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Effect of sex and dietary fat intake on the fatty acid composition of phospholipids and triacylglycerol in rat heart

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ABSTRACT

Variations in the fatty acid composition of lipids in the heart alter its function and susceptibility to ischaemic injury. We investigated the effect of sex and dietary fat intake on the fatty acid composition of phospholipids and triacylglycerol in rat heart. Rats were fed either 40 or 100 g/kg fat (9:1 lard:soybean oil) from weaning until day 105. There were significant interactive effects of sex and fat intake on the proportions of fatty acids in heart phospholipids, dependent on phospholipid classes. 20:4n-6, but not 22:6n-3, was higher in phospholipids in females than males fed a low, but not a high, fat diet. There was no effect of sex on the composition of triacylglycerol. These findings suggest that sex is an important factor in determining the incorporation of dietary fatty acids into cardiac lipids. This may have implications for sex differences in susceptibility to heart disease.

1. Introduction

The fatty acid composition of membranes is an important determinant of cell function. The relative proportions of the saturated, monounsaturated and long chain polyunsaturated fatty acids (PUFA), including arachidonic acid (20:4n-6) and docosahexaenoic acid (22:6n-3) [1,2], modify the biophysical properties of membranes and so change the activities of integral proteins. In addition, variation in phospholipid fatty acid composition may alter cell function by changing the composition of phospholipid-derived second messengers. Membrane phospholipid composition is the product of the specificity of synthesis *de novo*, acyl remodelling mechanisms and differential turnover of individual molecular species [3]. Together these processes maintain phospholipid class and cell type-specific differences in fatty acid composition.

A number of recent studies in humans and in animal models have shown that males and females differ in the composition of membrane and plasma phospholipids. For example, women have higher concentrations of 22:6n-3 in plasma phospholipids than men [4–7]. Female rats also have higher phospholipid 22:6n-3 in liver and plasma [8–10], and in erythrocytes [11] than males. The 20:4n-6 content of rat liver phosphatidylcholine (PC) was also higher in females, but decreased following ooectomy, while administration of oestrogen following orchidectomy to males increased hepatic PC 20:4n-6 [12]. Furthermore, pregnancy in rats was associated with a specific increase in liver PC16:0/22:6 due to decreased acyl remodelling of newly synthesised PC [13]. 20:4n-6 and 22:6n-3, which are substrates for synthesis of more complex lipids, can be synthesised from their respective essential fatty acid precursors 18:2n-6 and 18:3n-3 by sequential desaturation and carbon chain elongation reactions [14]. In humans, conversion of 18:3n-3 to 22:6n-3 is very limited in men, but substantially higher in women [15,16] and is greater still in women using an oestrogen-based oral contraceptive pill [16]. mRNA expression of the rate limiting enzyme in 20:4n-6 and 22:6n-3 synthesis, Δ6-desaturase, is higher in female than male liver [10]. Furthermore, we have shown recently the effect of feeding diets to adult rats with different amounts of total fat or different essential fatty acid contents on plasma and liver lipids differed between males and females [9]. Thus sex differences in phospholipid composition

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may reflect the effect of sex hormones on phospholipid biosynthesis, on specificity of synthesis of fatty acid substrates and on differential partitioning of dietary lipids between cellular lipid classes.

