective of their adult diet (Table 3).

Plasma Glucose, Insulin, and Lipids

Neither the maternal diet (ie, control or low protein) nor the adult diet (ie, LAD1, EFA, SAT) had any detectable effects on plasma glucose, triacylglycerol, or NEFA concentrations (Table 4). The low-protein animals had significantly lower plasma insulin concentrations than the controls ($P < .001$; Table 4). The adult diet had a significant effect on fasting plasma insulin levels, and the SAT groups had the highest insulin concentrations ($P < .01$). The maternal diet had no detectable effect on the plasma total cholesterol concentrations (Table 4). The adult diet had a significant effect on the plasma cholesterol concentrations; the EFA groups had the highest levels, followed by the SAT and then the LAD1 groups ($P < .001$). No interactions were detected between any of the independent variables on any plasma measurements.

Liver and Skeletal Muscle Membrane Phospholipid Fatty Acid Composition

Effects of Maternal Diet

Muscle and liver phospholipid fatty acid composition data are shown in Tables 5 and 6. The type of maternal diet was not associated with any changes in liver membrane phospholipid fatty acid composition. Effects of the maternal diet were detected in skeletal muscle; the low-protein groups had decreased proportions of 16:0 ($P < .01$), sum of saturated fatty acids ($P < .05$), 22:6n-3 ($P < .01$), and sum of n-3 fatty acids ($P < .01$) and increased proportions of 18:1n-9 ($P < .01$), 22:5n-6 ($P < .01$), and sum of n-6 fatty acids ($P < .01$). The raised proportions of 18:1n-9 and decreased proportions of 22:6n-3 and the sum of n-3 fatty acids were only apparent in the low-protein groups fed the SAT diets (interaction between effects associated with maternal and adult diets for 18:1n-9, $P < .01$; for 22:6n-3 and total n-3, $P < .05$; comparison between control and low-protein SAT-fed animals for 22:6n-3 and total n-3, $P < .05$; for 18:1n-9, $P < .01$). The increased proportions of

Table 3. Body Weights of 2-Day-, 28-Day-, and 3-Month-Old Rats

	Control			Low Protein		
Day 2*	6.94 (0.17) (n = 29)			6.21 (0.14) (n = 27)		
Day 28*	80.99 (1.75) (n = 29)			41.59 (1.42) (n = 27)		
	LAD1	EFA	SAT	LAD1	EFA	SAT
3 mo† (n = 8–10)	423.8 (6.0)	414.8 (13.3)	391.7 (13.9)	321.4 (7.6)	322.6 (12.4)	305.2 (12.8)

NOTE. Values are mean (SEM).

*Statistical significance of effect (Student *t* test), $P < .001$.

†Statistical significance of effect of maternal diet (2-way ANOVA), $P < .001$.

Table 4. Plasma Measurements of Fasted 3-Month-Old Rats

	Control			Low Protein		
	LAD1	EFA	SAT	LAD1	EFA	SAT
Glucose (mmol/L)	5.5 (5.0, 6.2)	5.3 (4.7, 6.0)	5.1 (4.6, 5.8)	5.4 (4.8, 6.0)	5.5 (4.9, 6.3)	5.2 (4.7, 5.6)
Insulin (pmol/L)*,†	111.7 (62.7, 199.1)	136.8 (86.3, 216.8)	237.7 (149.8, 378.4)	57.0 (38.2, 85.1)	71.5 (40.9, 124.7)	113.9 (71.0, 182.8)
Triacylglycerols (mmol/L)	0.38 (0.29, 0.50)	0.28 (0.19, 0.43)	0.24 (0.18, 0.33)	0.30 (0.19, 0.47)	0.28 (0.19, 0.42)	0.27 (0.19, 0.40)
Cholesterol (mmol/L)‡	1.5 (1.2, 1.8)	2.5 (2.2, 3.0)	2.2 (2.1, 2.4)	1.7 (1.3, 2.2)	2.4 (2.0, 2.8)	1.9 (1.7, 2.3)
NEFA (μmol/L)	1,191 (985, 1.439)	1,018 (768, 1,331)	1,250 (1,029, 1,518)	1,042 (812, 1,337)	1,018 (766, 1,354)	1,380 (1,196, 1,592)

NOTE. Values are geometric means (95% confidence intervals); n = 8 to 10 per group).

