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Membrane Fatty Acid Composition of Tissues is Related to Body Mass of Mammals

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Abstract. Phospholipids were extracted from tissues (heart, skeletal muscle, kidney cortex, liver and brain) of mammals representing a 9,000-fold range in body mass (mouse, rat, rabbit, sheep and cattle) and their fatty acid composition was determined. In heart, skeletal muscle and kidney cortex, there were significant allometric decreases in the Unsaturation Index (UI; average number of double bonds per 100 fatty acid molecules) with increasing body mass. There were significant inverse allometric relationships between body mass and the proportion of docosahexaenoic acid (22:6ω3) in heart and skeletal muscle. In heart, skeletal muscle and kidney cortex, larger mammals also had shorter fatty acid chains in their phospholipids and a higher proportion of monounsaturates. In liver, smaller mammals had a higher UI than larger mammals (except the rabbit, which had the lowest UI and very low proportions of ω3 fatty acids). The brain of all mammals maintained a high UI with similar levels of polyunsaturated fatty acids, especially 22:6ω3. Our results suggest that in heart, skeletal muscle and kidney cortex the activity of the elongases and desaturases are reduced in large mammals compared to small mammals. The allometric trends in membrane composition may be involved in modifying membrane permeability. It is proposed that the elevated degree of polyunsaturation in the membranes of several tissues from small mammals is related to their higher metabolic activity.

Key words: Mammals — Allometry — Cell membranes — Phospholipid — Fatty acids — Polyunsaturation

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Introduction

Membrane phospholipids provide a hydrophobic barrier between the cell and its environment as well as between various cellular compartments. They also provide a suitable physical environment for the molecules embedded in the membrane. The fatty acid composition of membrane phospholipids influences the physical behavior of the membrane bilayer as well as membrane permeability and the activities of many enzyme molecules embedded in it (White & Somero, 1982; Brenner, 1984; Spector & Yorek, 1985; Hoch, 1992). Membrane fatty acid composition appears to be largely under genetic control, being regulated through the activities of the elongase and various desaturase enzyme systems. Environment factors such as diet, temperature and hormones also influence the fatty acid composition of membrane bilayers. While dietary intake can influence the fatty acid pool from which phospholipids are made and thus influence membrane fatty acid composition, the desaturase and elongase enzyme systems modify membrane composition in response to dietary deficiencies and excesses. In ectotherms, where body temperature can vary dramatically, these enzyme systems are also involved in modifying membrane fatty acid composition for maintaining membrane homeoviscosity (Hazel, 1984). In endothermic mammals, because of their relatively constant body temperature, this factor is not as important an influence on the composition of membrane bilayers.

In an examination of the high levels of metabolism of endothermic mammals compared to ectothermic reptiles we have observed correlations between metabolic activity, membrane permeability and fatty acid composition (Hulbert & Else, 1989; Brand et al., 1991; Brand, Couture & Hulbert, 1994). The metabolic intensity of mammals varies in an allometric manner with body size, both intraspecifically and interspecifically (Brody, 1945; Kleiber, 1961); small mammals have a faster metabolic rate than large mammals. Mechanistically, the explana-

tion is that small mammals have relatively larger internal organs and that the tissues of small mammals exhibit a greater mass specific metabolic activity than tissues from larger mammals (Krebs, 1950; Holliday et al., 1967; Couture & Hulbert, 1995). Kidney cortex and liver metabolic activity was also found to be correlated to sodium pump activity, an indicator of plasma membrane sodium and potassium permeability, in different-sized mammals (Couture & Hulbert, 1995). Whether the tissue phospholipids of small mammals are more polyunsaturated than those of large mammals is not known.

A major stimulus for the work reported here was the findings of Gudbjarnason and coworkers (Gudbjarnason et al., 1978; Gudbjarnason, 1989) who reported a very strong relationship between the highly polyunsaturated phospholipid docosahexaenoic acid (22:6ω3) content and heart rate for mammals ranging in size from mice to whales. The higher the heart rate (which is an indicator of heart metabolic activity), the greater the 22:6ω3 content of the heart phospholipids. The significance of this finding has not yet been established. The present study was designed to confirm and possibly extend this observation. Since heart rate, like metabolic rate, is allometrically related to body mass in mammals, we hypothesized that the relationship reported by Gudbjarnason may be part of a broader relationship between body mass and membrane fatty acid composition. Specifically, we have tried to answer the following questions: Does the relative content of particular fatty acids in cardiac phospholipids systematically vary with the body mass of mammals? Is the extent of unsaturation of heart membrane phospholipid fatty acids related to body mass? Do other tissues show body size related systematic variations in the fatty acid composition of their phospholipids?

To our knowledge, no systematic investigation concerning possible body mass related variations in mammalian tissue phospholipid fatty acid composition has been conducted to date. To this end, tissues from mammals ranging from mouse to cattle were analyzed for their cell membrane phospholipid fatty acid composition. The species examined were mouse, rat, rabbit, sheep and cattle which represent a total range of approximately 9,000-fold in body mass and conveniently exhibit an approximate 10-fold increase in body mass with each next larger species. The mass-specific basal metabolic rate of a mouse is 11 times higher than that of a cattle (Kleiber, 1961). To obtain general information on the distribution of the various fatty acids in the tissues of different-sized mammals, total phospholipids from liver, kidney cortex, heart, skeletal muscle and brain were examined. In addition to describing the relative amount of specific fatty acids in the phospholipids from these tissues, we have used general indexes related to membrane fatty acid composition, such as the Unsaturation Index (UI) and the percentages of fatty acids in particular families. With this information, allometric trends in the activity of the desaturase and elongase enzyme systems have also been deduced.

Materials and Methods

Animals and Tissues

All animals used were adults, appeared to be in good health and no animal was obese or scraggy. Mice, rats and rabbits were weighed after death and their mean mass (±SEM, n = 4) was 42.1 ± 1.2 g, 581 ± 44 g and 4100 ± 737 g, respectively. The body mass of sheep and cattle was calculated using dressed carcass mass and assuming it to be 55% of total body mass (pers. comm., School of Wool and Pastoral Sciences, University of New South Wales). The body mass of these species was calculated to be 32.9 ± 0.8 Kg for sheep and 369 ± 34 Kg for cattle. The mice (*Mus musculus*, breed CBA/H), rats (*Rattus norvegicus*, breed Wistar) and rabbits (*Oryctolagus cuniculus*, breed New Zealand White) were killed by concussion and tissues immediately removed. The sheep (*Ovis aries*) and cattle (*Bos taurus*) were farm animals and their tissues were collected within 30 min after death at Yallah abattoir and transported back to the laboratory (20 km) in ice. Liver samples were taken randomly from the main lobe for all animals. Kidney cortex samples were taken randomly for all animals except in mice where the whole kidney cortex was used. Heart samples were excised from the ventricular muscles in sheep and cattle while the whole cardiac muscle was used for the smaller mammals. Brain samples were collected from the cortex. Skeletal muscles were the gastrocnemius from mice, rats and rabbits while from sheep and cattle the samples were from the inner thigh. The tissues were stored at −80°C for a period of time not exceeding 12 weeks. Previous trials in the laboratory have shown phospholipid fatty acid composition remained stable for this period. These experiments were approved by the University of Wollongong Animal Experimentation Ethics Committee.

Mice and rats were fed Rat and Mouse Cubes (Allied Feeds, NSW) while rabbits were fed rabbit pellets (Sieldorf Feed, NSW). Both types of chow were analyzed for essential fatty acid content and found to be adequate (*see* "Discussion"). For all species both water and food were available *ad libitum*.

Towards the end of this study, it appeared that the phospholipid fatty acid composition of tissues from the laboratory rabbits may be anomalous in that they contained unusually low levels of PUFA from the ω3 family and as a consequence, low UI. Tissues from feral rabbits shot in the wild became available and we also measured the phospholipid fatty acid composition of these tissues. The body mass of these wild rabbits was 1.185 ± 0.088 Kg [n = 4]. The fatty acid composition of phospholipids from wild rabbits was very similar to that of laboratory reared rabbits, with the exception of 22:5ω6 which in the brain of laboratory rabbits was 9.8 mol% and only 0.5 mol% for the wild rabbits. Thus, the data were not included in this article since this verification suggested that the data obtained from laboratory-reared rabbits were not an artifact of diet or strain selection and their inclusion would not have affected the interpretation of the results.

