PE1.11 Cellular changes in subcutaneous adipose tissue of landrace and meishan pigs during growth 108.00

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***Abstract*— Backfat thickness is one of the most important carcass traits in pork. However, it remains unclear how adipose tissue deposition is regulated during aging and why adipose tissue development is different among pig breeds. In this study, to address these questions, we examined the cellularity of developing subcutaneous adipose tissue in low backfat Landrace and high backfat Meishan pigs. As pigs aged, body weight of both breeds significantly increased, though Meishan pigs were smaller than Landrace pigs by 6 wk of age ($P < 0.01$). Meishan pigs tended to have thick backfats than Landrace pigs ($P = 0.06$). Fat cell size increased with age, and by 6 wk, osmium fixed adipocytes from both outer and inner layer of subcutaneous adipose tissue were larger in Meishan pigs than in Landrace pigs ($P < 0.01$). Furthermore, apparently larger fat cells were observed in Meishan pigs under light microscopy. Our data showed that cellular differences exists in subcutaneous adipose tissue of Landrace and Meishan pigs, and this cell size difference finally leads to the greater backfat accumulation in Meishan pig.**

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***Index Terms*—adipose cell, backfat, growth, pig**

I. INTRODUCTION

BACKFAT thickness is one of the most economically important traits in pig carcass. Regulating the amount of fat deposition has been a major goal in the continuing improvement of pork production for many years. The comparative study among greatly different genotypes may give us a clue to control the adipose tissue development. Chinese pigs such as Meishan breed provide a particularly interesting model to study this phenomenon. Meishan pigs are known to have the characteristics of slower growth, earlier puberty, and higher fat deposits especially in subcutaneous tissue than the conventional breeds of pigs [1, 2]. Although its genetic difference is quite obvious, it is still a puzzle for the reason why Meishan pigs become so thick with its backfat.

Experiments were designed to compare the adipocyte cellularitiy of subcutaneous adipose tissue during development between Landrace (low backfat) and Meishan (high backfat) pigs. Two to six female pigs of each breed were slaughtered at 1 wk, 3 wk, 6 wk, 3 mo and 5 mo of age. The following determinations were made on each animal: 1) live body weight, 2) backfat thickness, 3) adipose cell size measurements by fixation of adipose tissue with osmium tetraoxide, and 4) light microscopic observations.

II. MATERIALS AND METHODS

A. Animals

The animal protocol was in agreement with the Guide for the Care and Use of Experimental Animals and approved by the Animal Care Committee of the National Institute of Livestock and Grassland Science. A total of 53 female pigs of Landrace and Meishan breeds were used at 1 wk (n = 6), 3 wk (n = 6), 6 wk (n = 6), 3 mo (n = 6) and 5 mo (n = 3 and 2, respectively) of age. All of the pigs were fed under same conditions. The animals were weaned at 4 wk and then fed *ad libitum* a commercial diet. At slaughter, live weight was taken and backfat samples were collected immediately. The thickness of subcutaneous backfat was measured at middle region.

B. Histology

Tissue specimens were fixed in 4% paraformaldehyde, dehydrated, and then embedded in paraffin wax. Sections of 3 μm in thickness were stained with hematoxylin and eosin and observed using a Zeiss Axiophot 2 light microscope.

C. Cell counting and sizing

Adipose cells were prepared for cell size determination as described by Hirsch and Gallian [3]. Uniform slices of backfat tissue weighing approximately 50-80 mg were added to 12 ml of isotonic osmium tetraoxide in 50 mM collidine buffer with pH adjusted to 7.4. After 7-10 d of fixation at room temperature, the fixed cells were released from the tissue matrix by filtration through a 250-μm mesh nylon screen. The separated cells from screening were caught on a 25-μm mesh nylon screen where they were washed with copious

amounts of distilled water. Finally, the cells were then transferred to a 250-ml round-bottomed beaker, taken up to a known volume in 0.15 M NaCl, and counted using an electronic particle size/number analyzer (Multisizer 3 with AccuComp® software and a 560 -μm aperture; Beckman Coulter). In this procedure, cells with a diameter below 25 μm or above 250 μm are not counted.

D. Statistics

Data were analyzed for statistical significance using Student's t-test.

III. RESULTS AND DISCUSSION

As pigs aged, body weight of both breeds significantly increased. Meishan pigs grew more slowly than Landrace pigs; it was not different until 3 wk, but became lower by 6 wk (10.3 ± 1.0 kg and 13.7 ± 2.1 kg) as well as 5 mo (52.7 ± 0.8 kg and 74.8 ± 8.0 kg). The two layers of backfat (outer and inner) also increased in thickness during the growth with both breeds, and Meishan tended to have thicker subcutaneous adipose tissue than the Landrace. These biological characteristics of this Chinese breed followed the same pattern as described previously by other workers [4].

