Membrane fatty acid composition of rat skeletal muscle is most responsive to the balance of dietary n-3 and n-6 PUFA

Sarah K. Abbott  
*University of Wollongong*

P. L. Else  
*University of Wollongong, pelse@uow.edu.au*

A. Hulbert  
hulbert@uow.edu.au

Publication Details

Membrane fatty acid composition of rat skeletal muscle is most responsive to the balance of dietary n-3 and n-6 PUFA

Abstract
The present study quantifies the relationships between diet fatty acid profile and fatty acid composition of rat skeletal muscle phospholipids. Young adult male Sprague–Dawley rats were fed, for 8 weeks, on one of twelve moderate-fat diets (25 % of total energy) differing only in fatty acid profile. SFA content ranged from 8–88 % of total fatty acids, MUFA 6–65 %, total PUFA 4–81 %, n-6 PUFA 3–70 % and n-3 PUFA 1–70 %. Diet PUFA included only essential fatty acids 18 : 2 \( n-6 \) and 18 : 3 \( n-3 \). The balance between \( n-3 \) and \( n-6 \) PUFA (PUFA balance) in the diet ranged from 1 : 99 to 86 : 14 % \( n-3 \) PUFA: \( n-6 \) PUFA. The slope of muscle phospholipid composition plotted against diet composition quantifies the response of muscle membrane composition to dietary fat (0, no response; 1, complete conformity with diet). The resulting slopes were 0·02 (SFA), 0·10 (PUFA), 0·11 (MUFA), 0·14 (\( n-3 \) PUFA) and 0·23 (\( n-6 \) PUFA). The response to PUFA balance was biphasic with a slope of 0·98 below 10 % diet PUFA balance and 0·16 above 10 %. Thus, low diet PUFA balance has greater influence on muscle composition than 18-carbon \( n-3 \) or \( n-6 \) PUFA individually. Equations provided may allow prediction of muscle composition for other diet studies. Diet PUFA balance dramatically affects muscle 20 : 4 \( n-6 \) and 22 : 6 \( n-3 \). This may have significant implications for some disease states in human subjects.

Disciplines
Arts and Humanities | Life Sciences | Medicine and Health Sciences | Social and Behavioral Sciences

Publication Details
Membrane fatty acid composition of rat skeletal muscle is most responsive to the balance of dietary n-3 and n-6 PUFA

Sarah K. Abbott1,2*, Paul L. Else1,3 and A. J. Hulbert1,2
1Metabolic Research Centre, University of Wollongong, Wollongong, NSW 2522, Australia
2School of Biological Sciences, University of Wollongong, Northfields Avenue, Wollongong, NSW 2522, Australia
3School of Health Sciences, University of Wollongong, Wollongong, NSW 2522, Australia

(Received 22 April 2008 – Revised 27 August 2009 – Accepted 28 August 2009 – First published online 13 October 2009)

The present study quantifies the relationships between diet fatty acid profile and fatty acid composition of rat skeletal muscle phospholipids. Young adult male Sprague–Dawley rats were fed, for 8 weeks, on one of twelve moderate-fat diets (25 % of total energy) differing only in fatty acid profile. SFA content ranged from 8–88 % of total fatty acids, MUFA 6–65 %, total PUFA 4–81 %, n-6 PUFA 3–70 % and n-3 PUFA 1–70 %. Diet PUFA included only essential fatty acids 18:2n-6 and 18:3n-3. The balance between n-3 and n-6 PUFA (PUFA balance) in the diet ranged from 1:99 to 86:14 %. Diet PUFA included only essential fatty acids 18:2n-6 and 18:3n-3. The balance between n-3 and n-6 PUFA (PUFA balance) in the diet ranged from 1:99 to 86:14 %. Diet PUFA included only essential fatty acids 18:2n-6 and 18:3n-3. The balance between n-3 and n-6 PUFA (PUFA balance) in the diet ranged from 1:99 to 86:14 %. Diet PUFA included only essential fatty acids 18:2n-6 and 18:3n-3. The balance between n-3 and n-6 PUFA (PUFA balance) in the diet ranged from 1:99 to 86:14 %. Diet PUFA included only essential fatty acids 18:2n-6 and 18:3n-3. The balance between n-3 and n-6 PUFA (PUFA balance) in the diet ranged from 1:99 to 86:14 %.

The response of muscle membrane composition to dietary fat (0, no response; 1, complete conformity with diet). The resulting slopes were

- 0.02 (SFA), 0.10 (PUFA), 0.11 (MUFA), 0.14 (n-3 PUFA) and 0.23 (n-6 PUFA). The response to PUFA balance was biphasic with a slope of 0.08 below 10 % diet PUFA balance and 0.16 above 10 %. Thus, low diet PUFA balance has greater influence on muscle composition than 18-carbon n-3 or n-6 PUFA individually. Equations provided may allow prediction of muscle composition for other diet studies. Diet PUFA balance dramatically affects muscle 20:4n-6 and 22:6n-3. This may have significant implications for some disease states in human subjects.

n-3: n-6: Obesity: Arachidonic acid: DHA

Higher animals can synthesise both SFA and MUFA from non-lipid sources, but are unable to synthesise de novo both n-6 and n-3 PUFA. They are also incapable of interconverting these two classes of polyunsaturates and consequently both n-6 and n-3 PUFA are separate essential dietary components. Indeed, in the food chain, both n-6 and n-3 PUFA are synthesised de novo by plants as 18-carbon fatty acids and are further elongated and desaturated by animals to produce longer chain n-6 and n-3 PUFA. The dietary importance of n-6 and n-3 PUFA derives from the fact that they are both essential constituents of membrane lipids of higher animals. The PUFA composition of cell membrane bilayers has important effects on the functionality of the great variety of proteins (e.g. hormone receptors, neuroreceptors, membrane pumps) that are embedded in cellular membranes. Also, as constituents of membrane lipids, PUFA are the precursors of important signalling molecules (e.g. eicosanoids and endocannabinoids).