Chemicals

All solvents used in the extraction and purification of the fatty acids from tissue phospholipids were of nanograde purity and were purchased from Mallinckrodt, except for methanol, which was obtained from BDH. Various sources of commercial fatty acid methyl ester standards were used. Individual fatty acid methyl ester standards were purchased from Sigma Chemicals, except 22:1ω9, purchased from Nucheck Prep. Commercial mixtures of standard fatty acid methyl esters used were PUFA 2 (Supelco), Standard Lipids Mixture ME82 and Qualmix Fish (Larodan).

Analysis of Total Membrane Phospholipid Fatty Acid Composition

The extraction and purification of fatty acid methyl esters from mammalian tissue phospholipids were performed as described elsewhere (Hulbert & Else, 1989). Analysis of sample fatty acid composition was performed using a Varian 3300 gas chromatograph with a 25 m BPX70 fused silica capillary column (internal diameter: 0.22 mm and 0.25 μm coating thickness) purchased from SGE Scientific. The column was connected to a flame ionization detector coupled to a Shimadzu C-R3A chromatopac integrator. The temperature program was sufficient to elute all fatty acid methyl esters. All fatty acids are described by their number of carbon atoms: number of double bonds followed by their family in the case of unsaturated fatty acids.

Only those peaks that were more than 1% of total are reported in Figs. 1–5 but all fatty acids that constituted more than 0.1% of total were used in calculations and considered in Figs. 6–9 and in the tables. Fatty acids were identified by matching their retention time to that of standards, except for 17:1ω7 which was identified by relating its retention time to that of other monounsaturated fatty acid standards of the ω7 family. Each of the unidentified peaks constituted less than 0.75% of the total peak area except for an unknown appearing once in a sheep and once in a cattle liver and which represented 2.9 and 1.0% of the sample respectively. Unknowns were ignored in the composite analysis of the results.

Calculations and Statistics

The unsaturation index (UI) represents the average number of double bonds per 100 membrane fatty acid molecules. It was calculated by summing the products of the proportion (mol%) of each unsaturated fatty acid multiplied by its number of double bonds. To calculate the average chain length, the chain length of each fatty acid was multiplied by the fraction of this fatty acid in the sample and these values summed. All other composition data are expressed in mol%.

Interspecific differences for the indexes reported in Tables 1 to 5 were tested for significance using a Mann-Whitney U test (Porkess, 1988) and a significance level of 5% for a two-tail test. All allometric relationships were plotted on double logarithmic plots and the linear regression equations were obtained with Cricket Graph (version 1.3.1) software. The correlations with body mass were tested using the Pearson product moment correlation coefficient (r) and a two-tail test. The level of significance is indicated in each case. These regressions were determined and tested for significance using the mean values for each species (i.e., n = 5). This method is a rigorous test (when compared with the alternative method of using the 20 individual data points) highlighting only very significant allometric relationships.

Results

Figures 1 to 5 provide a description of all fatty acids in mammalian heart, skeletal muscle, kidney cortex, liver and brain phospholipids found to constitute more than 1% of the total phospholipid fatty acids. Within each species, fatty acids were grouped into four categories: saturates, monounsaturates (also including for convenience 20:3ω9), ω6, and ω3 polyunsaturates. Within each category, fatty acids were organized such that they increase in degree of unsaturation and/or chain length from left to right. A fatty acid profile shifted to the right within a category compared to the same category from other species was considered indicative of a higher activity of the desaturase and elongase enzyme systems. Nonessential fatty acids (NEFA) included all the saturated and monounsaturated fatty acids as well as the polyunsaturate 20:3ω9. Essential fatty acids (EFA) comprised all the ω6 and ω3 polyunsaturates. Polyunsaturates were mostly EFA, since 20:3ω9, the only NEFA polyunsaturate present in the mammalian tissue phospholipids examined, never represented more than 1.1 mol% (in mouse liver, Fig. 4) of the total tissue phospholipid fatty acid composition. Intraspecific variability was typically very low, as illustrated by the error bars in Figs. 1–5.

The fatty acid composition of heart phospholipids for the species examined is presented in Fig. 1. As can be seen from this figure, the smaller the mammalian species the more the relative distribution of ω3 and ω6 fatty acids is shifted to the right. A significant allometric decrease in 22:6ω3 content was found (Fig. 6, $P < 0.05$), with values ranging from 22.3 mol% in mouse compared to 0.5 mol% in cattle, the greatest allometric range found in this study for an individual fatty acid. The tendency of smaller mammals to exhibit more polyunsaturated PUFA led to a significant allometric decrease of the UI (Fig. 7, $P < 0.01$), mouse heart phospholipids containing 44% more double bonds per fatty acid molecule than found in cattle (Table 1). Heart phospholipid fatty acids of mouse were also significantly longer than for rat and these species had longer fatty acids than found in larger mammals (Table 1, $P < 0.05$), leading to a significant allometric decrease in average chain length (Fig. 8, $P < 0.01$). This relationship reflects the highly significant linear relationship between average chain length and UI for the pooled data for all tissues (n = 25, $P < 0.01$, *not shown*). Within both the saturates and the monounsaturates there was no apparent size-related change in profile although a significant allometric increase in the total monounsaturate content with body mass was found (Table 1 and Fig. 9, $P < 0.05$), ranging from 16 to 26% in the mouse to cattle comparison.

Skeletal muscle showed highly similar allometric changes in tissue phospholipid fatty acid composition as found in heart. The same significant allometric trends that were observed in heart, discussed above, were found in skeletal muscle (Figs. 2 and 6–9, Table 2). The allometric relationship of the UI was more pronounced in skeletal muscle than in heart (Fig. 7, $P < 0.02$), with mouse skeletal muscle phospholipids containing 76% more double bonds per fatty acid molecule than for sheep and cattle. The proportion of monounsaturates increased from 15 to 44% between mouse and cattle (Table 2). In both heart and skeletal muscle, there was a highly significant increase in the proportion of ω7 fatty acids with body mass ($P < 0.01$, *not shown*). Thus, ω7 fatty acids were largely responsible for the allometric increase in the total monounsaturates in both types of muscle.

