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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 SCD expression. 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.

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 anti-oxidants (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 sub-cutaneous 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 2×3 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. The diets contained 14 MJ digestible energy per kg. These formulations were achieved by changing the diet ingredients as shown in Table 1. The fatty acid composition of the oils used in the diets is presented in Table 2. The animals were fed *ad libitum* and slaughtered at an average live weight of 100 ± 10 kg.

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 N_2 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 $-20°C$.

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-H_2SO_4 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 ($50 \times 0·25$ 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).

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

Ingredients	Type of diet					
	PKO Con	PKO RPD	SBO Con	SBO RPD	PO Con	PO RPD
Barley	500	475	500	475	500	475
Wheat	173	292	173	292	173	292
Soyabean meal	239	120	239	120	239	120
Prairie meal	25	50	25	50	25	50
Palm kernel oil	28	28	–	–	–	–
Palm oil	–	–	–	–	28	28
Soyabean oil	–	–	28	28	–	–
Limestone	17·5	17·5	17·5	17·5	17·5	17·5
Salt	4	4	4	4	4	4
Vitamin–mineral premix	13·5	13·5	13·5	13·5	13·5	13·5
Vitamin E supplement	0·25	0·25	0·25	0·25	0·25	0·25

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

Fatty acid	Palm kernel oil	Soyabean oil	Palm oil
C8:0	1·43	nd	nd
C10:0	2·45	nd	Trace
C12:0	42·06	nd	0·23
C14:0	14·32	Trace	1·06
C16:0	12·33	11·03	45·14
C16:1	Trace	0·14	0·16
C18:0	17·41	4·05	4·30
C18:1n-9	5·95	21·40	37·72
C18:1n-7	0·28	1·97	0·78
C18:2n-6	1·07	52·91	8·50
C18:3n-3	0·06	6·80	0·07
C20:1	0·05	nd	0·16

nd, not detected.
For details of diets and procedures, see p. 610.

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 2 M-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 Schenkman & 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 inhibitors of proteolytic enzymes (1·5 μM-pepstatin + 1·5 μM-antipain + 2 μM-leupeptin). The post-mitochondrial fraction, containing cytosol and microsomes, was obtained by centrifugation at 12 000 g for 10 min. Cytosol and microsomes were then separated by centrifugation at 20 000 g for 30 min in the presence of 8 mM-CaCl$_2$. The resulting 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, 1·0 mM-dithiothreitol 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.

Determination of stearoyl-CoA desaturase activity

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-^{14}C]-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 M-potassium phosphate buffer (pH 7·4), 5 mM-MgCl$_2$, 7·2 mM-ATP, 0·5 mM-NADH and 0·044 mM-[1-^{14}C]palmitoyl-CoA (specific activity 60·0 μCi/mmol). 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-H$_2$SO$_4$, 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 redistilled *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 Rack-Beta 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 M-sucrose (pH 7·4), using a Polytron (Kinematica, Lucerne, Switzerland) at low speed. The infranatant obtained after spinning at 10 000 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 × 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
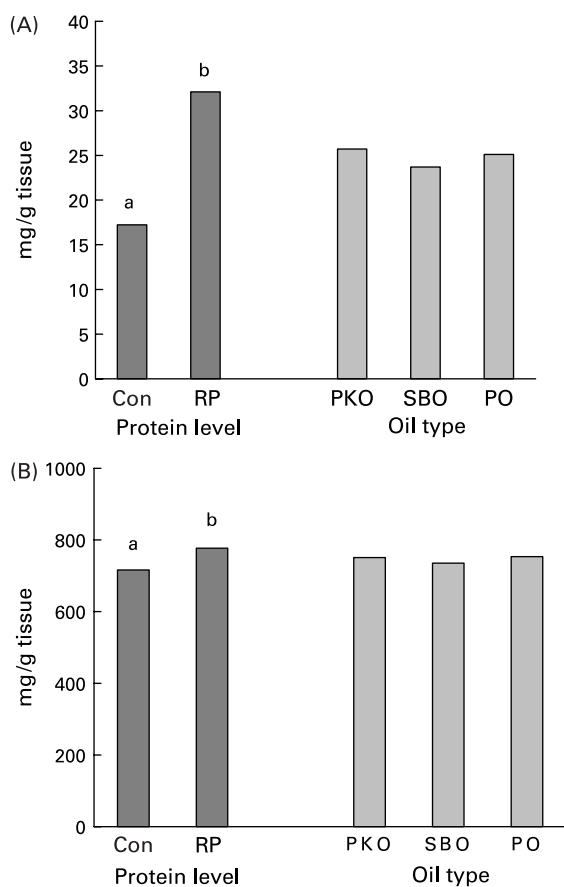

