Cellularity of porcine adipose tissue: effects of growth and adiposity

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Abstract Adipose tissue, from two depots in pigs of three breeding groups with different propensities to fatten, was characterized in terms of weight of the adipose tissue organ, adipose cell number, and mean cell volume as determined by electronic counting of adipose cells fixed with osmium tetroxide. Perirenal and extramuscular adipose tissue growth was accompanied by progressive adipose cell enlargement along with an increase in cell number. By approximately 18–20 weeks of life, adipose tissue growth in both lean Hampshire × Yorkshire and fat Minnesota 3 × 1 pigs occurred exclusively by cellular hypertrophy. By 24 weeks of life (37 kg), hyperplasia was complete in Hormel Miniature pigs, which contained about one-third as many extramuscular adipose cells as the conventional pigs. Adiposity in the pig was due to cellular hypertrophy rather than cellular hyperplasia, since during growth, the leaner conventional pigs (30.6% extramuscular fat) contained more adipose cells than the fatter pigs (46.6% extramuscular fat). The number of adipose cells per animal or per adipose organ was directly related to the true body size (weight of fat-free carcass) of the animal. Fat Minnesota 3 × 1 pigs had fewer adipose cells than lean Hampshire × Yorkshire pigs at an equivalent live weight due to the smaller true body size of these animals. In young animals (28 and 54 kg), growth rate was positively correlated with adipose cell number. However, growth rate was unrelated to the total number of cells in the more mature animals (83 and 109 kg). Therefore a slow, normal growth rate may delay but not alter the final cell number.

Supplementary key words adipose cell · cell size · cell number · Coulter counter · subcutaneous fat · perirenal fat · total extramuscular fat · miniature and conventional pigs · true body size

Genetic and nutritional influences along with experimental treatments may alter the size of adipose tissue depots (1–3). Several investigations (1, 4, 5) have shown that increased size of the adipose tissue organ during growth is associated with an increase in both cell number and cell size. Hyperplasia is complete in the rat by the 15th week of life (1, 4). Moderate obesity in the adult is associated with cellular hypertrophy (6, 7), whereas in cases of severe obesity hyperplasia becomes increasingly important (2, 7, 8).

By prolonged fixation of adipose tissue with osmium tetroxide and the subsequent separation of the fixed cells, Hirsch and Gallian (9) were able to size and count fixed adipose cells in a suspension using a Coulter electronic counter. This technique has been used to study normal cellular growth and development in porcine (5) and rat adipose tissue (1), in abnormal cellular growth in experimentally obese rats (1) and man (10), and in genetically obese rats (2). Cellularity of adipose tissue from various anatomical locations has also been reported in swine (11) and six strains of genetically obese mice (3).

This communication describes the application of the technique of Hirsch and Gallian (9) to study changes in adipose cell size and number during the growth and development of adipose tissue in three breeding groups of swine that show different propensities to fatten.

MATERIALS AND METHODS

Animals

Twenty-four Minn 3 × 1 barrows (male castrate pigs) and 24 H × Y barrows were assigned to four weight groups of six per breeding group and studied at 28, 54, 83, and 109 kg, live weight. Twenty-four HM barrows, six per weight group, were studied at 28, 37, 45, and 54 kg, live weight. Minn 3 × 1 and H × Y barrows were fed a conventional swine diet (16% protein and 3.2% fat) from weaning to a weight of 45.5 kg and then changed to a second diet (13% protein and 3.5% fat) until each pig had reached its designated slaughter weight. HM barrows were changed to the second diet at an age similar to that of conventional pigs weighing 45.5 kg.

The Minnesota No. 1 breed was developed 35 years ago from pigs of the Landrace and Tamworth

Abbreviations: Minn 3 × 1, Minnesota No. 3 × Minnesota No. 1; H × Y, Hampshire × Yorkshire; HM, Hormel Miniature; OSQ, outer subcutaneous; MSQ, middle subcutaneous.

breeds (12) whereas the Minnesota No. 3 breed was started 15 years ago as a result of the combination of eight breeds of pigs of diverse genetic origin (13). Development of the HM breed, a breed selected for small size, was initiated in 1949 as a result of a joint project at the University of Minnesota and the Hormel Institute (14).

