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Original article

The cellularity of developing adipose tissues in Pietrain and Meishan pigs

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Summary — The development of external, internal, inter- and intramuscular adipose tissues was compared in Pietrain (lean) and Meishan (obese) pigs during their growth, at 0, 7, 30 and 90 days of age. Up to 1 month of age, the total lipid content of external and internal adipose tissues as well as mean adipocyte diameter increased similarly in both breeds of pigs. The percentage of plurilocular adipocytes decreased in both breeds; it remained higher in Meishan up to 30 days of age, which would indicate a delayed maturity of adipose tissue in this pig. Adipocyte hypertrophy characterizing the Meishan pig starts between 1 and 3 months of age. At 90 days, adipocyte diameters were about 50% higher in Meishan than in Pietrain. Muscle lipid content was higher in Pietrain than in Meishan until 1 month of age, whereafter it became lower than that of Meishan. Intramuscular adipocytes appeared later than those of other tissues in both breeds and were larger in Meishan than in Pietrain.

pig / adipose tissue / fat cell / muscle / development

Résumé — Développement cellulaire des tissus adipeux chez les porcs Piétrain et Meishan. Le développement des tissus adipeux externes, internes, inter- et intramusculaires est comparé chez les porcs Piétrain (maigres) et Meishan (obèses) au cours de la croissance à 0, 7, 30 et 90 j. Jusqu'à l'âge de 1 mois, les contenus en lipides totaux des tissus adipeux externes et internes, ainsi que les diamètres moyens des adipocytes augmentent de manière semblable chez les deux races de porcs. Les pourcentages d'adipocytes pluriloculaires diminuent chez les deux races, mais restent supérieurs chez le Meishan jusqu'à 30 j, ce qui traduirait une maturité retardée du tissu adipeux chez ce porc. L'hypertrophie des cellules adipeuses commence dès 1 mois chez le porc Meishan. À 90 j, les diamètres adipocytaires sont plus élevés de 50 % environ chez le Meishan par rapport au Piétrain. Les contenus des muscles en lipides sont plus élevés chez le Piétrain jusqu'à l'âge de 1 mois, puis ils deviennent plus faibles que ceux du Meishan. Les adipocytes intramusculaires apparaissent plus tardivement que dans les autres tissus adipeux et ils sont plus volumineux chez le Meishan que chez le Piétrain.

porc / tissu adipeux / adipocyte / muscle / développement

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INTRODUCTION

In pig, the setting of adipose depots occurs according to the sequence: subcutaneous, mesenteric and perirenal, intermuscular, intramuscular (Anderson and Kaufman, 1971; Lee et al, 1973; Henry, 1977). The first elements of adipose tissue appear in the pig foetus at about day 70 in periepididymal and pericardic depots, and at about day 106 in mesenteric depot (Vodovar et al, 1971; Desnoyers and Vodovar, 1974). At birth, the adipose tissues are small since they represent less than 2% of the body mass (Le Dividich et al, 1991). The development of adipose depots would then occur in three phases, including mainly hyperplasia between 1 and 2 months of age, both hyperplasia and hypertrophy between 2 and 5 months of age, and mostly hypertrophy thereafter (Anderson et al, 1972; Henry, 1977; Hood and Allen, 1977). The activity of enzymes for lipid synthesis shows maximal values between 40 and 60 kg body weight in fast growing pigs, with marked differences according to the anatomical localization of the fat depot (Mourot et al, 1995) and breeds (Hood and Allen, 1973).

The aim of this study was to compare the rate of maturity of several adipose depots in two very different porcine breeds: Pietrain, which is recognized as one of the leanest breeds (Bidanel et al, 1994) and Meishan, which exhibits one of the highest degrees of adiposity (Yen et al, 1991).

MATERIAL AND METHODS

Animals and tissue removal

The Pietrain pigs originated from a local breeder (Melin, Jodoigne, Belgium) and the Meishan pigs were bred at the Station de recherches porcines Inra (St Gilles, France). Pigs were killed at birth, 7, 30 and 90 days of age. Three animals were killed at each age. The backfat (sampled at the level of the 15–16th costal area), the leaf fat, the intermuscular fat inserted in *Quadriceps femoris*, as well as muscles (*Quadriceps femoris, Longissimus dorsi* and *Latissimus dorsi*) were removed immediately after slaughtering.