Fig. 1. Total fatty acids in muscle (A) and subcutaneous adipose tissue (B) of pigs fed a control (Con) and a reduced protein (RP) diet, containing palm kernel oil (PKO), soyabean oil (SBO) or palm oil (PO). For details of diets and procedures see Tables 1 and 2 and p. 610. Values are means derived from general linear models using protein level and oil type as factors. [a,b] Mean values within the protein level with unlike superscript letters were significantly different (Tukey–Kramer test; *P* values are given on this page).

MUFA: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 × 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 × 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 ($P = 0.007$ and $P = 0.002$, respectively). Since the increase was similar for both, 18 : 0 and 18 : 1*n*-9 (by 13·8 % and 13·6 %, 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 ($P = 0.015$, $P < 0.001$ and $P = 0.003$, 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 (by 120·4 %), when compared with the control diet ($P < 0.001$). Oil

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†

(Mean values with their standard errors‡)

Fatty acids and ratios	Protein level			Oil type				
	Control	RPD	P	PKO	SBO	PO	SEM	P
Muscle								
16:0	3·70	7·63	<0·001	5·95	5·43	5·61	0·735	0·871
16:1	0·56	1·19	<0·001	0·91	0·82	0·90	0·133	0·861
16:1:16:0	0·15	0·15	0·82	0·15	0·15	0·16	0·008	0·859
18:0	1·84	3·47	<0·001	2·89	2·60	2·47	0·315	0·611
18:1n-9	5·56	12·87	<0·001	9·57	8·50	9·57	1·291	0·791
18:1n-9:18:0	3·03	3·68	0·002	3·23	3·19	3·65	0·166	0·103
Subcutaneous adipose tissue								
16:0	162·2	181·5	0·005	184·3[b]	159·5[a]	171·8[ab]	5·55	0·015
16:1	17·12	18·5	0·237	21·84[b]	15·47[a]	16·12[a]	1·02	<0·001
16:1:16:0	0·10	0·10	0·537	0·12[b]	0·10[a]	0·09[a]	0·005	0·003
18:0	83·45	94·96	0·007	98·53[b]	83·1[a]	85·99[a]	3·446	0·008
18:1n-9	244·5	277·8	0·002	248·9[a]	238·6[a]	295·9[b]	8·135	<0·001
18:1n-9:18:0	2·97	2·96	0·946	2·55[a]	2·89[a]	3·47[b]	0·14	<0·001

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

[a,b] Mean values within a row with unlike superscript letters were significantly different (*post hoc* Tukey–Kramer test for unbalanced data; $P < 0.05$).

† For details of procedures, see p. 610.

‡ Mean values derived from general linear models using protein level and oil type as factors and including an interaction term.
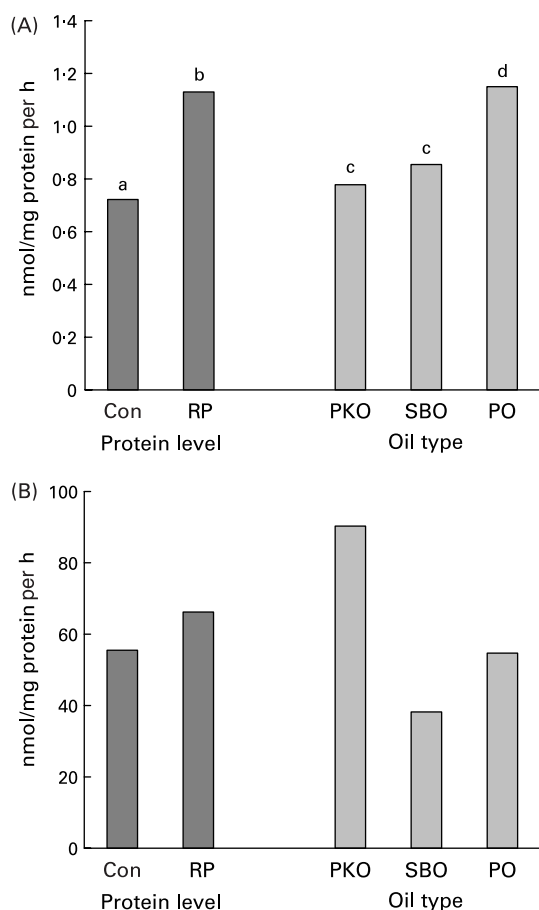