Pigs of the Minn 3 × 1 strain were selected because of their propensity to deposit fat and were compared to H × Y pigs, which are less adipose than Minn 3 × 1 pigs at the same carcass weight. Pigs of the HM breed were included in the comparison because of their small body size at maturity.

Definitions

Perirenal: This term is used to describe the adipose tissue surrounding the kidneys and lining the inner abdominal walls.

Carcass: The carcass includes all parts of the animal excluding the head, viscera, and perirenal adipose tissue.

True body size: The term true body size represents the weight of the fat-free carcass.

Total extramuscular fat: The term total extramuscular fat represents all the fat present outside the muscles of the porcine carcass.

Outer subcutaneous (OSQ) and middle subcutaneous (MSQ) backfat layers: In the pig the subcutaneous adipose tissue is separated by connective tissue into two anatomically distinguishable layers.

Carcass fat

After a 24 hr postmortem chilling period, eight paired muscles (from the hind limb: biceps femoris, quadriceps, semimembranosus and semitendinosus; from the loin: longissimus dorsi and psoas major; from the shoulder: biceps brachii and triceps brachii) were dissected from the weighed carcass. The percent lipid as determined in duplicate by ether extraction of oven-dried samples from these 16 pooled muscles was considered to be representative of the total percent intramuscular lipid. The remainder of the carcass was ground through a whole body grinder (Hobart Model 415b, 15 horsepower) fitted with a 10 mm plate. The ground portion was frozen in a plastic bag, cut with a saw into 5 × 5 cm strips and reground once through a 10 mm plate and twice through a 5 mm plate. The well-mixed and ground carcass was sampled from several locations and percent lipid was determined after three ether extractions. From the weight and percent lipid of the 16 muscles and the carcass (minus 16 muscles), the corrected percent carcass lipid was calculated. Adipose tissue and bone have a low moisture content, therefore most of the water in a porcine carcass is associated with the muscle. A is the weight of moisture in the muscle tissue of the carcass, which was calculated assuming that 90% of the weight of moisture in the carcass is associated with muscle tissue. Knowing the average percent moisture (B) in the 16 muscles, the total weight of muscle was calculated by 100 A/B and the weight of intramuscular lipid was estimated as (100 A/B) × $C/100$ or $A \times C/B$, where C equals the average percent lipid in 16 muscles. By subtraction of the intramuscular lipid weight from the total carcass lipid weight, the weight and percent of extramuscular lipid were calculated.

Tissue isolation and preparation

Adipose tissue samples from the anatomically distinguishable OSQ and MSQ backfat layers were sampled, immediately after exsanguination, from the left side dorsal to the last rib and 5 cm from the midline. All backfat thicknesses were measured at the midline and perpendicular to the last rib. The perirenal sample was taken adjacent to the left kidney, just before evisceration (20–30 min after exsanguination).

Fresh adipose tissue slices (<1.0 mm) were prepared and washed in warm (37°C) isotonic (0.15 M) saline to remove surface fat resulting from cell breakage during slicing. The tissue slice was dried on filter paper and weighed. The adipose tissue slice (100–150 mg) was placed in a 2.5 × 5.0 cm vial (scintillation counting vial) containing 3 ml of 50 mM collidine HCl buffer at pH 7.4. (9) and 5 ml of 3% osmium tetroxide in collidine buffer. Fixation was allowed to proceed in a well-ventilated fume hood for 72 hr. Separation and isolation of the osmium-fixed adipose cells was achieved by a procedure similar to that described by Hirsch and Gallian (9) and the suspended cells were sized and counted on a Coulter counter.

Adipose cell diameter and volume

A Coulter counter (model B) fitted with a 400 μm aperture was used and adjusted to measure the number of cells in 2 ml of suspension. Other permanent instrument settings used were: matching switch, 32H; gain, 100; and upper threshold, infinity. The calibration constant was calculated using a program, written for an IBM 360 computer, to calculate and interpret raw data from the Coulter counter.

The difference between the number of cells measured at two consecutive settings is the number of adipose cells within that diameter range (usually 8–14 μm in width). With this information for the complete adipose cell population, the average cell volume for the sample was calculated using the mean volume

of the adipose cells within a narrow class over the whole range of adipose cells. The total volume of the osmium tetroxide-fixed cells within a class was calculated by multiplying the mean class volume by the number of fixed adipose cells within each diameter range. Addition of these products for each diameter range yields an arbitrary total volume. Division of the total volume by the total number of cells counted gives the mean adipose cell volume for the population of cells counted.