Measurements

The tissue samples were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer pH 7.4, postfixed in 2% osmium tetroxide in the same buffer, dehydrated in a graded series of ethanol concentrations and embedded in epoxy resin (Fraselle-Jacobs et al, 1987). In each tissue sample, semi-thin sections (1 μm) were cut at three different levels and stained with 1% (w/v) toluidine blue in phosphate buffer. On each section, the diameters of 300 adipocyte profiles were measured at a × 200 or × 800 magnification, with an electronic planimeter (Kontron, Messgerate, Germany). The percentage of plurilocular adipocytes was calculated on the same preparations. Ultrathin sections were cut with a diamond knife (Diatome, Bienne, Switzerland) on a Ultracut microtome (Reichert-Jung, Wien, Austria), stained with uranyl acetate and lead citrate, and observed in a Jeol 100C transmission electron microscope.

The lipid content was determined by using the method of Folch et al (1957). The cell number was assessed by dividing the quantity of lipids per gram of tissue by the lipid content of one adipocyte, calculated from the mean adipocyte volume and taking into account a density of 0.92 (Di Girolamo et al, 1971). The means were compared by using a Student's *t*-test.

RESULTS

The weights of the animals are indicated in table I. In Pietrain, the total lipid content of backfat and leaf fat (fig 1) increased between 7 and 30 days and decreased at day 90. In intermuscular tissue, the lipid content rose until 3 months of age. In muscles (fig 1), the lipid content decreased continuously up to 3 months of age in Pietrain pig. In Meishan, the total lipid content increased with age in all the tissues. It was higher in Meishan pig than in Pietrain pig, except for the intramuscular tissue, at 1 week of age. No

Table I. Body weights (kg) of the Meishan and Pietrain pigs at the different ages (means ± sd).

Age (days)	*Meishan*	*Pietrain*
0	0.88 ± 0.37	1.23 ± 0.12
7	1.31 ± 0.12	2.07 ± 1.08
30	7.06 ± 0.28	9.48 ± 0.95*
90	35.47 ± 0.93	27.30 ± 2.42**

$n = 3$; * $P < 0.05$; ** $P < 0.01$.

clear differences were noted at 1 month of age, whereas the lipid content was higher in backfat and leaf fat at 90 days in Meishan pig compared to Pietrain pig.

The adipocyte size was measured in subcutaneous adipose tissue, leaf fat, intermuscular tissue and in the fattest muscle of the series, ie, the *Latissimus dorsi* when that measure became possible (fig 2). In addition, the percentage of plurilocular adipocytes was calculated as a mark of the maturation of the tissue, since the plurilocular adipocyte is a well-known step in the differentiation process of the adipocyte (fig 3). In all tissue, with the exception of muscle, the adipocytes increased in size in both porcine breeds. In Pietrain pigs, the diameters reached 50–60 μm at the age of 1 month; they were smaller at 3 months of age. In muscle, the adipocytes were smaller