It has been long known that the fatty acid composition of the diet can influence membrane fatty acid composition. Most studies compare a very small number of diets (normally only two diets) and to our knowledge there are no studies that have mathematically quantified the relationship between diet and membrane composition. Studies in animals show that membrane fatty acid composition is influenced via the process of constant membrane remodelling, whereby fatty acids that constitute membrane lipids are being constantly removed and replaced by a complex series of membrane-bound enzymes. Acyltransferases, an important group of enzymes involved in this process, are highly selective for polyunsaturates but do not discriminate well between n-6 PUFA and n-3 PUFA(1). Consequently, membrane fatty acid composition is likely, a priori, very responsive to the balance between n-6 and n-3 PUFA in the diet.

To quantify the relationship between the fatty acid composition of the diet and membrane lipids, one recent analysis has applied the ‘conformer/regulator’ paradigm to literature data for the laboratory rat. It showed that, while membrane composition is relatively unresponsive to the SFA and MUFA content of the diet, it is most responsive to the ratio of n-3 PUFA to n-6 PUFA. This conclusion was, however, based on the comparison of four to five diets of which only one of the diets was high in n-3 PUFA (see Fig. 2 of Hulbert et al. (2)) and consequently lacked robustness.

The present study was designed to overcome this problem. It compares the effect of twelve isoenergetic diets, identical except for fatty acid composition, on the fatty acid composition of muscle phospholipids of the rat. All diets had a moderate fat content (25 % of total energy). To quantify the diet–membrane relationship accurately, the twelve diets

Abbreviations: B, beef dripping; C, coconut oil; % en, percentage energy; F, flaxseed oil; O, olive oil; S, safflower oil.
* Corresponding author: Sarah K. Abbott, fax +61 2 4221 4135, email ska454@uow.edu.au
were designed to supply a wide range of relative content of SFA, MUFA, n-6 PUFA and n-3 PUFA. Only 18-carbon n-3 and n-6 PUFA were provided as these are the dominant PUFA in the normal diet of rats. Differences in lipid metabolism exist between rodents and human subjects, so caution must be taken when extrapolating results from an animal model to human conditions. These differences will be discussed later.

Unlike the earlier analysis that used the ‘n-3 PUFA:n-6 PUFA’ ratio\(^2\), here we use in its place what we have called ‘PUFA balance’ (defined as ‘n-3 PUFA as percentage of total PUFA’) to analyse the interaction between these two types of PUFA in the diet on membrane composition. PUFA balance is analogous to a proportion (or a ratio where the two values sum to 100%). We discuss the advantages of this approach later in this paper. In the present study, the diet n-3 PUFA:n-6 PUFA ranged from 1.99 (Diet 1) to 86:14 (Diet 12) and was found to be the most influential on membrane fatty acid composition. This may have significant implications for some disease states in human subjects.

Materials and methods

**Animals**
All experiments were approved by the University of Wollongong Animal Ethics Committee and were conducted in conformity with the Public Health Service policy on Humane Care and Use of Laboratory Animals. Eight-week-old male Sprague–Dawley rats (Gore Hill Research Laboratories, Sydney) were housed individually under a 12:12 h light:dark schedule. The room was maintained at 23°C and 40% relative humidity. Before dietary manipulation, all rats were fed *ad libitum* on commercial rodent diet (Y.S. Feeds, Young, Australia), the composition of which was 13% en (percentage energy) fat, 22% en protein and 65% en carbohydrate.

Rats were randomly assigned to either the initial group (n 6) or one of the twelve dietary groups (n 6 per group). Rats initially weighed 295.7 (SE 2.5) g (n 68) and following diet treatment weighed 528.2 (SE 7.5) g (n 72). An initial group of rats were euthanised at 8 weeks of age to give an indication of muscle fatty acid composition before dietary intervention. Following diet treatment, rats were euthanised via intraperitoneal injection (200 mg/kg body mass) of Lethabarb (sodium pentobarbitone, Virbac, Peakhurst, NSW, Australia). The skeletal muscle (*medial gastrocnemius*) was immediately removed, frozen and stored at −80°C until analysis.

**Diets**
Rats were fed *ad libitum* for 8 weeks on complete diets differing only in fatty acid profile (Tables 1 and 2). The average fatty acyl chain length was 17.0 (SE 0.2) carbons for all diets. No 20-carbon or 22-carbon PUFA were included in the diets. Diets were designed to cover a wide range of SFA, MUFA, n-3 PUFA, n-6 PUFA and PUFA balance, which was achieved by mixing various proportions of the following oils: flaxseed oil (F; Nutralive Hi-Omega, Moorabbin, NSW, Australia); safflower oil (S; Healthy Life Cold Pressed, Milperra, NSW, Australia); olive oil (O; Lupi Cold Pressed Extra Virgin, Gosford, NSW, Australia); coconut oil (C; Aclara Health Organic Gourmet Cold Pressed Extra Virgin, Mount Crosby, QLD, Australia); beef dripping (B; Allowrie Prime, Rowville, VIC, Australia).

