A reduced protein diet induces stearoyl-CoA desaturase protein expression in pig muscle but not in subcutaneous adipose tissue: relationship with intramuscular lipid formation

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A reduced protein diet (RPD) is known to increase the level of intramuscular lipid in pig meat with a smaller effect on the amount of subcutaneous adipose tissue. This might be due to tissue-specific activation of the expression of lipogenic enzymes by the RPD. The present study investigated the effect of a RPD, containing palm kernel oil, soyabean oil or palm oil on the activity and expression of one of the major lipogenic enzymes, stearoyl-CoA desaturase (SCD) and on the level of total lipids and the fatty acid composition of muscle and subcutaneous adipose tissue in pigs. The RPD significantly increased SCD protein expression and activity in muscle but not in subcutaneous adipose tissue. The level of MUFA and total fatty acids in muscle was also elevated when the RPD was fed, with only small changes in subcutaneous adipose tissue. A positive significant correlation between SCD protein expression and total fatty acids in muscle was found. The results suggest that an increase in intramuscular but not subcutaneous adipose tissue fatty acids under the influence of a RPD is related to tissue-specific activation of expression of SCD. It is suggested that the SCD isoform spectra in pig subcutaneous adipose tissue and muscle might be different.

Stearoyl-CoA desaturase: Intramuscular lipid: Pig: Reduced protein diet

The amount and type of fat in the diet has a major impact on human health. High levels of total fat intake have been shown to be risk factors for cancer and obesity (for review see Ferguson et al. 2004). High consumption of saturated fatty acids (SFA) raises plasma LDL-cholesterol, which is a major risk factor for atherosclerosis and CHD (Mensink & Katan, 1992; Clarke et al. 1997; Wolfram, 2003). In contrast, MUFA and PUFA are beneficial for human health. PUFA have been shown to protect against CHD by lowering plasma LDL-cholesterol (Sacks & Katan, 2002). MUFA are also considered to have a hypocholesterolaemic effect (Duckett et al. 1993) and, in addition, have been found to have an antithrombogenic effect (Smith et al. 2003).

One of the most consumed meats in Europe is that from pigs (Mourot & Hermier, 2001). Strategies that would decrease the amount of subcutaneous fat (about 70% total fat) would improve the healthiness of pig meat from a consumer perspective. However, the increasing trend towards the production of leaner meat has resulted not only in a reduction of subcutaneous fat, but also of intramuscular lipids (IML), the fat located within the structure of muscle. IML has been shown to be positively related to the juiciness, tenderness and overall sensory quality of pork (Fernandez et al. 1999; Van Barneveld, 2003). De Vol et al. (1988) suggested that acceptable pork eating quality requires a minimum IML of 2.5%. Reduction of IML below 2% may have a deleterious effect on characteristics of eating quality (Eikelenboom & Hoving-Bolink, 1994). In contrast to bovine meat, IML in pork is not often visible (except in the Duroc breed) (Mourot & Hermier, 2001), and hence an increase in IML should not result in rejection of the meat by consumers on the basis of ‘marbling’. Therefore, production of pig meat with higher amounts of IML without an increase in subcutaneous fat would be advantageous for the pig industry.

Earlier studies have shown that the amount of IML can be increased, with less effect on subcutaneous fat deposition, by the feeding of low protein diets (Wood et al. 2004). The mechanism of this process is not clear. One of the possible explanations could be that lower dietary protein levels stimulate an expression of muscle lipogenic enzymes, and hence increase de novo fatty acid synthesis. One of the key lipogenic enzymes is stearoyl-CoA desaturase (SCD), which catalyses the cellular biosynthesis of MUFA (Enoch et al. 1976). Da Costa et al. (2004) have recently shown that a low lysine (low protein) diet increases SCD transcriptional rate in pig muscles. However, it remains unknown whether the increase in the transcriptional rate is followed by an increase in SCD protein expression and activity. The effect of a reduced protein diet (RPD) on SCD expression in pig adipose tissue has not yet been studied.