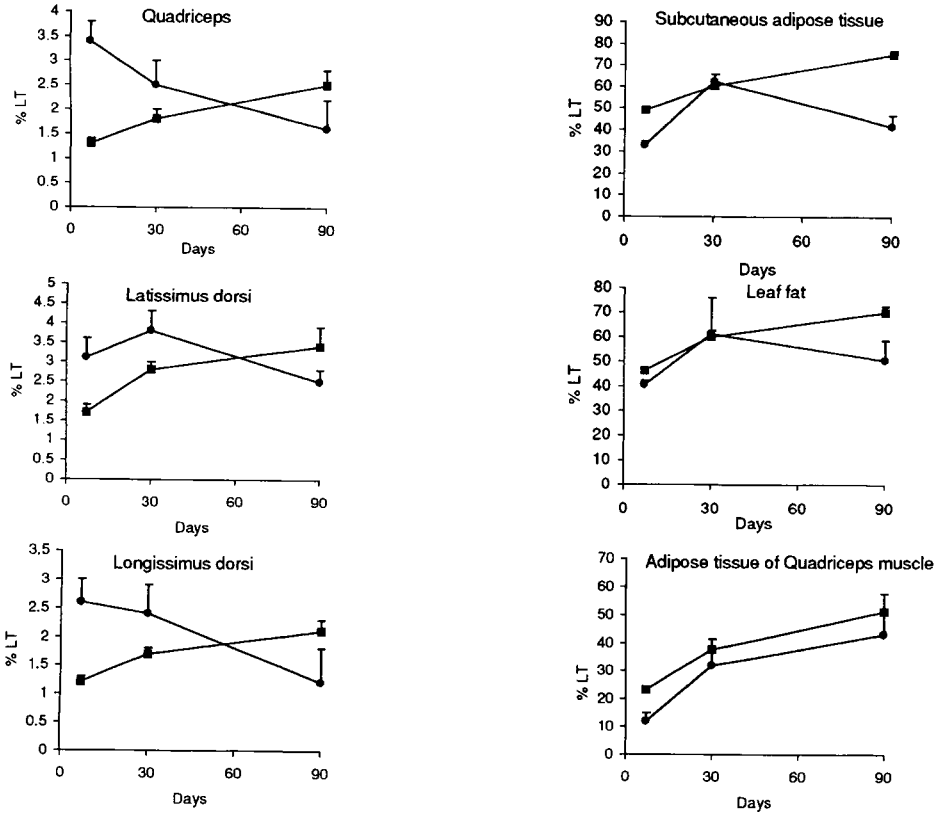

Fig 1. Total lipid contents of the tissues (% of wet weight) in the different localizations, in Pietrain (●) and Meishan (■) pigs at different ages (means ± sd, $n = 3$).

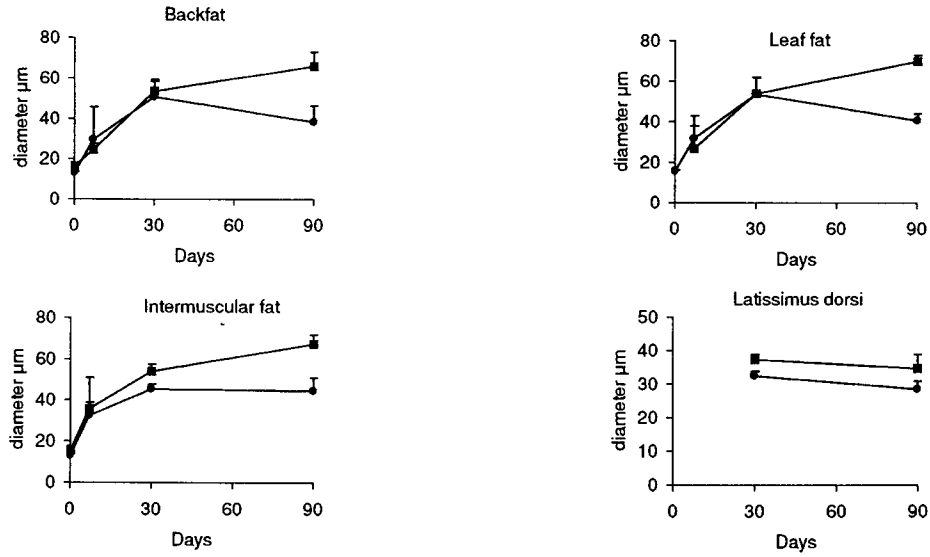

Fig 2. Comparison of mean adipocyte diameters (µm) in adipose depots of Pietrain (●) and Meishan (■) pigs at different ages (means ± sd, $n = 3$).

