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Original article

Effect of a high linoleic acid diet on Δ9-desaturase activity, lipogenesis and lipid composition of pig subcutaneous adipose tissue

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Abstract – The effects of three diets were compared: a high linoleic acid diet (diet M containing 4 % maize oil), diet T containing 4 % beef tallow, and C, a conventional control diet, on Δ9-desaturase activity and lipogenesis in pig subcutaneous adipose tissue. Diet M increased lipogenesis (estimated from the activities of acetyl-CoA-carboxylase, malic enzyme and glucose-6-phosphate dehydrogenase), and decreased Δ9-desaturase activity, in comparison to the other diets. Linoleic acid content was higher in the pigs fed diet M than in the other pigs (amounting to 26 % of total tissue fatty acids versus 15 %, respectively). The lower monounsaturated fatty acid content in adipose tissue of pigs fed diet M compared to pigs fed other diets could be associated with the lower Δ9-desaturase activity and the lower oleic acid content of diet M. The present study suggests that Δ9-desaturase could be involved in the regulation of monounsaturated fatty acid content and hence in the quality of pig adipose tissue. © Inra/Elsevier, Paris

pig / linoleic acid / Δ9-desaturase / lipogenic enzymes / adipose tissue

Résumé – Effet d'un régime à teneur élevée en acide linoléique sur l'activité de la Δ9-désaturase, la lipogenèse et la composition lipidique du tissu adipeux de porc. Nous avons étudié les effets d'un régime à teneur élevée en acide linoléique (régime M, renfermant 4 % d'huile de maïs) sur l'activité de la Δ9-désaturase et la lipogenèse dans le tissu adipeux sous-cutané de porc, en comparaison avec un régime T contenant 4 % de suif et un régime témoin C. Comparé aux autres régimes, le régime M a augmenté la lipogenèse (estimée par les activités de l'acétyl-CoA-carboxylase, de l'enzyme malique et de la glucose-6-phosphate déhydrogénase) et diminué l'activité de la Δ9-désaturase. Le tissu adipeux des porcs soumis au régime M était plus riche en acide linoléique que celui des porcs soumis aux autres régimes (26 % des acides gras totaux du

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tissu contre 15 %, respectivement). Nous avons observé une plus faible teneur en acides gras mono-insaturés du tissu adipeux des porcs soumis au régime M, due à la fois à une plus faible activité de la Δ9-désaturase chez ces porcs et à une plus faible teneur en acide oléique du régime M, par rapport aux autres régimes. Cette étude suggère un rôle potentiel de la Δ9-désaturase dans la régulation de la teneur en acides gras mono-insaturés du tissu adipeux de porc et par conséquent dans sa qualité. © Inra/Elsevier, Paris

porc / acide linoléique / Δ9-désaturase / enzymes lipogéniques / tissu adipeux

1. INTRODUCTION

Oleic acid, the most abundant fatty acid in mammals, is synthesized from stearoyl-CoA by Δ9-desaturase (stearoyl-CoA-desaturase, EC 1.14.99.5). The activity of this enzyme is therefore an important determinant of the amounts of stearic acid and oleic acid, the most abundant fatty acid in pig tissue lipids [2, 14]. The adipose tissue is the only significant site of fatty acid synthesis in weaned pigs [22] and is also the main site of stearoyl-CoA desaturation [11]. The energy content of the diets used in swine production can be increased by adding fats, and such diets often present a high content of polyunsaturated fatty acids, especially linoleic acid. The dietary fatty acid composition is known to influence the quality of lean and fat tissues in pigs (review by Madsen et al. [15]). However, the effets of the high polyunsaturated fatty acid content of the diet on lipogenesis in the pig are poorly documented. Much is known about the repression of Δ9-desaturase by dietary linoleic acid in the rodent liver (review by Clarke and Jump [7]). However, only one study has been carried out on the effect of dietary fatty acids on Δ9-desaturase activity in pig adipose tissue [2], and these authors failed to show any significant effect of dietary linoleic acid on Δ9-desaturase activity in such tissue.