Abbreviations: ACC, acetyl-CoA carboxylase; FAS, fatty acid synthase; IML, intramuscular lipids; PKO, palm kernel oil; PO, palm oil; RPD, reduced protein diet; SBO, soyabean oil; SCD, stearoyl-CoA desaturase; SFA, saturated fatty acids.

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The expression of lipogenic enzymes can also be regulated by dietary oils. Soyabean oil (SBO), rich in PUFA, is known to repress SCD gene expression in different tissues of a number of species (Ntambi, 1999; Bene et al. 2001; Lefevre et al. 2001; Kim et al. 2002). There is an increasing interest in using tropical oils, in particular palm oil (PO) and palm kernel oil (PKO), as feed supplements, because of their low cost and high level of antioxidants (Ebong et al. 1999; Perez et al. 2000). Whether these tropical oils, rich in SFA, affect SCD expression is not clear. To the best of our knowledge, the effect of a RPD in combination with dietary oil supplements on SCD expression has not been studied.

The present research investigated the effect of a RPD in combination with three different types of oil (PKO, SBO and PO) on the activity and expression of SCD protein in pig muscle and subcutaneous adipose tissue, and the relationships between SCD expression and levels of intramuscular and subcutaneous adipose tissue lipids.

Materials and methods

Animals and diets

Intact male pigs (0.5 Duroc, 0.25 Large White, 0.25 Landrace) were used in this experiment. The animals were reared and slaughtered in compliance with regulations for the humane care and use of animals in research as operated at Harper Adams University College and at the University of Bristol. Out of an original group of thirty pigs, only twenty-six were available for the trial. The pigs were reared from 40 kg live weight in groups of four or five on one of the six diets. There was a 2 x 3 factorial experimental design with two levels of protein and three types of vegetable oil (PKO, SBO or PO). The diets were formulated to contain 21 or 18 % crude protein (called control diet and RPD, respectively) and 5 % oil on a dry weight basis. The calculated values for non-structural carbohydrates (including starch) were 68 % and 71 % for the control diet and RPD, respectively, and 5 % oil on a dry weight basis. The calculated values for non-structural carbohydrates (including starch) were 68 % and 71 % for the control diet and RPD, respectively. The calculated values for non-structural carbohydrates (including starch) were 68 % and 71 % for the control diet and RPD, respectively.

Table 1. Composition of the diets (g/kg)

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>PKO Con</th>
<th>PKO RPD</th>
<th>SBO Con</th>
<th>SBO RPD</th>
<th>PO Con</th>
<th>PO RPD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barley</td>
<td>500</td>
<td>475</td>
<td>500</td>
<td>475</td>
<td>500</td>
<td>475</td>
</tr>
<tr>
<td>Wheat</td>
<td>173</td>
<td>292</td>
<td>173</td>
<td>292</td>
<td>173</td>
<td>292</td>
</tr>
<tr>
<td>Soyabean meal</td>
<td>239</td>
<td>120</td>
<td>239</td>
<td>120</td>
<td>239</td>
<td>120</td>
</tr>
<tr>
<td>Prairie meal</td>
<td>25</td>
<td>50</td>
<td>25</td>
<td>50</td>
<td>25</td>
<td>50</td>
</tr>
<tr>
<td>Palm kernel oil</td>
<td>28</td>
<td>28</td>
<td>-</td>
<td>-</td>
<td>28</td>
<td>-</td>
</tr>
<tr>
<td>Palm oil</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>28</td>
<td>-</td>
</tr>
<tr>
<td>Salt</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Vitamin–mineral premix</td>
<td>13.5</td>
<td>13.5</td>
<td>13.5</td>
<td>13.5</td>
<td>13.5</td>
<td>13.5</td>
</tr>
<tr>
<td>Vitamin E supplement</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
</tr>
</tbody>
</table>

Con, control diet (calculated to provide 21 % protein, 5 % fat); PO, palm oil; PKO, palm kernel oil; RPD, reduced protein diet (calculated to provide 18 % protein, 5 % fat); SBO, soyabean oil.

For details of diets and procedures, see this page.