*Statistical significance of effect of maternal diet (2-way ANOVA), $P < .001$.

†Statistical significance of effect of adult diet (2-way ANOVA), $P < .01$.

‡Statistical significance of effect of adult diet (2-way ANOVA), $P < .001$.

the sum of n-6 fatty acids were apparent only in the low-protein groups fed the EFA diets (interaction between effects associated with maternal and adult diets, $P < .05$; comparison between control and low-protein EFA-fed animals, $P < .05$).

Effects of Adult Diet

Saturated fatty acids. In liver, the animals fed the LAD1 diet had increased proportions of 16:0 and decreased proportions of 18:0, followed by the EFA and then the SAT groups (both $P < .001$). Therefore, no dietary effect was detected on the sum of saturated fatty acids. In muscle, no dietary effect was detected in the proportions or the sum of saturated fatty acids.

Monounsaturated fatty acids and n-9 polyunsaturated fatty acids. In both liver and muscle phospholipids, the proportions of 16:1, 18:1n-9, and 20:3n-9 were increased in the animals fed the SAT diet compared with those fed the LAD1 and EFA diets (all $P < .001$).

Table 5. Liver Microsomal Membrane Phospholipid Fatty Acid Composition and Δ5-Desaturase Activity in 3-Month-Old Rats

	Control			Low Protein		
	LAD1	EFA	SAT	LAD1	EFA	SAT
16:0*	18.63 (0.39)	17.64 (0.28)	17.37 (0.38)	19.02 (0.46)	18.28 (0.38)	16.53 (0.50)
18:0*	22.94 (0.71)	23.74 (0.66)	26.19 (0.65)	22.55 (0.80)	23.88 (0.61)	25.49 (0.59)
Total SAT	42.23 (0.65)	42.12 (0.65)	44.24 (0.70)	42.44 (1.19)	43.04 (0.67)	42.69 (0.85)
16:1*	1.02 (0.11)	0.61 (0.12)	1.78 (0.15)	0.76 (0.14)	0.60 (0.14)	1.56 (0.21)
18;1 n-9*	5.87 (0.49)	5.71 (0.45)	9.77 (0.41)	5.73 (0.35)	5.83 (0.34)	10.92 (0.49)
20:3 n-9*	0.26 (0.02)	0.22 (0.02)	7.08 (0.62)	0.22 (0.03)	0.20 (0.04)	7.57 (0.35)
18:2 n-6*	11.22 (0.42)	8.28 (0.40)	3.83 (0.20)	11.62 (0.40)	8.63 (0.30)	3.88 (0.15)
20:3 n-6*	0.82 (0.11)	0.25 (0.03)	1.06 (0.09)	0.79 (0.09)	0.27 (0.02)	1.00 (0.07)
20:4 n-6*	26.50 (0.63)	34.53 (0.32)	21.39 (0.55)	26.42 (0.51)	33.07 (0.57)	22.10 (0.34)
22:5 n-6*	0.39 (0.04)	3.06 (0.33)	4.01 (0.36)	0.38 (0.06)	3.28 (0.24)	4.00 (0.26)
20:4 n-6/20:3 n-6*	37.38 (5.10)	147.58 (11.35)	21.46 (1.86)	36.67 (4.21)	125.87 (6.66)	22.69 (1.28)
Total n-6*	39.44 (0.42)	47.07 (0.30)	30.76 (0.49)	39.63 (0.77)	46.19 (0.50)	31.52 (0.45)
20:5 n-3*	0.76 (0.05)	0.03 (0.02)	0.19 (0.03)	0.72 (0.10)	0.16 (0.10)	0.16 (0.03)
22:5 n-3*	1.15 (0.05)	0.42 (0.04)	0.33 (0.04)	1.19 (0.06)	0.36 (0.04)	0.31 (0.03)
22:6 n-3*	9.12 (0.31)	3.73 (0.18)	5.63 (0.11)	9.15 (0.32)	3.48 (0.28)	5.10 (0.18)
22:6 n-3/22:5 n-3*	8.01 (0.41)	9.34 (0.30)	19.12 (2.13)	7.92 (0.33)	10.64 (1.89)	17.86 (1.77)
Total n-3*	11.18 (0.35)	4.26 (0.19)	6.33 (0.09)	11.19 (0.33)	4.13 (0.28)	5.74 (0.17)
Unsaturation index*	207.0 (1.3)	206.2 (1.6)	187.9 (2.0)	206.7 (3.8)	201.3 (2.4)	189.7 (3.0)
Δ5-Desaturase (pmol/min/mg)*,†,‡	81.8 (5.1)	61.6 (1.9)	75.0 (4.4)	113.4 (9.2)	78.4 (9.8)	71.0 (5.1)

NOTE. Results are expressed as percentage of total fatty acids. Values are mean (SEM); n = 8 to 10 per group.