In kidney cortex, Fig. 3 reveals that both ω3 and ω6

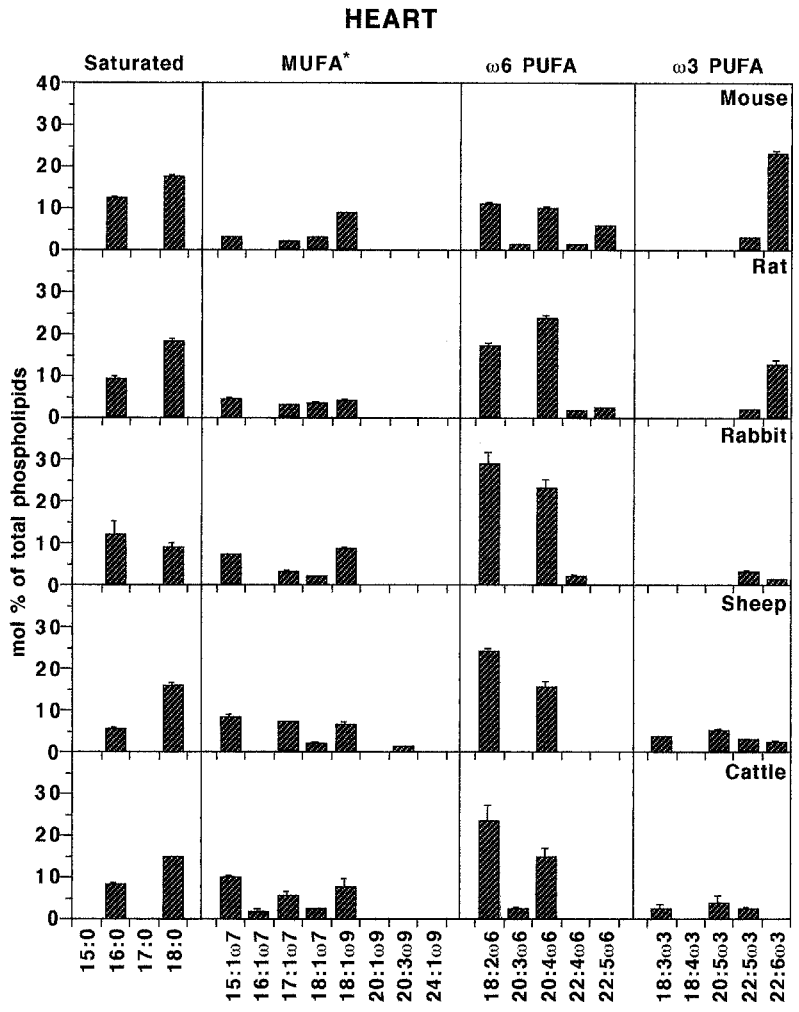

Fig. 1. Fatty acid composition (mol% of total phospholipids) of heart phospholipids in five mammalian species. Results are as mean ± SE (n = 4). *Also included is the nonessential PUFA 20:3ω9.

fatty acids were shorter and less polyunsaturated in larger mammals. An allometric decrease in the UI in kidney cortex was found (Fig. 7, $P < 0.05$), mouse kidney cortex cell membranes containing 53% more double bonds per fatty acid molecule than cattle. There was an allometric increase in the proportion of monounsaturates in this tissue (Fig. 9, $P < 0.05$), from 17 to 24% from mouse to cattle (Table 3). The ω9 fatty acids (in particular 18:1ω9, the major ω9 fatty acid) were mostly responsible for the allometric increase in monounsaturates observed for this tissue. Although mouse kidney cortex exhibited 16.6 mol% 22:6ω3, compared with 1.3 mol% in cattle, the allometric trend for this fatty acid was not significant. The trend of decrease in average chain length in larger mammals (Table 3) was not significant at the 5% level for kidney cortex membrane fatty acids.

The phospholipid fatty acid composition of liver did not vary as much allometrically as in heart, skeletal muscle and kidney cortex. Nevertheless, Fig. 4 shows that for the ω3 fatty acids, while mouse and rabbits accumulated only 22:6ω3, sheep and cattle accumulated shorter and less polyunsaturated fatty acids of this group instead. Rabbit are noteworthy in this tissue for the presence of only very small amounts of ω3 fatty acids (Table 4). By contrast, the latter species had more of the ω6 fatty acids than other mammalian species examined. Although the UI of mouse and rat liver was significantly lower than that in sheep and cattle (Table 4, $P < 0.05$), the inverse allometric relationship with the UI was not significant at the 5% level (Fig. 7). Nevertheless, mouse and rat had 17% more double bonds per fatty acid molecule than sheep and cattle in their liver cell membranes (Table 4, $P < 0.05$). Finally, there was a significant decrease in 16:0 ($P < 0.01$, not shown) and a proportional but non significant increase in 18:0 with increasing body mass.

Compared to the other tissues examined, brain phospholipid fatty acid composition was the most conservative interspecifically (Fig. 5, Table 5). No allometric trends were observed in this tissue. All five species maintained a high 22:6ω3 content, even rabbit, notoriously low in ω3 fatty acids in other tissues (Fig. 5). There was still a slightly lower 22:6ω3 content in rabbit brain compared to other species and this was com-

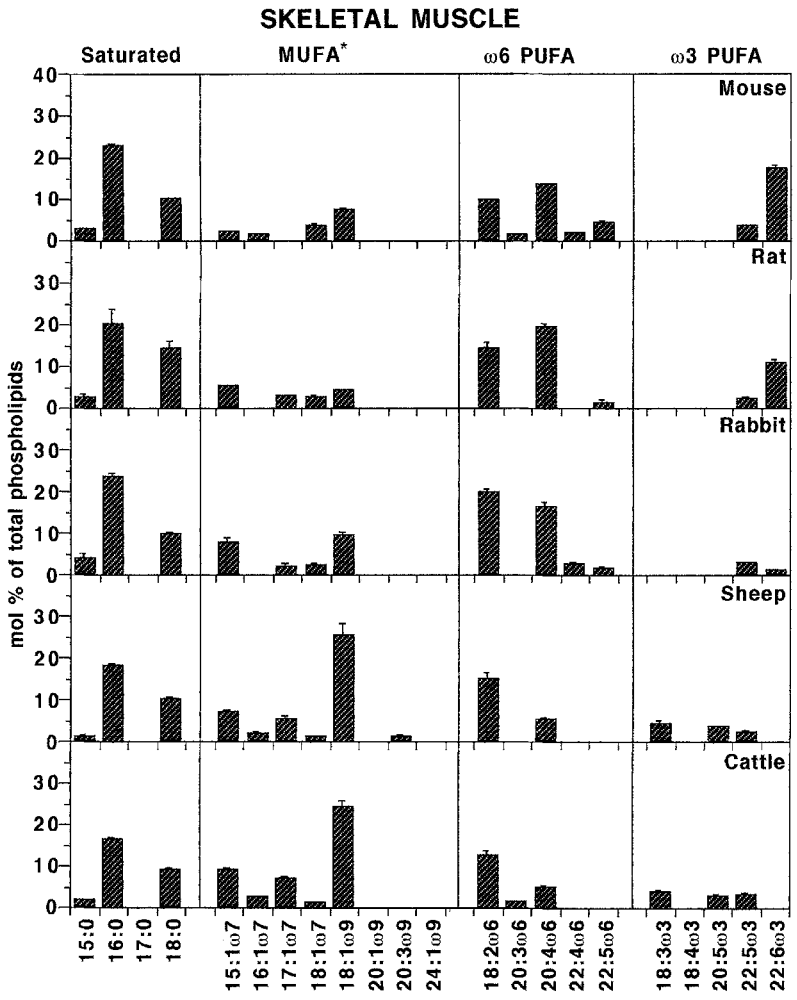

Fig. 2. Fatty acid composition (mol% of total phospholipids) of skeletal muscle phospholipids in five mammalian species. Results are as mean ± SE (n = 4). *Also included is the nonessential PUFA 20:3ω9.

pensated for by higher levels of 22:5ω6. In general though, brain contained lower levels of EFA than other tissues, especially fatty acids from the ω6 family.

In all tissues and species investigated, about two thirds of the fatty acids were unsaturated (from 58–59% in sheep and cattle liver to 79% in rabbit and sheep heart, Tables 1–5). There were no allometric variations of the % unsaturates in the mammalian tissue phospholipids examined, although heart and skeletal muscle of larger mammals had significantly more total unsaturates than heart and skeletal muscle of smaller mammals (Tables 1 and 2).

Monounsaturates belonged to 2 groups, the ω9 and ω7 monounsaturates (20:1ω11 was also detected in heart, kidney cortex and liver but was never more than 0.4 mol% of total fatty acid composition and was thus excluded from Figs. 1–5). Five ω9 fatty acids were present in mammalian tissues. Although the proportions of ω9 fatty acids varied interspecifically, a significant allometric relationship was only observed in kidney cortex, where the proportion of ω9 monounsaturates increased with body mass (Table 3). The smaller mammals (mouse, rat and rabbit) had higher proportions of 18:1ω9 in brain than in the other tissues. Using the pooled data from Figs. 1–5 (n = 25), there was a highly significant inverse relationship between the % PUFA (mainly EFA) and the % 18:1ω9 ($P < 0.01$, *not shown*), as well as between the % PUFA and the % MUFA ($P < 0.01$, *not shown*). The other ω9 fatty acids present in mammalian tissue phospholipids were present only in small proportions, not more than 2 mol%. The only nonessential PUFA, 20:3ω9, was found in all tissues and species but again only in small proportions, reaching 1.1 mol% of the total membrane fatty acid composition in mouse liver.