The aim of the present work was to investigate the effect of diets with high contents of linoleic acid (maize oil) or stearic acid (tallow) on the activity of enzymes involved in fatty acid synthesis and on Δ9-desaturase activity in pig adipose tissue.

2. MATERIALS AND METHODS

2.1. Animals and diets

Twenty-four castrated male pigs (Large White × Pietrain) were housed in individual pens in environmentally controlled buildings under normal husbandry conditions. The animals were reared in compliance with national regulations for the humane care and use of animals in research. At 40 kg live weight, the pigs were allocated within litter to one of three diets C, T or M ($n = 8$ per diet). The amount administered between 40 and 100 kg live weight was weight-based and ranged from 1.9 kg for 40 kg live weight to 2.8 kg feed/day for pigs weighing 75 kg or more. The diets were isocaloric (3 100 kcal ME/kg) and formulated to contain 17 % crude protein and 0.8 % lysine. The control diet C was purchased from a commercial feed manufacturer (Glon S.A., Pontivy 56 302, France). The experimental diets M (maize) and T (tallow) contained 4 % of maize oil and 4 % of tallow, respectively. Maize oil was chosen because of its high percentage of linoleic acid. The diet compositions are shown in *tables I* and *II*.

Table I. Composition of control and experimental diets.

	Treatment		
	Control (diet C)	Tallow (diet T)	Maize (diet M)
Composition, g/100 g			
Ground wheat	24.00	24.00	24.00
Ground barley	24.00	24.00	24.00
Soybean meal (48 % crude protein)	23.00	21.50	21.50
Ground yellow maize	15.00	—	—
Decorticated oat	—	17.50	17.50
Wheat bran	05.00	04.00	04.00
Treacle	03.00	—	03.00
Beef tallow '15'	02.00	04.00	—
Maize oil	—	—	04.00
Ground limestone	01.40	01.80	01.80
Dicalcium phosphate	01.20	01.70	01.70
Vitamin mix	00.50	01.00	01.00
Salt	00.50	00.50	00.50
L-HCl lysine	00.08	00.08	00.08

Table II. Proximate analysis of the experimental diets.

	Treatment		
	Control (diet C)	Tallow (diet T)	Maize (diet M)
Proximate analysis			
Crude proteins, %	17.2	17.1	17.0
Energy (kcal ME/kg)	3059	3109	3149
Fat, %	5.0	5.5	5.3
Cholesterol content (mg/100 g diet)	156	109	75
Fatty acid composition, g/100 g diet			
C14 : 0	0.04	0.10	trace
C16 : 0	0.96	1.16	0.58
C16 : 1	0.05	0.11	trace
C18 : 0	0.41	0.61	0.08
C18 : 1	1.52	1.71	1.19
C18 : 2	1.50	1.21	2.93
C18 : 3	0.11	0.07	0.09

2.2. Data collection

The pigs were electrically stunned and exsanguinated when they reached 100 kg live weight.

Immediately after slaughter, two samples were taken from the subcutaneous adipose tissue (at the 15th–16th rib level). One sample was immediately processed for enzyme activity measurements. The other sample was frozen in liquid nitrogen and stored at – 80 °C until lipid extraction and analysis.

2.3. Enzyme analysis

The activities of acetyl-CoA-carboxylase (ACC, EC 6.4.1.2), malic enzyme (ME, EC 1.1.1.40) and glucose-6-phosphate dehydrogenase (G-6-PDH, EC 1.1.1.49) in the subcutaneous adipose tissue were determined. Weighed amounts of adipose tissue were homogenized in 0.25 M sucrose buffer and centrifuged at 30 000 g for 40 min. The supernatants were analyzed for malic enzyme and glucose-6-phosphate dehydrogenase, using a modification [10] of the methods of Fitch et al. [8] and Hsu and Lardy [12], respectively. NADPH formation was measured at 37 °C by absorbance at 340 nm. Acetyl-CoA-carboxylase was assayed by the $H^{14}CO_3^-$ fixation method [3–5].