*Statistical significance of effect of adult diet (2-way ANOVA), $P < .001$.

†Statistical significance of effect of maternal diet (2-way ANOVA), $P < .01$.

‡Statistical significance of effect of interaction of maternal diet and adult diet (2-way ANOVA), $P < .05$.

Table 6. Skeletal Muscle (Tibialis Anterior) Membrane Phospholipid Fatty Acid Composition in 3-Month-Old Rats

	Control			Low Protein		
	LAD1	EFA	SAT	LAD1	EFA	SAT
16:0*	21.87 (0.32)	21.27 (0.43)	20.50 (0.43)	20.51 (0.39)	19.94 (0.42)	20.10 (0.42)
18:0	14.75 (0.73)	15.47 (0.75)	14.66 (0.60)	13.95 (0.63)	14.25 (0.53)	14.39 (0.88)
Total SAT†	38.21 (1.06)	38.30 (1.28)	37.13 (0.90)	36.16 (0.93)	35.55 (0.71)	35.74 (1.14)
16:1‡	1.14 (0.23)	1.08 (0.19)	4.91 (0.36)	1.35 (0.23)	0.90 (0.19)	5.42 (0.33)
18:1 n-9*,‡,§	8.14 (0.30)	8.31 (0.37)	18.59 (0.52)	7.90 (0.30)	8.72 (0.26)	21.24 (0.59)
20:3 n-9‡	0.11 (0.03)	0.18 (0.03)	4.93 (0.48)	0.12 (0.03)	0.15 (0.04)	5.60 (0.27)
18:2 n-6‡	16.05 (0.56)	16.01 (0.32)	10.45 (0.63)	16.63 (0.58)	17.40 (0.65)	9.82 (0.35)
20:3 n-6‡	0.62 (0.03)	0.53 (0.04)	0.76 (0.06)	0.62 (0.02)	0.53 (0.03)	0.78 (0.08)
20:4 n-6‡	10.68 (0.34)	22.01 (0.64)	13.43 (0.37)	11.14 (0.25)	22.77 (0.33)	13.31 (0.24)
22:5 n-6*,‡	0.33 (0.05)	4.69 (0.36)	1.94 (0.11)	0.83 (0.44)	6.02 (0.28)	2.17 (0.10)
20:4 n-6/20:3 n-6‡	17.42 (0.93)	43.79 (3.82)	18.82 (2.12)	17.97 (0.55)	44.05 (2.32)	19.74 (3.46)
Total n-6*,‡,§	27.88 (0.64)	45.13 (0.78)	27.49 (1.03)	29.64 (0.60)	48.53 (0.58)	26.97 (0.48)
20:5 n-3‡	0.63 (0.02)	0.03 (0.02)	0.15 (0.05)	0.69 (0.03)	0.01 (0.01)	0.14 (0.02)
22:5 n-3‡	2.53 (0.09)	1.66 (0.34)	1.03 (0.04)	2.58 (0.06)	1.30 (0.07)	0.76 (0.03)
22:6 n-3*,‡,§	21.15 (0.39)	5.45 (0.16)	5.63 (0.17)	21.20 (0.44)	4.74 (0.32)	3.98 (0.14)
22:6 n-3/22:5 n-3‡	8.48 (0.38)	4.22 (0.30)	5.54 (0.28)	8.25 (0.27)	3.68 (0.22)	5.28 (0.22)
Total n-3*,‡,§	24.50 (0.41)	6.90 (0.20)	6.90 (0.18)	24.62 (0.43)	6.10 (0.37)	5.19 (0.26)
UI‡	231.7 (3.3)	202.0 (4.2)	168.1 (2.5)	239.0 (3.2)	210.4 (2.3)	167.1 (6.7)

NOTE. Results are expressed as percentage of total fatty acids. Values are the mean (SEM); n = 8 to 10 per group.