Table 1. Composition of the diets (g/kg)

Fatty acid analysis

Lipids from muscle were extracted as follows. Samples of longissimus thoracis et lumborum muscle were trimmed of connective and adipose tissue before blending in a food processor. Duplicate 1 g sub-samples were hydrolysed for 2 h at 60°C with 5 M-KOH in 50 % aqueous methanol, containing hydroquinone as an antioxidant, and a known amount of C21:0 as an internal standard. After allowing the samples to cool, distilled water was added and non-saponifiable lipids were extracted into light petroleum ether (40–60°C) and discarded. The hydrolysates were acidified with 5 M-H2SO4 and fatty acids were extracted into petroleum ether. Methyl esters were prepared using diazomethane in diethyl ether, and analysed by GC. Analysis was performed using a temperature-programmed run on a Fisons Mega Series 5160 gas chromatograph equipped with a CP Sil88 WCOT capillary column (50 x 0.25 mm internal diameter; Chrompak UK Ltd, London, UK), a split/splitless injector set with a split of 50:1, and a flame-ionisation detector. He gas was used as a carrier, and fatty acids were identified by comparison with standards purchased from Sigma (Poole, UK). Quantification was achieved by use of the internal standard added prior to hydrolysis, and the linearity of the detector response was tested by using a reference monoenoic FAME mix (FAME#5, Thames Restek UK Ltd., Bucks., UK).

Samples of subcutaneous backfat and longissimus thoracis et lumborum muscle from the dorsal region at the level of the last rib were obtained within 5 min of slaughter and before the carcasses entered the scalding tank. They were quickly frozen in liquid N2 and kept at −80°C (for analysis of enzyme activity and expression). The expression of the enzymes investigated (SCD, fatty acid synthase (FAS) and acetyl-CoA carboxylase (ACC)) was similar in corresponding subcellular fractions isolated from fresh or frozen tissues. For analysis of fatty acid composition, samples were taken 24 h after slaughter from carcasses, which were stored at 1°C. Storage of the carcasses under these conditions did not cause oxidation or degradation of fatty acids. The level of PUFA (most vulnerable) is similar in tissue samples taken immediately or 24 h after slaughter (data not shown). The samples were frozen and subsequently stored at −20°C.
Lipids from adipose tissues were extracted into chloroform, containing 2,6-di-tert-butyl-p-cresol as an antioxidant, and anhydrous sodium sulphate added to remove water. After filtration, duplicate sub-samples were hydrolysed as described earlier for muscle except that 2M-KOH was used, and the samples were kept at 60°C for 1 h. Fatty acids were extracted and methyl esters prepared as described earlier, before analysis by GC.

**Isolation of subcellular organelles**

Microsomes and cytosol from subcutaneous adipose tissue and muscle were isolated by differential centrifugation using the procedure described by Schenkm a & Cinti (1978) with minor modifications. A sample of frozen muscle or subcutaneous adipose tissue (4 g) was homogenised in a Tris-sucrose buffer composed of 10 mM-Tris-HCl, 250 mM-sucrose and 0.3 M-sucrose containing cytosol and microsomes, was obtained by centrifugation on a sucrose gradient (Schenkman & Cinti, 1978). The mitochondria-enriched post-mitochondrial fraction, containing cytosol and microsomes, was obtained by centrifugation at 12000g for 30 min in the presence of 8 mM-CaCl2. The final pellet (microsomal fraction) was resuspended in the Tris-sucrose buffer at a concentration of about 20 mg protein/ml for muscles and 5 mg protein/ml for subcutaneous adipose tissue in the presence of the inhibitors of proteolytic enzymes at the concentrations shown earlier. When microsomes were used for SCD activity analysis, 10 mM-EDTA was added to the isolation buffer to prevent oxidation of protein thiols. In this case the microsomes were resuspended in 50 mM-potassium phosphate buffer, pH 7.4.

