Red meat from animals offered a grass diet increases plasma and platelet n-3 PUFA in healthy consumers


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Abstract
Red meat from grass-fed animals, compared with concentrate-fed animals, contains increased concentrations of long-chain (LC) n-3 PUFA. However, the effects of red meat consumption from grass-fed animals on consumer blood concentrations of LC n-3 PUFA are unknown. The aim of the present study was to compare the effects on plasma and platelet LC n-3 PUFA status of consuming red meat produced from either grass-fed animals or concentrate-fed animals. A randomised, double-blinded, dietary intervention study was carried out for 4 weeks on healthy subjects who replaced their habitual red meat intake with three portions per week of red meat (beef and lamb) from animals offered a finishing diet of either grass or concentrate (n 20 consumers). Plasma and platelet fatty acid composition, dietary intake, blood pressure, and serum lipids and lipoproteins were analysed at baseline and post-intervention. Dietary intakes of total n-3 PUFA, as well as plasma and platelet concentrations of LC n-3 PUFA, were significantly higher in those subjects who consumed red meat from grass-fed animals compared with those who consumed red meat from concentrate-fed animals (P<0.05). No significant differences in concentrations of serum cholesterol, TAG or blood pressure were observed between groups. Consuming red meat from grass-fed animals compared with concentrate-fed animals as part of the habitual diet can significantly increase consumer plasma and platelet LC n-3 PUFA status. As a result, red meat from grass-fed animals may contribute to dietary intakes of LC n-3 PUFA in populations where red meat is habitually consumed.

Key words: Red meat; Long-chain n-3 PUFA; Grass-fed animals

Red meat produced from grass-fed animals is recognised as a dietary source of long-chain (LC) n-3 PUFA(1–4). A ruminant diet of grass, compared with cereal-based concentrate feeds, is rich in α-linolenic acid (ALA) (18:3n-3), thereby allowing the elongation of LC n-3 PUFA and their incorporation into muscle tissue(5). A recent scientific opinion from the European Food Safety Authority (EFSA) panel indicates that 250 mg LC n-3 PUFA per d is an Adequate Intake for adults to reduce the risk of CVD(6–8). IP address: 54.202.195.117, on 12 Apr 2017 at 21:30:23, subject to the Cambridge Core terms of use, available at https://doi.org/10.1017/S0007114510003090

Abbreviations: ALA, α-linolenic acid; DPA, docosapentaenoic acid; FAME, fatty acid methyl esters; LC, long chain.

Key points:
- Red meat from grass-fed animals contains increased concentrations of n-3 PUFA.
- Consuming red meat from grass-fed animals can significantly increase consumer plasma and platelet LC n-3 PUFA status.
- Grass-fed red meat may contribute to dietary intakes of LC n-3 PUFA in populations where red meat is habitually consumed.

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Abbreviations: ALA, α-linolenic acid; DPA, docosapentaenoic acid; FAME, fatty acid methyl esters; LC, long chain.
investigate the effects of regular moderate consumption of beef and lamb from grass-fed animals on LC n-3 PUFA status among free-living healthy adults. The secondary aim was to investigate the possible effects on serum cholesterol, TAG and blood pressure.

**Methods**

**Subjects and study design**

The 4-week study was a double-blind, randomised dietary intervention in forty healthy and free-living volunteers (twenty males and twenty females). All the volunteers were recruited from staff and students at the University of Ulster. Exclusion criteria included those with high cholesterol (> 5.0 mmol/l)(20), high blood pressure (systolic > 140 and diastolic > 90 mmHg)(21), those on prescribed medication or taking dietary supplements containing PUFA, those who consumed oily fish or n-3 PUFA-enriched foodstuffs more than twice a month and those with a BMI < 18.5 or > 30 kg/m². A further exclusion criterion was with respect to volunteers who habitually consumed more than three portions of red meat per week. Participants were randomly allocated to one of two groups: to consume red meat from animals that had been offered a finishing diet of grass or to consume red meat from animals that had been offered a finishing diet of concentrate. During each week of the 4-week intervention, participants in each group were provided with, and required to consume in place of their habitual red meat intake, one portion of mince beef (250 g raw weight), one sirloin steak (200 g raw weight) and four small lamb medallion pieces (240 g raw weight). The weekly consumption of red meat consumed by study participants were estimated to be 469 and 67 g, respectively. Participants were instructed not to consume any oily fish during the 4-week study period, but were otherwise encouraged to follow their normal dietary habits. Meat was kept at −20°C until required for the human subject intervention study.

**Red meat characteristics**

Red meat was sourced from producers in Northern Ireland who offered diets to animals under experimental conditions. Eight beef cattle and forty-four lambs of a similar age, sex and breed were used in the study. Half of the animals were offered a finishing diet of fresh grass only, while the other half of the animals were offered a finishing diet of concentrate only for a minimum period of 6 weeks before slaughter. The typical composition of concentrate feeds offered in this region has been defined previously as a mixture of cereal, maize and soya with a vitamin/mineral mix(23). The compliance of the producers to strictly offer these diets to animals was monitored by the members of the research team throughout the 6 weeks. At the end of the pre-slaughter feeding regimen, animals were slaughtered according to routine practice at a commercial abattoir (Dunbia, Dungannon, Northern Ireland), after which strip loins were chilled for an ageing period of 16 d and lamb loins for 7 d. Beef topsides were used to prepare mince beef samples with the addition of a small amount of adipose tissue (5%), to produce a fat content similar to that which is commercially available. After ageing, lamb loins were cut into small boneless medallions and sirloin steaks were cut from the strip loins. All the portions were vacuum sealed and stored at −20°C until required for the human subject intervention study.