The fatty acid composition of cardiac muscle has been described a number of studies. In rats, heart total phospholipids contain predominately 18:0, 18:2n-6 and 20:4n-6, while triacylglycerol (TAG) was enriched in 16:0, 18:1n-9 and 18:2n-6 [17,18]. However, these studies focussed exclusively on males. Furthermore, although the fatty acid composition of heart lipids can be modified by dietary fat intake [17,18], to our knowledge there is no information as to whether variations in dietary fat induce the same changes in cardiac lipid composition in males and females. Understanding processes which affect the fatty acid composition of heart fatty acids is important because variations in phospholipid composition have been shown to alter cardiac function and susceptibility to disease. For example, increased intake of fish oil improves risk of heart disease and arrhythmia, although these effects are not entirely consistent between studies [19]. Feeding saturated fatty acids increased the incidence of arrhythmias in isolated rat heart exposed to ischaemia/reperfusion, while tachycardia and fibrillation were reduced by feeding corn oil or fish oil [20]. Increased incorporation of 20:5n-3 into heart phospholipids also improved recovery from ischaemia/ reperfusion in isolated rat heart [21]. Decreased 18:2n-6 accompanied by increased 20:4n-6 and 22:6n-3 in rat heart PC and phosphatidylethanolamine (PE) reduced the sensitivity and number of β-adrenoceptors in cardiac muscle [22]. However, stimulation of β-adrenoceptors in vivo decreased PE and PC 18:2n-6, but increased 20:4n-6 in heart PC and 22:6n-3 in PE which suggests that the PUFA content of cardiac phospholipids may be involved in regulating the activity of β-adrenoceptors [23]. In rat heart, exercise increased 18:2n-6 and decreased 20:4n-6 in total phospholipids, while TAG was essentially unaltered [24].

We, therefore, tested the hypothesis that the composition of lipids in rat heart differed between males and females, and that changes in the fatty acid composition of the heart induced by altering dietary fat intake differ between sexes.

2. Materials and methods

2.1. Materials

Diets were from Special Diets Services (Witham, Essex, UK). Solid phase extraction cartridges were from Varian (Oxford, UK). Solvents were from Fisher Scientific Ltd. (Loughborough, Leicestershire, UK). All other reagents were from Sigma (Poole, Dorset, UK).

2.2. Animal procedures

The study was carried out in accordance with the Home Office Animals (Scientific Procedures) Act (1986). The study from which the hearts were obtained has been described previously [8,25]. Briefly, Virgin female Wistar rats were mated and fed either a protein-sufficient or protein-restricted diet from conception until spontaneous delivery (Table 1). Litters were reduced to 8 pups at delivery (equal males and females). Dams were fed AIN-76G diet throughout lactation. The pups were weaned onto a diets containing either containing 40 g/kg (low fat, LF) or 100 g/kg fat (high fat, HF) on post-natal day 28 (Table 1). The fat component of the post-weaning diet was composed of lard:soybean oil (9:1, w/w). On postnatal day 105, food was withdrawn at about 08:00, but water was available *ad libitum*, and offspring were killed by asphyxiation with CO_2 6 h later. Hearts were exsanguinated with a hypodermic syringe, and then removed, frozen in liquid nitrogen and stored at −80 °C. Food intake and weight gain did not differ between dietary groups [25].

Table 1
Composition of the diets fed from weaning.

	Pregnancy		Lactation	Post-weaning diet	
	Diets		Diet	Low fat	High fat
Casein (g/kg)	180	90	AIN76G	180	180
Maize starch (g/kg)	425	425	150	455	425
Sucrose (g/kg)	213	213	500	243	213
Choline (g/kg)	2	2	2	2	2
Methionine (g/kg)	5	5	3	5	5
Vitamin mix (g/kg)	5	5	5	5	5
Mineral mix (g/kg)	20	20	20	20	20
Cellulose (g/kg)	50	50	50	50	50
Folic acid (mg/kg)	1	1	2	1	1
Maize oil (g/kg)	0	0	50		
Soybean oil (g/kg)	100	100	0	4	10
Lard (g/kg)	0	0	0	36	90
Energy (MJ/kg)	17.3	17.5	15.5	16.1	17.3
Fatty acids (g/kg)					
14:0	ND	ND	ND	0.6	1.5
16:0	11.6	11.6	11.6	9.6	24.0
18:0	4.3	4.3	1.6	5.8	14.5
18:1n-9	21.6	21.6	31	14.6	36.5
18:2n-6	55.4	55.4	55.1	7.3	18.3
18:3n-6	1.3	1.3	ND	0.1	0.3
18:3n-3	5.9	5.9	0.7	0.7	1.8