Fig. 2. Stearoyl-CoA desaturase activity in muscle homogenates (A) and isolated subcutaneous adipose tissue microsomes (B) of pigs fed a control (Con) and a reduced protein (RP) diet containing palm kernel oil (PKO), soyabean oil (SBO) or palm oil (PO). For details of diets and procedures see Tables 1 and 2 and p. 610. Values are means derived from general linear models using protein level and oil type as factors. [a,b] Mean values within the protein level with unlike superscript letters were significantly different; [c,d] mean values within oil type with unlike superscript letters were significantly different (Tukey–Kramer test; P values are are given on this page).

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 × 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
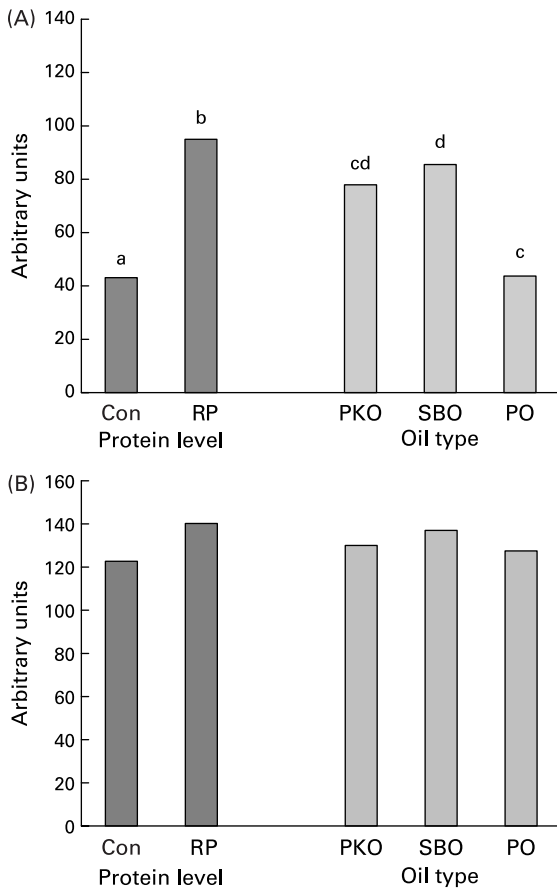
Fig. 3. Stearoyl-CoA desaturase protein expression in muscle (A) and subcutaneous adipose tissue (B) of pigs fed a control (Con) and a reduced protein (RP) diet, containing palm kernel oil (PKO), soyabean oil (SBO) or palm oil (PO). For details of diets and procedures see Tables 1 and 2 and p. 610. Values are means derived from general linear models using protein level and oil type as factors. [a,b] Mean values within the protein level with unlike superscript letters were significantly different; [c,d] mean values within oil type with unlike superscript letters were significantly different (Tukey–Kramer test; *P* values are are given on p. 613).