**Lipid extraction**

Additional samples (n = 31) were taken from meat from each treatment group for confirmatory fatty acid analysis where possible. Meat samples were thawed and dissected to separate lean tissue, removing adipose tissue and discarding bone and connective tissue components. Total lipid was extracted from the lean tissue according to an adaptation of the Folch et al.(24) method. Sub-samples of lean and adipose tissue were homogenised in a chloroform–methanol (2:1, v/v) mixture, antioxidant (butylated hydroxytoluene, 20 mg/ml) was added and homogenised samples were then filtered. Filtrate from lean and adipose tissue was mixed with 0.37% KCl and allowed to settle overnight. The lower phase containing the lipid component was re-filtered and further evaporated under N₂. Sub-samples were taken for total lipid estimation by oven drying at 40°C. Fatty acid methyl esters (FAME) were prepared using the transesterification method(25) by adding 5 % 2 m KOH in anhydrous methanol.

**Quantification of fatty acid methyl esters**

FAME were quantified using a Varian CP 3800 GC (Varian Associates Limited, Walton-on-Thames, Surrey, UK) equipped with a temperature programmable injector operated in the split mode and a flame ionisation detector. Separation of the FAME was performed on a BPX70 capillary GC column (SGE Analytical Science, Milton Keynes, Bucks,
using the Friedewald equation, formulated as

\[ \text{LDL} = \text{total cholesterol} - \text{HDL} - \frac{\text{TAG}}{2.2}. \]

**Blood samples**

Fasting blood samples were collected at baseline and post-intervention. Serum, plasma and platelets were extracted within 1 h. Plasma and serum aliquots were obtained by spinning whole blood at 2500 g for 15 min at 4°C. Platelets were extracted by centrifuging whole blood slowly at 150 g for 15 min and subsequently harvesting the top layer of platelet-rich plasma. The platelet-rich plasma was centrifuged at 2500 g for 15 min to obtain a pellet, which was washed with Tris–HCl (pH 7.6, 4°C) and re-suspended in 500 μl Tris–HCl. Prepared samples were then stored at −80°C until subsequent analyses.

**Biochemical analysis**

Total lipid was extracted from plasma and platelet tissue using a method adapted from Folch et al. Internal standard, heptadecanoic acid (C17:0), was added to all the samples before extraction at a concentration of 1 mg/ml. The lipid extracts were esterified with boron trifluoride in methanol (Sigma Aldrich Company Limited). FAME were quantified using an Agilent 5975C GC MS (Agilent Technologies UK Limited, Stockport, UK) operated in split mode with a BPX70 capillary GC column (SGE Analytical Science) (length 100 m, internal diameter 0.25 mm and film thickness 0.25 μm) and using He as the carrier gas. The samples were injected at a temperature of 160°C and the temperature was ramped by 2°C/min to 225°C, where it was held for 25 min. Fatty acids were identified by their retention time with reference to those of commercially available fatty acid standards (37 Supelco FAME mix; Sigma Aldrich Company Limited, Gillingham, Dorset, UK) and were quantified by use of internal standard C13:0 and C21:0 which were added before extraction (Sigma Aldrich Company Limited).

**Dietary assessment**

Dietary intake was assessed at baseline and at the 3-week point of the intervention using a 4 d food diary, which the subjects completed over two weekdays and two weekend days. The reported intake at 3 weeks was taken to represent the mean food and nutrient intakes during intervention. This assessment allowed mean daily macronutrient, micronutrient and fatty acid intake of each subject before and during the study period to be evaluated. Fatty acid measurements from meat consumed by the study participants were used to supplement the existing data for beef and lamb within food composition tables. Any meat consumed before the study and recorded in baseline food diaries was assumed to have a fatty acid composition comparable to that of meat from concentrate-fed animals.

The prevalence of under-reporting (kcal/d) of energy intake was determined using the formula energy intake reported:BMR <1:1, adapted from Goldberg et al. Subjects completed an additional meat diary on days where they consumed any of the meat portions to aid compliance by recording details of any leftovers.

**Anthropometric and blood pressure measures**

Weight (kg) and height (m) of the participants were measured at baseline (with weight also measured at post-intervention) using calibrated scales and a stadiometer, respectively. BMI was calculated as weight (kg)/height² (m²). Blood pressure was measured at both baseline and post-intervention using a blood pressure monitor (Omron, Milton Keynes, Bucks, UK).

**Statistical analysis**

Sample size was determined using power calculations based on a similar study where the consumption of beef significantly increased LC n-3 PUFA concentrations within plasma phospholipids of healthy subjects, with a difference between means of 0.59/100 g and with a standard deviation of 0.37. It was predicted that a sample of thirty subjects (n 15) would be required to find significant differences at a level of 5 % and with a power of 80 %. Therefore, it was decided that a sample size of forty (n 20) would allow for potential dropouts during the study.