Vitamin mix: Thiamine hydrochloride 2.4 mg/kg; riboflavin 2.4 mg/kg; pyridoxine hydrochloride 2.8 mg/kg; nicotinic acid 12.0 mg/kg; D-calcium pantothenate 6.4 mg/kg; biotin 0.01 mg/kg; cyanocobalamin 0.003 mg/kg; retinyl palmitate 6.4 mg/kg; DL-a-tocopherol acetate 79.9 mg/kg; cholecalciferol 1.0 g/kg; menaquinone 0.02 mg/kg.
Mineral mix: Calcium phosphate dibasic 11.3 g/kg; sodium chloride 1.7 g/kg; potassium citrate monohydrate 5.0 g/kg; potassium sulphate 1.2 g/kg; magnesium sulphate 0.5 g/kg; magnesium carbonate 0.1 g/kg; ferric citrate 0.1 g/kg; zinc carbonate 36.2 mg/kg; cupric carbonate 6.8 mg/kg; potassium iodate 0.2 mg/kg; sodium selenite 0.2 mg/kg; chromium potassium sulphate 12.5 mg/kg.
20:3n-6, 20:4n-6, 20:5n-3, 22:5n-3 and 22:6n-3 were not detected (ND).

2.3. Analysis of fatty acid composition

The fatty acid composition of ventricle PC, PE and TAG was measured by gas chromatography. Ventricles (approximately 100 mg) were homogenised in 0.9% (w/v) NaCl (0.8 ml). Total lipids were extracted with chloroform and methanol [26]. Individual lipid classes were isolated from the total lipid extracts by solid phase extraction using 100 mg aminopropylsilica cartridges (Varian Ltd., Oxford, UK) [27]. Fatty acid methyl esters (FAME) were prepared from purified lipid classes from liver by incubation with methanol containing 2% (v/v) sulphuric acid at 50 °C for 2 h [27]. FAME were resolved using a 6890 gas chromatograph (Agilent, Cheshire, UK) equipped with a 30 m × 0.25 μm × 0.25 mm BPX-70 fused silica capillary column (SGE, Milton Keynes, UK) and flame ionisation detection [27]. The proportions of individual fatty acids were determined by measurement of the peak area using ChemStation software (Agilent Ltd.).

2.4. Statistical analysis

Statistical analysis was carried out using SPSS software (SPSS Inc., Chicago, IL, USA). Tests for single factor and interactive effects were by a general linear model with Bonferroni's *post hoc* analysis.

3. Results

Analysis of the fatty acid compositions of individual heart lipid classes by the general linear model showed significant effects of offspring sex and the diet fed after weaning. However, there was no effect of maternal diet on the fatty acid composition of any of the lipid classes measured. Therefore, the data from the two maternal dietary groups were combined and reanalysed for the effects of offspring sex and post-weaning diet (PWD).

3.1. Heart phosphatidylcholine

There was a significant interaction between sex and post-weaning diet on the total saturated (SFA), monounsaturated (MUFA) and n-3 PUFA content of PC (Table 2). However, total n-6 PUFA was only affected by sex (Table 2). The proportion of 16:0 was decreased slightly in males fed the HF diet, but increased fat intake did not significantly alter PC 16:0 content in females. The proportion of 18:0 was higher in females than males fed a LF diet, but did not differ between sexes in animals fed the HF diet (Table 2). The 24:0 content of PC was higher in females than males, irrespective of fat intake. Neither sex nor fat intake altered PC 20:0 content. PC 18:1 content was increased in males and females fed the HF diet (Table 2). The proportions of 18:2n-6, 18:3n-3 and 20:3n-6 in heart PC from females was significantly lower than in males fed the LF diet irrespective of their fat intake. However, feeding the HF diet decreased the proportion of these fatty acids in males to a level similar to females (Table 2). The proportion of 20:4n-6 in heart PC was higher in females than males fed the LF diet irrespective of fat intake (Table 2). The HF diet increased the proportion of 20:4n-6 in males to a level similar to that of females. The proportion of 22:5n-3 was similar in LF males and females, while 22:6n-3 tended to be higher in females. Increased fat intake after weaning increased the proportion of 22:5n-3 and 22:6n-3 to a similar extent in males and females, while 18:3n-3 and 20:5n-3 contents were unaffected by sex or PWD (Table 2). There was no effect of sex on the ratio of 20:4n-6 to 22:6n-3 in heart PC. However, this ratio was lower in both males and females fed the HF diet compared to rats fed the LF diet.

