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Lipogenic enzyme activities in subcutaneous adipose tissue and skeletal muscle from neonatal pigs consuming maternal or formula milk

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Abstract — The influence of maternal and formula milk on lipid metabolism was studied in 7-day-old pigs. Lipid content, fatty acid composition, lipogenic enzyme activities and expression of GLUT4 mRNA were determined in subcutaneous adipose tissue and skeletal muscle from pigs that were bottle-fed formula milk (F) or sow milk (SM), or were sow-reared (SR). Bottle-fed pigs were isoenergetically fed and achieved similar daily body weight gain. SR pigs have a higher ($P < 0.05$) body weight gain than bottle-fed pigs. Lipid content of adipose tissue was lower ($P < 0.05$) in F than in SM and SR pigs. In muscle, lipid content did not differ significantly between groups. In adipose tissue, acetyl-CoA-carboxylase (CBX), fatty acid synthase (FAS), malic enzyme (ME), glucose-6-phosphate-dehydrogenase (G6PDH) and lipoprotein lipase (LPL) activities and GLUT4 mRNA levels were higher ($P < 0.05$) in SR than in bottle-fed pigs. In muscle, ME and G6PDH activities and GLUT4 mRNA were higher ($P < 0.05$) in F than in SM and SR pigs; LPL was not detected. The present study indicates that lipogenic enzyme activities and GLUT4 mRNA expression are regulated differently in subcutaneous adipose tissue and skeletal muscle in the neonatal pig.

lipogenic enzyme / milk / skeletal muscle / subcutaneous adipose tissue / pig

Résumé — Étude des enzymes lipogéniques dans le tissu adipeux sous-cutané et le muscle squelettique chez des porcelets recevant du lait maternel ou du lait artificiel. L'influence du lait maternel et du lait artificiel sur le métabolisme lipidique a été étudiée chez des porcelets âgés de 7 jours. La teneur en lipides, la composition en acides gras, les activités des enzymes lipogéniques et l'expression des ARNm codant pour GLUT4 ont été déterminées dans le tissu adipeux sous-cutané et le muscle squelettique de porcelets qui sont nourris au biberon avec du lait artificiel (F) ou du lait de truie (SM) ou qui sont élevés par leur mère (SR). Les porcelets nourris au biberon ont été alimentés de manière isoénergétique et ont eu un gain de poids identique. Les porcelets SR ont eu un gain

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de poids plus élevé ($P < 0,05$) que les porcelets nourris au biberon. Dans le tissu adipeux, la teneur en lipides est plus faible ($P < 0,05$) chez les porcelets F que chez les porcelets SM et SR. Dans le muscle, elle ne diffère pas significativement entre les 3 groupes. Dans le tissu adipeux, les activités de l'acétyl-CoA-carboxylase (CBX), de l'acide gras synthase (FAS), de l'enzyme malique (ME), de la glucose-6-phosphate-déshydrogénase (G6PDH) et de la lipoprotéine lipase (LPL), et l'expression des ARNm codant pour GLUT4 sont plus élevées ($P < 0,05$) chez les porcelets SR que chez les porcelets nourris au biberon. Dans le muscle, les activités de ME et de G6PDH et l'expression des ARNm codant pour GLUT4 sont plus élevées ($P < 0,05$) chez les porcelets F que chez les porcelets SM et SR ; l'activité de la LPL n'est pas détectable. Cette étude montre que les activités lipogéniques et l'expression des ARNm codant pour GLUT4 sont régulées différemment dans le tissu adipeux et le muscle pendant la période néonatale.

enzymes lipogéniques / lait / muscle squelettique / tissu adipeux sous-cutané dorsal / porc

ABBREVIATIONS USED

CBX, acetyl-CoA-carboxylase; C0, colostrum obtained during or soon after parturition; C12, colostrum obtained at 12 h after parturition; F, formula milk bottle-fed pigs; FAS, fatty acid synthase; FM, formula milk; FMI, formula milk supplemented with porcine immunoglobulins G; GLUT4, the insulin-regulated glucose transporter; G6PDH, glucose-6-phosphate-dehydrogenase; LPL, lipoprotein lipase; M, milk obtained at 6 days of lactation; ME, malic enzyme; SM, sow milk bottle-fed pigs, SR, sow-reared pigs.