**Fatty acid analysis**
All solvents used were HPLC grade (Crown Scientific, Moorabbin, NSW, Australia) and contained 0.01% w/v butylated hydroxytoluene (Sigma Aldrich, Sydney, NSW, Australia). Total lipids were extracted from the skeletal muscle using chloroform–methanol (2:1, v:v) and glass/glass homogenisers as previously described\(^4\). Phospholipids were separated from total lipids using Sep-Pak\(^®\) Classic Silica Cartridge (Waters, Rydalmere, NSW, Australia). Phospholipids were transmethylated\(^5\) and fatty acid methyl esters were measured by GC (Shimadzu GC-17A, Rydalmere, NSW, Australia) using a Varian WCOT fused silica column (50 m × 0.25 mm internal diameter, CP7419, Sydney, New South Wales, Australia) with the following temperature program: 150°C initial temperature; 17.5°C/min to 170°C; 0.5°C/min to 178°C; 15°C/min to 222°C; 2°C/min to 232°C. Individual fatty acids were identified by comparison with an external standard (FAME Mix C4-C24, Sigma Aldrich, Sydney, Australia) and then expressed as mole percentage of total fatty acids.

**Table 1. Composition of experimental diets (see text for details)**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Amount (g/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oil mix</td>
<td>120</td>
</tr>
<tr>
<td>Sucrose</td>
<td>82</td>
</tr>
<tr>
<td>Maize starch</td>
<td>493</td>
</tr>
<tr>
<td>Casein</td>
<td>155</td>
</tr>
<tr>
<td>Fibre</td>
<td>50</td>
</tr>
<tr>
<td>Mineral mix</td>
<td>40</td>
</tr>
<tr>
<td>Vitamin mix</td>
<td>10</td>
</tr>
<tr>
<td>Gelatin</td>
<td>50</td>
</tr>
<tr>
<td>Energy contribution (% total energy)</td>
<td>55</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td></td>
</tr>
<tr>
<td>Protein</td>
<td>20</td>
</tr>
<tr>
<td>Fat</td>
<td>25</td>
</tr>
</tbody>
</table>

\(^2\) Unlike the earlier analysis that used the ‘n-3 PUFA:n-6 PUFA’ ratio, here we use in its place what we have called ‘PUFA balance’ (defined as ‘n-3 PUFA as percentage of total PUFA’) to analyse the interaction between these two types of PUFA in the diet on membrane composition. PUFA balance is analogous to a proportion (or a ratio where the two values sum to 100%). We discuss the advantages of this approach later in this paper. In the present study, the diet n-3 PUFA:n-6 PUFA ranged from 1.99 (Diet 1) to 86:14 (Diet 12) and was found to be the most influential on membrane fatty acid composition. This may have significant implications for some disease states in human subjects.

\(^3\) Phospholipids were separated from total lipids using Sep-Pak\(^®\) Classic Silica Cartridge (Waters, Rydalmere, NSW, Australia). Phospholipids were transmethylated\(^5\) and fatty acid methyl esters were measured by GC (Shimadzu GC-17A, Rydalmere, NSW, Australia) using a Varian WCOT fused silica column (50 m × 0.25 mm internal diameter, CP7419, Sydney, New South Wales, Australia) with the following temperature program: 150°C initial temperature; 17.5°C/min to 170°C; 0.5°C/min to 178°C; 15°C/min to 222°C; 2°C/min to 232°C. Individual fatty acids were identified by comparison with an external standard (FAME Mix C4-C24, Sigma Aldrich, Sydney, Australia) and then expressed as mole percentage of total fatty acids.
Data analysis was performed using JMP 5.1 (Statistical Analysis System Institute Inc., Cary, NC, USA). All results are expressed as means with their standard errors with $P<0.005$ set as the level of significance. Data were tested for normality using the Shapiro–Wilk $W$ test and homogeneity of variance using the O’Brien and Brown–Forsythe tests. Data not normally distributed were compared using non-parametric Wilcoxon/Kruskal–Wallis tests and any data showing unequal variance were compared using a Welch ANOVA. A one-way ANOVA was completed with diet as the independent variable and each fatty acid as the dependent variable. Means were then compared using the Tukey–Kramer honestly significant difference test. Linear regression was employed for plots comparing diet and membrane fatty acid composition followed by a one-way ANOVA as an indication of fit. Data for individual rats were used for all linear regression analyses ($n=72$ data points); however, only the mean and standard error for each diet group are plotted in each figure. Data for the initial group of rats are also plotted in all figures (shown as solid squares), but was not included in any linear regression analysis.

Results

Fatty acid composition of the skeletal muscle phospholipids is presented in Table 3. Significant differences were evident between the diet groups for all fatty acids, except $16:0$ and $18:0$ (Table 3). In the muscle phospholipids, $22:6\text{n-3}$ accounted for the majority of the $n-3$ PUFA content, while $18:2\text{n-6}$ and $20:4\text{n-6}$ provided the highest proportions in the total $n-6$ PUFA composition (Table 3).

The skeletal muscle results were analysed using the classic conformer/regulator concept$^3$, with the muscle fatty acid composition plotted against the dietary fatty acid composition (Fig. 1). The slope of each relationship represents how responsive membrane composition is to diet composition. A slope of one indicates membrane composition conforms completely to diet fatty acid composition, while a value of zero indicates a membrane regulating a constant fatty acid composition irrespective of diet composition. The results of the linear regression analysis for each figure are provided in Table 4, which also includes some regression analyses of relationships not plotted in the figures.