*Statistical significance of effect of maternal diet (2-way ANOVA), $P < .01$.

†Statistical significance of effect of maternal diet (2-way ANOVA), $P < .05$.

‡Statistical significance of effect of adult diet (2-way ANOVA), $P < .001$.

§Statistical significance of effect of interaction of maternal diet and adult diet (2-way ANOVA), $P < .05$.

n-6 Polyunsaturated fatty acids:

Liver. The animals fed the LAD1 diet had the greatest proportions of 18:2n-6 and the lowest proportions of 22:5n-6, followed by the EFA and then the SAT groups (both $P < .001$). The animals fed the EFA diet had the lowest proportions of 20:3n-6 and the highest proportions of 20:4n-6, followed by the LAD1 and then the SAT groups (both $P < .001$). Therefore, the ratio of 20:4n-6 to 20:3n-6 (a possible index of 5-desaturase activity) was increased in the animals fed the EFA diet, followed by the LAD1 and then the SAT groups ($P < .001$). The sum of the proportion of n-6 fatty acids was also greatest in the animals fed the EFA diet, followed by the LAD1 and then the SAT groups ($P < .001$).

Muscle. The animals fed the SAT diet had lower proportions of 18:2n-6 than the LAD1 and EFA groups ($P < .001$). The animals fed the EFA diet had the lowest proportions of 20:3n-6, followed by the LAD1 and then the SAT groups ($P < .001$). The proportions of both 20:4n-6 and 22:5n-6 were highest in the groups fed the EFA diet, followed by the SAT and then the LAD1 groups (both $P < .001$). The animals fed the EFA diet also had increased ratios of 20:4n-6 to 20:3n-6, and the sum of the proportions of n-6 fatty acids compared with the LAD1 and SAT groups (both $P < .001$).

n-3 Polyunsaturated fatty acids and unsaturation index:

Liver. The proportions of both 20:5n-3 and 22:5n-3 were increased in the groups fed the LAD1 diet compared with the EFA and SAT groups (both $P < .001$). The animals fed the LAD1 diet also showed increased proportions of 22:6n-3 and sum of the proportions of n-3 fatty acids, followed by the SAT and then the EFA groups (both $P < .001$). The ratio of 22:6n-3 to 22:5n-3 was increased in the groups fed the SAT diet, followed by the EFA and then the LAD1 groups ($P < 0.001$). This ratio was traditionally considered to be an index of 4-desaturase activity. However, more recently it has been suggested that a specific 4-desaturase enzyme probably does not exist, and the conversion of 22:5n-3 to 22:6n-3 involves multiple steps.[22] The animals fed the SAT diet also had reduced unsaturation indexes (number of double bonds per 100 fatty acid molecules), followed by the EFA and then the LAD1 groups ($P < .001$).

Muscle. The animal groups fed the LAD1 diet had increased proportions of 20:5n-3, 22:6n-3, and sum of n-3 fatty acids compared with the EFA and SAT groups (all $P < .001$). The animal groups fed the LAD1 diet also had increased proportions of 22:5n-3, followed by the EFA and then the SAT groups ($P < .001$). The ratio of 22:6n-3 to 22:5n-3 was also increased in animals fed the LAD1 diet, followed by the SAT and then the EFA groups ($P < .001$). The animals fed the SAT diet had reduced unsaturation indexes, followed by the EFA and then the LAD1 groups ($P < .001$).

In summary, the different adult diets (LAD1, EFA, and SAT) increased the proportions of fatty acids in membrane phospholipids from each of the 3 different polyunsaturated fatty acid families. The LAD1 groups showed increased proportions of the n-3 series of polyunsaturated fatty acids (ie, 22:6n-3), and the EFA groups showed increased proportions of the n-6 series (ie, 20:4n-6). The SAT groups showed increased proportions of the n-9 series (18:1n-9 and 20:3n-9) but reduced proportions of the n-3 series compared with the LAD1 groups and reduced proportions of the n-6 series compared with the EFA groups.