Δ9-desaturase activity was measured in microsomes extracted from the subcutaneous adipose tissue as described in the method of Kouba et al. [14]. The activity was determined by measuring the conversion of [^{14}C]stearic acid into [^{14}C]oleic acid.

2.4. Lipid extraction

The lipids were extracted from the subcutaneous adipose tissue and diets using chloroform/methanol (2:1), according to the method of Folch et al. [9]. The extracts were taken to dryness under vacuum on a rotary evaporator.

2.5. Fatty acid composition

Fatty acid methyl esters were prepared with boron fluoride methanol according to Morrison and Smith [19] and analysed on a Di 200 gas chromatograph (Delsi, Paris, France) (capillary column CW 20 M, temperature: 180 °C, hydrogen pressure: 0.5 bar), using margaric acid as internal standard. The oven, detector and injector temperatures were maintained at 180, 240 and 220 °C, respectively. Retention time and peak areas were determined using an ordinat software (Nelson Analytical, Inc, San Jose, U.S.A.).

2.6. Statistical analysis

The effect of the diet on the different parameters was tested using the one-way analysis of variance (ANOVA procedure of SAS [23]).

3. RESULTS AND DISCUSSION

We did not observe any differences in growth or at slaughter that could be attributed to diet.

Acetyl-CoA-carboxylase (ACC), malic enzyme, glucose-6-phosphate dehydrogenase and Δ9-desaturase activities are presented in *table III*.

ACC catalyses the first step in the fatty acid biosynthetic process. There is considerable evidence that this enzyme has a key role in the regulation of fatty acid biosynthesis in animal tissues and ACC is generally considered as a rate-limiting enzyme of lipogenesis in animals [21] especially in pigs [17, 24]. Malic enzyme and glucose-6-phosphate dehydrogenase are the main enzymes involved in supplying NADPH for the reductive biosynthesis of fatty acids [26, 27]. ACC and ME activities were significantly higher in the adipose tissue of pigs fed diet M which contained more linoleic acid. Total lipid content was also significantly higher ($P < 0.05$) in the adipose tissue of pigs fed diet M compared to diet C and T (69.44 ± 2.8 % versus 65.10 ± 3.12 and 63.66 ± 3.78 %, respectively). These results agreed with previous results obtained in the pig[1, 20, 25] and in rat adipose tissue ([25]; review by Chilliard [6]) but contrast with

Table III. Lipogenic enzyme activities and Δ9-desaturase activity in subcutaneous adipose tissue of pigs fed control or experimental diets.

Item	Treatments			RSD	Diet effect
	Control (diet C)	Tallow (diet T)	Maize oil (diet M)		
ACC[y]	9.00[a]	7.41[a]	12.1[b]	2.32	$P < 0.002$
ME[x]	241[a]	239[a]	435[b]	73	$P < 0.001$
G-6-PDH[x]	162 [a]	151[a]	182[a]	42	NS
Δ9-desaturase[w]	26.8[a]	28.7[a]	20.9[b]	3.41	$P < 0.02$

[y] Expressed as nmol $HCO3^-$ incorporated per min per mg protein; [x] expressed as nmol NADPH formed per min per mg protein; [w] expressed as nmol oleic acid formed per hour per mg protein.
Results are the mean of data from eight pigs.
Means in the same row with no superscripts in common differ ($P < 0.05$ or less); NS: not significant.

those obtained in the rodent liver ([25]; review by Chilliard [6]; review by Clarke and Jump [7]). This opposite effect of diet on lipogenesis in the adipose tissue compared to that in the liver suggests tissue-specific responses to dietary fat sources.