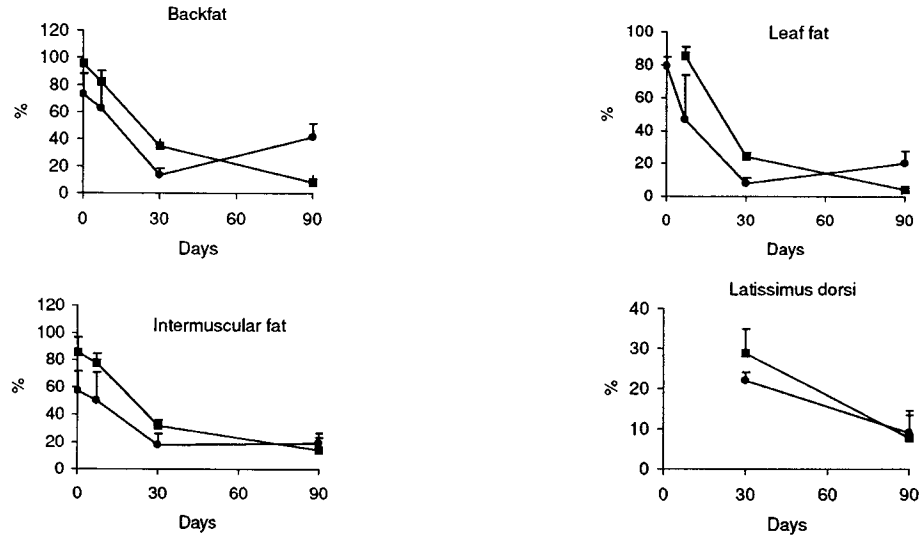

Fig 3. Comparison of percentage of plurilocular adipocytes (%) in adipose depots of Pietrain (●) and Meishan (■) pigs at different ages (means ± s.d., $n = 3$).

than in adipose tissues and their size did not increase between 30 and 90 days. In Meishan, the adipocyte size in adipose tissues increased rapidly from birth up to 30 days of age and then more slowly thereafter. In *Latissimus dorsi* muscle, the adipocytes remained about two times smaller than those of other adipose tissues. In the different depots, the adipocyte size of both breeds was similar up to 30 days of age, then became larger in Meishan pig, except intramuscular adipocytes.

The percentage of plurilocular adipocytes was high at birth in all tissues. In Meishan pig especially it varied between 80 and 95%. At 30 days of age, the percentage was markedly decreased, and continued to decrease in Meishan pig while it increased again in Pietrain pig, particularly in the backfat. The number of adipocytes calculated by gram of weight of adipose tissue doubled in backfat and leaf fat of Pietrain pig between 30 and 90 days of age, while it diminished to a similar extent in Meishan pig (Table II). In intermuscular tissue, this evolution of the adipocyte number was less marked.

Figures 4 and 5 illustrate the localization of lipid droplets in the *Latissimus dorsi* muscle. The droplets were found mainly inside muscle fibers in young animals (fig 4). At 1 month of age, the adipocytes develop in the inter- and intramuscular connective tissue (fig 5).

DISCUSSION

Development of adipose tissues

In our study, the mean body weight of Meishan piglets at birth was slightly lower (NS) than that of Pietrain piglets. The weights of Meishan were lower than that of Pietrain at 30 days of age. The Meishan pigs became heavier than the Pietrain pigs only at 90 days of age. These results confirm those of McLaren et al (1990) and Young (1990), reporting weights at birth in Meishan pigs of 0.9 versus 1.1–1.5 kg in Pietrain pigs, and weights at 30 days of age of, respectively, 5.6 versus 6–8 kg. The obese phenotype appears rather late in Meishan pigs and does not imply an early development of adipose tissue.

Up to 1 month of age, the development of adipose tissue in Meishan and Pietrain pigs appeared similar. The total lipid content of adipose tissues increased while the water content decreased (unpublished data), which corresponds to previous reports (Mersmann et al, 1976; Henry, 1977). However, an important divergence between Meishan and Pietrain appeared at 3 month of age, lipid content reduced in adipose tissues of the Pietrain, while it continued to increase in Meishan pig. In backfat, a transient decrease in lipid content was previously described in

Table II. Number of adipocytes (× 1 000) per gram of tissue in adipose depots of Meishan and Pietrain pigs at different ages (means ± sd).

Age (days)	*Tissue*	*Pietrain*	*Meishan*
30	subcutaneous	1091 ± 451	956 ± 510
	leaf fat	846 ± 185	839 ± 222
	intermuscular	731 ± 292	495 ± 38
90	subcutaneous	2106 ± 1686	559 ± 48
	leaf fat	1556 ± 267	475 ± 28**
	intermuscular	1091 ± 443	392 ± 46

$n = 3$; ** $P < 0.01$.