**Western blotting**

The expression of microsomal protein SCD, and the cytosolic proteins, ACC and FAS, were estimated by Western blotting. Proteins were separated by SDS–PAGE, electroblotted on to nitrocellulose membrane at a constant 100 V for 1 h and probed with one of the following antibodies: rabbit polyclonal anti-bovine adipose tissue SCD, sheep polyclonal anti-rabbit mammary gland ACC or sheep polyclonal anti-rabbit mammary gland FAS. After washing the membrane and re-probing with an appropriate commercial secondary antibody, the blots were developed using an ECL reagent (Amersham Pharmacia Biotech, Amersham, Bucks, UK). The films were scanned and the intensity of the corresponding bands was quantified using the ImageQuant program (Molecular Dynamics, Sunnyvale, CA, USA). One of the samples from a particular pig was present on each blot and the SCD, FAS or ACC content of this sample was taken as 100 arbitrary units throughout. The antibodies used were shown to recognise pig SCD, ACC or FAS. There was a linear relationship between the intensity of SCD, ACC and FAS signals and the amount of the protein loaded on the gel, when up to 15 μg adipose tissue protein or up to 50 μg muscle protein was used (the calibrations are not shown). For all subsequent Western blots the amount of protein used was 6 μg for subcutaneous adipose tissue and 20 μg for muscle, which fit on the linear parts of the calibrations and therefore would allow detection of up to 2.5-fold changes in the amount of SCD, ACC or FAS.

**Table 2. Fatty acid composition (% total fatty acids) of the oils used in the diets**

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Palm kernel oil</th>
<th>Soyabean oil</th>
<th>Palm oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>C8:0</td>
<td>1.43</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>C10:0</td>
<td>2.45</td>
<td>nd</td>
<td>Trace</td>
</tr>
<tr>
<td>C12:0</td>
<td>40.06</td>
<td>nd</td>
<td>0.23</td>
</tr>
<tr>
<td>C14:0</td>
<td>14.32</td>
<td>Trace</td>
<td>1.06</td>
</tr>
<tr>
<td>C16:0</td>
<td>12.33</td>
<td>11.03</td>
<td>45.14</td>
</tr>
<tr>
<td>C16:1</td>
<td>Trace</td>
<td>0.14</td>
<td>0.16</td>
</tr>
<tr>
<td>C18:0</td>
<td>17.41</td>
<td>4.05</td>
<td>4.30</td>
</tr>
<tr>
<td>C18:1n-9</td>
<td>5.95</td>
<td>21.40</td>
<td>37.72</td>
</tr>
<tr>
<td>C18:1n-7</td>
<td>0.28</td>
<td>1.97</td>
<td>0.78</td>
</tr>
<tr>
<td>C18:2n-6</td>
<td>1.07</td>
<td>52.91</td>
<td>8.50</td>
</tr>
<tr>
<td>C18:3n-3</td>
<td>0.06</td>
<td>6.80</td>
<td>0.07</td>
</tr>
<tr>
<td>C20:1</td>
<td>0.05</td>
<td>nd</td>
<td>0.16</td>
</tr>
</tbody>
</table>

nd, not detected.

For details of diets and procedures, see p. 610.

SCD activity in isolated microsomes from adipose tissue was determined by measuring the conversion of labelled SFA into MUFA in optimal conditions. Commercially available [1-14C]-palmitoyl-CoA was used as a substrate (Yang et al. 1999; Smith et al. 2002). The incubation medium (final volume 1·0 ml) contained 50 mM-potassium phosphate buffer (pH 7.4), 5 mM-MgCl2, 7·2 mM-ATP, 0·5 mM-NADH and 0·044 mM-[1-14C]-palmitoyl-CoA (specific activity 600 μCi/mmole). The assay was initiated by the addition of 1 mg microsomal protein and was carried out in a shaking water-bath at 37°C for 5 min before terminating by adding 2 ml ice-cold 10% KOH in methanol containing hydroquinone as an antioxidant. Carrier fatty acids (0·2 mg C16:0 and C16:1) were added to assay samples before hydrolysis at 60°C for 1 h. After acidification with 5·M-H2SO4, fatty acids were extracted three times with petroleum ether (40–60°C) and the extracts combined. Fatty acid methyl esters were prepared using diazomethane in diethyl ether and taken up in 100 μl distilled n-hexane. To quantify the incorporation of labelled substrate into labelled product, fatty acids were separated by TLC using silver nitrate-impregnated Polygram Sil-G plates (Camlab, Cambridge, UK). The plates were developed in hexane–ether (9:1) and the bands of fatty acids were visualised under UV light after spraying with 0·1% Rhodamine-6G (BDH, Poole, UK). The bands were cut out, and the radioactivity was counted using an LKB RackBeta liquid scintillation counter (Wallac, Turku, Finland). Desaturase enzyme activities were obtained by calculating the ratio of the activity in MUFA to SFA + MUFA, and multiplying this ratio by the amount of substrate added to the incubation, and expressed as nmol palmitoleic acid formed/mg protein per h.