Hepatic 5-desaturase activity. The liver Δ5-desaturase activities in 3-month-old rats are shown in Table 5. Overall, the low-protein animals had significantly increased Δ5-desaturase activities compared with controls ($P < .01$), largely because of the trend for increased activities in LAD1-fed animals (interaction between effects associated with maternal and adult diets, $P < .05$; comparison between control and low-protein LAD1-fed animals, $P = .06$). The adult diet also had a significant independent effect on the Δ5-desaturase activities, with the LAD1 groups having the highest levels ($P < .001$).

Associations between ratio of 20:4n-6/20:3n-6 (product to precursor ratio catalyzed by 5-desaturase) in liver microsomes, proportion of 20:4n-6 in liver microsomes, fasting plasma insulin levels, and microsomal 5-desaturase activity. Within each study group, the activity of the liver microsomal 5-desaturase enzyme was compared with the ratio of 20:4n-6 to 20:3n-6 in liver microsomal phospholipids, the proportion of 20:4n-6 in liver microsomal phospholipids, and fasting plasma insulin levels. No consistent significant correlations were found between measured and deduced 5-desaturase activity (20:4n-6 or ratio of 20:4n-6 to 20:3n-6 ratio) in all groups. A weak inverse relationship was found between the proportion of 20:4n-6 and measured 5-desaturase activity in the low-protein group fed the LAD1 diet ($P = .02$). No significant correlations were found between measured 5-desaturase activity and fasting plasma insulin levels in any of the groups.

DISCUSSION

An animal model of protein restriction during pregnancy and lactation with subsequent dietary fatty acid manipulation has been used to investigate (1) the possible association between poor early growth and defective unsaturated fatty acid handling and (2) the association between membrane phospholipid fatty acid composition and insulin sensitivity and the involvement of the 5-desaturase enzyme.

Effect of the Maternal and Adult Diets on the Membrane Phospholipid Fatty Acid Composition and 5-Desaturase Activity

Previous studies have shown that in the rat, the liver membrane phospholipid fatty acid composition can change within 6 days of dietary fatty acid manipulation.[23] In the present study, the proportions of oleic acid (18:1n-9) and mead acid (20:3n-9) increased dramatically in the membrane phospholipids of animals weaned onto a diet containing almost exclusively saturated fatty acids. This finding is consistent with previous studies showing that mead acid accumulates in tissue membranes of animals fed an essential fatty acid–deficient diet.[24,25] The EFA groups had increased proportions of 20:4n-6 levels, and the LAD1 groups had increased proportions of 22:6n-3.

The fatty acids 20:3n-9, 20:4n-6, and 22:6n-3 belong to different families of unsaturated fatty acids. Thus, the different adult diets increased the proportions of fatty acids from each of the 3 different polyunsaturated fatty acid families. Fatty acids from each family compete for the same elongase and desaturase enzymes, which have preference for n-3 fatty acids over n-6 and n-6 over n-9 fatty acids.[10-14] The type of unsaturated fatty acids, not than the sum of the proportions of saturated fatty acids in the tissue membrane phospholipids, changed within the adult dietary groups (LAD1, EFA, SAT). This was reflected in the unsaturation index of membranes, which were highest in the LAD1 groups, followed by the EFA and then the SAT groups.

Maternal diet was associated with a number of significant effects on muscle membrane phospholipid fatty acid composition. The low-protein animals had increased proportions of 18:1n-9 but reduced proportions of 22:6n-3. These differences were most pronounced in animals fed the SAT diet. This is consistent with a previous study suggesting that low-protein animals may have a defective ability to generate or incorporate 22:6n-3 in their membranes and that this difference is most apparent when this fatty acid is not present in the diet.[9] This is interesting in light of the finding that Pima Indians (a population with the highest prevalence of type 2 diabetes in the world) have reduced proportions of 22:6n-3 in their skeletal muscle membranes.[3]

