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Systematic differences in membrane acyl composition associated with varying body mass in mammals occur in all phospholipid classes: an analysis of kidney and brain

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SUMMARY

The acyl composition of membrane phospholipids in kidney and brain of mammals of different body mass was examined. It was hypothesized that reduction in unsaturation index (number of double bonds per 100 acyl chains) of membrane phospholipids with increasing body mass in mammals would be made-up of similar changes in acyl composition across all phospholipid classes and that phospholipid class distribution would be regulated and similar in the same tissues of the different-sized mammals. The results of this study supported both hypotheses. Differences in membrane phospholipid acyl composition (i.e. decreased omega-3 fats, increased monounsaturated fats and decreased unsaturation index with increasing body size) were not restricted to any specific phospholipid molecule or to any specific phospholipid class but were observed in all phospholipid classes. With increase in body mass of mammals both monounsaturates and use of less unsaturated polyunsaturates increases at the expense of the long-chain highly unsaturated omega-3 and omega-6 polyunsaturates, producing decreases in membrane unsaturation. The distribution of membrane phospholipid classes was essentially the same in the different-sized mammals with phosphatidylcholine (PC) and phosphatidylethanolamine (PE) together constituting ~91% and ~88% of all phospholipids in kidney and brain, respectively. The lack of sphingomyelin in the mouse tissues and higher levels in larger mammals suggests an increased presence of membrane lipid rafts in larger mammals. The results of this study support the proposal that the physical properties of membranes are likely to be involved in changing metabolic rate.

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Key words: fatty acids, lipids, lipid head group, metabolism, mass spectrometry, phospholipids, glycerophospholipids, lipid class, lipid rafts, ESI–MS, basal metabolic rate, lipidomics.

INTRODUCTION

The lipid composition of membranes has been suggested as a major factor in determining the metabolic rate of animals. This idea constitutes the Membrane Pacemaker Theory of Metabolism. This theory proposes that membrane lipids influence the rate of turnover of membrane bound proteins (i.e. molecular or molar activity, this is the rate of substrate turnover per protein molecule) including those that produce and use the energy of metabolism (Hulbert and Else, 1999; Hulbert and Else, 2000). This theory is based on measurements showing predictable systematic changes in membrane phospholipid acyl composition in tissues from animals with different metabolic rates (Hulbert et al., 2002a; Turner et al., 2003) plus experiments demonstrating changes in the rate of turnover of membrane enzymes associated with lipid composition and following species membrane cross-over experiments (Else and Wu, 1999; Wu et al., 2001; Wu et al., 2004).

The measurement of membrane phospholipid acyl composition in these previous experiments has been conducted using gas chromatography following hydrolysis of fatty acids from their glycerol backbone. This utilitarian method has one major disadvantage; it cannot identify the phospholipid class(es) within which this variation occurs. To gain this information requires a multidimensional chromatography approach that can reduce the sensitivity and reliability of the measurement. An alternative method for analysis of intact phospholipids is electrospray ionisation tandem mass spectrometry. The appeal of mass spectrometry is its ability to provide accurate, reliable, unambiguous identification and quantification of intact phospholipid molecules with high sensitivity (Ekroos et al., 2002; Han and Gross, 2003; Han and Gross, 2005; Pulfer and Murphy, 2003).

Recently we applied this new approach to examine the membrane lipids of a mammal (the rat, *Rattus norvegicus*) and a reptile (a lizard, *Trachydosaurus rugosus*) of similar body mass and preferred body temperature. The study (Mitchell et al., 2007) examined the molecular sources of the lower unsaturation index (the number of double bonds per 100 acyl chains), higher monounsaturation and lower polyunsaturation of membrane phospholipids previously described in ectothermic compared with endothermic vertebrates (Hulbert and Else, 1989). The study (Mitchell et al., 2007) found that all phospholipid classes contribute to the differences in acyl composition of membrane phospholipids and that the distribution of phospholipid classes within the same tissues of the mammal and reptile were essentially the same.

In this present study we used a similar method to examine the phospholipids responsible for the systematic differences in acyl composition reported for mammals of different body size (Hulbert et al., 2002b). These differences include decreases in the unsaturation index and decreases in the omega-3 (n-3) polyunsaturated fat content (particularly docosahexaenoic acid 22:6n-3) with increasing body mass in mammals. Kidney and brain

were chosen as the tissues for examination based on their apparent differences in mammals (Hulbert et al., 2002b). In kidney, membrane acyl composition has been found to vary with variation in body size, in line with that found in other tissues (e.g. liver, heart and skeletal muscle) and its dominant polyunsaturated fats are omega-6 (n-6) fats. By contrast, the brain does not show these large-scale changes in membrane acyl composition with changes in body mass, and its dominant polyunsaturated fats are omega-3 fats (Hulbert et al., 2002b).

Species selection for this study was based on those mammals most commonly used in body size (allometric) analysis and ready availability. It should be noted that because of the large volume of work involved in these measurements we were forced to limit our species selection to three mammal species only (i.e. mouse, sheep and cow). The previous results for the rat were not included since although similar, the methods employed were subtly different and so did not allow for reliable quantitative comparison. Furthermore, it should be noted that this study is not an allometric study nor has it the capacity to avoid some phylogenetic bias based on the number of species used. The animals used in the current study have already been shown to reflect the average membrane phospholipid acyl composition for body mass in mammals (Hulbert et al., 2002b). This study focuses on examining membrane phospholipid molecules in precise detail in three different-sized mammal species against the differences in acyl composition already well established for mammals and well represented by the mammals chosen for inclusion in this study (Hulbert et al., 2002b).

Based on our study of an ectotherm and endotherm, we hypothesized that the same rules would apply to differences in phospholipid acyl composition in mammals of varying body mass. We proposed that changes in acyl composition such as the reduction in unsaturation index and decrease in the more highly unsaturated omega-3 and omega-6 fats with increase in mass in mammals will be spread throughout all phospholipid classes and that phospholipid head group (i.e. class) will be regulated and similar in the different-sized mammals.

MATERIALS AND METHODS

Materials

All solvents used were HPLC grade and purchased from Crown Scientific (Moorebank, NSW, Australia). Analytical grade butylated hydroxytoluene (BHT) was purchased from Sigma Aldrich (Castle Hill, NSW, Australia). Phospholipid standards were synthesised by Avanti Polar Lipids (Alabaster, AL, USA) and purchased from Auspep (Parkville, Victoria, Australia).

Animals, organs and ethics

Sheep (*Ovis aries* L.; body mass ~38 kg) and cattle (*Bos taurus* L.; herein referred to as cow, body mass ~560 kg) kidney and brains were collected from the Wollondilly Abattoir (Picton, NSW, Australia) immediately following the death of each animal. Organs were transported on ice then stored at –80°C until used. Body masses for the sheep and cattle were determined from their individual carcass mass (i.e. that of slaughtered animal minus hide, head, tail, extremities and viscera) assumed to be 55% of body mass (as determined and used commercially at the abattoir). Male C57bl/6 mice (*Mus musculus* Linnaeus; body mass ~30 g) were obtained from the Animal Resource Centre (Perth, WA, Australia), kept under standard 12 h:12 h light:dark conditions and sacrificed by peritoneal injection of sodium pentobarbitone at a concentration of 0.6 mg g^{-1} body mass. The kidney and brains were collected from four individuals of the three animal species studied and stored at –80°C until used. The University of Wollongong Animal Ethics Committee approved all animal-based experimentation.