The ω7 fatty acids were all monounsaturates. Four ω7 fatty acids were found in every tissue of every species. The odd chained 15:1ω7 and 17:1ω7 were commonly more abundant than the even chained 16:1ω7 and 18:1ω7 (Figs. 1 to 5). There were usually lower proportions of ω7 fatty acids than of other groups of unsaturates. An exception to this was in heart phospholipids, where the larger mammals accumulated twice as much ω7 (mainly odd chained) as ω9 fatty acids. The even chained 16:1ω7 was of minor relative importance in all tissues (reaching 2.5% of total molar composition in cat-

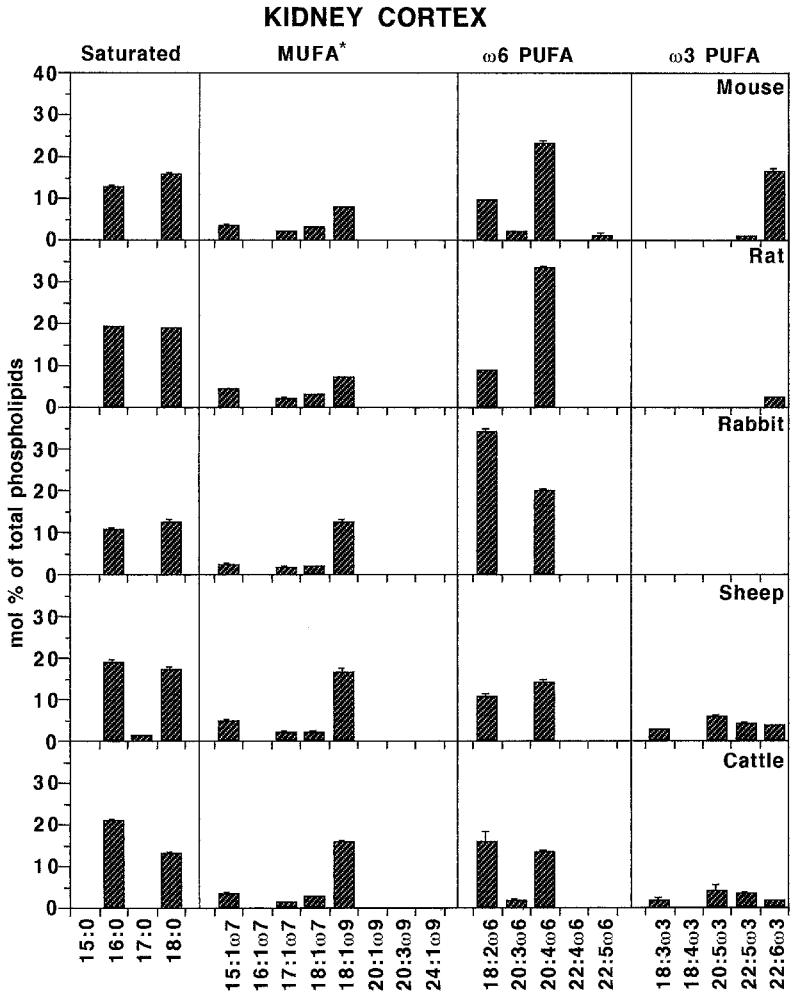

Fig. 3. Fatty acid composition (mol% of total phospholipids) of kidney cortex phospholipids in five mammalian species. Results are as mean ± SE (n = 4). *Also included is the nonessential PUFA 20:3ω9.

tle skeletal muscle). The even chained 18:1ω7 was generally slightly more abundant than 16:1ω7, although the highest concentrations found were only 3.7 mol% (in mouse and cattle brain).

Approximately two thirds of the unsaturated fatty acids contained two or more double bonds. Thus, on average in mammalian tissues, 40 to 50% of the fatty acids in membranes were polyunsaturated and came from the diet (or gut flora), as they were essential ω6 and ω3 PUFA. For all species, heart was generally richer in EFA than the other tissues. The proportion of EFA in mammalian cell membranes ranged from 59% in rat and rabbit heart and in rabbit kidney to about 30% in sheep and cattle skeletal muscle and in cattle brain. In general, lower proportions of EFA were found in the tissues of larger mammals. Nevertheless, a statistically significant allometric decline of EFA was found only in skeletal muscle ($P < 0.02$, *not shown*).

The majority of the EFA in mammalian tissues were ω6 PUFA (Tables 1 to 5). Larger mammals had less ω6 PUFA than smaller mammals in their skeletal muscle, kidney cortex and liver phospholipids. Rabbits were exceptional in having more of this group of EFA than the other species for all tissues, rendering several allometric trends nonsignificant. The accumulation of particular fatty acids from the ω6 family varied between tissues and in the present study appeared to be a species characteristic unrelated to body mass. Although six ω6 fatty acids were detected in mammalian tissues, only three (18:2, 20:4 and 22:5) were found in substantial quantities (more than 3 mol%), except in brain where 22:4ω6 accounted for 3 to 6 mol% of the total membrane fatty acid composition for all species (Fig. 5). Linoleic acid (18:2ω6) is a common diet component for all species examined and is the least unsaturated EFA. It accumulated in good proportions, at least 7 mol%, in all tissues except in brain where rabbits, with only 1.7 mol% of 18:2ω6, had about 3 times as much as the other species. Rabbits accumulated very high amounts of 18:2ω6 in all tissues compared to other species (up to 37 mol% in liver, Fig. 4). In their heart, larger mammals had significantly more 18:2ω6 than did the smaller mammals. Large amounts of arachidonic acid (20:4ω6) were found in the tissues of smaller mammals. Rats had more of this fatty acid than the other species in all tissues (up to 33 mol% in kidney cortex and liver, Figs. 3 and 4), except in brain where similar amounts were found in rabbits (Fig. 5). The most polyunsaturated ω6 fatty acid, 22:5ω6, was only present

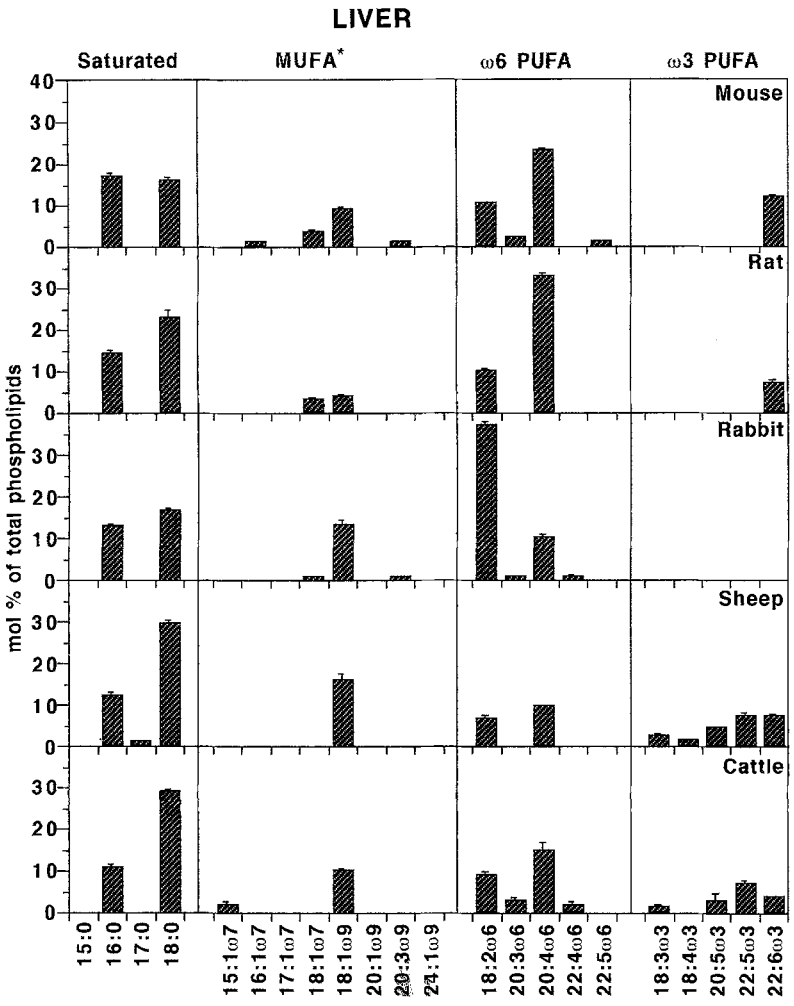

Fig. 4. Fatty acid composition (mol% of total phospholipids) of liver phospholipids in five mammalian species. Results are as mean ± SE (n = 4). *Also included is the nonessential PUFA 20:3ω9.

in substantial amounts in mouse heart and skeletal muscle (5.5 mol% and 4.2 mol%, respectively, Figs. 1 and 2) and in rabbit brain (9.8 mol%, Fig. 5). Thus rabbit brain had more of all major ω6 fatty acids than the brains of the other species examined.