The muscle composition was unresponsive to large changes in diet SFA with a slope of 0.02 (Fig. 1(a)). This also means that membrane composition is similarly unresponsive to the total unsaturated fatty acid content in the diet (as these are co-depandant variables). Muscle membrane composition was more responsive to diet MUFA content with a responsivity of 0.11 (Fig. 1(a)). Although muscle response to diet total PUFA was 0.10 (Fig. 1(a)), the response to the two separate classes of PUFA was much greater. The slope for the $n-3$ PUFA relationship was 0.14 (Table 4), and the greatest overall slope was for the response of muscle to diet $n-6$ PUFA with a value of 0.23 (Table 4).

The diet–membrane relationship with the highest slope was found for the PUFA balance (Fig. 1(b)) with a value of 0.98 at diet PUFA balance <10%. This value indicates that muscle PUFA balance changed by 98% for a 100% change in diet PUFA balance. The PUFA balance data do not seem to
Table 3. Fatty acid composition (percentage of total fatty acid) of skeletal muscle phospholipids from rats fed experimental and initial diets

(Mean values with their standard errors, n = 6)

<table>
<thead>
<tr>
<th>Diet</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>Initial</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>se</td>
<td>Mean</td>
<td>se</td>
<td>Mean</td>
<td>se</td>
<td>Mean</td>
<td>se</td>
<td>Mean</td>
<td>se</td>
<td>Mean</td>
<td>se</td>
<td>Mean</td>
</tr>
<tr>
<td>14:0</td>
<td>0.1</td>
<td>0.01</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.2</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.5</td>
<td>0.1</td>
<td>1.0</td>
</tr>
<tr>
<td>16:0</td>
<td>22.9</td>
<td>1.1</td>
<td>24.7</td>
<td>0.1</td>
<td>23.3</td>
<td>0.9</td>
<td>23.2</td>
<td>0.9</td>
<td>24.0</td>
<td>0.7</td>
<td>23.9</td>
<td>0.6</td>
<td>25.1</td>
</tr>
<tr>
<td>18:0</td>
<td>153</td>
<td>0.3</td>
<td>156</td>
<td>0.2</td>
<td>151</td>
<td>0.5</td>
<td>139</td>
<td>0.9</td>
<td>144</td>
<td>0.6</td>
<td>139</td>
<td>0.3</td>
<td>154</td>
</tr>
<tr>
<td>18:1n7</td>
<td>0.5</td>
<td>0.1</td>
<td>0.6</td>
<td>0.0</td>
<td>0.4</td>
<td>0.0</td>
<td>1.0</td>
<td>0.1</td>
<td>0.5</td>
<td>0.0</td>
<td>0.5</td>
<td>0.1</td>
<td>1.6</td>
</tr>
<tr>
<td>18:1n9</td>
<td>6.6</td>
<td>0.5</td>
<td>4.8</td>
<td>0.2</td>
<td>3.4</td>
<td>0.1</td>
<td>9.8</td>
<td>0.5</td>
<td>11.0</td>
<td>0.7</td>
<td>6.3</td>
<td>0.2</td>
<td>4.2</td>
</tr>
<tr>
<td>18:1n11</td>
<td>2.6</td>
<td>0.1</td>
<td>2.3</td>
<td>0.0</td>
<td>2.1</td>
<td>0.0</td>
<td>2.7</td>
<td>0.1</td>
<td>3.0</td>
<td>0.1</td>
<td>2.3</td>
<td>0.0</td>
<td>2.2</td>
</tr>
<tr>
<td>18:1n13</td>
<td>17.7</td>
<td>1.3</td>
<td>14.9</td>
<td>0.3</td>
<td>15.0</td>
<td>0.6</td>
<td>14.2</td>
<td>1.0</td>
<td>20.3</td>
<td>1.0</td>
<td>21.6</td>
<td>0.6</td>
<td>16.3</td>
</tr>
<tr>
<td>18:2n6</td>
<td>0.5</td>
<td>0.0</td>
<td>0.6</td>
<td>0.0</td>
<td>0.5</td>
<td>0.0</td>
<td>0.9</td>
<td>0.0</td>
<td>0.7</td>
<td>0.0</td>
<td>0.6</td>
<td>0.0</td>
<td>0.7</td>
</tr>
<tr>
<td>20:4n6</td>
<td>196</td>
<td>1.2</td>
<td>204</td>
<td>0.2</td>
<td>164</td>
<td>0.7</td>
<td>138</td>
<td>0.6</td>
<td>121</td>
<td>0.7</td>
<td>115</td>
<td>0.5</td>
<td>10.1</td>
</tr>
<tr>
<td>22:5n6</td>
<td>32.0</td>
<td>0.9</td>
<td>19.0</td>
<td>0.3</td>
<td>7.0</td>
<td>0.1</td>
<td>5.0</td>
<td>0.1</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.3</td>
</tr>
<tr>
<td>22:6n3</td>
<td>8.1</td>
<td>0.5</td>
<td>10.5</td>
<td>0.5</td>
<td>11.4</td>
<td>0.5</td>
<td>13.1</td>
<td>0.3</td>
<td>15.4</td>
<td>0.7</td>
<td>13.0</td>
<td>0.4</td>
<td>12.9</td>
</tr>
<tr>
<td>% SFA</td>
<td>38.8</td>
<td>0.9</td>
<td>41.0</td>
<td>0.5</td>
<td>39.5</td>
<td>0.8</td>
<td>39.5</td>
<td>0.7</td>
<td>38.3</td>
<td>0.8</td>
<td>39.1</td>
<td>0.8</td>
<td>39.7</td>
</tr>
<tr>
<td>% MUFA</td>
<td>9.8</td>
<td>0.5</td>
<td>7.8</td>
<td>0.2</td>
<td>6.0</td>
<td>0.2</td>
<td>14.0</td>
<td>0.6</td>
<td>14.8</td>
<td>0.7</td>
<td>9.2</td>
<td>0.0</td>
<td>7.3</td>
</tr>
<tr>
<td>% PUFA</td>
<td>51.4</td>
<td>0.5</td>
<td>51.3</td>
<td>0.5</td>
<td>51.1</td>
<td>0.4</td>
<td>46.6</td>
<td>0.4</td>
<td>46.9</td>
<td>0.8</td>
<td>51.7</td>
<td>0.6</td>
<td>47.9</td>
</tr>
<tr>
<td>n-6 PUFA</td>
<td>42.3</td>
<td>0.6</td>
<td>38.7</td>
<td>0.1</td>
<td>40.8</td>
<td>0.0</td>
<td>27.1</td>
<td>0.6</td>
<td>32.4</td>
<td>0.5</td>
<td>27.4</td>
<td>0.9</td>
<td>31.4</td>
</tr>
<tr>
<td>n-3 PUFA</td>
<td>9.1</td>
<td>0.4</td>
<td>12.6</td>
<td>0.6</td>
<td>13.7</td>
<td>0.4</td>
<td>16.0</td>
<td>0.3</td>
<td>19.8</td>
<td>0.6</td>
<td>19.2</td>
<td>0.3</td>
<td>20.5</td>
</tr>
<tr>
<td>PUFA balance</td>
<td>17.7</td>
<td>0.8</td>
<td>24.5</td>
<td>0.8</td>
<td>25.1</td>
<td>0.8</td>
<td>34.4</td>
<td>0.7</td>
<td>42.2</td>
<td>0.8</td>
<td>37.2</td>
<td>0.6</td>
<td>38.4</td>
</tr>
</tbody>
</table>