The adipocyte diameter distributions were determined by using a Coulter electronic counter (Multisizer 3) to size osmium tetraoxide-fixed adipocytes (Fig.1). There were significant age ($P < 0.01$) and breed ($P < 0.01$) effects for adipocyte mean diameter. At 1 wk, adipose cell diameter was similar for both breeds; by 3 mo, cell size in Meishan pigs constantly increased up to 1.7-fold in diameter (48.6 ± 2.5 to 82.7 ± 5.8 μm), whereas those in Landrace pigs increased 1.5-fold from 1 wk to 3 mo (48.2 ± 4.0 to 72.6 ± 4.5 μm), with inserting a kind of stationary period between 3 and 6 wk (53.4 ± 3.6 to 52.0 ± 2.2 μm) which did not show any enlargement in average cell diameter. Adipocytes in outer layer of subcutaneous adipose tissue were larger in Meishan pigs than in Landrace by 6 wk. Small cells were observed at all ages in both breeds. These findings were supported by the results from morphological observations of subcutaneous tissue sections: at 1 wk, fat cell size was similar for both breeds, and by 6 wk, Meishan pigs had larger adipocytes than in Landrace pigs. Finally at 5 mo, unilocular adipocytes of Meishan pigs were densely packed in a polyhedron shape, whereas those of Landrace pigs were in spherical shape which still seemed to have a room for lipid fillings. Taken together, our data indicate that greater cell size in adipocytes is a major contributing factor in increasing backfat tissue of Meishan pigs than those of Landrace pigs.

Some of the lipogenic gene expressions and enzyme activities concerning triglyceride lipid biosynthesis are known to change with adipocyte size [4, 5]. It is now generally accepted that human obesity can be characterized by adipocyte cell size with enlarged fat cells secreting more unfavorable cytokines than smaller cells [6]. We have established two clonal subcutaneous preadipocyte cell lines originated from crossbred pigs [7] and Meishan pigs [8]. These both cell lines will be helpful tools to study the adipose tissue metabolism and developmental mechanism from *in vitro* approaches.

IV. CONCLUSION

We have demonstrated that differences in subcutaneous adipose tissue mass in Landrace and Meishan pigs are due to differences in fat cell size.

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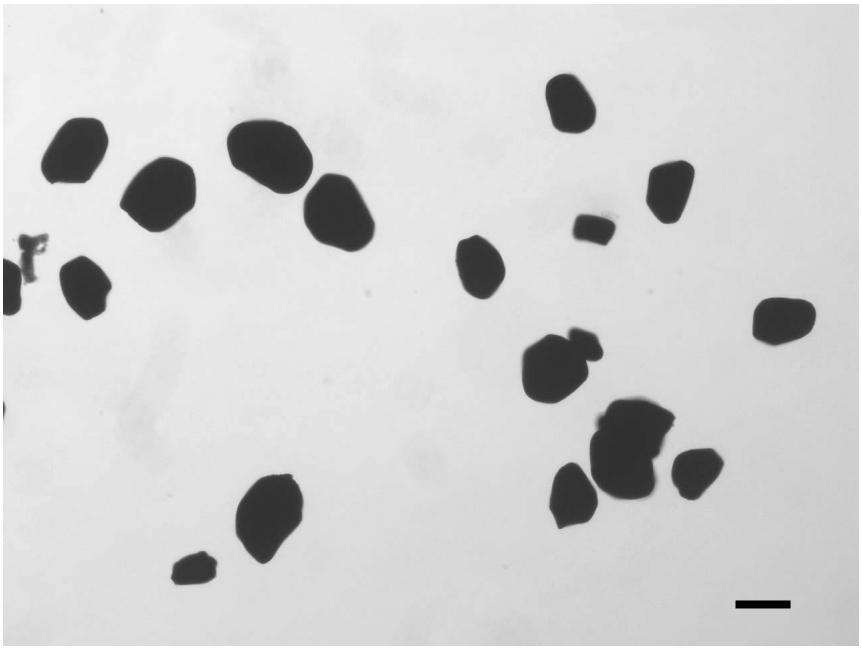

Fig.1. Example of osmium tetraoxide -fixed adipose cells which have been prepared for counting on a Coulter electronic counter (Multisizer 3). Adipocytes are from Landrace pig at 3 mo of age. Scale bar = 100 μm.