Seven different ω3 EFA were found in mammalian tissues (Figs. 1 to 5), with up to six co-occurring in heart, kidney cortex and liver. In contrast to the ω6 EFA, rabbits had remarkably low amounts of ω3 fatty acids in their tissues. There was no significant allometric trend in the total proportion of ω3 fatty acids accumulated in any tissue, but the proportion of 22:6ω3 varied inversely with body mass in the two striated muscles examined (*see* above). There was a clear tendency in all tissues but brain for large mammals to accumulate shorter and less unsaturated ω3 fatty acids than smaller mammals.

Discussion

The work presented here was largely motivated by the relationship between heart phospholipid docosahexaenoic acid (22:6ω3) proportion and the heart rate of mammals described by Gudbjarnason et al. (1978). Because heart rate is allometrically related to body mass in mammals it suggested the possibility of allometric scaling in the fatty acid profile of tissue phospholipids. To all three questions posed in the introduction we can answer yes. The fatty acid profile of cardiac phospholipids varies with body mass in mammals. Smaller mammals tend to have more polyunsaturated cardiac cell membranes. Such body mass related trends in membrane fatty acid composition were also observed in other (but not all) tissues. It appears that the correlation observed by Gudbjarnason et al. (1978) may be part of a more general trend of increasing desaturase and elongase activity in some tissues from small mammals.

Phospholipids from the heart, skeletal muscle, kidney cortex and liver of mice contained on average 44%, 73%, 53% and 17% more double bonds than those in the respective tissues of cattle. The higher UI found in some tissues of smaller mammals was mainly due to the presence of longer and more polyunsaturated ω3 fatty acids in these animals, but not to proportionally more of this fatty acid family as a whole. Such differences in relative membrane polyunsaturation could be explained by the

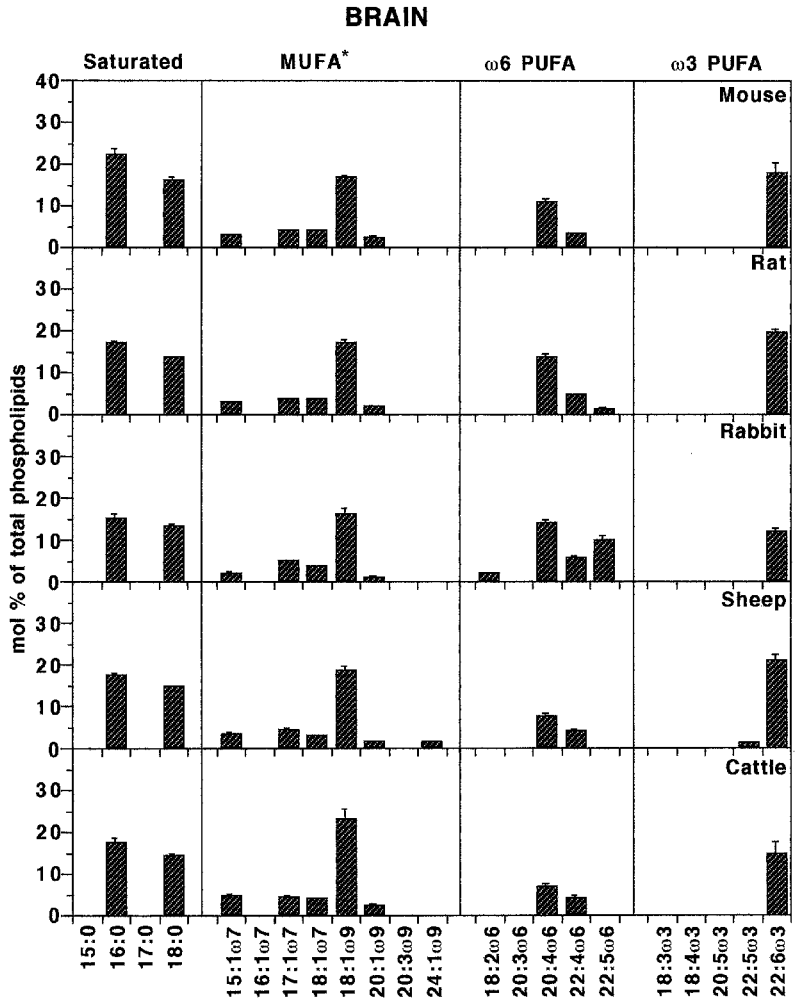

Fig. 5. Fatty acid composition (mol% of total phospholipids) of brain phospholipids in five mammalian species. Results are as mean ± SE (n = 4). *Also included is the nonessential PUFA 20:3ω9.

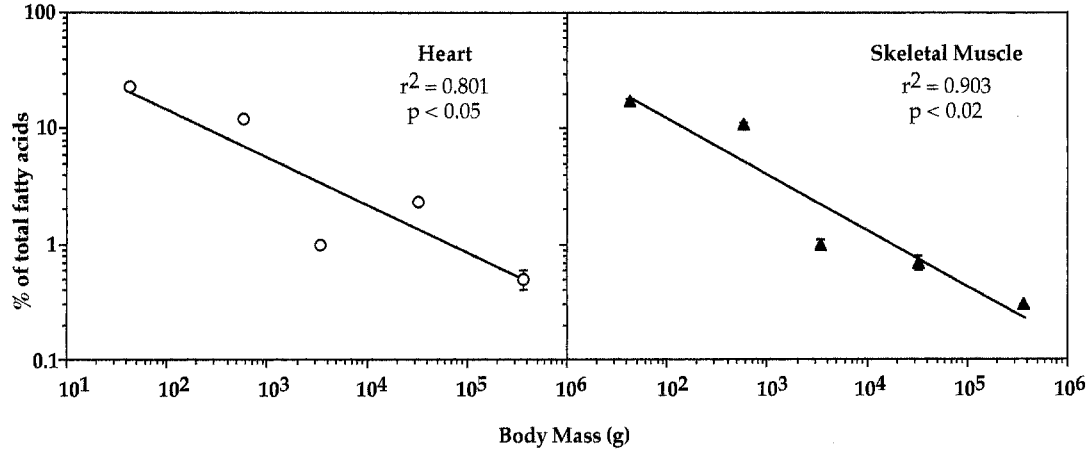

Fig. 6. Relationship between the proportions of 22:6ω3 and body mass in mammalian heart and skeletal muscle phospholipids. Points are mean ± SE (n = 4) (hidden by symbols when not visible).

relative activity of the desaturase and/or of the elongase enzyme systems in different-sized mammals. The substrate preference of desaturases is reported to be ω3>ω6>ω9 (Stubbs & Smith, 1984) and thus it is understandable that it is the ω3 polyunsaturated composition of tissue phospholipids that best demonstrate body size related trends in enzyme activity. It should be noted that such size-related trends appear to apply to only some

desaturases. The enzyme complex responsible for desaturating the saturated fatty acids (the Δ9 desaturase) is different from the enzyme systems that further desaturate unsaturated fatty acids (the Δ6, Δ5 and Δ4 desaturases) and it is only these latter desaturases that we suggest vary in activity with body mass in mammals.