*Mean values for each fatty acid without a common superscript in each row are significantly different (P < 0.0001).
follow a single linear function, and this is illustrated in Fig. 1(b) where the data points are plotted in a biphasic manner. This plot reveals that at diet PUFA balance, <10% of the muscle PUFA balance is drastically reduced with a slope of 0.98 (Fig. 1(b)). Above 10%, however, the muscle PUFA balance displays a diet responsivity of 0.16 (Fig. 1(b)).

In the present experiment, both n-6 and n-3 PUFA were supplied in the diet as 18-carbon PUFA molecules; 18:2n-6 and 18:3n-3, respectively (Table 2). Both n-6 and n-3 PUFA in muscle phospholipids included substantial amounts of more unsaturated, 20- and 22-carbon acyl chains (Table 3), with the 20:4n-6 and 22:6n-3 predominant longer-chain PUFA present. In Fig. 2, we have analysed the abundance of 20:4n-6 and 22:6n-3 in muscle phospholipids relative to (i) the amount of the respective 18-carbon precursor fatty acid in the diet (left-hand graphs) and (ii) the PUFA balance of the diet (right-hand graphs). The regression equations and statistical data for similar analysis of the other 20- and 22-carbon PUFA are provided in Table 4.

For muscle 20- and 22-carbon n-6 PUFA, 20:4n-6 predominated with only low levels of 22:4n-6 and 22:5n-6 present (Table 3). Muscle 20:4n-6 increased in response to diet 18:2n-6 (Fig. 2(a)), but decreased in response to the PUFA balance of the diet (Fig. 2(b)). Muscle 22:4n-6 content was very low (0–11%) and showed only minor changes in response to both diet 18:2n-6 content and

![Table 4](image)

**Table 4.** Results for all linear regression analyses including relationships plotted in Figs. 1–2 and additional relationships (graphs not shown).