The SPSS version 11.5 (Chicago, IL, USA) statistical package was used for all data analysis. Data were initially tested for normality. Comparisons of baseline characteristics, platelet fatty acid status and serum lipids between groups were analysed by one-way ANOVA. Where there were significant differences between values at baseline, differences in plasma fatty acid status, nutrient intake and meat fatty acid composition between groups at post-intervention were analysed by ANCOVA, adjusting for appropriate covariates with Bonferroni correction. All the data are presented as means and standard deviations or means...
Results

Of the forty volunteers who were recruited, two withdrew as a result of being unable to commit to the study requirements. Therefore, eighteen subjects in the group who consumed meat from grass-fed animals and twenty subjects in the group who consumed meat from concentrate-fed animals successfully completed the study by consuming all portions of provided beef and lamb per week for 4 weeks. None of the subjects reported difficulty with compliance, and meat intakes during the intervention were not significantly different from baseline. No subjects reported in their food diaries of having consumed either oily fish or any n-3 PUFA-enriched foodstuffs during the week before the study commenced or during the

Table 1. Basal characteristics of the study participants (n=38)
(Mean values with their standard deviations, except for age (Mean (range)))

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Grass group (n=18)</th>
<th>Concentrate group (n=20)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (n)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>9</td>
<td>10</td>
<td>-†</td>
</tr>
<tr>
<td>Female</td>
<td>9</td>
<td>10</td>
<td>-†</td>
</tr>
<tr>
<td>Age (years)</td>
<td>25 (19–41)</td>
<td>26 (18–41)</td>
<td>-†</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22·90</td>
<td>23·50</td>
<td>0·688</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>117·94</td>
<td>121·55</td>
<td>0·207</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>76·61</td>
<td>79·70</td>
<td>0·223</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>4·07</td>
<td>4·74</td>
<td>0·414</td>
</tr>
<tr>
<td>LDL (mmol/l)</td>
<td>2·16</td>
<td>2·50</td>
<td>0·123</td>
</tr>
<tr>
<td>HDL (mmol/l)</td>
<td>1·44</td>
<td>1·54</td>
<td>0·354</td>
</tr>
<tr>
<td>TAG (mmol/l)</td>
<td>0·99</td>
<td>1·16</td>
<td>0·315</td>
</tr>
</tbody>
</table>

SBP, systolic blood pressure; DBP, diastolic blood pressure.
* Significance in mean values between groups at baseline in one-way ANOVA (P<0·05).
† Not calculated.

Table 2. Fatty acid composition of plasma at baseline and post-intervention according to study group (% of total fatty acids)
(Adjusted mean values with their standard errors)

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>Grass group (n=18)</th>
<th>Concentrate group (n=20)</th>
<th>P*</th>
<th>P†</th>
</tr>
</thead>
<tbody>
<tr>
<td>C14:0</td>
<td>1·01±0·09</td>
<td>1·19±0·12</td>
<td>1·24±0·13</td>
<td>1·28±0·11</td>
</tr>
<tr>
<td>C16:0</td>
<td>24·33±0·75</td>
<td>25·44±1·44</td>
<td>26·99±2·13</td>
<td>27·67±1·34</td>
</tr>
<tr>
<td>C16:1c</td>
<td>1·91±0·34</td>
<td>2·48±0·36</td>
<td>2·66±0·20</td>
<td>2·13±0·13</td>
</tr>
<tr>
<td>C18:0</td>
<td>6·34±0·24</td>
<td>8·05±0·38</td>
<td>7·23±0·68</td>
<td>6·89±0·36</td>
</tr>
<tr>
<td>C18:1c</td>
<td>24·23±0·64</td>
<td>20·68±2·32</td>
<td>22·97±1·42</td>
<td>22·44±1·29</td>
</tr>
<tr>
<td>C18:2n-6 (LA)</td>
<td>27·85±0·83</td>
<td>25·29±2·38</td>
<td>26·98±1·75</td>
<td>27·54±2·25</td>
</tr>
<tr>
<td>C18:3n-3 (ALA)</td>
<td>1·31±0·09</td>
<td>1·64±0·17</td>
<td>1·35±0·07</td>
<td>1·04±0·16</td>
</tr>
<tr>
<td>C18:11(11) (TVA)</td>
<td>0·43±0·04</td>
<td>3·45±1·39</td>
<td>0·43±0·04</td>
<td>0·54±1·32</td>
</tr>
<tr>
<td>C18:2n-6 (LA)</td>
<td>0·97±0·03</td>
<td>2·24±0·53</td>
<td>0·81±0·09</td>
<td>2·09±0·49</td>
</tr>
<tr>
<td>C20:4n-6 (AA)</td>
<td>0·06±0·26</td>
<td>7·65±0·82</td>
<td>7·08±0·61</td>
<td>7·05±0·77</td>
</tr>
<tr>
<td>C20:5n-3 (EPA)</td>
<td>0·70±0·09</td>
<td>0·95±0·13</td>
<td>0·95±0·08</td>
<td>0·70±0·13</td>
</tr>
<tr>
<td>C22:5n-3 (DPA)</td>
<td>1·01±0·12</td>
<td>1·08±0·12</td>
<td>1·01±0·09</td>
<td>0·86±0·11</td>
</tr>
<tr>
<td>C22:6n-3 (DHA)</td>
<td>0·81±0·09</td>
<td>1·57±0·22</td>
<td>1·29±0·19</td>
<td>0·69±0·20</td>
</tr>
<tr>
<td>SFA‡</td>
<td>31·68±0·91</td>
<td>34·55±2·02</td>
<td>35·46±2·88</td>
<td>36·25±1·91</td>
</tr>
<tr>
<td>MUFA§</td>
<td>26·14±0·67</td>
<td>22·14±2·39</td>
<td>24·64±1·41</td>
<td>23·65±2·28</td>
</tr>
<tr>
<td>Total n-6 PUFA‖</td>
<td>35·91±0·79</td>
<td>33·06±2·57</td>
<td>34·06±1·98</td>
<td>34·47±2·43</td>
</tr>
<tr>
<td>LC n-3 PUFA†</td>
<td>2·68±0·29</td>
<td>3·61±0·39</td>
<td>3·29±0·29</td>
<td>2·23±0·38</td>
</tr>
<tr>
<td>Total n-3 PUFA**</td>
<td>3·93±0·31</td>
<td>5·39±0·49</td>
<td>4·53±0·31</td>
<td>3·14±0·46</td>
</tr>
<tr>
<td>n-6:n-3††</td>
<td>9·18±0·47</td>
<td>6·21±1·19</td>
<td>8·20±0·79</td>
<td>12·87±1·09</td>
</tr>
</tbody>
</table>