Adipose cell number

The number of adipose cells present in 1 g of fat was calculated from the specific gravity (0.95) of fat and the average cell volume. The number of cells greater than 25 μm was also determined in a known weight of tissue. The latter method is dependent on the complete extraction of all adipose cells from the tissue sample. Reproducibility was considerably better using the first method and, for this reason, this technique was used in all cell number calculations. The number of adipose cells per total extramuscular and perirenal depot was then determined from the respective weights of extramuscular fat and perirenal fat and the number of adipose cells per g of fat. The number of cells per g of extramuscular fat was determined by taking into consideration the relative thickness of the OSQ and MSQ backfat layers at the assay site.

Statistical analyses

All statistical analyses were conducted as described by Steele and Torrie (15). Differences between treatment means were determined by comparing each treatment with all other treatments using a simple F-test with two treatments.

RESULTS

Adipose tissue growth

Various aspects of the growth and development of adipose tissue are shown in **Table 1** and **Fig. 1**. The percent and weight of perirenal and extramuscular carcass fat increased significantly ($P < 0.05$) with live weight. The weight of extramuscular carcass fat (Fig. 1b) for the two conventional groups (Minn 3 × 1 and H × Y) was similar at 28 kg live weight, but, as weight and age increased, the difference in the deposition of fat became progressively larger. At the heavier

TABLE 1. Effect of breeding group and live weight on carcass traits for three groups of swine

Trait	Breed Group	Live Weight Group (kg) 28	37	45	54	83	109
Age (days)	H × Y	$79.8_x^a \pm 3.0$			$117.3_x^b \pm 3.7$	$142.3_x^c \pm 5.0$	$167.8^d \pm 4.6$
	3 × 1	$70.0_y^a \pm 2.3$			$99.7_y^b \pm 2.6$	$122.8_y^c \pm 3.8$	$159.6^d \pm 6.9$
	HM	$158.8_z^a \pm 13.6$	168.5 ± 14.3	193.0 ± 14.2	$200.7_z^b \pm 10.8$		
Carcass weight (kg)	H × Y	$18.4^a \pm 0.4$			$38.7^b \pm 0.2$	$60.5_x^c \pm 1.0$	$82.5_x^d \pm 0.4$
	3 × 1	$17.2^a \pm 0.5$			$37.3^b \pm 0.7$	$57.4_y^c \pm 0.6$	$79.6_y^d \pm 0.8$
	HM	$17.8^a \pm 0.3$	$24.8^b \pm 0.1$	$31.3^c \pm 0.2$	$38.8^d \pm 0.3$		
True body size[e] (kg)	H × Y	$14.1_x^a \pm 0.2$			$28.1_x^b \pm 0.4$	$42.9_x^c \pm 0.8$	$54.7_x^d \pm 0.9$
	3 × 1	$12.8_y^a \pm 0.4$			$24.0_y^b \pm 0.2$	$32.7_y^c \pm 0.6$	$39.7_y^d \pm 0.8$
	HM	$12.2_y^a \pm 0.3$	$16.9^b \pm 0.4$	$19.8^c \pm 0.1$	$22.1_z^d \pm 0.4$		
Perirenal fat (%)	H × Y	$0.7_x^a \pm 0.1$			$1.3_x^b \pm 0.1$	$1.7_x^c \pm 0.1$	$2.3_x^d \pm 0.1$
	3 × 1	$0.8_x^a \pm 0.1$			$1.4_x^b \pm 0.1$	$2.1_y^c \pm 0.1$	$3.2_y^d \pm 0.2$
	HM	$2.2_y^a \pm 0.2$	$2.8^b \pm 0.1$	$4.2^c \pm 0.6$	$4.0_y^c \pm 0.1$		
Extramuscular carcass fat (%)	H × Y	$21.0_x^a \pm 1.1$			$24.9_x^b \pm 1.1$	$26.1_x^b \pm 0.7$	$30.6_x^c \pm 1.1$
	3 × 1	$22.7_x^a \pm 0.6$			$31.7_y^b \pm 0.9$	$40.1_y^c \pm 1.3$	$46.6_y^d \pm 0.8$
	HM	$28.1_y^a \pm 1.0$	$31.5^b \pm 1.4$	$34.2^c \pm 0.5$	$39.7_z^d \pm 1.2$		
Intramuscular carcass fat (%)	H × Y	$2.4_x^a \pm 0.1$			$2.3_x^a \pm 0.1$	$3.0_x^b \pm 0.2$	$3.1_x^b \pm 0.3$
	3 × 1	$3.0_y^a \pm 0.1$			$3.3_y^a \pm 0.3$	$2.9_x^a \pm 0.2$	$3.5_x^a \pm 0.2$
	HM	$3.2_y^a \pm 0.2$	$2.8^a \pm 0.1$	$2.5^a \pm 0.2$	$2.9_y^a \pm 0.1$		
Outer subcutaneous fat thickness (cm)	H × Y	$0.41_x^a \pm 0.05$			$0.71_x^b \pm 0.03$	$0.66_x^b \pm 0.03$	$0.89_x^c \pm 0.08$
	3 × 1	$0.56^a \pm 0.05$			$0.69_x^a \pm 0.08$	$1.07_y^b \pm 0.08$	$1.27_y^c \pm 0.10$
	HM	$0.64_y^a \pm 0.03$	$0.71^a \pm 0.04$	$0.76^b \pm 0.03$	$0.94_y^c \pm 0.10$		
Middle subcutaneous fat thickness (cm)	H × Y	$0.43_x^a \pm 0.03$			$0.79_x^b \pm 0.08$	$0.76_x^b \pm 0.03$	$1.32_x^c \pm 0.15$
	3 × 1	$0.58_y^a \pm 0.04$			$0.91_x^b \pm 0.05$	$1.55_y^c \pm 0.13$	$2.10_y^d \pm 0.15$
	HM	$0.69_z^a \pm 0.03$	$1.04^b \pm 0.05$	$1.22^b \pm 0.08$	$1.50_y^c \pm 0.13$		