1. INTRODUCTION

A better understanding of the regulation of lipid deposition in muscle and other tissues is required for a better control of meat quality in the pig. Nutrition is known to affect adipose tissue [1, 31]. There is also increasing evidence that early nutrition may have long-term effects on growth and metabolism of some organs and tissues. The characteristics of neonates fed maternal or formula milk have received increasing interest in recent years in different species including pigs [5, 14, 37]. In humans, some clinical studies suggest that breast-fed infants are generally leaner than formula-fed infants whereas other studies fail to show such an effect [14, 37]. In pigs, as in other species, little is known about the influence of maternal milk on lipid metabolism.

Lipid deposition increases rapidly after birth in the pig, which is born with extremely low amounts of body fat [26]. Body fat in the neonate is thought to be primarily derived from dietary fat since it is usually accepted that fatty acid synthesis is negligible in suckling newborn mammals [4, 15, 38]. Nevertheless, the detection of lipogenic enzymes in adipose tissue [27] and in skeletal muscle [28] of suckling pigs suggests that the activities of these enzymes may be significant for fatty acid synthesis. Lipid deposition is regulated mainly through effects on fatty acid intake and synthesis. It is therefore related to lipogenic enzyme activities and the amount of substrate available. Glucose is the main substrate for fatty acid synthesis and glucose transport rate is a limiting step of glucose utilisation in adipose tissue and muscle. In these tissues, the predominant isoform is the insulin-regulated glucose transporter (GLUT4) [20].

The present study was undertaken to compare the effect of maternal milk and formula milk on lipid metabolism in subcutaneous adipose tissue and skeletal muscle in the neonatal pig. Formula milk and sow milk differ in their composition in nutrients and bioactive peptides [34]. Lipid content, fatty acid composition and lipogenic enzyme

activities were examined in pigs that were bottle-fed colostrum and sow milk or formula milk or that were sow-reared.

2. MATERIALS AND METHODS

2.1. Animals

Eighteen Large White-Landrace × Piétrain pigs (*Sus scrofa*) from six litters were used. Unsuckled newborn pigs were carefully dried, ear notched and placed in a temperature-controlled room maintained at 34 °C. About 6 h after birth, within each litter, three pigs of similar body weight were allotted to one of the three groups of diet. Pigs were bottle-fed sow colostrum then sow milk (SM) or formula milk (F), or they were sow-reared (SR, 10 pigs per litter) for a 7-day period. SM and F pigs were placed in individual metal wire cages (40.5 × 40.5 × 50.0 cm) in a temperature-controlled room under conditions of thermal neutrality by reducing the ambient temperature gradually from 34 to 30 °C between birth and 7 days of age. Relative humidity was between 30 and 40%.

The chemical composition of colostrum and milk is given in Table I. Colostrum and milk that were allocated to SM pigs were

Table I. Average gross chemical composition of colostra, sow milk and formula.

	SM			F	
	C0	C12	M	FMI	FM
Chemical analysis					
Dry matter (%)	21.90	21.75	20.25	23.99	19.90
Crude protein (%)[1]	14.28	11.53	6.29	7.90	5.28
Lactose (%)	3.31	3.41	4.87	6.70	6.60
Total fat (%)	3.91	6.28	8.22	4.57	4.33
Fatty acid composition (% of total fatty acids)					
8:0	0.4	0.3	0.1	3.3	7.1
10:0	0.0	0.0	0.0	1.9	2.8
12:0	0.0	0.0	0.0	9.2	14.0
14:0	2.1	2.0	2.8	6.6	7.5
16:0	25.1	25.0	27.9	23.1	21.2
16:1 n-9	5.5	6.0	7.6	3.1	1.7
18:0	5.6	5.9	5.7	13.1	14.4
18:1 n-9	38.1	41.3	42.3	29.1	27.5
18:2 n-6	21.9	17.7	13.0	7.3	3.5
18:3 n-3	1.3	1.1	0.6	0.8	0.3
20:0	0.0	0.0	0.0	1.1	0.0
20:1 n-9	0.0	0.0	0.0	1.4	0.0
20:4 n-6	0.0	0.7	0.0	0.0	0.0
Gross energy ($kJ \cdot kg^{-1}$)	5 254	5 663	5 425	5 613	4 384
Insulin ($\mu UI \cdot mL^{-1}$)	867	782	194	33	33
IGF-I ($ng \cdot mL^{-1}$)	894	817	19	51	11

[1] Colostra, sow milk and formula crude protein was calculated as N × 6.38.