3.2. Heart phosphatidylethanolamine

There was no effect of sex or fat intake on the SFA content of heart PE, nor an interactive effect of these factors on the n-3 PUFA content (Table 3). However, there was a significant interactive effect of sex and fat intake on PE total MUFA and n-3 PUFA content. There was no effect of the HF diet on the proportion of 16:1n-7 in females, which was similar to LF males (Table 3). However, the HF diet decreased the level of 16:1n-7 in males. 18:1n-7, but not 18:1n-9, was lower in females than LF males, irrespective of fat intake, while this difference was lost in HF males (Table 3). There was no significant difference in the proportion of 20:4n-6 between LF males and females, while feeding the HF diet decreased the level of 20:4n-6 in females, but not males (Table 3). There was no significant effect of sex or fat intake on the proportions of the other n-6 PUFA measured. The HF diet increased the proportions of 22:5n-3 and 22:6n-3 in males and females, while 18:3n-3 and 20:5n-3 levels did not differ between groups. The 20:4n-6 to 22:6n-3 ratio was lower in females fed the LF diet than males (Table 3). However, feeding males the HF diet decreased this ratio, while there was no significant effect in females.

3.3. Heart triacylglycerol

There was no effect of sex on the proportions of any of the total lipid classes or individual fatty acid measured in heart TAG. TAG SFA, MUFA, n-3 PUFA contents of heart TAG were also not altered by fat intake, although specific fatty acids within these classes were affect by post-weaning diet (Table 4). Total n-6 PUFA content was altered significantly by fat intake. The HF diet decreased the proportion of 14:0 and 16:1n-7, which the level of 18:2n-6 was higher in the males and females fed the HF diet

Table 2
Effect of post-weaning fat intake and sex on phosphatidylcholine fatty acid composition.

n	Proportion of total fatty acids (%)						
	Male		Female		GLM (*P*)		
	LF 20	HF 11	LF 18	HF 18	Sex	PWD	Interaction
14:0	0.14 ± 0.02	0.1 ± 0.01	0.1 ± 0.02	0.1 ± 0.03			
16:0	14.2 ± 0.6[a]	13.4 ± 0.5[b]	13.9 ± 0.6[a]	13.8 ± 0.7[a]		0.002	0.025
18:0	25.9 ± 0.8[a]	28.1 ± 0.6[b]	27.5 ± 0.8[b]	27.7 ± 0.9[b]	0.006	< 0.0001	< 0.0001
20:0	0.12 ± 0.03	0.1 ± 0.01	0.1 ± 0.03	0.1 ± 0.02			
24:0	1.4 ± 0.4[a]	1.2 ± 0.3[a]	1.6 ± 0.3[b]	1.5 ± 0.4[b]	0.006		
16:1n-7	0.2 ± 0.04	0.1 ± 0.02	0.2 ± 0.03	0.2 ± 0.1			
18:1n-7	4.7 ± 0.5[a]	3.3 ± 0.3[b]	3.9 ± 0.5[a]	3.5 ± 0.4[b]	0.015	< 0.0001	< 0.0001
18:1n-9	6.4 ± 1.2[a]	4.3 ± 0.7[b]	5.2 ± 0.6[ab]	4.5 ± 0.9[b]	0.022	< 0.0001	0.006
18:2n-6	12.0 ± 2.5[a]	9.5 ± 2.4[b]	8.8 ± 1.1[b]	8.9 ± 1.9[b]	0.001	0.019	0.014
18:3n-6	0.07 ± 0.02[a]	0.04 ± 0.02[b]	0.05 ± 0.01[b]	0.05 ± 0.01[b]		< 0.0001	0.001
20:3n-6	0.4 ± 0.1[a]	0.3 ± 0.07[b]	0.3 ± 0.03[b]	0.3 ± 0.07[b]	< 0.0001	< 0.0001	0.01
20:4n-6	25.3 ± 2.2[a]	27.2 ± 1.3[b]	27.6 ± 1.3[b]	27.2 ± 1.4[b]		0.008	0.012
22:4n-6	0.8 ± 0.01[a]	0.9 ± 0.1[a]	1.0 ± 0.1[b]	1.0 ± 0.1[b]	< 0.0001		
18:3n-3	0.04 ± 0.02	0.04 ± 0.01	0.03 ± 0.02	0.04 ± 0.01			
20:5n-3	0.08 ± 0.06	0.1 ± 0.2	0.07 ± 0.05	0.08 ± 0.03			
22:5n-3	1.0 ± 0.2[a]	1.7 ± 0.2[b]	1.1 ± 0.3[a]	1.5 ± 0.3[b]		< 0.0001	0.007
22:6n-3	7.0 ± 1.2[a]	9.4 ± 1.5[bc]	8.3 ± 0.9[ab]	9.4 ± 1.1[c]	0.042	< 0.0001	0.035
20:4n-6:22:6n-3	3.6 ± 0.5[a]	2.9 ± 0.4[b]	3.4 ± 0.5[a]	2.9 ± 0.3[b]		< 0.0001	
SFA	41.8 ± 1.1[a]	42.8 ± 0.8[b]	43.3 ± 0.8[b]	43.2 ± 0.9[b]	< 0.0001	0.025	0.021
MUFA	11.3 ± 1.5[a]	7.7 ± 0.9[b]	9.3 ± 0.9[c]	8.1 ± 1.2[b]	0.011	< 0.0001	< 0.0001
n-6 PUFA	38.8 ± 1.2[a]	38.1 ± 1.4[ab]	37.9 ± 1.1[ab]	37.7 ± 1.1[b]	0.036		
n-3 PUFA	8.1 ± 1.3[a]	11.3 ± 1.6[b]	9.5 ± 1.2[c]	11.0 ± 1.4[b]		< 0.0001	0.016