Each value is the average of six animals ± SEM.

[a,b,c,d] Mean values on the same line with different superscripts (a,b,c,d) are significantly different ($P < 0.05$). No superscript indicates no significant difference.

[e] Weight of the fat-free carcass.

[x,y,z] Mean values having different subscripts for a trait (x,y,z) and in the same column are significantly different ($P < 0.05$). No subscript indicates no significant difference.

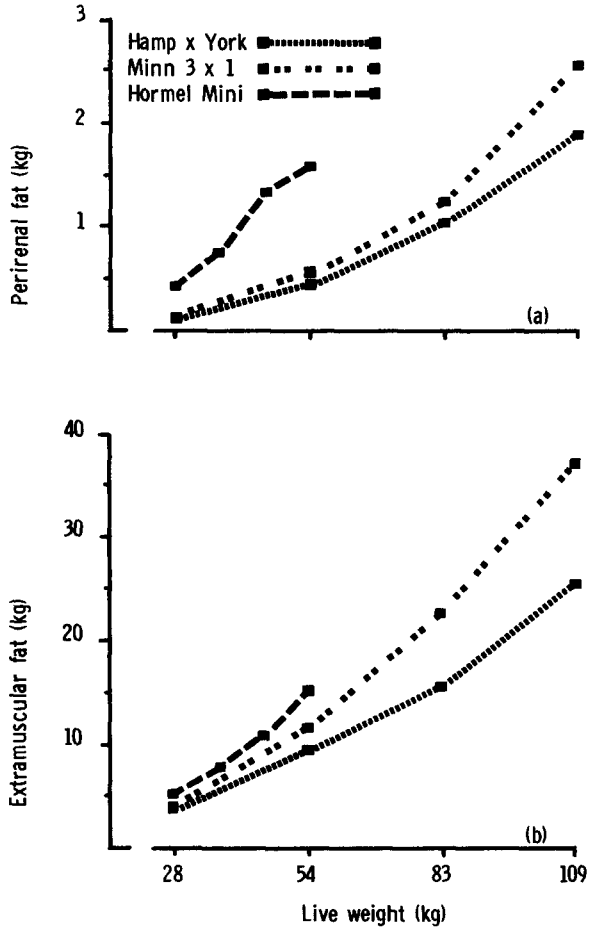

Fig. 1. Effect of breeding group and growth on the weight of perirenal and extramuscular carcass fat in the pig.