SM, sow milk; FM, formula milk; C0, colostrum obtained during or soon after parturition; C12, colostrum obtained at 12 h after parturition; M, milk obtained at 6 days of lactation; FMI, formula milk supplemented with porcine immunoglobulins G.

obtained from several sows by manual expression during or soon after parturition (C0), at approximately 12 h after parturition (C12) and at 6 days of lactation (M). For C12 and M collection, each sow was i.v. injected with 20 IU of oxytocin to initiate colostrum and milk letdown. The collected products were subdivided into 50 to 60 g portions and kept at –20 °C until used. Sow milk replacement formula (FM) was a commercial formula available as a complete milk replacer for pigs (Toniporc, Agralco, France). SM and F pigs were isoenergetically bottle-fed every 2 h from 07:00 h to 23:00 h and only once during the night at 03:00 h. To provide adequate immunity, F pigs were fed a formula milk supplemented with purified porcine immunoglobulins G (FMI) for 24 h. SM pigs were bottle-fed C0 for 18 h, C12 for 18 h and M until the end of the experiment. They were fed 25 g of maternal milk per kg of body weight per meal during the daytime. To compensate for the long interval between meals, the size of the 03:00 h meal was increased by 50%. Milk allocated to SM and F pigs was adjusted daily according to body weight. Pigs were weighed approximately 90 min after the 09:00 h meal. Milk intake was determined by weighing the bottle (± 0.10 g) before and after each feeding. It was 2.50 ± 0.16 $MJ \cdot day^{-1}$ and 2.45 ± 0.21 $MJ \cdot day^{-1}$ for SM and F pigs respectively. Ninety minutes after the last meal, pigs were weighed, anaesthetised using halothane and killed by exsanguination. Samples of dorsal subcutaneous adipose tissue and skeletal muscle (*longissimus*) were immediately frozen in liquid nitrogen and were stored at –70 °C until lipogenic enzyme analyses and RNA isolation.

2.2. Enzyme analyses

Lipogenic enzyme activities were determined in adipose tissue and muscle. Tissues were homogenised in 0.25 M sucrose buffer and centrifuged at 30 000 *g* for 40 min at 4 °C. The supernatants were analysed for malic enzyme (ME, EC 1.1.1.40) and glucose-6-phosphate-dehydrogenase (G6PDH, EC 1.1.1.49) activities using previously described methods [16, 24] with modification [19]. Fatty acid synthase activity (FAS, EC 2.3.1.85) was determined using the method of Lavau et al. [25]. The tissues were also homogenised in ammoniac heparin buffer (NH_4Cl 50 mM, pH 8.2, heparin 2.5 $UI \cdot mL^{-1}$) [9] and centrifuged at 700 *g* for 10 min at 4 °C. The supernatants were analysed for lipoprotein lipase activity (LPL, EC 3.1.1.34) [42]. Acetyl-CoA-carboxylase (CBX, EC 6.4.1.2) was assayed by the $H^{14}CO_3^-$ fixation method [6, 7]. Protein contents were determined by the method of Lowry et al. [30] using bovine serum albumin as the standard.

2.3. Lipid content and fatty acid composition

Lipid content of colostra, milks, adipose tissue and muscle were determined by the method of Folch et al. [17]. The methyl esters were prepared with boron trifluoride methanol [32] and fatty acids were determined by gas chromatography (Delsi Instruments, Argenteuil, France; capillary column, 30 m in length, split/splitless injector, hydrogen pressure: 0.45 bar).