The hepatic Δ5-desaturase activity measured in this study was increased in the low-protein animals compared with controls, especially in those fed the LAD1 diet. The type of adult diet also had an effect on the activity of the 5-desaturase enzyme, being highest in the LAD1 groups. This is consistent with previous animal studies showing that diets rich in 18:2n-6 (similar to the EFA diet) and diets rich in saturated fatty acids (similar to the SAT diet) decrease the activity of the 5-desaturase enzyme.[26,27] The increased Δ5-desaturase activity seen in the low-protein animals contrasts with the results of a previous study by Ozanne et al (1998)[9], which found decreased hepatic Δ5-desaturase activity in the low-protein animals. The discrepancy may be attributable to the different rat strains used in the studies. Our study used Sprague-Dawley rats, and Ozanne et al used Wistar rats. It is known that Sprague-Dawley rats have reduced Δ5-desaturase activity compared with Wistar rats.[28] Consistent with this observation, measured 5-desaturase activity was lower in this current study than in the previous study. Further, it has also been suggested that because the desaturase activities and the microsomal fatty acid composition are different in each rat strain, the lipid metabolic response to different stimuli (such as disease, hormones, and dietary treatment) may also be dependent on strain.[28]

Relationship Between Measured and Deduced 5-Desaturase Activity

In the present study, microsomal Δ5-desaturase activity did not correlate with the ratio of 20:4n-6 to 0:3n-6 in liver microsomal membrane phospholipids or the proportion of 20:4n-6 within each dietary group. In addition, the highest 5-desaturase activity was seen in the LAD1 groups, whereas the highest ratios of 20:4n-6 to 20:3n-6 in both liver and skeletal muscle membrane phospholipids were seen in the EFA groups. In contrast, the LAD1 groups had both the greatest proportion of 20:5n-3 (product of 5-desaturase from the n-3 pathway) and the greatest measured 5-desaturase activity. These findings are consistent with previous studies, some of which show that desaturase activity influences phospholipid composition[29,30] and some of which suggest they play a minor role.[28]

Relationship Between Dietary Factors, Membrane Phospholipid Fatty Acid Composition, and Fasting Plasma Insulin Levels

Consistent with previous studies, animals that were weaned onto the diet rich in saturated fatty acids (SAT) had increased fasting plasma insulin levels compared with animals fed the diets rich in unsaturated fatty acids (EFA and LAD1).[31-33] This finding suggests that the SAT diet results in some degree of insulin resistance. In agreement with studies that have shown a positive correlation between the degree of membrane unsaturation and insulin sensitivity,[1,3] in the present study animals that were weaned onto the SAT diet had the lowest degree of membrane unsaturation. The diets that were used in the present study were low in fat, constituting approximately 10% of calories, compared with 40% to 60% in similar studies reported so far. This suggests that the quality of fat has important consequences for insulin sensitivity, even if it is consumed in low quantities. The type of adult diet had no effect on levels of plasma lipids, with the exception of cholesterol. Plasma cholesterol levels were highest in the EFA groups, followed by the SAT and then the LAD1 groups. This is consistent with findings of previous animal studies, in which diets rich in 18:2n-6 resulted in increased plasma cholesterol levels.[34-37]

In this study, a range of 20:4n-6/20:3n-6 ratios were achieved without the changes in insulin sensitivity that would be expected from the results of human epidemiologic studies. For example, the EFA groups, which had 5- to 10-fold higher ratios of 20:4n-6 to 20:3n-6 in their membrane phospholipids than the other dietary groups, appeared not to be supersensitive to insulin compared with the other dietary groups. This lack of consistency with the human epidemiologic studies could be attributed to a number of reasons: (1) differences between rats and humans; (2) greater ratios of 20:4n-6 to 20:3n-6 created in membrane phospholipids in this study (which varied from 12 to 150) than those described in the human studies (which were in the range 6 to 12)[1,3] and the possibility such ratio are determining factors of insulin sensitivity only when they are below a certain value; and (3) the possibility that the membrane phospholipid fatty acid composition only influences insulin sensitivity when tissues such as skeletal muscle are loaded with triglycerides because the diets used in this study were of low fat content.

At this age (3 months), no interaction was detected between maternal diet and adult diet with respect to fasting plasma insulin levels. If early growth–restricted animals had a defect in unsaturated fatty acid metabolism or incorporation into membranes, it would have been predicted that early growth–restricted animals fed the EFA diet and SAT diet would show signs of deterioration of insulin sensitivity. These results suggest that maternal protein restriction in the rat results in changes in the membrane phospholipid fatty acid composition that are associated with insulin resistance in humans. However, no evidence of insulin resistance was apparent in the early growth–restricted rats, suggesting that such changes in fatty acid composition are not sufficient to precipitate insulin resistance.

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