Lipid extraction

Samples of brain and kidney were homogenised (at 0.25 g ml^{-1}) in glass–glass tissue homogenisers in 2:1 chloroform:methanol containing 0.01% butylated hydroxytoluene and 4 µl mg^{-1} tissue of a stock solution of phospholipid internal standards [phosphatidylcholine (PC) 19:0/19:0, 250 µmol l^{-1}; phosphatidylethanolamine (PE) 17:0/17:0, 188 µmol l^{-1}; phosphatidylserine (PS) 17:0/17:0, 125 µmol l^{-1}; phosphatidic acid (PA) 17:0/17:0, 25 µmol l^{-1}; and phosphatidylglycerol (PG) 17:0/17:0, 25 µmol l^{-1}, in methanol:chloroform) was added. Total lipid extracts were then place on a rotating platform that turned the samples upside down overnight in order to maximise lipid extraction. This was followed by an acid wash (1 mol l^{-1} H_2SO_4) to further enhance extraction of acidic phospholipids and standard procedures followed thereafter as previously described (Folch et al., 1957). All extracts were stored at –80°C until analysed.

Mass spectrometry

Electrospray ionisation mass spectrometry (ESI–MS) analysis was performed on a Waters QuattroMicro™ (Waters, Manchester, UK) equipped with a z-spray electrospray ion source and controlled by Micromass Masslynx version 4.0 software. Capillary voltage was set to 3000 V, source temperature 80°C and desolvation temperature 120°C. Cone voltage was set to 50 V and –35 V in negative and positive ion mode, respectively. Nitrogen was used as the drying gas at a flow rate of 320 l h^{-1}. Phospholipid extracts were diluted to an estimated final concentration of 40 µmol l^{-1} with the addition of methanol:chloroform (2:1 v:v). For negative ion analyses, ammonia (28%) was added to adjust the pH to 10. Samples were infused into the electrospray ion source at a flow rate of 10 µl min^{-1} using the instrument's on-board syringe pump. In all precursor ion, neutral loss and product ion scans, argon was used as the collision gas at a pressure of 3 mTorr and the collision energy was set between 22–50 eV depending upon on the scan being performed. Phosphatidic acid (PA), phosphatidylglycerol (PG), phosphatidylinositol (PI), phosphatidylethanolamine (PE) and phosphatidylserine (PS) phospholipids readily form $[M–H]^-$ anions in negative ion mode and were detected in the mass range of *m/z* 640–940. By contrast phosphatidylcholine (PC) and sphingomyelin (SM) phospholipids were detected as $[M+H]^+$ ions in positive ion mode at *m/z* 640–850 (Brugger et al., 1997). PE and PS phospholipids also formed abundant $[M+H]^+$ ions in positive ion mode that were used here for quantification of these two classes. Overall a combination of precursor ion and neutral loss scans in both positive and negative ion modes were used to identify the head-group of each phospholipid as previously described. For phospholipid classes observed in negative ion mode, precursor ion scans of the fatty acid carboxylate anions in negative ion mode allowed for the identification of fatty acids associated with each individual phospholipid. To allow for the identification of the acyl chains in PC phospholipids, neutral loss scans for loss of the lithiated fatty acids were performed in positive ion mode after aqueous lithium acetate was added to the lipid extract (to a final concentration of 200 µmol l^{-1} in methanol:chloroform 2:1; v:v). A full description of all scans types performed in this study and corresponding instrumental parameters is provided in Table S1 in supplementary material. Where two or more isomeric phospholipids of the same class were identified (e.g. PE 18:1/18:1 and PE 18:0/18:2) a product ion spectrum was obtained. The abundances of the pairs of fatty acid carboxylate anions arising

from each isomer in this spectrum were summed and normalized to the abundance of all fatty acid carboxylate anions to obtain the relative contribution of all isomers to the abundance of the parent ion (e.g. the contribution of PE 18:0/18:2 to the precursor ion at *m/z* 742 is given by the correction factor $[I_{283}+I_{279}]/[I_{283}+I_{279}+I_{281}]$, where I_{283}, I_{281} and I_{279} are the intensities of the 18:0, 18:1 and 18:2 fatty acid carboxylate anions, respectively, in the product ion spectrum of *m/z* 742 in negative ion mode). It should be noted that neither the relative position of the acyl chains on the glycerol backbone (sometimes called the *sn*-position) (Ekroos et al., 2003) nor the position of double bonds (Thomas et al., 2007) can be rigorously assigned from these data and discussions in this paper are based on naturally abundant regioisomers.

For quantification purposes, head-group-specific neutral loss and precursor ion mass spectra (see Table S1 in supplementary material) were obtained from averaging a minimum of 200 scans. Phospholipids were quantified by comparing their peak areas with the peak area of an appropriate internal standard for each phospholipid class following corrections for isotope contributions as described by Han and Gross (Han and Gross, 2005). Briefly, the increasing natural abundance of ^{13}C-isotope ions with increasing carbon chain length distributes the ion abundance from a single phospholipid toward isotopologues of higher mass. For accurate quantification, the contributions of all isotopologues were summed to provide a single, comparable measure of abundance for each lipid; independent of size. To achieve this, the isotopic ion distribution of each phospholipid was calculated from isotope models and the area of the monoisotopic peak was multiplied by the calculated correction factor. For example, the calculated isotopic abundances for PE 18:2/18:2 are M=1.0, M+1=0.475, M+2=0.126 and M+3=0.024 and thus for quantification, the abundance of the monoisotopic ion is corrected by a factor of 1.63. Conversely, the contribution of phospholipid isotopologues to the area of the monoisotopic peak of a lipid of higher *m/z* needs to be subtracted for accurate quantification. Isotopic corrections of this type were carried out sequentially from low to high *m/z* such that any contribution of isotopologues from low mass lipids were subtracted from phospholipids of greater *m/z*, prior to isotopic correction of the abundance of the heavier ion (as above). For example, if both PE 18:2/18:2 and PE 18:1/18:2 were found to be present in a positive ion mass spectrum, the abundance of the latter lipid ($A_{\text{PE 18:1/18:2}}$) was obtained from the corrected intensity of the peak at *m/z* 742 (I_{742}) *via* $A_{\text{PE 18:1/18:2}}=I_{742}-0.126\times A_{\text{PE 18:2/18:2}}$, where $A_{\text{PE 18:2/18:2}}$ is the already corrected abundance of PE 18:2/18:2.

As no appropriate internal standards for SM or PI where available, their concentrations where derived from internal standards from related lipid classes. Specifically, sphingomyelins were quantified by comparison with the PC internal standard and multiplication by a correction factor of 3.15 that takes into account the underestimation of SM abundance in precursor ion scans of *m/z* 184 due to the differing fragmentation efficiency of these two choline-bearing lipid classes. The correction factor was determined by comparison of signal response of SM 16:0 and SM 18:1 with PC 16:0/18:1 and PC 18:1/18:1 in *m/z* 184 precursor ion scans over a concentration range of 0.8–1.6 μmol l^{-1}. An approximate quantification of PI was achieved *via* comparison of the most abundant phosphatidylinositol, PI 18:0/20:4, to the mean abundance of PA and PG internal standards in a negative ion mass spectrum. This empirical correction for relative ionization efficiencies is derived from Koivusalo et al. (Koivusalo et al., 2001). The relative abundance of other PIs were then derived by comparison with PI 18:0/20:4 in an *m/z* 241 precursor ion scan undertaken in negative ion mode. Given the approximations associated with the quantification of both SM and PI, these data are treated separately to those of other phospholipid classes in the following discussion.

Statistical analysis

Data were analysed by a one-way ANOVA using species as a fixed factor, with a Student's *t*-test for comparison of means. For unequal variances, a Welch one-way ANOVA was undertaken. Normality of data was assumed from previous GC studies (Hulbert et al., 2002b). *P* values of <0.05 were considered statistically significant, and all values are expressed as means ± s.e.m. Statistical analyses were completed using JMP 5.1 (SAS Institute, NC, USA).