weights, the faster growing Minn 3 × 1 pigs deposited significantly ($P < 0.05$) more fat than the leaner H × Y pigs. However, if the growth of nonfat tissue (Table 1), that is, true body size, is compared, the Minn 3 × 1 pigs grew at a rate similar to that of the H × Y pigs at first, and later, at approximately 120 days of life, the Minn 3 × 1 pigs grew slower, ending up with a smaller body size. Hormel Miniature (HM) pigs contained more fat ($P < 0.05$) than the two conventional groups at equivalent live weights. At equivalent ages, HM and H × Y pigs had similar percentages of extramuscular fat, but, in the fatter Minn 3 × 1 pigs, the value was higher. The growth and development pattern of the perirenal depot (Fig. 1a) was similar to the deposition of extramuscular carcass (Fig. 1b). The OSQ and MSQ backfat layers (Table 1) generally increased in thickness in the three groups with increasing live weight. The MSQ layer was the thickest and fastest growing layer, particularly in the Minn 3 × 1 and the HM pigs. Consequently, this backfat layer makes the major contribution to the accumulation of excess fat in the pig. The total weight of intramuscular carcass fat increased with increasing live weight. However, when intramuscular fat was expressed as a percentage of the carcass (Table 1), 83 and 109 kg H × Y pigs were the only animals to have significantly ($P < 0.05$) more intramuscular fat than lighter animals of the same breeding group.

Cell size in adipose tissue depots

All three depots studied in the two conventional groups contained adipose cells of similar size at 28 kg live weight. However, the MSQ adipose tissue layer contained larger adipose cells than the OSQ layer, particularly in the heavier live weight groups (**Table 2**). In general the perirenal adipose tissue from all three groups of pigs contained cells of a size similar to those in the MSQ adipose tissue of the respective groups (Table 2). The perirenal depot in young 28 kg pigs from the two conventional groups contained adipose cells of smaller size than those found in the OSQ and MSQ backfat layers. Brook (16) has reported that in children adipose cells from deep sites were significantly smaller than those from subcutaneous sites. At equivalent weights, the HM pigs had a higher ($P < 0.05$) percent of perirenal fat (Table 1) than either H × Y or Minn 3 × 1 pigs. This was reflected in the larger cell size ($P < 0.05$) at these live weights (Table 2). A similar pattern existed between these two parameters when the Minn 3 × 1 and H × Y breeds were compared. However, the Minn 3 × 1 pigs had significantly ($P < 0.05$) more perirenal fat and larger perirenal adipose cells than the H × Y pigs only at 83 and 109 kg live weight.

Cellularity during growth and development

The relative contributions of adipose cell size and cell number to extramuscular adipose tissue growth are shown in **Fig. 2.** Considering the two conventional groups, a general increase in the number of adipose cells was observed during growth (Fig. 2a), and was most evident between 54 and 83 kg live weight. During this period of growth, when cell number was increasing at a faster rate, no significant increase in average cell volume (Fig. 2b, Table 2) was observed. However, this does not mean that during this period the increase in adipose tissue mass was due solely to an increase in the number of adipose cells, but rather that the contribution made by hypertrophy of existing cells was masked by the presence of smaller adipose cells (greater than 25 μm in diameter), which were now measurable on the Coulter counter. This counting technique has some shortcomings, since adipose cells smaller than 25 μm in diameter and cells with a low lipid content, which are insufficiently fixed to be separable from tissue debris,

TABLE 2. Effect of breeding group, live weight, and anatomical location on the cellularity of porcine adipose tissue