LA, linoleic acid; ALA, a-linolenic acid; TVA, trans-vaccenic acid; CLA, conjugated linoleic acid; AA, arachidonic acid; DPA, docosapentaenoic acid.
* Significance in mean values between groups at baseline.
† Significance in mean values between groups at post-intervention with baseline value as covariate in ANCOVA (P<0·05).
‡ SFA: sum of 14:0, 16:0 and 18:0.
§ MUFA: sum of 16:1c and 18:1c.
‖ Total n-6 PUFA: sum of LA and AA.
¶ Total n-3 PUFA: sum of EPA, DPA and DHA.
** Total n-3 PUFA: sum of ALA, EPA, DPA and DHA.
†† n-6:n-3: total n-6/total n-3.
4-week study period. Results of the meat diaries showed subjects reported consuming almost all provided portions of mince (97%), steak (99%) and lamb (98%) throughout the 4 weeks. No subjects reported difficulty in consuming any of the meat provided. Dietary data were available for thirty-seven of the thirty-eight subjects.

**Anthropometry**

Mean values for the subjects’ age, height, weight, BMI, blood pressure and lipid profiles at baseline are presented in Table 1. There were no significant differences with respect to any subject characteristics between the two groups at baseline.

**Plasma and platelet fatty acids**

Table 2 shows plasma fatty acid composition at baseline and post-intervention for each study group (expressed as % total fatty acids). There were significant differences between groups at baseline for EPA (P<0.04) and DHA (P=0.04). In response to the intervention, stearic acid (18:0), ALA, DHA, LC n-3 PUFA and total n-3 PUFA were significantly increased (P<0.005), and the n-6:n-3 ratio was significantly decreased (P<0.01) within the group that consumed meat from grass-fed animals compared with the group that consumed meat from concentrate-fed animals.

Table 3 shows platelet fatty acid composition at baseline and post-intervention for each study group (% total fatty acids). There were no significant differences in fatty acid data between groups at baseline. In response to the intervention, EPA, DPA, DHA, LC n-3 PUFA and total n-3 PUFA were significantly increased (P<0.05) and the n-6:n-3 ratio was significantly decreased (P<0.001) within the group that consumed meat from grass-fed animals compared with the group that consumed meat from concentrate-fed animals.

**Serum lipids and lipoproteins**

In response to the intervention, there were no significant differences in serum lipids, lipoproteins, TAG or blood pressure between the study groups (data not shown).

**Nutrient intakes**

Under-reporting was identified in fourteen of the seventy-four completed food diaries, using the equation energy intake: BMR <1.1, adapted from Goldberg et al.(27). Removing these diaries did not result in any notable changes to group intakes of energy, macronutrients or fatty acids; therefore, it was decided not to exclude them from the analysis. At baseline, arachidonic acid (20 : 4n-6) and DPA intakes (P=0.04) were significantly greater within the group consuming meat from grass-fed animals.