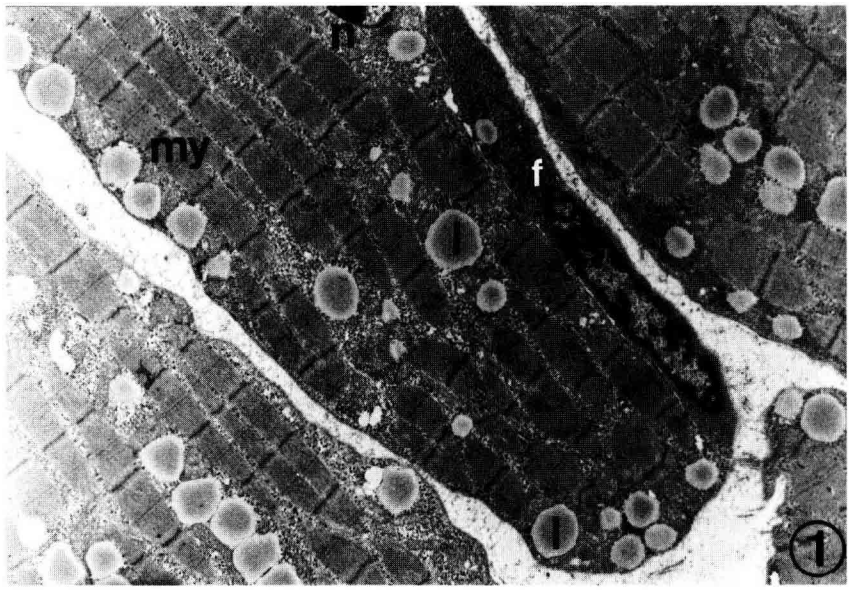

Fig 4. Electron micrograph of muscle tissue in 7-day-old Pietrain pig. The lipid droplets (l) are found inside muscle fibers; n: nucleus of muscle fiber; my: myofibrils; f: fibroblastoid cell. Magnification: × 5 000.

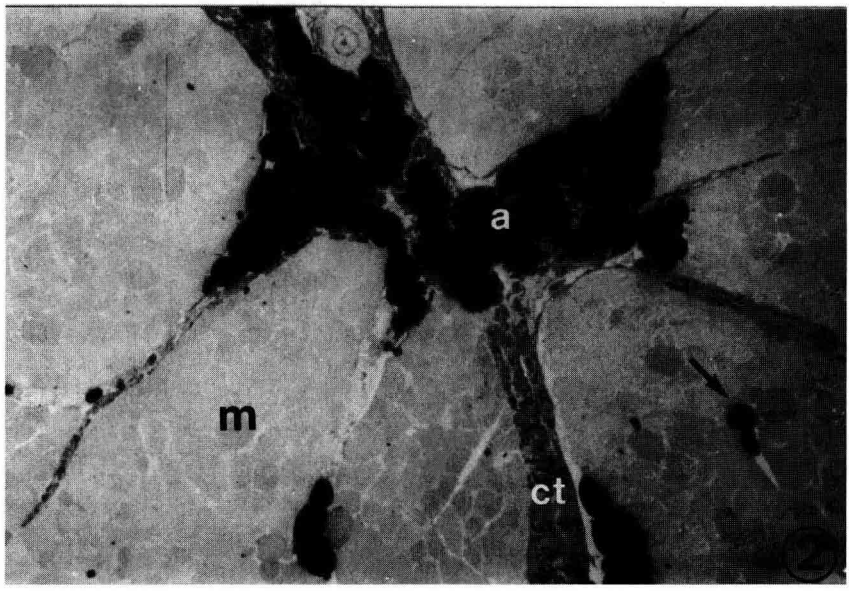

Fig 5. Light micrograph of semithin section in muscle tissue of 30-day-old Pietrain pig. The adipocytes (a) stained in black are located in the connective tissue (ct); m: muscle fibers. The arrow indicates isolated intramuscular adipocytes. Magnification: × 100.

Meishan pig, but at an earlier age of about 40 days, whereas the lipid content remained roughly constant between 20 and 80 days of age in Large White pig (Camara et al, 1994).