SCD activity in muscle homogenates was determined as described by Kouba et al. (1999). Muscle homogenates were prepared by dicing and blending 5 g muscle in 50 mM-potassium phosphate buffer plus 0·25 mM-sucrose (pH 7.4), using a Polytron (Kinematica, Lucerne, Switzerland) at low speed. The infranatant obtained after spinning at 10000 g for 40 min at 4°C was assayed for SCD activity by adding 0·2 ml infranatant to the incubation mixture and proceeding as described earlier.
Protein assay
Protein was determined by the Bradford method using bovine serum albumin as a standard.

Statistics
The data were analysed using either ANOVA (if data were balanced) or general linear models (if data were unbalanced) with protein level and type of oil as factors and including an interaction term. \( P<0.05 \) was considered statistically significant.

Results
Muscle fatty acids
The RPD significantly increased (by 86·3 %) the total fatty acid content in muscle when compared with the control diet \((P<0.01)\) (Fig. 1(A)). There was no significant effect of oil type on the total fatty acid content of muscle \((P=0.87)\). The oil \( \times \) protein interaction was not significant \((P=0.51)\).

The levels of individual SFA and MUFA, which are substrates, and their respective products in the SCD-catalysed reaction were measured and the results are presented in Table 3. The ratio of MUFAs:SFA (desaturation index), which is frequently used for indirect estimation of SCD activity (Yang et al. 1999; Miyazaki & Ntambi, 2003), was also calculated. The RPD led to a 106·2 % increase in the levels of saturated palmitic fatty acid \((16:0)\) \((P<0.001)\) with a similar \((112.5 \%) \) increase in its corresponding MUFA, palmitoleic \((16:1)\) \((P<0.001)\). As a result, the desaturation index \((16:1:16:0)\) remained unchanged. This diet also led to an increase in the level of the other SFA, stearic fatty acid \((18:0)\) \((P<0.001)\) and its corresponding MUFA, oleic fatty acid \((18:1\n-9)\) \((P<0.001)\). Since the level of \(18:1\n-9\) was increased by 131·5 %, while the level of \(18:0\) was raised only by 88·6 %, the desaturation index \((18:1\n-9:18:0)\) was significantly increased \((P=0.002)\). No significant differences in the levels of major SFA and MUFA and their ratios were observed in the presence of different types of oil (in all cases \(P>0.1\), see Table 3 for individual \( P \) values). There was no significant protein \( \times \) oil interaction \((P>0.05)\).

Fatty acids in subcutaneous adipose tissue
Feeding the RPD increased the total fatty acid content of the adipose tissue only by 8·5 %, when compared with the control diet \((P=0.002)\) (Fig. 1(B)). There was no significant effect of oil type on the total fatty acid content \((P=0.67)\). The protein \( \times \) oil interaction was not significant either \((P=0.72)\).

The effects of the RPD and type of oil on the major SFA and MUFA in subcutaneous adipose tissue are presented in Table 3. The RPD led only to a small \((11·9 \%) \) increase in the level of \(16:0\) \((P=0.005)\) without a significant effect on the level of \(16:1\) \((P=0.237)\). The ratio \(16:1:16:0\) remained unchanged. The levels of \(18:0\) and \(18:1\n-9\) were significantly increased by the RPD \((P=0.007\text{ and }P=0.002\text{, respectively})\). Since the increase was similar for both, \(18:0\) and \(18:1\n-9\) \((by13:8\%\text{ and }13:6\%,\text{ respectively})\), no change in the ratio \(18:1\n-9:18:0\) was observed.