Δ9-desaturase activity was lower in the adipose tissue of pigs fed diet M rather than diet T. To our knowledge, this is the first report of an inhibitory effect of linoleic acid on Δ9-desaturase in pig adipose tissue, although the inhibition of Δ9-desaturase activity by dietary linoleic acid in rodent liver is well established ([13]; review by Clarke and Jump [7]). Buller and Enser [2] failed to show any significant repressive effect of dietary linoleic acid on Δ9-desaturase activity in pig adipose tissue probably because the duration of treatment was too brief (6 d) in their experiment. However, Δ9-desaturase activity in pig adipose tissue seems to be less sensitive to dietary linoleic acid than the enzyme activity in rodent liver. In fact, the 27 % decrease in Δ9-desaturase activity observed in the present study is much lower than the 70–80 % decrease in desaturase activity obtained in the rat liver under similar experimental conditions [13]. It should however be noted that the pigs in our study were subjected to diet M for more than 8 weeks, whereas the rats had received an equivalent diet for 2 weeks. So, it is possible that there was more suppression of the enzyme activity earlier.

The influence of the linoleic acid content of the diet on the fatty acid composition of the subcutaneous adipose tissue is illustrated in *figure 1*. The fatty acid composition of pig adipose tissue is known to reflect the fatty acid composition of the diet [18, 20] and as expected, the linoleic acid content of the adipose tissue of pigs fed diet M was higher than that of the adipose tissue of pigs fed other diets (26 % versus 15 %, respectively). Moreover, the oleic acid content in the adipose tissue of pigs fed diet M was lower than that of pigs receiving the other diets (36.5 % versus 46 %, respectively). This result agreed with previous results [16, 20]. This lower percentage of oleic acid could be related both to the lower oleic acid content of diet M and to the lower Δ9-desaturase activity in the adipose tissue of pigs fed diet M.

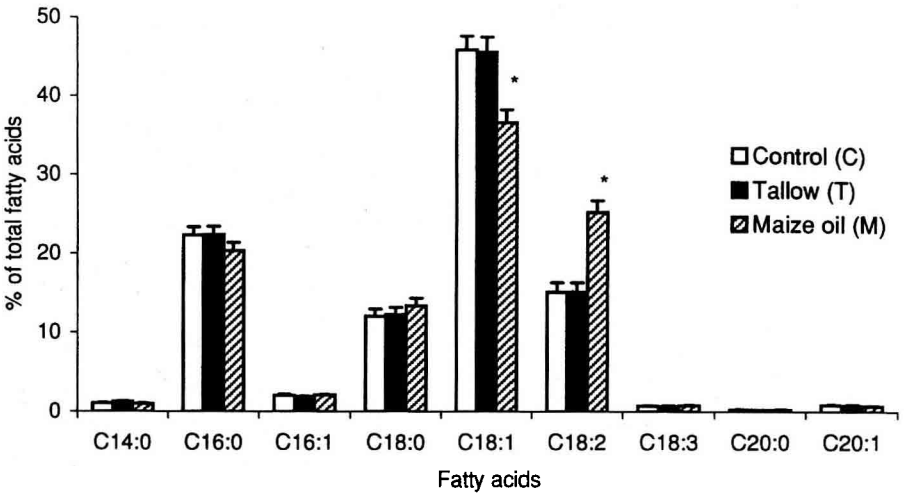

Figure 1. Fatty acid composition of subcutaneous adipose tissue lipids of pigs fed control or experimental diets. Results are the mean ± SD of data from eight pigs; * $P < 0.05$, comparison of diet M with diets C and T.

4. CONCLUSION

In summary, our results indicated that the lipogenic response of pig adipose tissue to dietary linoleic acid contrasted with the response observed for Δ9-desaturase activity.

The present study suggested that Δ9-desaturase may be involved in the regulation of monounsaturated fatty acid content and hence in the quality of pig adipose tissue.

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