Mammals are unable to synthesize ω3 and ω6 polyunsaturated fatty acids although they can both elongate and further desaturate them. This fact together with the finding that these essential fatty acids make up 30–60% of membrane fatty acids suggests diet will influence the composition of cell membranes in mammals. Modification of dietary PUFA is central to the regulation of membrane lipid composition and if the diet is deficient in such fatty acids relatively unusual PUFAs are synthesized. The presence of a PUFA which can be synthesized in cases of EFA deficiency, mead acid (20:3ω9), has become diagnostic of essential fatty acid deficiency. Moore, Richardson & Deluca (1969) reported that EFA-deficient rats had 16% 20:3ω9 in heart, kidney and liver pooled, whereas rats on a normal diet had around 1%. The low levels of 20:3ω9 fatty acids found here in mammalian phospholipids (maximum 1.1%) suggests that the relatively low amounts of EFA found in the tissues of larger mammals were not associated with dietary EFA deficiency but are instead a regulated phenomenon. To be sure of sufficient levels of dietary EFA, we analyzed the diet of the laboratory mammals examined here and found them to contain ample EFA (*results not shown*). We were unable to do such analyses of the food of the large mammal species and for the wild rabbits examined but, given the very low levels of 20:3ω9 in their tissues (undetectable except in brain, where 0.3 mol% were found) it is unlikely they were EFA deficient. The fatty acids found in forage crops or in grains are mainly the polyunsaturated 18:3ω3 and 18:2ω6, respectively (Christie, 1981).

Two questions must be addressed in relation to diet to validate our interpretation of the data presented here. The first question is whether the high concentrations of the longest and most polyunsaturated PUFA from the ω3 family found in some tissues from small mammals is the result of dietary input or of the activity of the elongase and desaturase systems. Since the diets examined for the laboratory animals did not contain 22:6ω3 but did contain 18:3ω3, the presence of 22:6ω3 in important proportions in tissues of smaller mammals implies a higher activity of the elongase and desaturase systems. The second question is whether the proportion of PUFA in the diet of different-sized mammals explains the inverse allometric relationship observed for the extent of polyunsaturation in heart, skeletal muscle and kidney cortex. While we do not argue that smaller mammals in the wild have a diet which is richer in PUFA than larger mammals (the former in the wild eat seeds while ruminants prefer grass), we believe that each animal has evolved to select

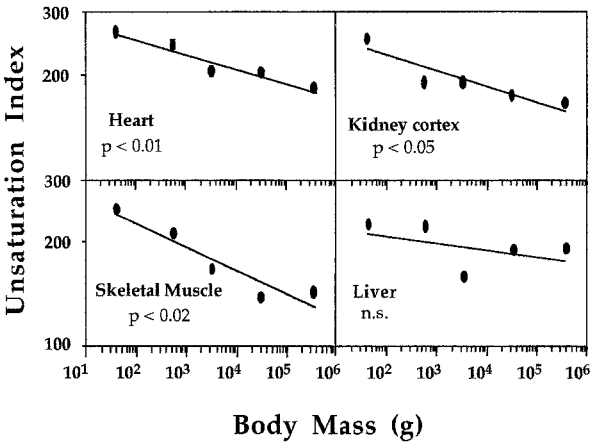

Fig. 7. Unsaturation Index *vs.* body mass in four tissues of mouse, rat, rabbit, sheep and cattle. Points are mean ± SE (n = 4) (hidden by symbols when not visible).

a diet which suits its biochemical needs, according to environmental constraints and to its metabolic activity. We postulate that the observation presented here, that smaller mammals possess more polyunsaturated cell membrane phospholipid fatty acids in some tissues, is the result of diet and of the activity of the elongase and desaturase system and is related to the mass of these mammals, itself determinant for tissue metabolic rate.

Our results show that the body mass-related compositional changes are clearly similar in heart and skeletal muscle, in agreement with the findings for the marmoset monkey (Charnock et al., 1992). However, the proportion of EFA in heart remained constant in different-sized mammals but decreased in skeletal muscle of larger mammals. As a result, fatty acid composition was similar for heart and skeletal muscle from small mammals but differences increased in larger mammals. These findings also suggest that there is a general requirement for a high proportion of PUFA in the heart of mammals of all size, in contrast to skeletal muscle. Nevertheless, the extent of polyunsaturation of the PUFA was related to body mass in both tissues.

The results presented here for mammalian brain depict this tissue as very conservative in terms of membrane fatty acid composition. Even though in all species examined brain maintained lower amounts of polyunsaturated fatty acids than the other tissues, mammals of all species showed a similar UI in their brain phospholipids, of around 210. There were only two prevalent EFA in this tissue, namely 20:4ω6 and 22:6ω3 (except for laboratory rabbit, *see below*). While larger mammals had only small amounts of 22:6ω3 in other tissues, all species maintained substantial amounts of this fatty acid in brain. The comparatively low levels of 22:6ω3 in laboratory rabbit brain compared to other species were accompanied by very high proportions of 22:5ω6. This was not due to dietary deficiency (*see above*) but more likely to

Table 1. Unsaturation Index (average number of double bonds per 100 fatty acid molecules), average chain length (number of carbon atoms) and proportion (mol%) of fatty acid types in mammalian heart

	Mouse	Rat	Rabbit	Sheep	Cattle
Unsat. Index	264 ± 2[a]	243 ± 8[a]	204 ± 7[b]	202 ± 4[b]	183 ± 3[c]
Chain length	19.2 ± 0.0[a]	18.8 ± 0.1[b]	18.3 ± 0.0[c]	18.2 ± 0.0[c]	18.0 ± 0.0[c]
% unsaturates	70.4 ± 0.6[c]	72.9 ± 1.5[bc]	79.4 ± 2.3[ab]	78.7 ± 0.2[a]	76.5 ± 0.5[ab]
% monounsat.	16.2 ± 0.6[b]	14.4 ± 0.6[b]	20.5 ± 1.0[a]	24.0 ± 0.9[a]	26.0 ± 2.8[a]
% polyunsat.	54.2 ± 0.1[b]	58.5 ± 1.8[a]	58.9 ± 3.3[ab]	54.7 ± 1.1[ab]	50.4 ± 3.0[ab]
% ω9	8.9 ± 0.3[a]	4.2 ± 0.1[b]	8.7 ± 1.2[a]	7.3 ± 0.9[a]	8.2 ± 2.2[ab]
% ω7	7.5 ± 0.3[c]	10.3 ± 0.6[b]	11.9 ± 0.3[b]	17.6 ± 0.3[a]	18.5 ± 1.1[a]
% ω6	28.2 ± 0.6[c]	44.0 ± 0.7[a]	54.2 ± 3.9[a]	40.1 ± 1.1[b]	40.7 ± 5.8[abc]
% ω3	25.6 ± 0.6[a]	14.3 ± 1.0[b]	4.4 ± 0.6[c]	13.6 ± 0.7[b]	9.0 ± 2.9[bc]

Results are average ± SE (n = 4). Indexes in a row with common exponent letters do not differ significantly ($P < 0.05$).

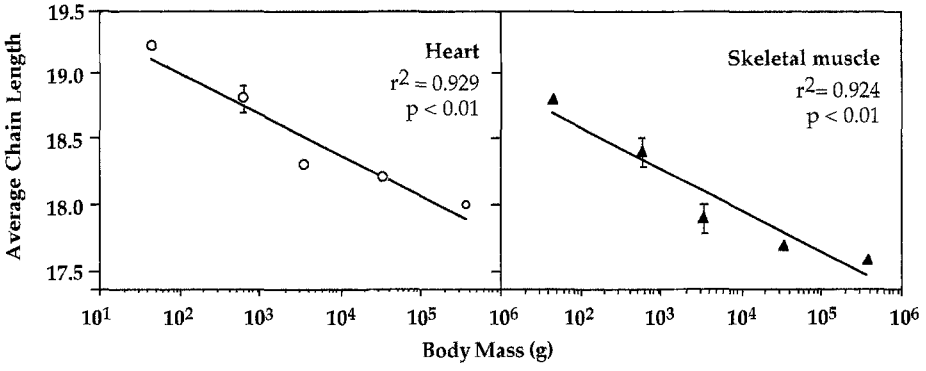

Fig. 8. Relationship between average fatty acid chain length (number of carbon atoms) and body mass (g) in mammalian heart and skeletal muscle. The data are presented as mean ± SE (n = 4) (hidden by symbols when not visible).