The oil type also had a significant effect on the SFA and MUFA levels in subcutaneous adipose tissue. The levels of \(16:0\), \(16:1\) and the ratio \(16:1:16:0\) were highest for PKO when compared to SBO and PO \((P=0.015\text{, }P<0.001\text{ and }P=0.003\text{, respectively})\). The level of \(18:0\) was also highest for PKO \((P=0.008)\), whilst the \(18:1\) level and the ratio \(18:1\n-9:18:0\) were significantly higher in the case of PO \((P<0.001)\).

Stearoyl-CoA desaturase activity and protein expression in muscle and subcutaneous adipose tissue
The results of measurements of SCD activity in muscle and subcutaneous adipose tissue are presented in Fig. 2(A, B). Feeding the RPD resulted in higher SCD activity in muscle \((56.5 \%) \) increase) when compared with the control diet \((P<0.001)\). A significant effect of oil type on SCD activity in muscle was also observed: the activity was about 40 % higher in the case of PO when compared with PKO or SBO \((P=0.009)\).

In subcutaneous adipose tissue there was no significant effect of the RPD \((P=0.58)\) or oil source used \((P=0.11)\) on SCD activity \((Fig. 2(B))\).

To determine whether the increase in the SCD enzyme activity in muscles is due to an increase in SCD expression, the SCD protein level was estimated by Western blotting. Figure 3(A) shows that the RPD increased SCD protein expression in muscle \((by120.4\%)\), when compared with the control diet \((P<0.001)\). Oil...
type also significantly affected the SCD protein expression, which was the lowest in the presence of PO (P<0.05).

No significant effect of the RPD or oil type on SCD expression was observed for subcutaneous adipose tissue (Fig. 3(B); P=0.35 and P=0.91, respectively).

Figure 4 shows that there was a statistically significant positive correlation (r=0.73, P<0.001) between SCD protein expression in muscle and the level of total fatty acids in this tissue (which represents the level of IML).

**Acetyl-CoA carboxylase and fatty acid synthase protein expression**

ACC and FAS are major enzymes involved in de novo synthesis of SFA. ACC and FAS expression was investigated in muscle in order to determine whether de novo fatty acid synthesis contributes to the increased level of SFA, which was observed when the RPD was fed (Table 3).

The results in Table 4 show that the RPD increased the expression of ACC and FAS proteins by 31.7% (P=0.011) and 189.5% (P<0.001), respectively, when compared with the corresponding control groups. There was a significant protein level x oil type interaction for ACC protein expression (Table 5): ACC expression was increased only in the case of the RPD containing PO but not PKO or SBO.

There was a significant effect of oil type on FAS protein expression (Table 4). The FAS level was highest in the case of PO when compared with the two other oil types.

**Discussion**

The effects of dietary protein level on IML formation have been studied previously. It was shown that pigs fed a low protein diet (13% crude protein) had a higher intramuscular fat content when compared with diets containing 18.5% or 21% protein (Adeola & Young, 1989; Karlsson et al. 1993).

Reduction of dietary protein from 20% to 16% also significantly increased intramuscular fat in growing pigs (Da Costa et al. 1988).

**Table 3. Effects of a reduced protein diet (RPD) and oil type on the levels (mg/g tissue) of the major saturated fatty acids and MUFA†**

<table>
<thead>
<tr>
<th>Fatty acids and ratios</th>
<th>Protein level</th>
<th>Oil type</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>RPD</td>
</tr>
<tr>
<td>16:0</td>
<td>3.70</td>
<td>7.63</td>
</tr>
<tr>
<td>16:1</td>
<td>0.56</td>
<td>1.19</td>
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<td>16:1:16:0</td>
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<td>0.15</td>
</tr>
<tr>
<td>18:0</td>
<td>1.84</td>
<td>3.47</td>
</tr>
<tr>
<td>18:1:9</td>
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<td>12.67</td>
</tr>
<tr>
<td>18:1:9:18:0</td>
<td>3.03</td>
<td>3.68</td>
</tr>
</tbody>
</table>

PKO, palm kernel oil; PO, palm oil; SBO, soyabean oil.