As with lipid content, fat cell sizes were similar in both pig breeds up to 1 month of age. The percentage of plurilocular fat cells was continuously higher in Meishan pig, indicating a slower maturity rate of adipose tissue in this breed. In Pietrain pig, the decrease in lipid content at 90 days of age was related to a decrease in adipocyte size and to an increase in the percentage of small plurilocular fat cells. These observations may correspond to a step of commitment of precursors to adipocyte differentiation.

At 3 months of age, the differences between the two breeds were obvious: adipocytes were bigger, the percentage of plurilocular fat cells in adipose tissues was lower and the lipid content was higher in Meishan pig than in Pietrain pig. The late hypertrophy of adipocytes has been observed in other breeds of fatty or obese pigs. At birth, Ossabaw (obese) pig shows smaller adipocytes than those of Yorkshire (lean) pig (Hausman and Martin, 1981) and the same is true for Large White pig compared to Pietrain (Moody et al, 1978). Later in life, after 45 days, the adipocyte size in Meishan pig is larger than that of Large White (Camara et al, 1994). This effect may be due to a slow differentiation or lipid filling of adipose tissue cell in obese pigs, or to a later commitment to adipocyte differentiation. This remains an open question.

Asynchronous maturation of intramuscular adipose tissue

Intramuscular fat is a late-developing adipose tissue, which differs from non-muscular ones both as far as cellularity and metabolic capacity are concerned. This delay is not exclusive to porcine species (Anderson et al, 1972; Lee and Kauffman, 1974; Hood and Allen, 1977; Moody et al, 1978) and was observed also in bovine species (Chakrabarty and Romans, 1972; Lin et al, 1992).

Morphological analysis of muscular tissue in Pietrain pig showed that, during the early postnatal period, the muscle fibers were particularly rich in lipid inclusions and that preadipocytes were rare. After 30 days, muscle fibers did not show intracellular lipid droplets, whereas intramuscular fat cells increased in number. Our results agree with those of Lee and Kauffman (1974) and Moody et al (1978), who showed an elevation of the lipid content in muscles of newborn piglets related to lipid accumulation in the fibers. Concomitant with muscle development, the lipid partition changed. The intrafiber lipid droplets decreased and the number of adipocytes increased, so that the total amount of intramuscular lipids remained unchanged (Chora et al, 1995). This modification in lipid partition could be linked to the decrease in the proportion of muscle oxidative fibers (or type I) in the growing young animal for the benefit of glycolytic fibers (Lefaucheur and Vigneron, 1986).

The specific development of intramuscular adipocytes compared to that of adipose tissues may result from the competition between the adipocyte and muscle fiber for the uptake of blood fatty acids. Both adipocyte and muscle fibers synthesise lipoprotein lipase, the regulation of which differs according to the cell type (Robinson and Speake, 1989; Pollare et al, 1991). The two cells contain different types of fatty acid binding proteins (Veerkamp et al, 1991; Xu et al, 1991). In addition, we suggest that fatty acids are captured more intensively by muscle cells, while the amount of fatty acids entering preadipocytes is lower, delaying their differentiation. Since the long chain fatty acids are known to induce expression of lipogenic enzymes and fatty acid bind-

ing proteins like aP2 (Amri et al, 1991, Ailhaud et al, 1995). The latter proteins facilitate the flow of fatty acids into adipocytes and then the triglyceride biosynthesis (Veerkamp et al, 1991; Xu et al, 1991).

In conclusion, up to 30 days of age the adipocytes of external, internal and intermuscular adipose tissues of Pietrain pig appeared to mature more rapidly than those of Meishan pig, whereas the total lipid content and adipocyte size increased more rapidly in Meishan pig. The maturity of intramuscular tissue is delayed compared to the other depots and lipid content features a continuous lowering in lean pig while increasing in obese pig.

Since the meat flavor, juiciness and tenderness largely depend on the meat fat content, an adequate amount of fat cells is required, which implies a specific development of extra- and intramuscular fat depots. The control of different adipose tissue development must then be planned by taking into account each individual depot and not only the total carcass fat content.

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