2.4. RNA isolation and RNase protection assays

Total RNA was isolated using the guanidium thiocyanate method [11] with modification [29] for adipose tissue. The quantity and quality of isolated total RNA were evaluated spectrophotometrically and confirmed with agarose gel electrophoresis. The abundance of GLUT4 mRNA was quantified using a sensitive solution hybridisation-RNase protection assay [12]. Porcine GLUT4 cDNA [10] was kindly provided by Dr TD Etherton (Penn State University, PA, USA). To generate antisense riboprobe,

the plasmid was linearised with *Eco*RI and transcribed with a Promega kit using T7 polymerase (Promega, Madison, WI, USA) in the presence of [α^{32}P]CTP (NEN, Paris, France). To check for possible differences in quantification and/or loading, samples were also assayed for 18S RNA using human 18S cDNA (pT7 RNA 18S) obtained from Ambion (Austin, TX, USA). The relative intensities of the protected bands were quantified using PhosphorImager (STORM, Molecular Dynamics S.A., Bondoufle, France). The data were then normalised to the abundance of 18S RNA.

2.5. Statistical analysis

All data were expressed as mean ± SEM. Data were analysed by analysis of variance using the generalised linear model of SAS [39]. The model included the effect of feeding and litter. When appropriate, multiple comparisons of the means was performed using the Duncan test.

3. RESULTS

3.1. Body weight gain

The birth weight of the three groups of pigs did not differ significantly. They were 1 494 ± 105 g, 1 504 ± 135 g and 1 453 ± 130 g for SM, F and SR pigs respectively. During the 7-day experimental period, SM and F pigs achieved similar daily body weight gain (136 ± 10 vs. 132 ± 11 g) which was lower ($P < 0.05$) than the 173 ± 17 g achieved in SR pigs. From the assumption that the ratio of sow milk intake (g):body weight gain (g) is similar in SM and SR pigs, it was calculated that SR pigs consumed 28% more milk than SM pigs.

3.2. Lipid content and fatty acid composition

The content of lipid in adipose tissue was much higher in pigs fed sow milk (SM and SR groups; $P < 0.05$) than in pigs fed formula (Fig. 1). It did not differ between SM and SR groups. In muscle, the difference was not significant between maternal milk-fed and F pigs. Fatty acid composition of adipose tissue and muscle differed between maternal milk-fed pigs and F pigs. In adipose tissue, F pigs have more ($P < 0.05$) 10:0, 12:0, 14:0, 18:0 and less ($P < 0.01$) 18:1 n-9, 18:2 n-6 than SM and SR pigs (Tab. II). In muscle, F pigs have more ($P < 0.001$) 12:0, 14:0, 18:0 and less ($P < 0.01$) 16:0, 16:1 n-9 and 18:2 n-6 than SM and SR pigs.

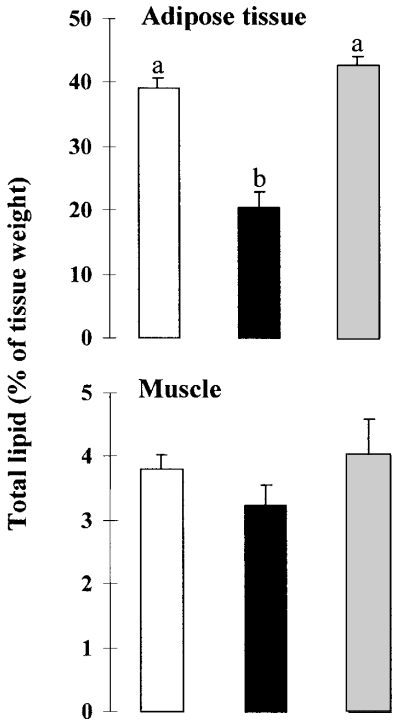

Figure 1. Lipid content of adipose tissue and muscle from SM (□), F (■) and SR (▭) pigs. Data represent means ± SEM, $n = 6$. Within the same tissue, means with different letters are significantly different ($P < 0.05$).

Table II. Average fatty acid composition of adipose tissue and muscle in 7-day-old pigs.