RESULTS

Phospholipid class distribution and sphingomyelin concentration

The concentration and distribution of the six different glycerophospholipid classes (PC, PE, PS, PA, PG and PI) and SM were measured in kidney and brain of mouse, sheep and cow. The relative distribution of these phospholipids is shown in Table 1. A relatively similar distribution within each tissue in each of the mammals is apparent. PC was the major glycerophospholipids in kidney and brain (71% and 68%, respectively), PE the second (19–20%) and PS the third (5–7% in kidney and 10% in brain) most abundant glycerophospholipid. PA was present, but at low levels (1.3–2.5%) and PG was detected but at very low levels (less than 0.4%). PI concentration was negligible (the highest value found was in kidney at approximately 0.14 μmol g^{-1}). No PI was found in mouse kidney whereas in sheep and cow 44 and 42%, respectively, were PI 18:0/20:4. The second most abundant PI molecule was 18:0/20:3 at 9 and 13% in sheep and cow kidney, respectively. In brain the dominant PI molecules was PI 18:0/20:4 representing 74, 66 and 67% of all PIs in mouse, sheep and cow,

Table 1. Class distribution of glycerophospholipids in kidney and brain of mouse, sheep and cow

Glycerophospholipid	Mouse	Sheep	Cow
Kidney			
PC (%)	72.5±0.9^{c}	72.1±0.4^{c}	68.4±1.6^{m,s}
PE (%)	19.6±0.5^{c}	18.0±0.5^{c}	23.5±0.9^{m,s}
PS (%)	5.4±0.2^{s}	7.4±0.2^{m}	6.3±0.9
PA (%)	2.5±0.3	2.1±0.1	1.7±0.4
PG (%)	ND	0.4±0.2	0.2±0.1
SM (μmol g^{-1} tissue)	ND	5.98±0.63	5.9±0.8
Brain			
PC (%)	66.7±2.4	69.4±0.7	69.3±0.9
PE (%)	21.6±2.8	18.1±1.6	18.2±1.0
PS (%)	10.2±2.6	10.8±1.4	10.5±0.3
PA (%)	1.3±0.1	1.6±0.2	2.0±0.3
PG (%)	0.2±0.1	0.2±0.1	0.1±0.1
SM (μmol g^{-1} tissue)	ND	4.0±0.9^{c}	9.3±1.8^{s}

PC, phosphatidylcholine; PE, phosphatidylethanolamine: PS, phosphatidylserine; PA, phosphatidic acid; PG, phosphatidylglycerol; SM, sphingomyelin.

Total glycerophospholipid concentrations (PC, PE, PS, PA) in mouse, sheep and cow kidney were 34.7, 32.8 and 40.7 μmol g^{-1} tissue, and for brain were 74.7, 96.7 and 101.3 μmol g^{-1} tissue. Phosphatidylinositol concentration was negligible.

Values are mean ± s.e.m. (*N*=4). ND, not detected. Superscript letters indicate significant differences between species at *P*<0.05: m, mouse; s, sheep; c, cow.

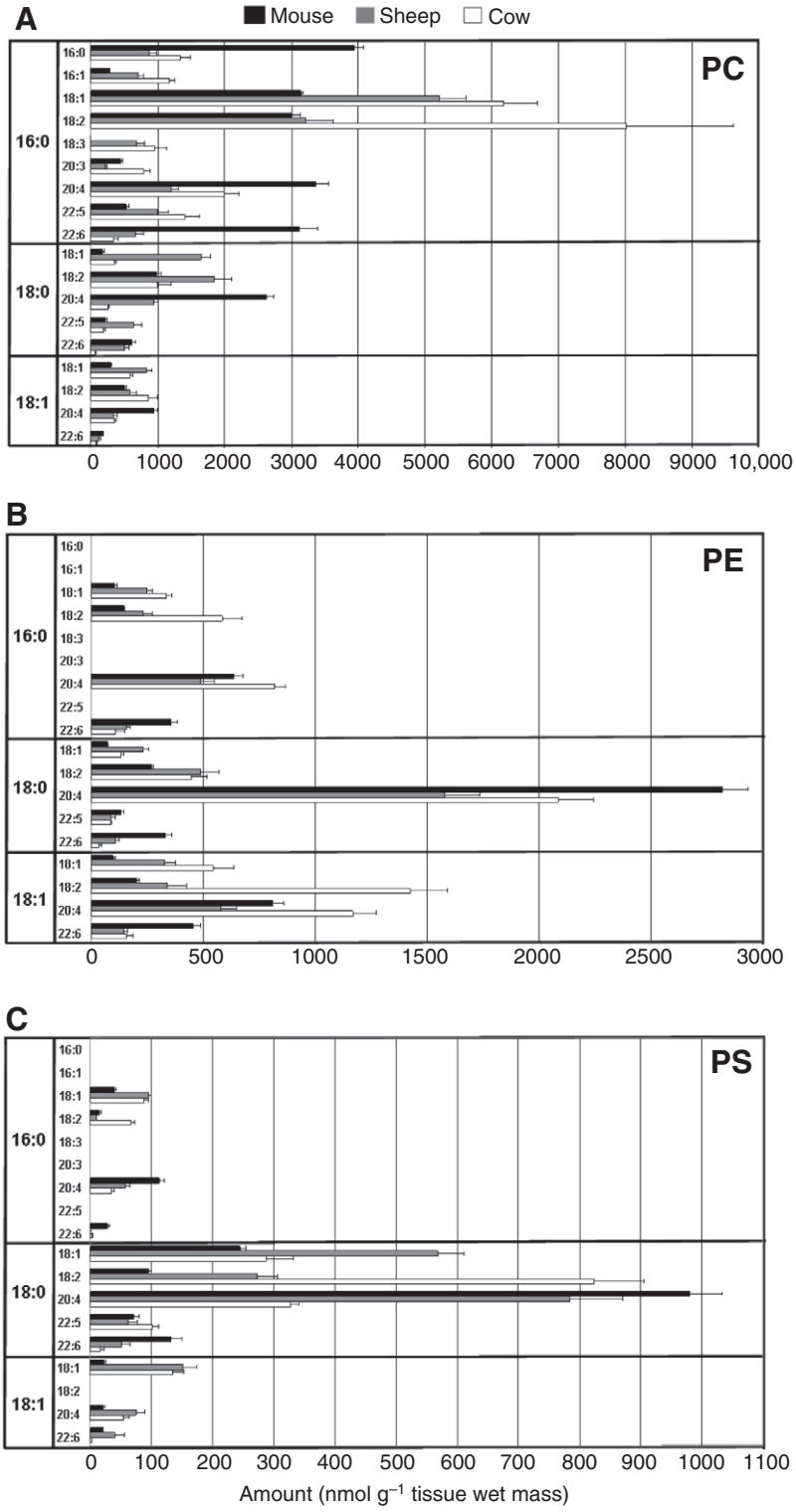

Fig. 1. A comparison of the type and concentration of the three most common glycerophospholipid classes (A) phosphatidylcholine (PC), (B) phosphatidylethanolamine (PE) and (C) phosphatidylserine (PS) present in mouse, sheep and cow kidney. Statistical analysis for differences in the concentration of these phospholipids in kidney between the three mammal species is provided in Table 2. Values are means ± s.e.m. (*N*=4).

Phospholipid molecules

Kidney

The distribution of phospholipid molecules belonging to the three most abundant classes, PC, PE and PS, in kidney and brain are provided in Fig. 1 (kidney) and Fig. 2 (brain). Statistical analysis of the results is presented in Table 2. Comparing the species, the kidney displayed a large number of differences whereas the brain showed relatively few. The diversity of phospholipid molecules was far greater in the kidney (82–120 different molecular combinations detected) compared with the brain (64–66 different combinations, i.e. including all PC, PE, PS, PA, PG, PI, SM). The two PC molecules with the highest concentration in kidney were PC 16:0/18:1 and PC 16:0/18:2 in sheep and cow, whereas in the mouse the PC with the highest concentration was PC 16:0/16:0 (followed by 16:0/18:1, 16:0/18:2, 16:0/20:4, 16:0/22:6, 18:0/20:4, at similar levels). This difference emphasises the lower levels of monounsaturated and less unsaturated polyunsaturated fats and higher levels of highly polyunsaturated fats in the membrane of the smallest mammal studied. In all three animals examined, the major PC phospholipid molecules associated with kidney are dominated by molecular combinations involving 16:0 (72, 61 and 83% of all PC molecules contained 16:0 in mouse, sheep and cow, respectively).