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>Grass group (n 18)</th>
<th>Concentrate group (n 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>SD</td>
</tr>
<tr>
<td>14 : 0</td>
<td>1.44</td>
<td>0.31</td>
</tr>
<tr>
<td>16 : 0</td>
<td>18.39</td>
<td>3.19</td>
</tr>
<tr>
<td>16 : 1c</td>
<td>4.96</td>
<td>1.15</td>
</tr>
<tr>
<td>18 : 0</td>
<td>11.22</td>
<td>2.09</td>
</tr>
<tr>
<td>18 : 1c</td>
<td>15.33</td>
<td>6.71</td>
</tr>
<tr>
<td>18 : 2n-6 (LA)</td>
<td>9.19</td>
<td>2.17</td>
</tr>
<tr>
<td>18 : 3n-3 (ALA)</td>
<td>6.61</td>
<td>1.53</td>
</tr>
<tr>
<td>18 : 11f (TFA)</td>
<td>3.30</td>
<td>0.76</td>
</tr>
<tr>
<td>18 : 2c9, f1 (CLA 9,11)</td>
<td>4.96</td>
<td>1.15</td>
</tr>
<tr>
<td>20 : 4n-6 (AA)</td>
<td>8.72</td>
<td>0.54</td>
</tr>
<tr>
<td>20 : 5n-3 (EPA)</td>
<td>4.96</td>
<td>1.15</td>
</tr>
<tr>
<td>22 : 5n-3 (DPA)</td>
<td>6.61</td>
<td>1.53</td>
</tr>
<tr>
<td>22 : 6n-3 (DHA)</td>
<td>4.96</td>
<td>1.14</td>
</tr>
<tr>
<td>SFA†</td>
<td>30.42</td>
<td>5.16</td>
</tr>
<tr>
<td>MUFAl‡</td>
<td>20.29</td>
<td>5.85</td>
</tr>
<tr>
<td>Total n-6 PUFA</td>
<td></td>
<td>17.91</td>
</tr>
<tr>
<td>LC n-3 PUFA†</td>
<td>16.52</td>
<td>5.52</td>
</tr>
<tr>
<td>Total n-3 PUFA**</td>
<td>23.13</td>
<td>5.34</td>
</tr>
<tr>
<td>n-6:n-3††‡‡</td>
<td>0.83</td>
<td>0.36</td>
</tr>
</tbody>
</table>

LA, linoleic acid; ALA, α-linolenic acid; TFA, trans-vaccenic acid; CLA, conjugated linoleic acid; AA, arachidonic acid; DPA, docosapentaenoic acid.

† Significance in mean values between groups at baseline.

‡‡ Significance in mean values between groups at post-intervention in one-way ANOVA (P<0.05).

SFA: sum of 14 : 0, 16 : 0 and 18 : 0.

MUFAl: sum of 16 : 1c and 18 : 1c.

Total n-6 PUFA: sum of LA and AA.

LC n-3 PUFA: sum of EPA, DPA and DHA.

Total n-3 PUFA: sum of ALA, EPA, DPA and DHA.

n-6:n-3: total n-6/total n-3.
than those consuming meat from concentrate-fed animals (Table 4); therefore, baseline intakes of each fatty acid were adjusted for in subsequent analyses between groups. During the intervention, arachidonic acid (20 : 4 \(n \)-6) (\(P=0.01\)) and DPA intakes (\(P<0.001\)) were significantly increased in the group consuming meat from grass-fed animals compared with intakes in the group consuming meat from concentrate-fed animals. There were no other significant differences between groups in response to the intervention. The mean total daily intake of LC \(n\)-3 PUFA in subjects in the group consuming meat from grass-fed animals during the intervention was 65 mg/d compared with 44 mg/d in the group consuming meat from concentrate-fed animals. Dietary analysis showed that red meat and other meats were responsible for contributing 94 and 6% of total LC \(n\)-3 PUFA during the intervention within the group consuming meat from concentrate-fed animals and 87 and 13% within the group consuming meat from concentrate-fed animals.

Fatty acid composition of meat portions

Table 5 shows the concentrations of fatty acids in meat portions from grass-fed and concentrate-fed animals (mg/100 g muscle). Focus has been given to the fatty acid composition of muscle, as it is common to remove adipose tissue before consumption; therefore, the fatty acid composition of muscle should have a greater impact on status \(28^\) Results show that the total fat content was significantly increased in all meat portions from concentrate-fed animals than that from grass-fed animals (\(P<0.001\)). Beef steaks from grass-fed animals had significantly higher concentrations of ALA, EPA, LC \(n\)-3 PUFA and total \(n\)-3 PUFA (\(P<0.05\)) than steaks from concentrate-fed animals. Mince beef from grass-fed animals had significantly lower concentrations of linoleic acid (18:2\(n\)-6), arachidonic acid and total \(n\)-6 PUFA, with significantly higher ALA, EPA, LC \(n\)-3 PUFA and total \(n\)-5 PUFA than mince from concentrate-fed animals (\(P<0.01\)). Lamb from grass-fed animals had significantly lower concentrations of total SFA, linoleic acid and arachidonic acid (\(P<0.001\)) and significantly higher conjugated linoleic acid (18:2\(n\)-9, \(c\)), DPA, LC \(n\)-3 PUFA and total \(n\)-3 PUFA than lamb from concentrate-fed animals (\(P<0.05\)). Ratios of \(n\)-6: \(n\)-3 were significantly lower in all meat portions from animals offered grass compared with those offered concentrate (\(P<0.001\)). The sum of (total \(n\)-6, total \(n\)-3):SFA ratio was significantly higher in lamb from grass-fed animals (\(P<0.001\)). DHA was not detected in the beef mince samples in meat from either grass-fed or concentrate-fed animals, and it was not significantly increased within steaks or lamb from grass-fed animals possibly owing to limited elongation of this LC \(n\)-3 PUFA \(29^\). Steaks, mince and lamb from grass-fed animals contained 25–97, 28–38 and 36–94 mg of LC \(n\)-3 PUFA per 100 g muscle, respectively, compared with 18–69, 16–86 and 28–94 mg per 100 g muscle from concentrate-fed animals.