	Adipose tissue					Muscle				
	SM	F	SR	Rsd	Effect	SM	F	SR	Rsd	Effect
10:0	0.0^{a}	0.2^{b}	0.0^{a}	0.1	$P < 0.01$	0.0	0.0	0.0	0.03	NS
12:0	0.2^{a}	5.5^{b}	0.9^{a}	2.0	$P < 0.001$	0.0^{a}	4.7^{b}	0.0^{a}	1.0	$P < 0.001$
14:0	2.6^{a}	8.2^{b}	3.5^{a}	2.0	$P < 0.001$	2.3^{a}	5.9^{b}	2.6^{a}	0.7	$P < 0.001$
16:0	27.2	26.4	27.2	0.7	NS	33.3^{a}	27.4^{b}	33.1^{a}	1.8	$P < 0.001$
16:1 n-9	8.1	6.3	8.3	2.0	NS	5.6^{a}	3.9^{b}	5.3^{a}	0.8	$P < 0.01$
18:0	5.6^{a}	8.5^{b}	6.4^{ab}	1.7	$P < 0.05$	8.9^{a}	12.4^{b}	8.2^{a}	1.0	$P < 0.001$
18:1 n-9	42.8^{a}	39.5^{b}	42.7^{a}	1.7	$P < 0.01$	31.5	35.2	33.5	3.7	NS
18:2 n-6	11.7^{a}	3.6^{b}	9.0^{a}	2.4	$P < 0.001$	13.2^{a}	5.57^{b}	11.8^{a}	2.3	$P < 0.001$
18:3 n-3	0.7	0.6	0.5	0.2	NS	1	0.6	0.7	0.4	NS
20:0	0.6	0.6	0.7	0.2	NS	0.3	0.5	0.3	0.2	NS
20:1 n-9	0.0	0.2	0.1	0.1	NS	0.7	1	1	0.3	NS
20:4 n-6	0.5	0.4	0.6	0.02	NS	3.2	2.5	3.5	1.2	NS

[a, b] Within the same tissue and the same line, means with different letters are significantly different ($P < 0.05$).
* Percentage of total fatty acids. Rsd: Root standard deviation.

3.3. Enzyme activities

Enzyme activities among the three dietary groups were compared in adipose tissue and skeletal muscle (Figs. 2 to 4). In adipose tissue, CBX and FAS activities were higher ($P < 0.05$) in SR than in SM and F pigs (Fig. 2). In the muscle, CBX and FAS activities were low and did not differ between the three groups (Fig. 2). In adipose tissue, ME and G6PDH activities were higher ($P < 0.05$) in SR than in SM and F pigs whereas in the muscle, activities were higher ($P < 0.05$) in F than in SM and SR pigs (Fig. 3). LPL activity was greater ($P < 0.05$) in SR than in SM and F pig adipose tissue, while LPL activity was not detected in muscle (Fig. 4).

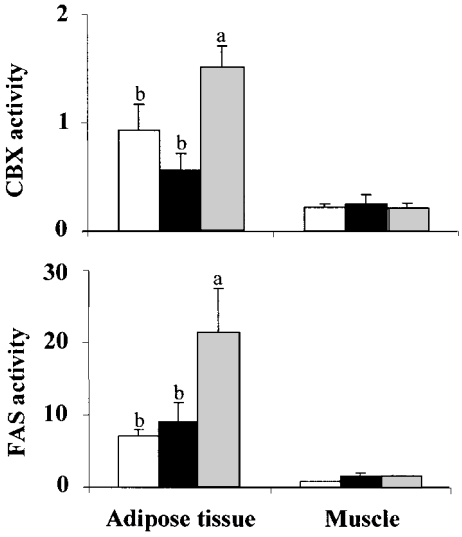

Figure 2. Acetyl-CoA-carboxylase (CBX) and fatty acid synthase (FAS) activities in adipose tissue and muscle from SM (□), F (■) and SR (▭) pigs. CBX activity is expressed as nmol HCO_3^- incorporated·min^{-1}·mg^{-1} of protein. FAS activity is expressed as nmol NADPH disappeared·min^{-1}·mg^{-1} of protein. Data represent means ± SEM, $n = 6$. Within the same tissue, means with different letters are significantly different ($P < 0.05$).

3.4. GLUT4 mRNA expression

GLUT4 mRNA was expressed in both adipose tissue and muscle (Fig. 5). In adipose tissue, the expression of GLUT4 mRNA was higher ($P < 0.01$) in SR than in SM and F pigs, whereas in muscle, the