Among kidney PE phospholipids, the molecule with the highest concentration in all three mammalian species was PE 18:0/20:4. The only other PE molecule present in moderately high abundance (in all three species) was PE 18:1/20.4. The PS phospholipids in highest concentration in both the mouse and sheep was PS 18:0/20:4, whereas in the cow PS 18:0/18:2 was most abundant, followed by PS 18:0/20:4. Among the other phospholipid classes there were no common trends with the most common PA molecule being PA 18:0/20:4 in the mouse, PA 16:0/18:1 in the sheep and PA 16:0/18:2 in the cow. In mouse kidney, 24 different molecular combinations of PA were detected whereas the variety of PA molecules in the sheep and cow was almost half that amount (11 and 14 PA molecules, respectively). This probably reflects a higher and therefore more detectable level of PA molecules in the mouse kidney. For PG the only detectable molecule was PG 16:0/18:1 and this was only found in sheep and cow kidney. SMs were not detected in the mouse kidney but were detected in both the sheep and cow kidney with the same nine SM combinations found: SM 16:0 was found at several times higher concentration (2.7 and 2.1 μmol g^{-1} tissue) than other SMs.

respectively. SM concentration in kidney varied from being undetectable in the mouse to about 6 μmol g^{-1} in sheep and cow kidney (this equals 18 and 14 SM molecules per 100 glycerophospholipid molecules in the sheep and cow kidney). In brain, SM concentration appeared to increase with body mass, being undetectable in the mouse but at 4.0 and 9.3 μmol g^{-1} (4 and 9 SM molecules per 100 glycerophospholipid molecules) in the brain of the sheep and cow, respectively.

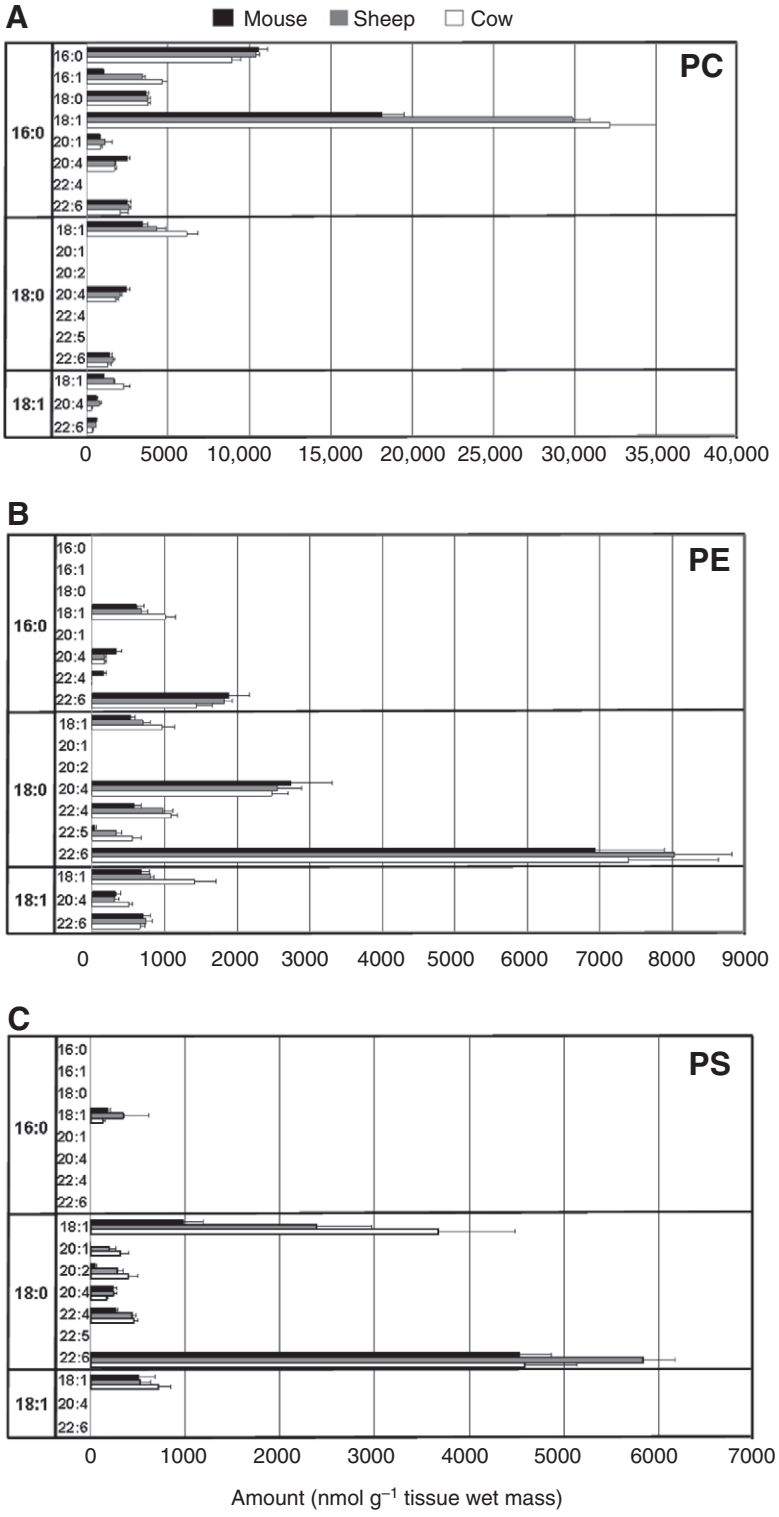

Fig. 2. A comparison of the type and concentration of the three most common glycerophospholipid classes (A) phosphatidylcholine (PC), (B) phosphatidylethanolamine (PE) and (C) phosphatidylserine (PS) present in mouse, sheep and cow brain. Statistical analysis for the differences in the concentration of these phospholipids in brain between the three mammal species is provided in Table 2. Values are means ± s.e.m. (*N*=4).

Of the SMs present in both the sheep and cow kidney, 80–85% contained a saturated fatty acid (mainly 16:0 or 22:0).

Brain

PC 16:0/18:1 constituting 24, 31 and 32% of all glycerophospholipids in the brains of the mouse, sheep and cow, respectively, must be considered as the primary 'house keeping' phospholipid of the brain, being at levels two to four times higher than the next most abundant PC molecule (PC 16:0/16:0). Most PC phospholipid molecules in the brain were partnered with 16:0 (80–82%, as also found for kidney). Among the 18:0-containing PC phospholipids, 18:0/18:1, 18:0/20:4 and 18:0/22:6 were present in relatively high abundance in all three mammals. The high level of PC 18:0/18:1 in the brain was a property not shared by the kidney. Other interesting PC phospholipids present in higher concentrations in the brain of all three mammals were 16:0/22:6 and 16:0/20:4 that shared similar levels in the brain (~2.4–5% of PC molecules).

Among brain PE molecules, PE 18:0/22:6 was the most abundant being 2–5 times more concentrated than the next most abundant molecules PE 18:0/20:4 and PE 16:0/22:6. PE molecules were highly polyunsaturated (80–87% of molecules). Although PE molecules represented only 18–21% of brain glycerophospholipids they served as the major source of polyunsaturated fats raising the unsaturation index (i.e. the number of double bonds per 100 acyl chains) from 83, 76 and 68 without PE to 144, 138 and 128 with PE in mouse, sheep and cow brain, respectively. PS 18:0/22:6 was the most common phosphatidylserine molecule and the major contributor to membrane docosahexaenoic acid (DHA) concentration. Among PA and PG molecules, PA 18:0/22:6 and PG 16:0/18:1 were the best represented, being present in all three animal species at low levels.