Values are mean ± SD proportions of fatty acids in each class. The single and interactive effects were determined by a general linear model with Bonferroni's *post hoc* analysis. Significantly different values ($P < 0.05$) are indicated by different superscripts.

Table 3
Effect of post-weaning fat intake and sex on phosphatidylethanolamine fatty acid composition.

n	Proportion of total fatty acids (%)						
	Male		Female		GLM (*P*)		
	LF 20	HF 11	LF 18	HF 18	Sex	PWD	Interaction
14:0	0.3 ± 0.07	0.2 ± 0.08	0.2 ± 0.1	0.3 ± 0.1			
16:0	11.3 ± 0.9	10.9 ± 1.1	11.4 ± 1.0	11.9 ± 1.3			
18:0	22.4 ± 1.9	22.6 ± 1.5	23.9 ± 5.4	23.6 ± 5.3			
20:0	0.2 ± 0.1	0.l ± 0.04	0.2 ± 0.1	0.2 ± 0.1			
24:0	2.6 ± 0.6	2.3 ± 0.6	3.0 ± 0.8	2.7 ± 0.8			
16:1n-7	0.7 ± 0.2^{a}	0.3 ± 0.1^{b}	0.5 ± 0.2^{a}	0.5 ± 0.3^{a}		0.001	0.004
18:1n-7	4.4 ± 0.4^{a}	3.5 ± 0.3^{b}	3.8 ± 0.6^{b}	3.5 ± 0.3^{b}	0.012	< 0.0001	0.011
18:1n-9	9.0 ± 1.3	7.1 ± 1.5	7.9 ± 1.7	7.8 ± 2.9			
18:2n-6	9.3 ± 1.4	9.4 ± 2.5	8.2 ± 2.1	8.3 ± 1.6			
18:3n-6	0.04 ± 0.01	0.1 ± 0.04	0.05 ± 0.03	0.1 ± 0.1			
20:3n-6	0.3 ± 0.05	0.3 ± 0.04	0.3 ± 0.06	0.2 ± 0.1			
20:4n-6	24.2 ± 2.0^{a}	22.1 ± 1.5^{ab}	22.7 ± 2.8^{ab}	20.9 ± 3.1^{b}	0.043	0.003	
22:4n-6	1.3 ± 0.2	1.4 ± 0.2	1.5 ± 0.3	1.5 ± 0.3			
18:3n-3	0.1 ± 0.05	0.1 ± 0.04	0.1 ± 0.04	0.1 ± 0.1			
20:5n-3	0.1 ± 0.03	0.1 ± 0.06	0.1 ± 0.1	0.1 ± 0.1			
22:5n-3	1.3 ± 0.2^{a}	2.2 ± 0.3^{b}	1.4 ± 0.3^{a}	1.8 ± 0.5^{c}	0.012	< 0.0001	0.007
22:6n-3	12.4 ± 2.2^{a}	17.2 ± 2.9^{b}	14.6 ± 2.5^{a}	16.3 ± 2.9^{b}		< 0.0001	0.024
20:4n-6:22:6n-3	2.0 ± 0.4^{a}	1.6 ± 0.3^{b}	1.3 ± 0.3^{b}	1.3 ± 0.2^{b}	0.005	< 0.0001	0.010
SFA	36.8 ± 2.4	36.1 ± 1.5	38.8 ± 5.9	38.6 ± 5.5			
MUFA	14.1 ± 1.5^{a}	10.9 ± 1.7^{b}	12.2 ± 2.4^{ab}	11.8 ± 3.3^{b}		0.004	0.025
n-6 PUFA	35.2 ± 2.1^{a}	33.4 ± 3.0^{ab}	32.9 ± 3.9^{ab}	31.2 ± 3.6^{b}	0.007	0.039	
n-3 PUFA	13.9 ± 2.3^{ab}	19.6 ± 3.0^{c}	16.1 ± 2.7^{b}	18.3 ± 3.2^{bc}		< 0.0001	0.016