† For details of procedures, see p. 610.
Therefore the increase in the level of total muscle fatty acids, which was observed in animals fed a RPD in the present study, is consistent with earlier reports. In our present experiment, the large (86.3 %) increase in total muscle fatty acids due to the RPD was accompanied by only a small (8.5 %) increase in subcutaneous adipose tissue lipids. Wood et al. (2004) also found that intramuscular fat formation did not follow the same pattern as carcass fat in pigs fed a protein-restricted diet. The distribution of fat between different fat depots also varies between breeds. For example, Duroc and Large White breeds have similar amounts of subcutaneous fat, but the Duroc is much higher in intramuscular fat (Sellier & Monin, 1994; Candek-Potokar et al. 2002). This indicates that fat distribution between different fat depots also varies between breeds. For example, Duroc and Large White breeds have similar amounts of subcutaneous fat, but the Duroc is much higher in intramuscular fat (Sellier & Monin, 1994; Candek-Potokar et al. 2002). This indicates that fat distribution between different fat depots also varies between breeds. For example, Duroc and Large White breeds have similar amounts of subcutaneous fat, but the Duroc is much higher in intramuscular fat (Sellier & Monin, 1994; Candek-Potokar et al. 2002). This indicates that fat distribution between different fat depots also varies between breeds. For example, Duroc and Large White breeds have similar amounts of subcutaneous fat, but the Duroc is much higher in intramuscular fat (Sellier & Monin, 1994; Candek-Potokar et al. 2002). This indicates that fat distribution between different fat depots also varies between breeds. For example, Duroc and Large White breeds have similar amounts of subcutaneous fat, but the Duroc is much higher in intramuscular fat (Sellier & Monin, 1994; Candek-Potokar et al. 2002). This indicates that fat distribution between different fat depots also varies between breeds. For example, Duroc and Large White breeds have similar amounts of subcutaneous fat, but the Duroc is much higher in intramuscular fat (Sellier & Monin, 1994; Candek-Potokar et al. 2002). This indicates that fat distribution between different fat depots also varies between breeds. For example, Duroc and Large White breeds have similar amounts of subcutaneous fat, but the Duroc is much higher in intramuscular fat (Sellier & Monin, 1994; Candek-Potokar et al. 2002). This indicates that fat distribution between different fat depots also varies between breeds. For example, Duroc and Large White breeds have similar amounts of subcutaneous fat, but the Duroc is much higher in intramuscular fat (Sellier & Monin, 1994; Candek-Potokar et al. 2002). This indicates that fat distribution between different fat depots also varies between breeds. For example, Duroc and Large White breeds have similar amounts of subcutaneous fat, but the Duroc is much higher in intramuscular fat (Sellier & Monin, 1994; Candek-Potokar et al. 2002). This indicates that fat distribution between different fat depots also varies between breeds. For example, Duroc and Large White breeds have similar amounts of subcutaneous fat, but the Duroc is much higher in intramuscular fat (Sellier & Monin, 1994; Candek-Potokar et al. 2002). This indicates that fat distribution between different fat depots also varies between breeds. For example, Duroc and Large White breeds have similar amounts of subcutaneous fat, but the Duroc is much higher in intramuscular fat (Sellier & Monin, 1994; Candek-Potokar et al. 2002). This indicates that fat distribution between different fat depots also varies between breeds. For example, Duroc and Large White breeds have similar amounts of subcutaneous fat, but the Duroc is much higher in intramuscular fat (Sellier & Monin, 1994; Candek-Potokar et al. 2002).
Oil type also showed a tissue-specific effect on SCD activity. SCD activity was significantly increased in muscle (but not in subcutaneous adipose tissue) in the case of PO when compared with PKO or SBO. This increase in SCD activity was not due to activation of SCD protein expression.

A tissue-specific increase in expression of genes involved in fat synthesis has previously been reported for rats (Maloney et al. 2003). It has been shown that a maternal low protein diet (8 %) increases the expression of ACC and FAS in liver but not in subcutaneous adipose tissue of offspring. The tissue-specific response of ACC on a low protein diet in this case was explained by the existence of different ACC isoforms, which are tissue-specifically expressed. Therefore, it is possible that in the present study, the different response of muscle and subcutaneous adipose tissue SCD expression on a RPD is due to a different pattern of SCD gene isoforms in these tissues. Four SCD genes have been identified and characterised in mice (see Miyazaki et al. 2003), two in rats (Thiede et al. 1986; Miyazaki & Ntambi, 2003), and one in man (Zhang et al. 1998). The expression of SCD gene isoforms is tissue-specific.