Contribution of individual glycerophospholipid molecules and phospholipid classes to the unsaturation index

The glycerophospholipids that contributed most to the unsaturation index (UI) of the kidney were PC 16:0/22:6 (27.0) in mouse and PC 16:0/18:2 (9.8 and 19.7) in the sheep and cow (see Table 2). Owing to the higher UI in the mouse kidney, other major contributors to UI in this species included PC 16:0/20:4 (19.4), PE 18:0/20:4 (16.2) and PC 18:0/20:4 (15.1) all contributing more than 15 UI units. In sheep and cow kidneys the only other phospholipid that contributed significantly to the UI was PE 18:0/20:4 (at 9.6 and 10.2, respectively). In the brain the major contributors to UI were PE 18:0/22:6, PS 18:0/22.6, PC 16:0/18:1 and PC 16:0/22:6, in all three species.

Tables 3 and 4 compare the contributions of the four major phospholipid classes (PC, PE, PS and PA) to the UI of the kidney and brain of mouse, sheep and cow. The tables also show the contributions to UI of the primary unsaturated acyl chains (18:1n-9, 18:2n-6, 20:4n-6 and 22:6n-3) plus the sum of saturated, monounsaturated, omega-3 and omega-6 polyunsaturated fats within each class of phospholipid in the kidney and the brain of the three mammals. For kidney, UI decreases as body mass

Table 2. Statistical significance of differences between mouse, sheep and cow in the levels of major glycerophospholipids present in kidney and brain as presented in Fig. 1 and Fig. 2

Molecular species		Kidney			Brain		
		PC	PE	PS	PC	PE	PS
16:0	16:0	MC***			MC*		
		MS***			MS		
		SC*			SC*		
	16:1	MC***			MC***		
		MS**			MS***		
		SC**			SC**		
	18:1	MC***	MC***	MC***	MC***	MC*	MC
		MS**	MS***	MS***	MS**	MS	MS
		SC	SC**	SC	SC	SC	SC
	18:2	MC**	MC***	MC***	MC***		
		MS	MS	MS	MS**		
		SC**	SC**	SC***	SC*		
	20:4	MC***	MC*	MC***	MC***	MC*	
		MS***	MS	MS***	MS**	MS*	
		SC*	SC**	SC	SC	SC	
	22:6	MC***	MC***	MC***	MC	MC	MC*
		MS***	MS**	MS***	MS	MS	MS
		SC	SC	SC	SC	SC	SC
18:0	18:1	MC	MC*	MC	MC**	MC*	MC*
		MS***	MS***	MS***	MS	MS	MS
		SC***	SC***	SC***	SC*	SC	SC
	18:2	MC	MC	MC***	MC*		
		MS*	MS**	MS*	MS		
		SC*	SC	SC***	SC		
	20:4	MC***	MC**	MC***	MC*	MC	MC
		MS***	MS***	MS*	MS	MS	MS
		SC***	SC*	SC***	SC	SC	SC
	22:4		MC***	MC**		MC*	MC
			MS***	MS***		MS*	MS
			SC	SC		SC	SC
	22:5	MC	MC*	MC		MC**	
		MS**	MS	MS		MS*	
		SC**	SC	SC		SC	
	22:6	MC***	MC***	MC***	MC	MC	MC
		MS***	MS***	MS**	MS	MS	MS
		SC	SC*	SC	SC	SC	SC
18:1	18:1	MC**	MC***	MC***	MC**	MC*	MC
		MS***	MS*	MS***	MS*	MS	MS
		SC**	SC*	SC	SC	SC*	SC
	18:2	MC*	MC***		MC***		
		MS	MS***		MS**		
		SC	SC		SC		
	20:4	MC***	MC**	MC*	MC*	MC	
		MS***	MS	MS**	MS**	MS	
		SC	SC***	SC	SC	SC	
	22:6	MC**	MC***	MC	MC*	MC	MC
		MS*	MS***	MS	MS	MS	MS
		SC	SC	SC*	SC	SC	SC

M, mouse; S, sheep; C, cow; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine.

***P<0.001, **0.001>P<0.01, *0.01>P<0.05, no asterisks indicates not significant at P>0.05.

Phospholipid molecular species that occurred only in one animal species or in one phospholipid class at very low concentration were excluded to minimize the number of data columns within the table. These phospholipid molecules included 16:0/18:3, 16:0/20:3 and 16:0/22:5 in kidney and 18:0/20:1 plus 18:0/20:2 in brain.

increases in all four classes of phospholipid (Table 3). In the brain the decrease in UI with increasing body mass only holds for PC and the change is small (but significant, because of the very low variance present in the values; see Table 4). In kidney, all four phospholipid classes examined showed relatively similar UI level in all three species. In brain, the UI of PE and PS was high (compared with PC and PA), primarily due to high docosahexaenoic acid (22:6n-3) levels and were the major contributors to the overall UI of the tissue. The major contributor to the UI in the kidney (in all four phospholipid classes) was arachidonic acid, in brain it was docosahexaenoic acid, emphasising the omega-6 and omega-3 dominance of kidney and brain, respectively.

Body mass variation and phospholipid molecules

As shown in Fig. 1 and Fig. 2 many of the glycerophospholipids show body mass trends. In kidney, the major PC molecules, 16:0/18:1 and PC 16:0/18:2, both increased with increasing body

mass whereas PC 16:0/22:6, 16:0/20:4 and 18:0/20:4 each with a long, highly unsaturated acyl chain tended to decrease in concentration with increasing body mass. The reduction in arachidonic acid (20:4n-6) with increasing body mass in PC of kidney, however, did not cause a fall in the overall omega-6 content of the PC lipids since the shorter-chained, less unsaturated linoleic acid made up the difference, with increasing concentrations of PC 16:0/18:2. The main PE and PS molecules in the kidney also showed some body mass changes with molecular combinations, with monounsaturates (18:1) and linoleic acid (18:2) increasing (e.g. PE 16:0/18:1, 18:1/18:1, 16:0/18:2 and 18:1/18:2 and PS 16:0/18:2 and 18:0/18:2) with body mass and those molecules with long, highly unsaturated acyl chains (20:4 and 22:6) decreasing (e.g. PE 16:0/22:6, 18:0/22:6 and 18:1/22:6 and PS 16:0/20:4, 18:0/20:4, 18:0/22:6; see Fig. 1 and Table 2 for statistical analysis of phospholipid species). Basically, as mammals increased in body mass the acyl chain of the kidney phospholipids became less unsaturated with fewer phospholipid molecules containing 22:6 and 20:4 and more contained 18:1 and 18:2 (Fig. 1; Table 3). Therefore, reductions in unsaturation index (UI) were underpinned by mass-dependent changes in every major phospholipid class.

In brain, there were fewer differences in the concentration of phospholipids, with those containing either arachidonic (20:4) or docosahexaenoic (22:6) acid being more similar between the species. There was a tendency for brain phospholipid molecules containing 18:1 to increase with body mass, notably among these was PC 16:0/18:1. Few brain phospholipid molecules contained linoleic acid (18:2) presumably as a result of it being converted to the longer chained arachidonic acid (20:4n-6). Few body mass trends seemed to be apparent in the brain with phospholipids displaying a more uniformed acyl phospholipid composition among the different-sized mammals.