<table>
<thead>
<tr>
<th>Muscle composition (y)</th>
<th>Diet composition (x)</th>
<th>Equation</th>
<th>Slope standard error</th>
<th>R</th>
<th>P</th>
<th>Fig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>SFA</td>
<td>SFA</td>
<td>$y = 38.80 + 0.02x$</td>
<td>0.008 0.31 0.0076</td>
<td>1(a)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MUFA</td>
<td>MUFA</td>
<td>$y = 6.73 + 0.11x$</td>
<td>0.015 0.65 &lt;0.0001*</td>
<td>1(a)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PUFA</td>
<td>PUFA</td>
<td>$y = 46.72 + 0.10x$</td>
<td>0.008 0.83 &lt;0.0001*</td>
<td>1(a)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PUFA balance†</td>
<td>PUFA balance</td>
<td>$y_1 = 18.70 + 0.98x$</td>
<td>0.233 0.72 0.0007*</td>
<td>1(b)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PUFA balance‡</td>
<td>PUFA balance</td>
<td>$y_2 = 31.62 + 0.16x$</td>
<td>0.028 0.62 &lt;0.0001*</td>
<td>1(b)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20:4n-6</td>
<td>18:2n-6</td>
<td>$y = 8.86 + 1.77$</td>
<td>0.189 0.59 &lt;0.0001*</td>
<td>2(a)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20:4n-6</td>
<td>18:3n-3</td>
<td>$y = 18.85 + 0.15x$</td>
<td>0.008 0.91 &lt;0.0001*</td>
<td>2(b)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>22:6n-3</td>
<td></td>
<td>18:3n-3</td>
<td>$y = 9.03 + 3.666$</td>
<td>1.00 0.65 0.0037*</td>
<td>2(c)</td>
<td></td>
</tr>
<tr>
<td>22:6n-3†</td>
<td>18:3n-3</td>
<td>$y = 14.89 + 0.49x$</td>
<td>0.075 0.67 &lt;0.0001*</td>
<td>2(c)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>22:6n-3‡</td>
<td>PUFA balance</td>
<td>$y_1 = 8.24 + 0.46x$</td>
<td>0.104 0.75 0.0004*</td>
<td>2(d)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>22:6n-3‡</td>
<td>PUFA balance</td>
<td>$y_2 = 15.19 - 0.03x$</td>
<td>0.014 0.31 0.0213</td>
<td>2(d)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>n-6 PUFA</td>
<td>n-6 PUFA</td>
<td>$y = 26.99 + 0.23x$</td>
<td>0.017 0.86 &lt;0.0001*</td>
<td>2(a)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>22:4n-6</td>
<td>18:2n-6</td>
<td>$y = 0.02 + 0.09x$</td>
<td>0.015 0.59 &lt;0.0001*</td>
<td>2(a)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>22:4n-6</td>
<td>18:3n-3</td>
<td>$y = 0.78 - 0.11x$</td>
<td>0.001 0.84 &lt;0.0001*</td>
<td>2(a)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>22:5n-6</td>
<td>18:2n-6</td>
<td>$y = -0.06 + 0.20x$</td>
<td>0.043 0.49 &lt;0.0001*</td>
<td>2(a)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>22:5n-6†</td>
<td>PUFA balance</td>
<td>$y_1 = 3.27 + 0.36x$</td>
<td>0.037 0.92 &lt;0.0001*</td>
<td>2(a)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>22:5n-6‡</td>
<td>PUFA balance</td>
<td>$y_2 = 0.35 + 0.00x$</td>
<td>0.001 0.48 0.0002*</td>
<td>2(a)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>n-3 PUFA</td>
<td>n-3 PUFA</td>
<td>$y = 15.56 + 0.14x$</td>
<td>0.020 0.64 &lt;0.0001*</td>
<td>2(a)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20:5n-3</td>
<td>18:3n-3</td>
<td>$y = 0.30 + 0.22x$</td>
<td>0.022 0.76 &lt;0.0001*</td>
<td>2(a)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>22:5n-3</td>
<td>18:3n-3</td>
<td>$y = 2.67 + 0.44x$</td>
<td>0.055 0.69 &lt;0.0001*</td>
<td>2(a)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20:5n-3</td>
<td>PUFA balance</td>
<td>$y = -0.22 + 0.02x$</td>
<td>0.001 0.91 &lt;0.0001*</td>
<td>2(a)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>22:5n-3</td>
<td>PUFA balance</td>
<td>$y = 1.45 + 0.05x$</td>
<td>0.003 0.89 &lt;0.0001*</td>
<td>2(a)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

All fatty acid compositions are expressed as percentage total fatty acids, except for 18:2n-6 and 18:3n-3, which are expressed as g/100 g diet, and PUFA balance, which is expressed as n-3 PUFA as % total PUFA.

* The slope of the line of fit is significantly different from zero.
† Linear regression plotted below 10% diet PUFA balance.
‡ Linear regression plotted above 10% diet PUFA balance.
§ Linear regression plotted below 1 g/100 g diet 18:3n-3.
|| Linear regression plotted above 1 g/100 g diet 18:3n-3.
PUFA balance (Table 4). Muscle 22:5\textsubscript{n-6} responded to diet composition to a greater extent than 22:4\textsubscript{n-6}, but showed huge variability in response to diet 18:2\textsubscript{n-6} content (Table 4). The response to diet PUFA balance was biphasic, with a dramatic increase in muscle 22:5\textsubscript{n-6} as diet PUFA balance decreased below 10%, while above this dietary PUFA balance muscle 22:5\textsubscript{n-6} remained relatively constant (Table 4).

The 20- and 22-carbon n-3 PUFA in muscle phospholipids were predominantly 22:6\textsubscript{n-3}, with only small amounts of 20:5\textsubscript{n-3} and 22:5\textsubscript{n-3} present (Table 3). Small increases in both 20:5\textsubscript{n-3} and 22:5\textsubscript{n-3} were evident in muscle phospholipids with increases in both diet 18:3\textsubscript{n-3} content and PUFA balance (Table 4). The response of muscle 22:6\textsubscript{n-3} to diet composition was biphasic (Fig. 2(c) and (d)). Muscle 22:6\textsubscript{n-3} showed a steep relationship at low diet 18:3\textsubscript{n-3} contents (<1 g/100 g), with a similar response to diet PUFA balance <10% (Fig. 2(c) and (d)). Increasing the amount of diet 18:3\textsubscript{n-3} (above 2 g/100 g) led to a small decline in muscle 22:6\textsubscript{n-3} (Fig. 2(c)). Levels of muscle 22:6\textsubscript{n-3} also showed a small decline as diet PUFA balance increased above 10% (Fig. 2(d)).