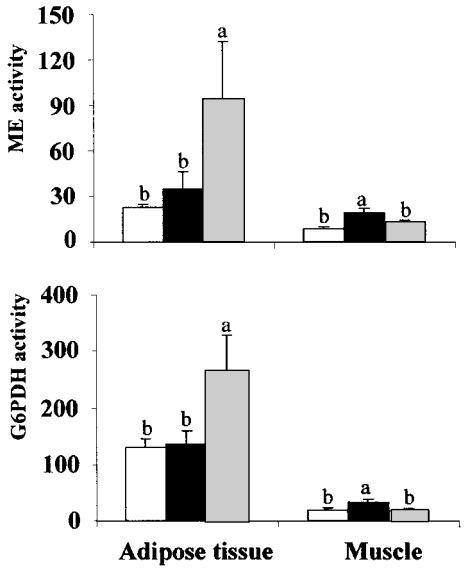

Figure 3. Malic enzyme (ME) and glucose-6-phosphate-dehydrogenase (G6PDH) activities in adipose tissue and muscle from SM (□), F (■) and SR (▭) pigs. Activities are expressed as nmol NADPH produced·min^{-1}·mg^{-1} of protein. Data represent means ± SEM, $n = 6$. Within the same tissue, means with different letters are significantly different ($P < 0.05$).

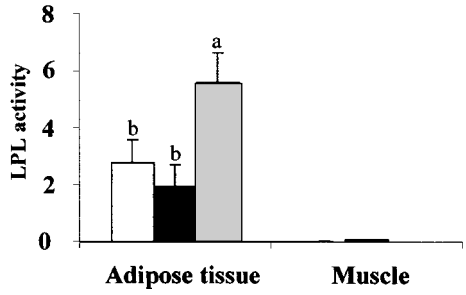

Figure 4. Lipoprotein lipase activity in adipose tissue and muscle from SM (□), F (■) and SR (▭) pigs. Activity is expressed as nmol oleate produced·min^{-1}·mg^{-1} of protein. Data represent means ± SEM, $n = 6$. Within the same tissue, means with different letters are significantly different ($P < 0.05$).

expression of GLUT4 mRNA was higher ($P < 0.01$) in F than in SM and SR pigs.

4. DISCUSSION

The present study is the first to clearly show the effect of maternal and formula milk on lipid metabolism in a well controlled environment. Although it is usually accepted that fatty acid synthesis is limited in suckling newborn mammals [4, 15, 38], the present data indicate that lipogenesis is not limited by enzyme activities or glucose transporter and that these parameters are regulated by early nutrition. The activities of lipogenic enzymes are lower in this study than in weaned pigs [3, 28] but they are significant. In adipose tissue, the activities of CBX, ME and G6PDH represent at least 50–80% of the activities found in 20 kg pigs [23, 33, 36]. The detection of GLUT4 mRNA associated with the finding that adipose tissue from neonatal pigs has the capacity to incorporate glucose into lipids [43] indicates that glucose transport is not a limiting step. The lack of substrate may contribute to the low rate of lipogenesis. Indeed, the estimated amount of glucose ingested is lower than the amount needed. For example, F pigs ingested 21 g glucose per 24 h, the highest amount ingested in the 3 groups, whereas the needs in glucose are estimated to be 36–40 g per 24 h for 7-day-old pigs [35]. This may not be, however, the only explanation because it has been shown that plasma glucose concentrations approaching the hyperglycaemic threshold do not stimulate lipogenesis in 1-day-old pigs [27].

The observation that fatty acid composition of adipose tissue and muscle of 7-day-old pigs reflects mainly milk fatty acid composition is consistent with a previous report that indicates that fat deposition is primarily dietary fat in origin [27]. Nevertheless, some observations support the hypothesis that lipogenesis may be significant in adipose tissue of the neonatal pig. Despite the absence of 12:0 in maternal