Table 3. Kidney fatty acid characterisation showing the distribution of saturated, monounsaturated, omega-3 and omega-6 polyunsaturated fatty acids plus the contribution to unsaturation index of the major fatty acids within each phospholipid classes

Phospholipid	Mouse	Sheep	Cow
Phosphatidylcholine			
ΣSFA	54.4±0.9	47.8±4.6	49.5±6.1
ΣMUFA	11.5±0.2^{s,c}	23.1±2.1^{m}	19.8±1.6^{m}
ΣPUFA n-6	26.1±0.8	21.8±2.4	27.6±4.5
ΣPUFA n-3	8.0±0.7^{c}	7.2±1.0	3.1±0.3^{m}
UI	144.7±3.5^{s,c}	122.2±2.4^{m,c}	107.7±1.0^{m,s}
UI (18:1 n-9)	10.6±0.1^{s,c}	21.2±0.5^{m,c}	17.4±1.1^{m,s}
UI (18:2 n-6)	19.2±0.9^{s,c}	24.7±1.6^{m,c}	35.6±3.5^{m,s}
UI (20:4 n-6)	54.9±1.8^{s,c}	21.3±1.8^{m}	18.8±0.8^{m}
UI (22:6 n-3)	47.7±3.1^{s,c}	16.4±2.5^{m,c}	5.0±0.4^{m,s}
Phosphatidylethanolamine			
ΣSFA	37.3±1.6^{c}	35.0±3.9	27.7±1.9^{m}
ΣMUFA	14.9±0.9^{c}	21.9±3.3	27.8±1.5^{m}
ΣPUFA n-6	39.4±1.7	34.2±4.3	38.4±3.4
ΣPUFA n-3	8.4±0.7^{c}	9.1±1.3	6.2±0.5^{m}
UI	215.0±1.5^{s,c}	189.5±2.0^{m}	185.3±1.8^{m}
UI (18:1 n-9)	14.9±0.4^{s,c}	21.4±0.5^{m,c}	26.2±0.9^{m,s}
UI (18:2 n-6)	9.2±0.4^{s,c}	18.0±1.5^{m,c}	28.0±1.5^{m,s}
UI (20:4 n-6)	125.7±0.4^{s,c}	92.4±3.9^{m}	90.7±1.3^{m}
UI (22:6 n-3)	50.4±1.6^{s,c}	21.1±1.0^{m,c}	9.4±1.8^{m,s}
Phosphatidylserine			
ΣSFA	48.3±2.2	42.4±4.3	39.5±3.2
ΣMUFA	9.8±0.4^{s,c}	25.1±2.8^{m}	21.9±2.3^{m}
ΣPUFA n-6	36.5±1.6	28.2±3.4	37.1±2.8
ΣPUFA n-3	5.4±0.5^{c}	4.2±1.0	1.5±0.4^{m}
UI	183.0±1.4^{s,c}	146.4±3.1^{m,c}	134.2±4.1^{m,s}
UI (18:1 n-9)	9.9±0.3^{s,c}	24.7±0.3^{m}	20.1±1.2^{m}
UI (18:2 n-6)	5.9±0.3^{s,c}	14.0±0.4^{m,c}	43.7±1.2^{m,s}
UI (20:4 n-6)	118.2±2.0^{s,c}	75.4±1.5^{m,c}	34.2±2.4^{m,s}
UI (22:6 n-3)	28.3±0.05^{s,c}	10.9±1.8^{m,c}	2.4±0.8^{m,s}
Phosphatidic acid			
ΣSFA	34.6±4.6	34.4±3.4	28.8±9.1
ΣMUFA	13.1±2.2^{s}	35.1±3.5^{m}	35.9±8.5
ΣPUFA n-6	48.7±6.2^{s}	24.7±4.7^{m}	33.0±7.9
ΣPUFA n-3	4.2±0.5^{c}	5.8±1.5	2.3±0.6^{m}
UI	181.0±0.6^{s,c}	143.1±10.1^{m}	133.8±5.4^{m}
UI (18:1 n-9)	12.6±0.6^{s,c}	35.4±1.6^{m}	36.8±1.8^{m}
UI (18:2 n-6)	44.7±0.9^{s,c}	11.2±2.1^{m,c}	26.5±5.5^{m,s}
UI (20:4 n-6)	86.3±1.3^{c}	74.7±10.7^{c}	31.1±0.8^{m,s}
UI (22:6 n-3)	18.7±1.3^{s,c}	0.0±0.0^{m}	0.0±0.0^{m}

SFA, saturated fatty acid ; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; Σ, total, UI, unsaturation index (the number double bonds per 100 fatty acid molecules).

Superscript letters indicate significant differences between the values derived for mouse (m), sheep (s) and cow (c) at a minimum of $P<0.05$. n-3, n-6, n-9 denote the location of the first double bond from the methyl end of the molecule. Values are mean ± s.e.m. ($N=4$).

DISCUSSION

This study supports the proposal that differences in membrane phospholipid acyl composition associated with differences in body mass in mammals are a product of systematic generalised changes in the composition of the acyl chains across all phospholipid classes. It shows that differences in membrane phospholipid acyl composition are not restricted to any particular type of phospholipid molecule or to any specific phospholipid class. The results agree with those of another recent study showing the same generalised differences in acyl composition spread throughout phospholipid classes in an endothermic mammal (rat) and ectothermic reptile (lizard) (Mitchell et al., 2007). The results of the study also support the proposition that membrane phospholipid class distribution is regulated in tissues irrespective of body size. The distribution of membrane phospholipid classes within kidney and brain were essentially the same in the three different-sized mammals. The combined percentage of phosphatidylcholine (PC) and phosphatidylethanolamine (PE) was ~91% and ~88% of all glycerophospholipid molecules in the kidney and brain, respectively, in the three mammal species. These values are comparable to 88 and 86% of all glycerophospholipid molecules for PC and PE found in kidney and brain of another mammal and a reptile previously examined (Mitchell et al., 2007).

Many different types of phospholipid molecules within each class of phospholipid showed body-mass-related differences in their acyl composition. These differences account for the previously described

Table 4. Brain fatty acid characterisation showing the distribution of saturated, monounsaturated, omega-3 and omega-6 polyunsaturated fatty acids plus the contribution to unsaturation index of the major fatty acids within each phospholipid classes

Phospholipid	Mouse	Sheep	Cow
Phosphatidylcholine			
ΣSFA	61.9±3.9	58.4±0.9	57.4±2.2
ΣMUFA	27.5±2.0	33.9±1.1	36.2±3.2
ΣPUFA n-6	6.2±0.4^{s,c}	4.1±0.2^{m}	3.8±0.2^{m}
ΣPUFA n-3	4.5±0.4^{c}	3.6±0.2	2.6±0.6^{m}
UI	78.7±1.3^{s,c}	70.7±0.7^{m}	67.3±2.6^{m}
UI (18:1 n-9)	25.2±0.2^{s,c}	29.9±0.5^{m}	32.3±1.5^{m}
UI (18:2 n-6)	0.5±0.1^{s,c}	1.1±0.1^{m,c}	1.5±0.1^{m,s}
UI (20:4 n-6)	22.5±1.1^{s,c}	13.7±1.0^{m}	11.3±1.3^{m}
UI (22:6 n-3)	27.1±0.6^{s}	21.4±1.2^{m}	16.3±3.5
Phosphatidylethanolamine			
ΣSFA	45.2±7.7	45.1±4.6	43.4±4.4
ΣMUFA	11.4±1.8	11.7±1.0	16.7±2.8
ΣPUFA n-6	13.4±2.9	12.7±1.7	13.4±1.3
ΣPUFA n-3	30.0±4.2	30.6±2.9	26.5±4.2
UI	246.1±1.8	247.1±1.1	230.0±7.9
UI (18:1 n-9)	11.0±0.3	11.5±0.2	16.0±2.2
UI (18:2 n-6)	0±0	0±0	0±0
UI (20:4 n-6)	41.9±1.9^{s}	34.1±2.1^{m}	34.7±3.2
UI (22:6 n-3)	187.0±3.9	184.6±4.4	158.0±13.6
Phosphatidylserine			
ΣSFA	45.2±7.9	47.2±6.2	46.5±3.8
ΣMUFA	18.8±7.6	19.4±5.2	26.2±5.6
ΣPUFA n-6	5.0±1.4	5.1±0.6	5.5±0.7
ΣPUFA n-3	31.1±2.8	28.4±1.6	21.8±2.7
UI	230.9±12.0	211.3±11.6	178.2±19.7
UI (18:1 n-9)	16.9±3.2	17.5±2.3	24.1±3.8
UI (18:2 n-6)	0±0	0±0	0±0
UI (20:4 n-6)	16.1±0.9^{c}	13.5±1.8	12.1±1.3^{m}
UI (22:6 n-3)	196.0±11.2	175.3±13.3	135.0±24.0
Phosphatidic acid			
ΣSFA	43.6±2.5	37.9±3.8	44.9±6.4
ΣMUFA	27.2±2.7	42.5±9.9	40.2±8.7
ΣPUFA n-6	7.0±1.3	6.1±0.8	2.8±0.7
ΣPUFA n-3	22.2±0.7	13.6±1.9	12.0±2.4
UI	188.7±2.2	150.9±10.9	130.3±19.9
UI (18:1 n-9)	27.0±1.0	41.1±4.4	39.0±4.4
UI (18:2 n-6)	0±0	0±0	0±0
UI (20:4 n-6)	27.5±3.3^{c}	26.3±5.8	10.7±1.4^{m}
UI (22:6 n-3)	134.2±5.9	83.4±10.8	80.6±24.4

SFA, saturated fatty acid ; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; Σ, total; UI, unsaturation index (the number double bonds per 100 fatty acid molecules).