**Discussion**

Using the conformer/regulator concept\(^3\), we have quantified how responsive the fatty acid composition of rat muscle phospholipids differs from dietary fat profile. Slopes close to zero indicate membrane composition changes little in response to changes in diet fat profile (as with SFA, and less so with PUFA and MUFA), while increased slopes indicate increased responsiveness of membrane composition to dietary fat profile (e.g. n-3 PUFA, n-6 PUFA and PUFA balance). These results confirm that although the fatty acid composition of muscle phospholipids is largely a regulated parameter, the muscle composition of the essential dietary fatty acids, n-3 and n-6 PUFA, is most influenced by dietary composition. Furthermore, it is the dietary balance between these two types of PUFA that has the greatest influence on the fatty acid composition and, in some cases, that diet PUFA balance is the best predictor of membrane composition.

We have used PUFA balance (\(n-3\) PUFA as percentage of total PUFA) because, as a 'proportion', it provides advantages over the more common use of 'ratios'. For example, using the n-3:n-6 ratio, when n-6 PUFA dominates in a mixture of the two, the value of the ratio will be between 0 and 1; however, when n-3 is the dominant PUFA in the mixture, the range of values will be 1–\(\infty\). Comparing (or averaging) ratios can be complicated, whereas the advantage of the PUFA balance is that the range of values are more even, ranging from 0 to 49% when n-6 PUFA dominate a mixture and 51–100% when n-3 PUFA are dominant. Another advantage of using PUFA balance is that it can be more readily used to average out dietary intake. For example, if a subject consumes two meals both with the same total PUFA content, but one having...
a PUFA balance of 10% (n-3:n-6 ratio = 0.11) and the other
90% (n-3:n-6 ratio = 9.0), then the average PUFA balance of
these meals is 50%, which is the actual situation of equal con-
sumption of both PUFA types. However, averaging the n-3:n-6
ratios (=4.6) does not give the same conclusion. These con-
siderations suggest that PUFA balance has benefits in easier
analysis of diets from the combinations of foods and meals.

An important feature of Fig. 2 is the greater strength of the
linear relationships when 20- and 22-carbon PUFA are plotted
against diet PUFA balance rather than when plotted against
their 18-carbon precursors. This is most pronounced for
muscle 20:4n-6, for example, the value of $R^2$ 0.35 when plotted
against diet 18:2n-6 content compared with $R^2$ 0.83
when plotted against diet PUFA balance. Thus, variation in
diet PUFA balance can explain 83% of variation in muscle
20:4n-6 content, while variation in diet 18:2n-6 amount
explains only 35% of muscle 20:4n-6 variation. Similarly
for n-3 PUFA, variation in diet PUFA balance explains 84%
of variation in muscle 20:5n-3 content and 80% of the vari-
muscle 22:5n-3 content, while the respective values for diet 18:3n-3 content are only 58 and 48%. This
means that diet PUFA balance is a better predictor of
muscle 20- and 22-carbon PUFA content than the amount of
each essential fatty acid precursor in the diet.

Comparison of the effects on muscle composition of the diet
level v. the balance between n-3 and n-6 PUFA can also be
illustrated by a comparison of some individual diets. For
example, diets 11 and 12 contained very different amounts
of 18-carbon PUFA, but similar PUFA balances. Rats on
these two diets had the same muscle phospholipid n-3
PUFA content even though there was >3-fold difference in
the amount of 18-carbon n-3 PUFA in the diet. Muscle
PUFA balance was not significantly different reflecting the
similar PUFA balance of the two diets. Similarly, comparison
of diets 8 and 9, which had very different total amounts of
PUFA but the same PUFA balance, resulted in PUFA balance
of muscle phospholipids that were not significantly different.
Conversely, diets 1 and 9 had the same total PUFA content,
but very different PUFA balances, and the PUFA balance of
the muscle phospholipids was also very different. Fur-
more, the muscle composition of the initial rats appears to
conform to the relationships determined from the experimental
rats, even though they were fed a diet with a lower fat content
(13 v. 25% en). This also supports the importance of relative
composition of fatty acids in the diet compared to absolute
amount of fat in the diet in determining membrane fatty acid
composition.

In the present study, rats were only provided with 18-carbon
PUFA in the diet. This is a realistic natural diet for both rats
and human subjects, with human subjects recorded to consume
the majority of their n-3 PUFA in the form of 18:3n-3
(1.3 g/d) and only a very small amount in the form of the
20- and 22-carbon n-3 PUFA (approximately 0.1 g/d) .
There are a number of processes involved in the regulatory
pathway between diet essential fatty acids and membrane
PUFA composition. These include (i) conversion of
the 18-carbon PUFA to longer chain PUFA by elongases
and desaturases (both located in the endoplasmic reticulum),
(ii) a peroxisomal processing step involving a single cycle
of $\beta$-oxidation for highly unsaturated PUFA (i.e. 22:6n-3
and 22:5n-6) and (iii) the deacylation–reacylation processes
of membrane remodelling (involving phospholipases and acyl-
transferases).