Discussion

The present study demonstrates that moderate consumption of red meat from grass-fed animals can contribute to increased plasma and platelet LC \(n\)-3 PUFA concentrations among healthy individuals. Sinclair et al. \(19^\) previously reported that 500 g/d of lean beef could increase plasma concentrations of LC \(n\)-3 PUFA compared with an intake of 30–100 g/d of beef. In the present study, the approximate daily intake of red meat (67 g) is similar to the quantity which 88% of the Irish population are presently consuming \(12^\), suggesting that it may be possible to modify total LC \(n\)-3 PUFA intakes in this population

### Table 4. Fat and fatty acid intakes at baseline and post-intervention according to study group (mg/d)

(Adjusted mean values with their standard errors)

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Baseline</th>
<th>Post-intervention</th>
<th>Baseline</th>
<th>Post-intervention</th>
<th>(P^*)</th>
<th>(P^\dagger)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total fat (g/d)</td>
<td>58.86</td>
<td>4.18</td>
<td>77.26</td>
<td>4.53</td>
<td>66.63</td>
<td>4.72</td>
</tr>
<tr>
<td>SFA (g/d)</td>
<td>22.94</td>
<td>1.65</td>
<td>30.76</td>
<td>2.05</td>
<td>29.71</td>
<td>1.82</td>
</tr>
<tr>
<td>MUFA (g/d)</td>
<td>17.71</td>
<td>1.32</td>
<td>25.81</td>
<td>1.88</td>
<td>20.77</td>
<td>1.48</td>
</tr>
<tr>
<td>PUFA (g/d)</td>
<td>7.55</td>
<td>0.79</td>
<td>9.14</td>
<td>0.92</td>
<td>9.21</td>
<td>0.81</td>
</tr>
<tr>
<td>18:2(n)-6 (LA)</td>
<td>881.12</td>
<td>170.02</td>
<td>648.00</td>
<td>228.01</td>
<td>621.05</td>
<td>171.23</td>
</tr>
<tr>
<td>18:3(n)-3 (ALA)</td>
<td>92.34</td>
<td>15.02</td>
<td>136.45</td>
<td>21.45</td>
<td>82.32</td>
<td>20.01</td>
</tr>
<tr>
<td>20:4(n)-6 (AA)</td>
<td>31.27</td>
<td>3.15</td>
<td>43.11</td>
<td>3.02</td>
<td>20.71</td>
<td>3.00</td>
</tr>
<tr>
<td>20:5(n)-3 (EPA)</td>
<td>20.38</td>
<td>3.25</td>
<td>28.78</td>
<td>3.15</td>
<td>22.46</td>
<td>3.20</td>
</tr>
<tr>
<td>22:5(n)-3 (DPA)</td>
<td>17.01</td>
<td>1.00</td>
<td>22.14</td>
<td>1.31</td>
<td>15.06</td>
<td>2.00</td>
</tr>
<tr>
<td>22:6(n)-3 (DHA)</td>
<td>9.53</td>
<td>7.00</td>
<td>14.24</td>
<td>7.05</td>
<td>10.09</td>
<td>2.00</td>
</tr>
<tr>
<td>LC (n)-3 PUFA</td>
<td>46.91</td>
<td>9.00</td>
<td>65.15</td>
<td>9.78</td>
<td>47.61</td>
<td>6.00</td>
</tr>
<tr>
<td>Total (n)-3 PUFA</td>
<td>139.25</td>
<td>18.00</td>
<td>201.61</td>
<td>18.25</td>
<td>129.93</td>
<td>21.30</td>
</tr>
<tr>
<td>Total (n)-6 PUFA</td>
<td>913.35</td>
<td>204.12</td>
<td>558.12</td>
<td>202.13</td>
<td>641.75</td>
<td>152.01</td>
</tr>
<tr>
<td>n-6:n-3</td>
<td>6.61</td>
<td>0.78</td>
<td>2.77</td>
<td>2.42</td>
<td>4.98</td>
<td>1.41</td>
</tr>
</tbody>
</table>

LA, linoleic acid; ALA, \(\alpha\)-linolenic acid; AA, arachidonic acid; DPA, docosapentaenoic acid.

*Significance in mean values between groups at baseline.