Superscript letters indicate significant differences between the values derived for mouse (m), sheep (s) and cow (c) at a minimum $P<0.05$. n-3, n-6, n-9 denote the location of the first double bond from the methyl end of the molecule. Values are mean ± s.e.m. ($N=4$).

decrease in membrane unsaturation associated with increasing body mass in mammalian species measured using gas chromatography (Hulbert et al., 2002b). This body-mass-dependent decrease in membrane unsaturation does not occur by decreases in the percentage of unsaturated acyl chains but alternatively by alterations in the type of unsaturates present. This includes increases in the use of monounsaturates (MUFA) and shorter, less unsaturated molecules at the expense of long-chain omega-3 (n-3) and omega-6 (n-6) polyunsaturated (PUFA) fats. This substitution was clearly evident in the kidney, with an increase in MUFA and a decrease in the long-chained, highly unsaturated omega-3 and omega-6 PUFAs with increasing body mass. In brain, the mass-dependent differences in phospholipid acyl composition were far less pronounced (as indicated by the reduction in the number of significant differences between the mammals) than in the kidney (see Table 2), in agreement with the previous work of Hulbert and colleagues (Hulbert et al., 2002b).

The domination of omega-6 in kidney and omega-3 in brain was found to be spread throughout the phospholipid classes and was not restricted to any one particular phospholipid class. In kidney all the PC molecules showed high omega-6 PUFA content as a percentage of their overall fatty acid composition (PC; 26–28%, PE; 34–39%, PS; 28–37%, PA; 25–48%). However, the makeup of these omega-6 fats in kidney moved from primarily arachidonic acid to linoleic acid in PC, PE and PS with increasing body mass. The single largest contributor to the arachidonic acid content of kidney membranes was PE 18:0/20:4, followed by PC 16:0/20:4, whereas the major contributor to linoleic acid was PC 16:0/18:2 (see Fig. 1). In the brain, the omega-3 domination of PUFA was also found spread throughout all phospholipid classes except PC, which had slightly more omega-6 than omega-3 in all three mammals measured. In brain the dominant omega-3 acyl chain was the long-chained docosahexaenoic acid (22:6n-3) with both PE 18:0/22:6 and PS 18:0/22:6 being the major contributors (64–79%). The brain also displayed less diversity of phospholipid molecules (64–66) compared with the kidney (82–120; including all PC, PE, PS, PA, PG and SM molecules). This greater phospholipid molecular diversity in the kidney was also found in an analysis of tissues of an ectothermic lizard (Mitchell et al., 2007).

The measurement of the fatty acid acyl composition of kidney and brain membrane glycerophospholipids by gas chromatography (GC) and mass spectrometry (MS) showed some clear differences. These differences included higher estimations for 16:0 and a lower estimation for 20:4n-6 and sometimes 22:6n-3 using MS than using GC (Table 5). These differences were also found in a previous study (Mitchell et al., 2007) and points to the trend for a lower estimation of unsaturation index using MS versus GC (in the kidney) primarily due to the effect of 20:4n-6 and 22:6n-6 upon UI.

The present study also found a lack of sphingomyelin phospholipids in both the kidney and brain of the mouse, a condition not apparent in tissues of the large mammal species. In sheep and cow, nine different types of sphingomyelin molecules were detected in the kidney and five in the brain, with the major molecules in both tissues being SM16:0 and SM18:0, respectively. Since lipid rafts/caveolae structures are primarily composed of sphingomyelin and cholesterol (Simons and Vaz, 2004; Slotte and Ramstedt, 2007) and the cholesterol to phospholipid ratio is similar in different-sized mammals (Starke-Peterkovic et al., 2005) it seems likely that the composition of these lipid structures varies with body mass, with the cholesterol to sphingomyelin ratio

Table 5. A comparison of the percentage of major fatty acids in kidney and brain of mouse, sheep and cow, as measured by gas chromatography and mass spectrometry

	Mouse		Sheep		Cow	
Phospholipid	GC	MS	GC	MS	GC	MS
Kidney						
16:0	12.9±0.2*	34.5±0.4^{s}	18.5±0.6*	25.9±0.4^{m,c}	20.7±0.5*	33.4±0.9^{s}
18:0	15.8±0.4	15.6±0.2^{s,c}	16.8±0.4	18.2±0.1^{m,c}	12.9±0.2*	8.8±0.5^{m,s}
18:1	7.8±0.1*	11.7±0.2^{s,c}	16.0±0.9*	22.0±0.4^{m}	15.7±0.3	17.8±1.1^{m}
18:2n-6	9.5±0.3	8.6±0.4^{c}	10.1±0.7	11.2±0.7^{c}	15.6±2.4	17.5±1.3^{m,s}
20:4n-6	23.3±0.5*	18.3±0.3^{s,c}	13.4±0.7*	9.8±0.5^{m}	13.1±0.4*	9.1±0.3^{m}
22:5n-6	1.1±0.3	1.6±0.1^{s,c}	–	3.0±0.2^{m}	–	2.6±0.2^{m}
22:6n-3	16.6±0.6*	7.8±0.4^{s,c}	3.1±0.2	2.7±0.3^{m,c}	1.3±0.1	1.0±0.1^{m,s}
UI	249±4*	161±3^{s,c}	172±3*	136±2^{m,c}	163±5*	128±1^{m,s}
Σn-6	36.5±0.6*	28.3±0.2^{s}	24.2±1.1	21.6±0.4^{m,c}	30.6±0.6	27.9±1.1^{s}
Σn-3	17.7±0.7*	9.4±0.4^{c}	14.1±0.5*	10.1±0.6^{c}	10.0±2.4	6.4±0.6^{m,s}
ΣUNSAT	71.0±0.6*	49.8±0.3^{s,c}	63.4±0.5*	55.1±0.4^{m,c}	65.3±0.5*	56.7±0.5^{m,s}
ΣMUFA	16.5±0.2*	12.2±0.2^{s,c}	24.4±0.8	23.4±0.4^{m}	24.3±0.8	22.4±1.2^{m}
ΣPUFA	54.6±0.8*	37.6±0.4^{s,c}	39.0±1.1*	31.6±0.5^{m,c}	41.1±1.0*	34.2±0.7^{m,s}
Brain						
16:0	21.9±1.6*	36.5±1.0	17.2±0.7*	35.9±0.6	17.4±1.1*	34.5±0.7
18:0	15.8±0.8*	19.5±0.6	13.0±0.3*	18.0±0.6	14.0±0.8*	18.0±0.4
18:1	20.2±0.5	21.6±0.6^{c}	21.4±1.0*	25.5±0.5	26.8±2.4	28.4±2.0^{m}
20:4n-6	10.5±0.7*	6.4±0.4^{s,c}	7.3±0.6*	4.2±0.4^{m}	6.7±0.5*	3.6±0.3^{m}
22:6n-3	17.7±2.1	13.0±0.4^{c}	21.0±1.2*	11.3±0.1	14.7±2.7	9.1±1.3^{m}
UI	196±15	144±5	218±7*	138±3	182±13	128±4
Σn-6	14.7±1.0*	7.6±0.5^{s,c}	12.7±0.8*	5.8±0.4^{m}	12.1±0.5*	5.6±0.4^{m}
Σn-3	18.2±1.9	13.0±0.4^{c}	22.8±1.1*	11.4±0.1	16.1±2.6	9.1±1.3^{m}
ΣUNSAT	62.2±2.1*	43.7±0.5^{c}	68.1±0.9*	45.6±0.4	68.3±1.8*	46.6±0.9^{m}
ΣMUFA	29.2±1.0*	23.1±0.6^{s,c}	32.0±1.3	28.4±0.8^{m}	39.5±3.4	31.8±2.1^{m}
ΣPUFA	33.0±3.0	20.6±1.0^{s,c}	36.1±1.5*	17.2±0.5^{m}	28.8±2.7*	14.8±1.3^{m}