An interaction between dietary n-3 PUFA and n-6 PUFA at
the level of desaturases and elongases was first conclusively
demonstrated by Mohrhauser & Holman . In their seminal
study, the fatty acid composition of total lipids from liver,
heart and depot fat was compared between rats provided a
fat-free diet supplemented with daily oral doses of very
small amounts of 18:2n-6 and 18:3n-3 as their sole source
of dietary fat. They compared an impressive eighteen combi-
nations of these two essential fatty acids. Intakes of 18:2n-6
ranged from 0.07 to 0.73% en, while 18:3n-3 intakes ranged
from 0.05 to 1.92% en. Thus, the range of total
PUFA intake was from 0.13 to 2.59% en (compared to 25%
en from fat in the present study). They conclusively
demonstrated that the conversion of 18:2n-6 to 20:4n-6 (from the
combination of elongase, $\Delta 6$ and $\Delta 5$ desaturase activity)
was inhibited by 18:3n-3. We have calculated and plotted
the PUFA balance of both diet and tissue lipids from their data
(results not shown), and there is an impressive correlation
coefficient of 0.98 for liver lipids and 0.92 for heart lipids. We
have also plotted the relationship between diet PUFA balance
and tissue 20:4n-6 content from their data (results not shown),
and the correlation coefficients were 0.95 and 0.88 for liver
and heart lipids, respectively. These values are similar to the
0.91 correlation coefficient we found for the relationship
between diet PUFA balance and 20:4n-6 content of muscle
phospholipids, and suggest that diet PUFA balance may be
a valuable predictor of membrane fatty acid composition
irrespective of the amount of fat in the diet.

The final step in the biosynthesis of the highly polyunsat-
rated 22:6n-3 and 22:5n-6 involves the peroxisomes, where
enzymes catalyse a single cycle of $\beta$-oxidation of 24-carbon
PUFA to produce these 22-carbon PUFA . We know little
about the regulation of their production, but the present results
suggest it differs from their shorter chain less-unsaturated pre-
cursors. For example, at diet PUFA balances >10%, there
appears to be no increase in 22:6n-3 content of muscle phos-
pholipids and negligible change in 22:5n-6 content. How-
however, at diet PUFA balance <10%, there is a dramatic increase
in 22:5n-6 and a decrease in 22:6n-3. The level of 22:5n-6
and 22:6n-3 combined is constant irrespective of changes in
diet PUFA balance. This suggests that the peroxisomal path-
way may have a set capacity, which produces a steady total
amount of these 22-carbon highly PUFA. It preferentially
converts the available n-3 PUFA to 22:6n-3, but will substi-
tute n-6 PUFA to make 22:5n-6 when there is inadequate n-3
PUFA for 22:6n-3 production.

One of the most interesting findings was the greater predict-
ability of 20:4n-6 content of muscle phospholipids by diet
18-carbon PUFA balance compared with diet 18:2n-6
content. The incorporation of 20:4n-6 into rat liver and
brain phospholipids appears to primarily occur during the
membrane remodelling (i.e. deacylation and reacylation)
phase rather than during de novo synthesis of phospholi-
pids , however, it is not definitely known whether this also
applies to muscle phospholipids. Membrane lipid
remodelling is performed by the combined action of phos-
pholipases and acyltransferases. Lands` has demonstrated
that acyltransferases show no discriminating ability for the
incorporation of n-3 and n-6 PUFA. This may be the
mechanism responsible for the greater predictability of diet PUFA balance than diet 18 : 2n-6 content on muscle 20 : 4n-6. To extrapolate these results in rodents to human subjects, one must take into account differences in lipid metabolism. Rodents have a higher capacity than human subjects in converting 18-carbon PUFA to their longer chain metabolites due to increased Δ5-desaturase activity. Dietary 18 : 3n-3 has been shown to increase mice plasma levels of 20 : 5n-3 to a greater extent than in human subjects; however, the impact on plasma 22 : 6n-3 and 20 : 4n-6 was similar for mice and human subjects. This study concluded that rodent diets including n-3 PUFA based on a percentage of total energy are most suitable for determining equivalent doses for human subjects. The range of percentage energy from dietary 18 : 3n-3 in the present study was 0–17.3% en, with half of the experimental diets below the achievable human doses of 18 : 3n-3 (<2% en). The diets with achievable human equivalent doses in the present study include diets with PUFA balances ranging from 0 to 50%, which provides quite an extensive range. Furthermore, the total fat content of the diet is equivalent to the 20–35% recommended intake for human subjects, so that some extrapolation to human conditions from this model is appropriate.

There are some implications of the present findings for the current human diet. Dietary intake studies have shown that the fat intake of the US averages a PUFA balance of approximately 9.5% (17). If the present results in rats also apply to human subjects, an average PUFA balance of 9.5% in the modern human diet is of considerable concern, as it indicates that rodent diets including n-3 PUFA based on a percentage of total energy are most suitable for determining equivalent doses for human subjects. The range of percentage energy from dietary 18 : 3n-3 in the present study was 0–17.3% en, with half of the experimental diets below the achievable human doses of 18 : 3n-3 (<2% en). The diets with achievable human equivalent doses in the present study include diets with PUFA balances ranging from 0 to 50%, which provides quite an extensive range. Furthermore, the total fat content of the diet is equivalent to the 20–35% recommended intake for human subjects (14–16), so that some extrapolation to human conditions from this model is appropriate.

Acknowledgements

We gratefully acknowledge the technical assistance provided from Adam Zieba, Taleitha Atkins and Jessica Nealon. The present research was supported by a Discovery Project grant from the Australian Research Council. All authors disclose that there are no conflicts of interest. The study was conceived and planned by all three authors; the experiments were carried out by S. K. A. and the manuscript was written by all the three authors.

References