† Significance in mean values between groups at post-intervention with baseline value as covariate in ANCOVA (\(P<0.05\)).
Table 5. Fat content and fatty acid composition of meat portions from animals fed a diet of grass or concentrate (mg/100 g muscle) (Adjusted mean values with their standard errors)

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>Beef steaks</th>
<th>Beef mince</th>
<th>Lamb</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Grass (n 5)</td>
<td>Concentrate (n 4)</td>
<td>Grass (n 3)</td>
</tr>
<tr>
<td></td>
<td>Mean (SEM)</td>
<td>Mean (SEM)</td>
<td>Mean (SEM)</td>
</tr>
<tr>
<td>Total fat (g/100g)</td>
<td>2·49 (0·39)</td>
<td>5·31 (0·98)</td>
<td>7·76 (0·35)</td>
</tr>
<tr>
<td>SFA†</td>
<td>1409·02 (44·09)</td>
<td>1384·58 (64·13)</td>
<td>3747·94 (90·85)</td>
</tr>
<tr>
<td>MUFA‡</td>
<td>1416·48 (40·41)</td>
<td>1349·4 (58·77)</td>
<td>3741·79 (77·73)</td>
</tr>
<tr>
<td>18:1t1 (TV)</td>
<td>69·99 (22·53)</td>
<td>86·99 (24·19)</td>
<td>203·03 (14·46)</td>
</tr>
<tr>
<td>18:2c9,t11 (CLA)</td>
<td>15·30 (1·99)</td>
<td>18·27 (2·14)</td>
<td>64·19 (7·26)</td>
</tr>
<tr>
<td>18:2n-6 (LA)</td>
<td>87·62 (16·11)</td>
<td>161·45 (23·44)</td>
<td>145·41 (15·83)</td>
</tr>
<tr>
<td>20:4n-6 (AA)</td>
<td>19·36 (1·25)</td>
<td>25·83 (1·82)</td>
<td>19·70 (1·15)</td>
</tr>
<tr>
<td>Total n-6 PUFA§</td>
<td>106·98 (16·81)</td>
<td>187·28 (24·45)</td>
<td>165·11 (16·01)</td>
</tr>
<tr>
<td>18:3n-3 (ALA)</td>
<td>33·19 (1·08)</td>
<td>17·52 (1·57)</td>
<td>70·04 (3·53)</td>
</tr>
<tr>
<td>20:5n-3 (EPA)</td>
<td>13·24 (0·66)</td>
<td>6·39 (0·96)</td>
<td>203·03 (14·46)</td>
</tr>
<tr>
<td>22:5n-3 (DPA)</td>
<td>11·84 (0·88)</td>
<td>10·33 (1·29)</td>
<td>13·91 (1·35)</td>
</tr>
<tr>
<td>22:6n-3 (DHA)</td>
<td>0·35 (0·31)</td>
<td>0·99 (0·36)</td>
<td>ND</td>
</tr>
<tr>
<td>Total n-3 PUFA¶</td>
<td>25·97 (1·30)</td>
<td>18·69 (1·89)</td>
<td>28·38 (2·02)</td>
</tr>
<tr>
<td>Total n-3 PUFA††</td>
<td>59·16 (1·64)</td>
<td>36·21 (2·39)</td>
<td>98·42 (5·34)</td>
</tr>
<tr>
<td>n-6:n-3**</td>
<td>2·29 (0·57)</td>
<td>8·39 (0·83)</td>
<td>1·73 (0·29)</td>
</tr>
<tr>
<td>P:S††</td>
<td>0·14 (0·01)</td>
<td>0·18 (0·02)</td>
<td>0·10 (0·01)</td>
</tr>
</tbody>
</table>

TVA, trans-vaccenic acid; CLA, conjugated linoleic acid; LA, linoleic acid; AA, arachidonic acid; ALA, α-linolenic acid; DPA, docosapentaenoic acid; ND, not detected.

* Significance in mean values between treatment groups with total fat content as covariate in ANCOVA (P<0·05).
† SFA: sum of 10 : 0,12 : 0,14 : 0,16 : 0,17 : 0,18 : 0.
‡ MUFA: sum of 14 : 1c,15 : 1c,16 : 1c,16 : 1c,17 : 1c,18 : 1c,18 : 1c,11.
§ Total n-6 PUFA: sum of LA and AA.
¶ LC n-3 PUFA: sum of EPA, DPA and DHA.
† Total n-3 PUFA: sum of ALA, EPA, DPA and DHA.
** n-6:n-3: total n-6/total n-3.
†† P:S: sum of (total n-6, total n-3):SFA.
Grass-fed red meat and n-3 PUFA status

without changing dietary habits. Furthermore, this intake
is below the upper limit of red meat consumption advised
by the World Cancer Research Fund(23), and, as such, is not
thought to cause any negative effect to health.

Animals were offered grass for a 6-week period before
slaughter. The LC n-3 PUFA concentrations found within
meat from grass-fed animals compared well with those
reported by others for beef(30,31) and lamb(2). Intake of
LC n-3 PUFA when red meat from grass-fed animals was
included in the diet was estimated at 65 mg/d, compared
to 44 mg/d when red meat from concentrate-fed animals
was consumed. The difference in LC n-3 PUFA intake
between groups attributed to the red meat consumed
was estimated at 18 mg/d, an acknowledge low intake
which was nonetheless shown to contribute to
increased plasma and platelet LC n-3 PUFA status. Fish
consumption can make it difficult to isolate and measure
the effect of meat consumption on n-3 PUFA status(32).
In the present study, however, the subjects omitted fish
from their diet for the 4-week study duration and were
infrequent consumers of n-3 PUFA-enriched foods. The
dietary data suggest that red meat was the primary
component responsible for the rise in blood concentrations
of LC n-3 PUFA within the group that consumed meat from
grass-fed animals compared with the group that consumed
meat from concentrate-fed animals. Limitations associated
with dietary analysis and food composition tables must
be considered in the interpretation of dietary intake data,
where LC n-3 PUFA data for many foods are lacking.