Thus, SCD1 is highly expressed in liver and adipose tissue (Ntambi et al. 1988; Kim et al. 2002), SCD2 is mainly expressed in the brain (Kaestner et al. 1989), SCD4 was found only in the heart (Miyazaki et al. 2003) and SCD3 is skin-specifically expressed (Zheng et al. 2001). So far only one SCD gene has been identified in pig adipose tissue (Ren et al. 2004), whereas the number of SCD isoforms in pig muscle is unknown. Miyazaki & Ntambi (2003) suggested that SCD isoforms have different substrate specificity.

According to Kim et al. (2000) the preferred substrate for SCD1 in mature 3T3-L1 adipocytes is 16:0, whilst 18:0 is a substrate for SCD1 or SCD2.

The tissue-specific effect of the RPD was also observed towards the SFA, which are the substrates for the SCD-catalysed reaction. SFA level was increased approximately 2-fold in muscle with only small changes (about 13 %) in subcutaneous adipose tissue. One of the possible reasons could be activation of de novo fatty acid synthesis in muscle under these conditions. This suggestion is supported by an increase in the level of FAS. The expression of ACC, the other major enzyme involved in biosynthesis of fatty acids, was also statistically increased under the RPD. However, the increase in ACC level was much smaller (only 13 %) than in the cases of FAS (189·6 %) or SCD (120·4 %), and it was significant only in the presence of PO. This is consistent with the report of Gondret & Lebret (2002) that ACC activity in *longissimus* muscle of pigs does not respond to feeding manipulation, including protein-restricted and energy-restricted diets. No changes in ACC activity were observed in muscles of fasting/feeding rats (Winder et al. 1995) and in feed-restricted rabbits, when compared with animals given *ad libitum* access to feed (Gondret et al. 2000). The expression of SCD and ACC genes was reported to be under different control in sheep (Barber et al. 2000). Winder et al. (1995) postulated that ACC expressed in skeletal muscle plays an important role in governing the rate of fatty acid oxidation during muscle contraction rather than exerting control over lipogenesis.

In conclusion, the present research shows, for the first time, that: (1) a RPD activates SCD protein expression and increases SCD activity in pig muscle but not in subcutaneous adipose tissue; (2) SCD protein expression in muscle correlates with the level of total intramuscular fatty acids.

Table 4. Effects of a reduced protein diet (RPD) and oil type on the expression of the acetyl-CoA carboxylase (ACC) and fatty acid synthase (FAS) proteins in muscle†

<table>
<thead>
<tr>
<th>Protein level</th>
<th>Oil type</th>
<th>PKO</th>
<th>SBO</th>
<th>PO</th>
<th>SEM</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RPD</td>
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<td></td>
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<td>P</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>ACC</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>FAS</td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

PKO, palm kernel oil; PO, palm oil; SBO, soyabean oil.

† Mean values within a row with unlike superscript letters were significantly different (* post hoc Tukey test for balanced data; *P* < 0·05).

‡ For details of procedures, see p. 610.

† Mean values derived from ANOVA using protein level and oil type as factors and including an interaction term.

Table 5. Interaction table (protein level × oil type) for acetyl-CoA carboxylase protein expression in muscle†

<table>
<thead>
<tr>
<th>Protein level</th>
<th>Oil type</th>
<th>PKO</th>
<th>SBO</th>
<th>PO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reduced protein</td>
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</tr>
</tbody>
</table>

PKO, palm kernel oil; PO, palm oil; SBO, soyabean oil.

† Mean values within a row with unlike superscript letters were significantly different (* post hoc Tukey test for balanced data; *P* < 0·05).

‡ For details of procedures, see p. 610.

† Mean values are the interaction means derived from ANOVA using protein levels and oil type as factors.

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References