MS, mass spectrometry; GC, gas chromatography [data from Hulbert et al. (Hulbert et al., 2002b)]. For the purpose of calculating Σn-6 and Σn-3 values the minor contributions of 20:3 were designated as predominantly omega-6 and those for 20:5 and 22:5 as omega-3 in kidney and 22:5 designated as predominantly omega-6 in brain based on the results of Hulbert et al. ΣUNSAT, total of unsaturated fatty acids; ΣMUFA, total monounsaturated fatty acids; ΣPUFA, total polyunsaturated fatty acids; Σn-6 and Σn-3, total polyunsaturated omega-6 and omega-3 fatty acids, respectively; UI, unsaturation index (the number double bonds per 100 fatty acid molecules).

Values are mean percentage ± s.e.m. (N=4). Superscript letters in the MS columns indicate significant difference between the values derived for mouse (m), sheep (s) and cow (c) at a minimum of P<0.05 whereas in the GC column the asterisk indicates a significant difference (P<0.05) between measurements made by GC [in the study of Hulbert et al. (Hulbert et al., 2002b)] and to those made by MS (the present study).

decreasing with increasing body mass in mammals. Sphingomyelin molecules, however, have been reported to be present in other mouse tissues, including mouse lens tissues, at up to 20% of total phospholipids [(Iwata et al., 1995); although lens tissue is known to have an extraordinarily high level of this particular phospholipid (Slotte and Ramstedt, 2007)]. These results suggest that the tissues of larger mammals may possess higher concentration or bigger raft structures. The potential functional implications of this finding and the involvement of these structures in modulating metabolism also remains an interesting unexplored possibility.

The primary idea of the membrane pacemaker theory is that membrane lipids provide an environment that can increase or decrease the activity of intrinsic proteins. The activity of membrane bound enzymes, such as Na^+/K^+-ATPase, is very different in mammals of different body mass. In the mouse, Na^+/K^+-ATPase molecular activities (turnover rate per enzyme) in the kidney and brain has been calculated at 23,000 and 28,800 ATP min^{-1}, respectively (Turner et al., 2005a; Turner et al., 2005b). In the cow the molecular activities of the Na^+/K^+-ATPase in kidney and brain is much lower at 6300 and 11,400 ATP min^{-1}, respectively (Starke-Peterkovic et al., 2005). These large differences in molecular activity have been linked to differences in the acyl composition of the membrane phospholipids including the level of membrane unsaturation and presence of long-chain highly polyunsaturated fats, especially DHA (Turner et al., 2003). The results of the present study suggest no single type of phospholipid molecule or phospholipid class will be responsible for determining the membrane properties associated with acyl composition between species since the difference is spread across the full range of phospholipids.

Na^+/K^+-ATPase molecular activity has also been shown to correlate more strongly with the lateral pressure of membrane lipid mixtures than with the composition of any individual fatty acid in the membrane (Wu et al., 2001). Na^+/K^+-ATPase appears to be distributed primarily in non-raft domains of the cell membrane (Atshaves et al., 2003; Gallegos et al., 2006) and is influenced by the transbilayer fluidity gradient (Schroeder et al., 2005; Schroeder and Sweet, 1988; Sweet and Schroeder, 1986a; Sweet and Schroeder, 1986b; Sweet and Schroeder, 1988). This suggests that differences in molecular activity are likely to be influenced by the physical properties of the bulk phase membrane lipids created by the acyl composition of the phospholipids. This may be explained by the fact that highly polyunsaturated fats have high level of dynamic motion and flexibility since they have more bisallylic bonds (those that occur on either side of the multiple double bonds present in highly polyunsaturated molecules). This can create high lateral pressures in the immediate environment of these acyl chains that may impinge upon the properties of neighbouring proteins and other lipids in the membrane (Carrillo-Tripp and Feller, 2005). In this

case, the present study suggests that all classes of phospholipids are likely to be involved in determining the physical properties of the membrane and any associated change in metabolism.

In summary, this study shows that differences in membrane phospholipid acyl composition associated with body mass in mammals are spread throughout the different classes of phospholipids, and that phospholipid class distribution is very similar in the same tissues of the different-sized mammals and is likely to be a regulated property of membranes in vertebrates. Furthermore, given that these same properties were found in a similar comparison in a different mammal (rat) and an ectothermic vertebrate (lizard) (Mitchell et al., 2007) it suggests that the physical properties of membranes may be an important determinant of the rate of metabolism in animal species.

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Table S1. Mass spectrometry scan parameters

Scan type	Ion mode	Fragment	Collision energzy (eV)	
Head-group scans				
PC and SM	Precursor ion	Positive	*m/z* 184.1	35
PE	Neutral loss	Positive	141.5 Da	25
PS	Neutral loss	Positive	185.4 Da	22
PA and PG	Precursor ion	Negative	*m/z* 153.0	50
Fatty acyl chain scans				
14:0	Precursor Ion	Negative	*m/z* 227.4	50
	Neutral loss	Positive (lithiated)	287.4 Da	35
15:0	Precursor ion	Negative	*m/z* 241.4	50
	Neutral loss	Positive (lithiated)	301.4 Da	35
16:0	Precursor ion	Negative	*m/z* 255.4	50
	Neutral loss	Positive (lithiated)	315.4 Da	35
16:1	Precursor ion	Negative	*m/z* 253.4	50
	Neutral loss	Positive (lithiated)	313.4 Da	35
17:0	Precursor ion	Negative	*m/z* 269.4	50
	Neutral loss	Positive (lithiated)	329.4 Da	35
18:0	Precursor ion	Negative	*m/z* 283.4	50
	Neutral loss	Positive (lithiated)	343.4 Da	35
18:1	Precursor ion	Negative	*m/z* 281.4	50
	Neutral loss	Positive (lithiated)	341.4 Da	35
18:2	Precursor ion	Negative	*m/z* 279.4	50
	Neutral loss	Positive (lithiated)	339.4 Da	35
18:3	Precursor ion	Negative	*m/z* 277.4	50
	Neutral loss	Positive (lithiated)	337.4 Da	35
20:1	Precursor ion	Negative	*m/z* 309.4	50
	Neutral loss	Positive (lithiated)	369.4 Da	35
20:2	Precursor ion	Negative	*m/z* 307.4	50
	Neutral loss	Positive (lithiated)	367.4 Da	35
20:3	Precursor ion	Negative	*m/z* 305.4	35
	Neutral loss	Positive (lithiated)	365.4 Da	35
20:4	Precursor ion	Negative	*m/z* 303.4	35
	Neutral loss	Positive (lithiated)	363.4 Da	35
20:5	Precursor ion	Negative	*m/z* 301.4	35
	Neutral loss	Positive (lithiated)	361.4 Da	35
22:4	Precursor ion	Negative	*m/z* 331.4	35
	Neutral loss	Positive (lithiated)	391.4 Da	35
22:5	Precursor ion	Negative	*m/z* 329.4	35
	Neutral loss	Positive (lithiated)	389.4 Da	35
22:6	Precursor ion	Negative	*m/z* 327.4	35
	Neutral loss	Positive (lithiated)	387.4 Da	35

PC, phosphatidylcholine; SM, sphingomyelin; PE, phosphatidylethanolamine; PS, phosphatidylserine; PA, phosphatidic acid; PG, phosphatidylglycerol; PI, phosphatidylinositol.
m/z, mass-to-charge ratio.