In the present study, an increase in DHA status occurred
within the consumers of meat from grass-fed animals.
The synthesis of DHA from ALA and EPA is known to be
relatively poor(33); however, it is probable that DPA could
be used to synthesise some DHA in consumers of red
meat. The rate of this synthesis has been proposed to be
37% in humans and was recently described in an animal
study where DPA supplementation increased DHA
status(16,34). As DHA synthesis occurs in a peroxisomal
reaction, it is also possible that this step may be independently
regulated from the typical LC n-3 PUFA elongation pathway(34).
While acknowledging the complexity of
DHA metabolism, it is possible that the observed increase
in DHA status within the consumers of meat from grass-
fed animals is a result of increased DPA intakes during
the intervention, which were significantly greater than
intakes within the consumers of meat from concentrate-
fed animals.

In the group that consumed meat from grass-fed
animals, the increase in LC n-3 PUFA concentrations
in platelets was more pronounced than in plasma. As
plasma is an effective short-term marker of LC n-3 PUFA
status(35), it is possible that some wash-out of LC n-3 PUFA
had occurred between the time of the last meal of
meat from grass-fed animals and blood collection at the
end of the intervention. In comparison, platelets are a
better reflection of long-term LC n-3 PUFA status(35), and
the significant increases observed in both plasma and
platelet measures confirm the bioavailability of LC n-3
PUFA from red meat from grass-fed animals. Plasma fatty
acid values measured in the present study compare well
to those of similar studies, albeit where plasma phospholi-
pids were measured(19,32,36,37).

It was not surprising to observe no significant differences
in serum concentrations of cholesterol, TAG or blood
pressure between groups. Firstly, there is inconsistent
evidence that low doses of LC n-3 PUFA can reduce total
or LDL-cholesterol serum concentrations(38–40). Generally,
the LC n-3 PUFA are recognised for their ability to decrease
TAG concentrations and this potential has been shown
predominantly with LC n-3 PUFA doses >450 mg/d(41).
Therefore, it is probable that a combined effect of the
low dose of LC n-3 PUFA received through the meat
from grass-fed animals, the short study duration and the
absence of hyperlipidaemia in subjects resulted in a lack
of effect on TAG concentrations in the present study. In
addition, there is a lack of evidence to show that LC n-3
PUFA can reduce blood pressure at low doses or in non-
hypertensive individuals(42). Nonetheless, it is important
to acknowledge the aspect that red meat consumption
had no effect on serum cholesterol, TAG or blood pressure
in the present study, as it concurs with other studies
showing moderate red meat consumption has no negative
effects to health(43,44).

Other means of increasing LC n-3 PUFA content of
meat include addition of oilseeds or fish oil in the animal
diet(45–47). For example, Medeiros et al.(46) showed
reduced concentrations of vascular cell adhesion mol-
ecule-1 in rats consuming beef from cattle offered a flax-
seed-supplemented diet compared to a typical diet of
maize. Moreover, another study showed human consump-
tion of linseed-enriched animal products to cause an
increase in plasma concentrations of LC n-3 PUFA(48).
However, the advantages of meat from grass-fed animals
are that the content of total fat, SFA or trans-fatty acids in
the meat are not simultaneously increased(49), the palatabil-
ity is not affected as natural levels of
\(\alpha\)-tocopherol in the grass reduce susceptibility to lipid peroxidation(29,50)
and offering animals a grass diet would be more cost-
effective to the producer and more sustainable with respect
to the environment than feeding concentrates to the ani-
mals. However, future studies should consider increasing
the length of the finishing period to allow greater
increments in LC n-3 PUFA concentrations in meat tissue
of grass-fed animals to occur.

Overall, the present study has shown that an animal diet
of grass before slaughter can help to increase the LC n-3
PUFA content of red meat. Furthermore, increases in
plasma and platelet concentrations of LC n-3 PUFA were
observed among consumers of this meat. This observation
may have implications for the red meat industry,
where increased production of red meat from grass-fed
animals would have greater appeal to the consumer,
adding marketable value to the product. Furthermore, the consumption of red meat from grass-fed animals may contribute to raising the overall LC n-3 PUFA intake closer to the recommended intake of 450 mg/d (6) without a change being made to dietary habits, which in turn would be beneficial for cardiovascular health.

Conclusions

The present study is novel in the sense that an animal diet of grass before slaughter has been shown to significantly have an impact on LC n-3 PUFA status in free-living healthy consumers of red meat and at a level of consumption similar to the present intakes among the Irish population. Overall, the results of the present study suggest that consumption of red meat from grass-fed animals may provide valuable amounts of LC n-3 PUFA to the consumer and increased production of red meat from grass-fed animals may thereby help to increase LC n-3 PUFA intakes of consumers.

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References

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