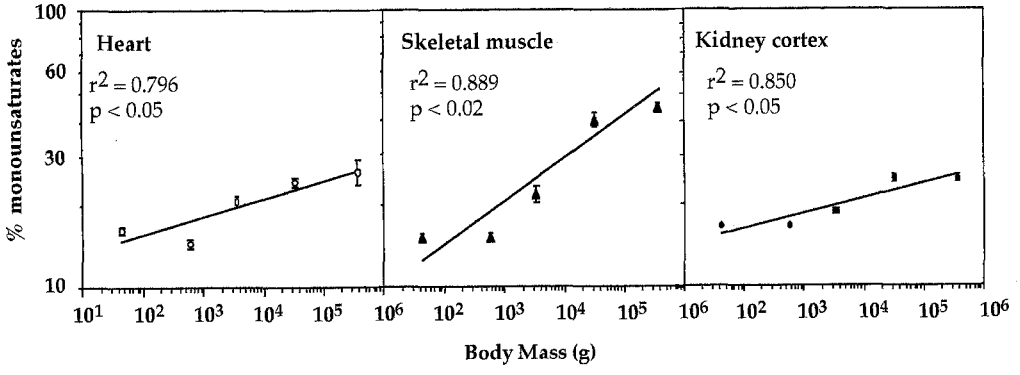

Fig. 9. Relationship between the proportion of monounsaturates in membrane phospholipids and body mass in mammalian kidney cortex, heart and skeletal muscle. The data are mean ± SE (n = 4) (hidden by symbols when not visible).

a reduced capacity for incorporating ω3 fatty acids in rabbits. The observation that 22:5ω6 compensated for lower levels of 22:6ω3 in rabbit brain is in agreement with the reciprocal relationship between the proportions of 22:5ω6 and 22:6ω3 established with rats under different circumstances following dietary manipulations (Stubbs & Smith, 1984).

The relatively constant polyunsaturation of brain tissue in all mammalian species examined distinguishes this tissue from others, in which larger mammals do not accumulate highly polyunsaturated fatty acids. Others have pointed to the requirement for highly polyunsaturated fatty acids in the brain of mammals (Stubbs & Smith, 1984), although reasons for this remain speculative. Docosahexaenoic acid (22:6ω3) occurs in high proportions in the phospholipids of excitable cells such as

Table 2. Unsaturation Index (average number of double bonds per 100 fatty acid molecules), average chain length (number of carbon atoms) and proportion (mol%) of fatty acid types in mammalian skeletal muscle

	Mouse	Rat	Rabbit	Sheep	Cattle
Unsat. Index	244 ± 4^{a}	210 ± 5^{b}	165 ± 4^{c}	137 ± 5^{d}	141 ± 2^{d}
Chain length	18.8 ± 0.0^{a}	18.4 ± 0.1^{b}	17.9 ± 0.1^{c}	17.7 ± 0.0^{d}	17.6 ± 0.0^{d}
% unsaturates	66.9 ± 0.4^{c}	65.0 ± 1.4^{c}	66.2 ± 0.3^{c}	71.4 ± 0.3^{b}	74.3 ± 0.4^{a}
% monounsat.	15.0 ± 0.6^{c}	15.1 ± 0.4^{c}	21.6 ± 1.5^{b}	39.9 ± 2.3^{a}	44.0 ± 1.4^{a}
% polyunsat.	51.9 ± 0.8^{a}	49.9 ± 1.6^{ab}	44.6 ± 1.2^{b}	31.5 ± 2.4^{c}	30.3 ± 1.5^{c}
% ω9	7.6 ± 0.5^{b}	4.4 ± 0.3^{c}	9.8 ± 0.5^{b}	26.3 ± 2.8^{a}	25.0 ± 1.2^{a}
% ω7	7.8 ± 0.1^{d}	11.0 ± 0.1^{c}	12.2 ± 1.2^{bc}	14.6 ± 0.7^{b}	19.6 ± 0.5^{a}
% ω6	30.5 ± 0.3^{b}	36.5 ± 1.2^{a}	40.5 ± 1.1^{a}	20.2 ± 1.7^{c}	19.0 ± 1.4^{c}
% ω3	20.9 ± 0.6^{a}	13.1 ± 0.7^{b}	3.6 ± 0.3^{c}	10.3 ± 1.0^{b}	10.7 ± 0.2^{b}

Results are average ± SE (n = 4). Indexes in a row with common exponent letters do not differ significantly ($P < 0.05$).

Table 3. Unsaturation Index (average number of double bonds per 100 fatty acid molecules), average chain length (number of carbon atoms) and proportion (mol%) of fatty acid types in mammalian kidney cortex

	Mouse	Rat	Rabbit	Sheep	Cattle
Unsat. Index	249 ± 4^{a}	185 ± 1^{b}	186 ± 3^{b}	172 ± 3^{c}	163 ± 5^{c}
Chain length	18.9 ± 0.0^{a}	18.3 ± 0.0^{b}	18.3 ± 0.0^{b}	18.2 ± 0.0^{c}	18.1 ± 0.0^{c}
% unsaturates	71.0 ± 0.6^{b}	62.1 ± 0.2^{d}	77.0 ± 0.9^{a}	63.4 ± 0.5^{cd}	65.3 ± 0.5^{c}
% monounsat.	16.5 ± 0.2^{c}	16.5 ± 0.1^{c}	18.6 ± 0.5^{b}	24.4 ± 0.8^{a}	24.3 ± 0.8^{a}
% polyunsat.	54.6 ± 0.8^{a}	45.6 ± 0.3^{b}	58.4 ± 1.2^{a}	39.0 ± 1.1^{c}	41.1 ± 1.0^{c}
% ω9	8.3 ± 0.1^{c}	7.2 ± 0.1^{d}	13.2 ± 0.4^{b}	16.6 ± 0.9^{a}	17.4 ± 0.6^{a}
% ω7	8.4 ± 0.2^{b}	9.6 ± 0.1^{a}	5.9 ± 0.2^{c}	8.5 ± 0.3^{b}	7.4 ± 0.3^{b}
% ω6	36.5 ± 0.6^{c}	43.1 ± 0.3^{b}	55.4 ± 1.2^{a}	24.2 ± 1.1^{d}	30.6 ± 2.9^{cd}
% ω3	17.7 ± 0.7^{a}	2.3 ± 0.0^{c}	2.2 ± 0.1^{c}	14.1 ± 0.5^{b}	10.0 ± 2.4^{b}

Results are average ± SE (n = 4). Indexes in a row with common exponent letters do not differ significantly ($P < 0.05$).

Table 4. Unsaturation Index (average number of double bonds per 100 fatty acid molecules), average chain length (number of carbon atoms) and proportion (mol%) of fatty acid types in mammalian liver

	Mouse	Rat	Rabbit	Sheep	Cattle
Unsat. Index	221 ± 4^{a}	216 ± 3^{a}	156 ± 5^{c}	186 ± 4^{b}	189 ± 2^{b}
Chain length	18.7 ± 0.0^{a}	18.7 ± 0.0^{a}	18.1 ± 0.0^{b}	18.6 ± 0.0^{a}	18.7 ± 0.0^{a}
% unsaturates	66.2 ± 0.5^{b}	62.2 ± 1.1^{bc}	69.9 ± 1.0^{a}	57.7 ± 0.5^{d}	59.0 ± 1.0^{cd}
% monounsat.	15.1 ± 0.2^{a}	8.9 ± 0.5^{b}	16.4 ± 1.3^{a}	17.9 ± 1.4^{a}	14.2 ± 0.7^{a}
% polyunsat.	51.1 ± 0.7^{a}	53.3 ± 0.6^{a}	53.5 ± 1.9^{a}	39.8 ± 1.5^{c}	44.9 ± 0.4^{b}
% ω9	10.4 ± 0.2^{b}	4.3 ± 0.4^{c}	14.9 ± 1.0^{a}	16.9 ± 1.5^{a}	10.6 ± 0.3^{b}
% ω7	5.4 ± 0.1^{a}	4.9 ± 0.2^{ab}	2.3 ± 0.3^{c}	1.6 ± 0.3^{c}	4.0 ± 0.4^{b}
% ω6	37.4 ± 0.3^{b}	44.7 ± 1.0^{a}	50.2 ± 1.8^{a}	16.8 ± 0.9^{d}	29.1 ± 3.0^{c}
% ω3	12.6 ± 0.4^{b}	8.1 ± 0.7^{c}	2.3 ± 0.2^{d}	22.3 ± 0.9^{a}	15.2 ± 2.6^{ab}

Results are average ± SE (n = 4). Indexes in a row with common exponent letters do not differ significantly ($P < 0.05$).

brain and retina (Gudbjarnason, 1989). Thus, in addition to other possible functions, there may be a specific role of this fatty acid in cell excitability. The statistically significant allometric decline of 22:6ω3 (Fig. 5) for both types of muscles examined (cardiac and skeletal) could thus indicate an allometric decline in the excitability of striated muscle in general.