Fig. 4. Relationship between stearoyl-CoA desaturase (SCD) protein expression and the level of total fatty acids in muscle. For details of procedures see p. 610. The relationship can be described by the equation $y\ 18.419x + 1364.7$, $r\ 0.73$, $P < 0.001$.

et al. 2004; Wood *et al.* 2004). 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 depots might be controlled by different mechanisms and possibly by different genes. A possibility of different biochemical control of fat deposition in the pig was also suggested by Mourot *et al.* (1995), who observed variations in the patterns of acetyl-CoA carboxylase, malic enzyme and glucose-6-phosphate dehydrogenase activities in different fat depots.
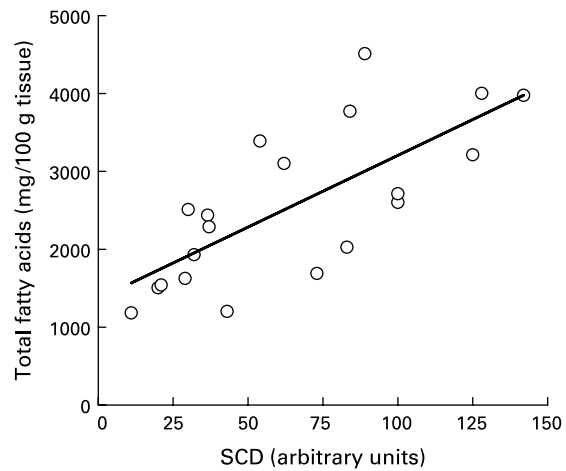
One of the key factors regulating fat deposition is expression of lipogenic enzymes. SCD is a key lipogenic enzyme, catalysing the formation of MUFA (mainly 16 : 1 and 18 : 1) from corresponding SFA (16 : 0 and 18 : 0) (Enoch *et al.* 1976). A number of publications have suggested an important role of SCD in the control of obesity (Smith *et al.* 1999; Cohen *et al.* 2002). The present research has shown an increase in SCD activity in pig muscle under a RPD. The increase in SCD activity in the present study was not always accompanied by a rise in the desaturation index. These results are consistent with observations of Smith *et al.* (2002), who reported a higher SCD activity in adipose tissue of pigs fed tallow when compared with maize-fed animals, without a significant difference in desaturation index. The authors concluded that desaturation index can be used in some cases as an indirect estimation of SCD activity but it is not an indicator of absolute activity. Hsieh & Kuo (2005) recently reported in a study on milkfish that there is a time-interval between changes in SCD activity and changes in desaturation index.

The most apparent reason for the increase in SCD activity is the activation of SCD expression, which is known to be highly regulated by dietary factors (Kim *et al.* 2002; Daniel *et al.* 2004; Singh *et al.* 2004). Indeed, SCD protein expression in muscle was significantly increased in our experiments under dietary protein reduction. These results are consistent with the observation of Da Costa *et al.* (2004) that dietary protein restriction increases the SCD transcriptional rate in the pig. The positive correlation between SCD protein expression and the amount of total fatty acids in muscle found in the present study suggests that SCD plays a significant role and might be a candidate gene for IML development in the pig. This does not exclude the possibility that other lipogenic enzymes might be involved in IML formation. Mourot & Kouba (1998) have shown a positive relationship between IML content and the activity of malic enzyme in studies on isolated porcine adipocytes.

One of the interesting findings in the present study is that the effect of the RPD on SCD activity and SCD protein expression was observed in muscle but not in subcutaneous adipose tissue.

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[†]

(Mean values with their standard errors[‡])

Enzyme	Protein level				Oil type			
	Control	RPD	P	PKO	SBO	PO	SEM	P
ACC	90.5	119.2	0.011	92.6	113.3	108.7	8.82	0.247*
FAS	53.1	153.8	<0.001	72.9[a]	89.7[a,b]	147.7[b]	19.38	0.034

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

[a,b] Mean values within a row with unlike superscript letters were significantly different (*post hoc* Tukey test for balanced data; $P < 0.05$).

* Protein level × oil type interaction was significant ($P = 0.001$).

† 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.

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.* 1999), cattle (Chung *et al.* 2000) and sheep (Ward *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/refeeding 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 5. Interaction table (protein level × oil type) for acetyl-CoA carboxylase protein expression in muscle[†,‡]

Protein level	Oil type		
	PKO	SBO	PO
Control	100·5[a,b]	108·1[a,b]	63[a]
Reduced protein	84·7[a]	118·4[a,b]	154·5[b]

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

[a,b] 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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