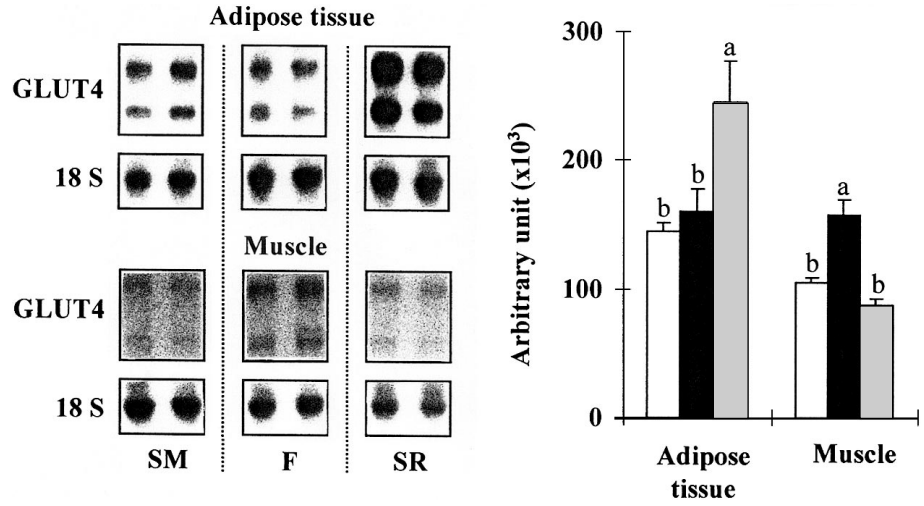

Figure 5. Expression of GLUT4 mRNA in adipose tissue and muscle in SM (□), F (■) and SR (▭) pigs. GLUT4 mRNA was quantified using the RNase protection assay as described in Materials and Methods. Hybridisation was performed using 20 µg total RNA. Left-hand panels show representative autoradiograms in adipose tissue and muscle. Right-hand panels show relative abundance of GLUT4 mRNA and show values of one experiment representative of a duplicate. Data represent means ± SEM, $n = 6$. Within the same tissue, means with different letters are significantly different ($P < 0.05$).

milk and in adipose tissue of neonatal pig at birth (data not shown), this fatty acid is detected in adipose tissue of maternal milk-fed pigs. The finding that 16:0 did not differ between the 3 groups in adipose tissue despite a lower level in formula milk supports also this hypothesis.

The present study clearly indicates that the potential of lipogenic enzyme activities and GLUT4 mRNA expression can be modulated by the diet in the neonatal pig. This study also shows for the first time that lipogenic enzyme, lipoprotein lipase and GLUT4 are regulated similarly within the same tissue in the pig. This suggests a relationship between the regulation of lipogenic enzymes and GLUT4. A question that arises is whether this relationship is secondary to glucose availability as suggested in the rat [22]. The regulation of lipogenic enzymes and GLUT4 is tissue-specific. Whereas enzyme activities and GLUT4 mRNA expression differed between sow-reared and bottle-fed pigs in adipose tissue, these parameters differed between maternal milk- and formula-fed pigs in skeletal muscle. In adipose tissue, the involvement of milk composition in the regulation of lipid metabolism is unlikely. Indeed, enzyme activities and GLUT4 mRNA levels differed significantly between maternal milk-fed groups. One can argue that these pigs did not consume milk of similar composition. However, the observation that the fatty acid profile in adipose tissue and muscle did not differ between these two groups suggests they were probably fed milk with similar composition. The difference between sow-reared and bottle-fed pigs is likely related to milk intake because SR pigs have a higher body weight gain than bottle-fed pigs. The possible effect of energy intake in the regulation of lipid metabolism in adipose tissue is supported by the observation that lipogenesis in

adipose tissue increases with the energetic level of the diet [41]. The influence of the amount of total fat or other nutrients ingested cannot be excluded although it has been reported that high–fat diets depressed lipogenesis in 21-day-old pigs that have been weaned at 14 days of age [2]. In muscle, the differences observed between formula milk and sow milk likely result from the difference in milk composition. The amount of proteins, lactose, lipids and milk-borne growth factors and the fatty acid composition differ between sow milk and formula milk. These latter nutrients may be involved because it has been demonstrated that they have the ability to affect lipogenesis in weaned rats and pigs [18, 21]. Further studies are however needed to identify the components involved. The different regulation of GLUT4 mRNA between adipose tissue and muscle is in accordance with previous studies in rats [8, 13]. The tissue-specific regulation of lipogenic enzymes and GLUT4 may be related to the different role of adipose tissue and muscle [28]. Whereas subcutaneous adipose tissue is a site of lipid storage, muscle adipose tissue is a source of energy [40].

In conclusion, the present study shows that the regulation of lipogenic enzymes and GLUT4 is similar within the same tissue but differs between adipose tissue and skeletal muscle in the neonatal pig. The mechanisms underlying this regulation remain to be elucidated. Further studies are also needed to evaluate the impact of maternal and formula milk on subsequent lipid metabolism.

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