Values are mean ± SD proportions of fatty acids in each class. The single and interactive effects were determined by a general linear model with Bonferroni's *post hoc* analysis. Significantly different values ($P < 0.05$) are indicated by different superscripts.

Table 4
Effect of post-weaning fat intake and sex on triacylglycerol fatty acid composition.

n	Proportion of total fatty acids (%)						
	Male		Female		GLM (*P*)		
	LF 20	HF 11	LF 18	HF 18	Sex	PWD	Interaction
14:0	2.2 ± 0.6^{a}	1.3 ± 0.4^{b}	1.8 ± 0.4^{a}	1.5 ± 0.4^{b}		< 0.0001	
16:0	28.7 ± 6.2	27.1 ± 1.1	26.9 ± 2.8	25.6 ± 5.4			
18:0	10.2 ± 4.0	12.4 ± 5.7	11.8 ± 5.8	10.5 ± 3.0			
16:1n-7	5.3 ± 2.0^{a}	2.6 ± 1.3^{b}	5.0 ± 1.7^{a}	3.2 ± 0.9^{b}		< 0.0001	
18:1n-7	6.6 ± 0.6	3.7 ± 0.5	4.1 ± 0.6	3.6 ± 0.4			
18:1n-9	36.2 ± 10.0	36.6 ± 4.3	39.6 ± 8.6	39.6 ± 4.2			
18:2n-6	8.2 ± 2.2^{a}	12.8 ± 1.7^{b}	9.1 ± 3.0^{a}	12.7 ± 2.7^{b}		< 0.0001	
20:3n-6	0.3 ± 0.2	0.5 ± 0.4	0.4 ± 0.2	0.4 ± 0.3			
20:4n-6	1.0 ± 0.4	1.4 ± 0.7	1.5 ± 0.7	1.4 ± 0.6			
22:5n-3	0.7 ± 0.9	0.6 ± 0.2	1.0 ± 2.1	0.7 ± 0.8			
22:6n-3	0.5 ± 0.4	0.9 ± 0.5	0.8 ± 0.6	0.8 ± 0.6			
20:4n-6:22:6n-3	2.4 ± 1.1	1.7 ± 0.4	2.1 ± 0.8	2.2 ± 1.1			
SFA	41.1 ± 7.8	40.9 ± 6.2	40.5 ± 6.4	37.6 ± 5.9			
MUFA	41.5 ± 9.7	39.2 ± 4.9	44.5 ± 8.7	42.8 ± 4.7			
n-6 PUFA	9.5 ± 2.3^{a}	14.8 ± 2.2^{b}	10.9 ± 3.0^{a}	14.4 ± 2.6^{b}		< 0.0001	
n-3 PUFA	1.3 ± 1.2	1.4 ± 0.7	1.8 ± 2.3	1.5 ± 1.3			

Values are mean ± SD proportions of fatty acids in each class. The single and interactive effects were determined by a general linear model with Bonferroni's *post hoc* analysis. Significantly different values ($P < 0.05$) are indicated by different superscripts.