The physiological significance of the scaling of membrane fatty acid composition in some mammalian tissues can only be conjectural at present. We have measured total tissue phospholipids and therefore cannot determine whether all subcellular membranes in these tissues show these allometric trends. However if only some subcellular membranes do show allometric trends in fatty acid composition while others do not, the allometric trends for such membranes will be even greater than reported here. Available evidence, discussed below, suggests that such body size related differences in

Table 5. Unsaturation Index (average number of double bonds per 100 fatty acid molecules), average chain length (number of carbon atoms) and proportion (mol%) of fatty acid types in mammalian brain

	Mouse	Rat	Rabbit	Sheep	Cattle
Unsat. Index	196 ± 15^{ab}	228 ± 4^{a}	233 ± 7^{ab}	218 ± 7^{ab}	182 ± 13^{b}
Chain length	18.6 ± 0.1^{b}	18.9 ± 0.0^{ab}	19.0 ± 0.0^{a}	18.9 ± 0.1^{ab}	18.6 ± 0.1^{b}
% unsaturates	62.2 ± 2.1^{c}	69.2 ± 0.7^{ab}	71.6 ± 0.6^{a}	68.1 ± 0.9^{b}	68.3 ± 1.8^{abc}
% monounsat.	29.2 ± 1.0^{b}	29.1 ± 0.9^{b}	27.3 ± 2.2^{ab}	32.0 ± 1.3^{ab}	39.5 ± 3.4^{a}
% polyunsat.	33.0 ± 3.0^{b}	40.0 ± 0.6^{a}	44.3 ± 1.8^{a}	36.1 ± 1.5^{b}	28.8 ± 2.7^{b}
% ω9	19.0 ± 0.7^{b}	19.1 ± 0.7^{b}	17.7 ± 2.0^{ab}	21.7 ± 1.0^{ab}	27.3 ± 2.7^{a}
% ω7	10.3 ± 0.3^{b}	10.1 ± 0.2^{b}	10.4 ± 0.4^{ab}	11.0 ± 0.3^{ab}	12.9 ± 0.7^{a}
% ω6	14.7 ± 1.0^{c}	20.1 ± 0.3^{b}	31.4 ± 1.4^{a}	12.7 ± 0.8^{c}	12.1 ± 0.5^{c}
% ω3	18.2 ± 1.9^{a}	19.8 ± 0.8^{a}	12.1 ± 0.6^{b}	22.8 ± 1.1^{a}	16.1 ± 2.6^{ab}

Results are average ± SE (n = 4). Indexes in a row with common exponent letters do not differ significantly ($P < 0.05$).

fatty acid composition affect membrane permeability and/or membrane fluidity. Membrane constituents other than phospholipids however, also play a role in membrane fluidity and permeability. For example, cholesterol is known to influence membrane fluidity (Spector & Yorek, 1985; Kimelberg & Papahadjopoulos, 1974; Kimelberg, 1975; Wiley & Cooper, 1975; Hazel, 1988; Lee, 1991) and permeability (De Gier, Mandersloop & van Deenen, 1968; Wiley & Cooper, 1975; Fettiplace & Haydon, 1980; Papahadjopoulos et al., 1973). Cholesterol content was not measured in the present study in which all subcellular membranes were pooled. Consideration of its relative presence will be most applicable to the consideration of plasma membrane function and not that of other subcellular membrane systems.

The concept of membrane fluidity is still relatively nebulous in relation to its implications for cell function. Within the range of compositions reported for mammalian cell membranes, differences in membrane fluidity are not currently considered to influence cell function (Stubbs & Smith, 1984; Lee, 1991). The first and second double bonds in fatty acids appear to be the most important contributors to fluidity in membranes (Stubbs & Smith, 1984). For example, Ehringer et al. (1990) found no difference in the fluidity of membranes containing 18:1ω9, 18:3ω3 or 22:6ω3, although such conclusions will be dependent on the method of measuring membrane fluidity and particularly on the position of the probe in the bilayer. Thus, since the presence, and not the number, of double bonds in individual fatty acids seem to be mostly determinant for membrane fluidity, variations in membrane fluidity in different-sized mammals should be reflected by the total % unsaturation in mammalian tissues. The absence of an allometric relationship for the % unsaturates in all tissues examined may suggest that the general fluidity of membranes does not vary systematically with body mass in mammals. The increase in monounsaturates in kidney cortex, heart and skeletal muscle of larger mammals could be aimed at maintaining membrane fluidity in the presence of lower levels of polyunsaturates in these tissues. However, conclusions regarding body mass-related differences in membrane fluidity await actual measurement of membrane fluidity at various depths in the bilayer.

Some evidence suggests that the allometric changes in membrane composition in heart, skeletal muscle and kidney cortex is associated with the modification of membrane permeability, possibly as a function of cellular metabolic activity and/or cell excitability. The major body mass-related trend in membrane composition was the increased proportion of the highly polyunsaturated species (such as 22:6ω3) in some tissues from the more metabolically active species. Recent studies have linked the presence of this fatty acid with increased membrane permeability for a variety of substances such as cytosolic components of tumor cells (Jensky et al., 1991) and erythritol and carboxyfluorescein in phospholipid vesicles (Stillwell, Ehringer & Jensky, 1993). As well, we found that vesicles prepared from the relatively more polyunsaturated rat liver mitochondrial phospholipids were more permeable to protons than those from the more monounsaturated reptilian liver mitochondrial phospholipids (Brand, Couture & Hulbert, 1994).

In another study (Couture & Hulbert, 1995), we have found for the same five mammalian species used here that kidney cortex and liver slices from smaller mammals had a higher metabolic rate and higher sodium pump activity and that the two parameters were related. Sodium pump activity is an indicator of sodium and potassium fluxes across the plasma membranes. The allometric variations of the phospholipid fatty acid profiles of some mammalian tissues may thus be related to membrane permeability differences which are proportional to tissue metabolic activity. As another example, Porter & Brand (1993) reported an inverse relationship between liver mitochondrial inner membrane proton permeability and body mass in mammals ranging from mouse to horse. Analysis of membrane phospholipids from their

mammalian liver mitochondria performed in our laboratory (*unpublished data*) showed a decrease in the extent of polyunsaturation for larger mammals.

Our study included only herbivores. The small mammals used were rodents, the intermediate-size mammal was a lagomorph and the larger mammals were both ruminants. One could argue that the choice of species used could have yielded differences in tissue membrane composition due solely to taxonomic differences rather than differences in body mass. This seems unlikely in that the relationship between 22:6ω3 and heart rate (Gudbjarnason et al., 1978) which was the stimulus for our study involved the mouse, rat, rabbit, man and whale but no ruminant. It thus suggests that the relationships we have described are more general ones between the degree of membrane polyunsaturation in some tissues and body mass, which are related to the well-known allometric relationship of metabolic rate in mammals. Nevertheless, the physiological and biochemical mechanisms linking metabolic rate to cell membrane phospholipid composition, may they involve membrane permeability, fluidity or cell excitability or yet other mechanisms, remain to be established.

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