(Table 4). There was no significant effect of sex or PWD on the 20:4n-6 to 22:6n-3 ratio.

4. Discussion

The findings of this study show that feeding diets with the same proportions of fatty acids at two levels of total intake to rats from weaning until adult induces specific changes in the composition of individual lipid classes in the heart which are contingent on the sex of the rats.

Previous studies have shown that sex is an important determinant of the fatty acid composition of liver, plasma and erythrocytes [8–11], and that incorporation of dietary fats into individual lipids classes in liver and plasma differs between males and females [9]. The present findings extend these observations to the heart. The fatty acid composition of both heart and liver [8] PC and PE, but not TAG, differed between males and females. 16:0 and 20:4n-6 were higher in females than males in both liver [8] and heart PC. 22:6n-3 only showed a trend towards higher concentration in female heart PC, but was significantly higher in female liver. Together, these findings imply that the effects of sex

on tissue fatty acid composition may be due in part to differences in the specificity of lipid metabolism in individual tissues as well as the supply of fatty acids from the diet or from blood.

We have shown recently that sex modifies the effects of differences in dietary fatty acid intake and the amount of fat consumed on the composition of hepatic phospholipids and TAG [9]. The present findings support the suggestion that sex is also an important determinant of the effects of dietary fat on the fatty acid composition of the heart. The ranking between lipid classes of number of fatty acids which were altered by the interaction of fat intake and sex was PC > PE > TAG. This may be counterintuitive because TAG represents a transient pool into which excess fatty acids not immediately required for β-oxidation are incorporated [28] and so may be expected to be modified by fat intake to a greater extent than phospholipids. The mechanism for this apparent difference in susceptibility to dietary change is not known. In heart TAG, increasing fat intake induced similar changes in the proportion of individual fatty acids in both males and females, for example PC 18:1n-7, PE 16:0 and TAG 14:0. However, in phospholipids, increasing fat intake tended to change the proportions of individual fatty acids in phospholipids in males to levels similar to females, while the proportions of fatty acids in females were essentially unchanged by fat intake. This suggests that heart phospholipid composition is more tightly regulated against variations in dietary fat intake in females than males, and thus males may be more susceptible to adverse effects of changes in fat intake, but possibly more amenable to therapeutic interventions such as fish oil.

Recent studies on the effect of sex on fatty acid composition of tissues in humans and in animals models have focussed on 22:6n-3 concentration particularly in liver and blood [4–11]. The proportion of 22:6n-3 in the heart showed a trend towards higher proportion in PC, and no difference between sexes in PE and TAG. However, 20:4n-6 was higher in PC in female heart compared to males. Since 20:4n-6 and 22:6n-3 were not provided in any of the diets, this suggests increased conversion from 18:2n-6 18:3n-3, respectively, presumably by the liver, and/or selective uptake and incorporation into heart PC. Higher ratio of 20:4n-6/22:6n-3 in heart phospholipids has been associated with fatal ventricular fibrillation in rats and risk of sudden cardiac death in humans [29]. PWD, but not sex, altered this ratio in PC, while there was a significant interaction between sex and PWD on PE. This indicates that dietary fat intake may be a primary determinant of the 20:4n-6 to 22:6n-3 ratio in PC and so may contribute to risk of arrhythmia. However, it is possible that the interactive effect of sex and dietary fat may modify such risk through changes in the 20:4n-6 to 22:n-3 ratio in PE which, in turn, may alter the activities of ion channel and receptors.

The fatty acid composition of the heart has been shown the modify its response to hormones, its electrical activity and its susceptibility to ischaemia [19–22]. If the findings of the present study were replicated in humans, one possible implication is that variations in membrane fatty acid composition may contribute to differences in susceptibility to cardiovascular disease between men and women [30].

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