	Breed Group	Live Weight Group (kg)					
		28	37	45	54	83	109
OSQ adipose cell volume ($\mu m^3 \times 10^{-4}$)	H × Y	$17.7_x^a \pm 1.1$			$32.4_x^b \pm 1.3$	$30.4_x^b \pm 1.2$	$41.0_x^c \pm 2.3$
	3 × 1	$19.8_x^a \pm 1.1$			$41.0_y^b \pm 2.3$	$51.7_y^c \pm 2.1$	$72.9_y^d \pm 2.6$
	HM	$41.0_y^a \pm 2.8$	$40.9^a \pm 3.8$	49.1 ± 4.7	$65.5_z^b \pm 6.8$		
MSQ adipose cell volume ($\mu m^3 \times 10^{-4}$)	H × Y	$18.3_x^a \pm 1.0$			$35.9_x^b \pm 2.7$	$33.3_x^b \pm 2.1$	$50.4_x^c \pm 3.2$
	3 × 1	$20.2_x^a \pm 1.7$			$58.9_y^b \pm 3.7$	$64.6_y^b \pm 2.8$	$90.5_y^c \pm 4.8$
	HM	$43.9_y^a \pm 4.6$	$49.2^{ab} \pm 4.3$	$59.6^b \pm 5.2$	$88.3_z^c \pm 6.0$		
Perirenal adipose cell volume ($\mu m^3 \times 10^{-4}$)	H × Y	$15.2_x^a \pm 1.3$			$33.8_x^b \pm 1.6$	$31.4_x^b \pm 2.7$	$54.0_x^c \pm 6.1$
	3 × 1	$16.8_x^a \pm 1.6$			$41.5_y^b \pm 2.5$	$55.2_y^c \pm 4.4$	$87.2_y^d \pm 6.7$
	HM	$50.3_y^a \pm 3.1$	$58.9^a \pm 7.3$	$63.3^a \pm 8.7$	$94.5_z^b \pm 11.0$		
Total number of extramuscular adipose cells ($\times 10^{-9}$)	H × Y	$25.3_x^a \pm 1.8$			$33.2_x^b \pm 1.8$	$58.5_x^c \pm 3.4$	$64.5_x^c \pm 3.7$
	3 × 1	$23.0_x^a \pm 1.6$			$29.7_x^b \pm 1.8$	$46.2_y^c \pm 2.3$	$52.4_y^c \pm 2.4$
	HM	$13.8_y^a \pm 0.8$	$20.0^b \pm 1.5$	$23.3^b \pm 2.0$	$23.7_y^b \pm 2.4$		
Total number of perirenal adipose cells ($\times 10^{-9}$)	H × Y	$1.1_x^a \pm 0.2$			$1.7_x^b \pm 0.1$	$3.9_x^c \pm 0.3$	$4.4_x^c \pm 0.5$
	3 × 1	$1.0_x^a \pm 0.1$			$1.5_x^b \pm 0.1$	$2.6_y^c \pm 0.1$	$3.5_x^d \pm 0.3$
	HM	$0.9_x^a \pm 0.1$	$1.5^b \pm 0.2$	$2.7^c \pm 0.4$	$2.2_y^c \pm 0.4$		

Each value is the average of six animals ± SEM.

^{a,b,c,d} Mean values on the same line with different supercripts are significantly different ($P < 0.05$). No superscript indicates no significant difference.

^{x,y,z} Mean values having different subscripts for a trait and in the same column are significantly different ($P < 0.05$). No subscript indicates no significant difference.

are not counted (1). This situation is particularly true in young or starved animals. During periods where increases in adipose cell number were minimal (28–54 kg and 83–109 kg), large increases in cell volume were observed (Fig. 2a and b).

The results indicate that adipose tissue increases in the young pig by both cellular hypertrophy and cellular hyperplasia. By the time 83 kg live weight was attained (20 weeks of life for the H × Y pigs and 18 weeks for the Minn 3 × 1 pigs), no further significant increase in cell number was observed in the extramuscular adipose tissue. Considering the perirenal adipose tissue as a complete depot, no significant ($P > 0.05$) increase in the number of adipose cells was observed beyond 83 kg for H × Y pigs (Table 2). However, in the Minn 3 × 1 pigs a significant ($P < 0.05$) increase (2.6×10^9 to 3.5×10^9) in the number of perirenal adipose cells was observed between 83 and 109 kg live weight.

At 28 kg live weight, the Minn 3 × 1 and H × Y pigs had carcasses with a similar content of extramuscular fat (Table 1) containing a similar number of adipose cells of similar size (Fig. 2). During growth and development from 28 kg, the Minn 3 × 1 pigs had a greater propensity to fatten than H × Y pigs (Fig. 1). This increase in adipose tissue was accompanied by an increase in adipose cell size and cell number. At 109 kg live weight, H × Y and Minn 3 × 1 pigs contained 65×10^9 and 52×10^9 extramuscular adipose cells, respectively (Table 2). There-

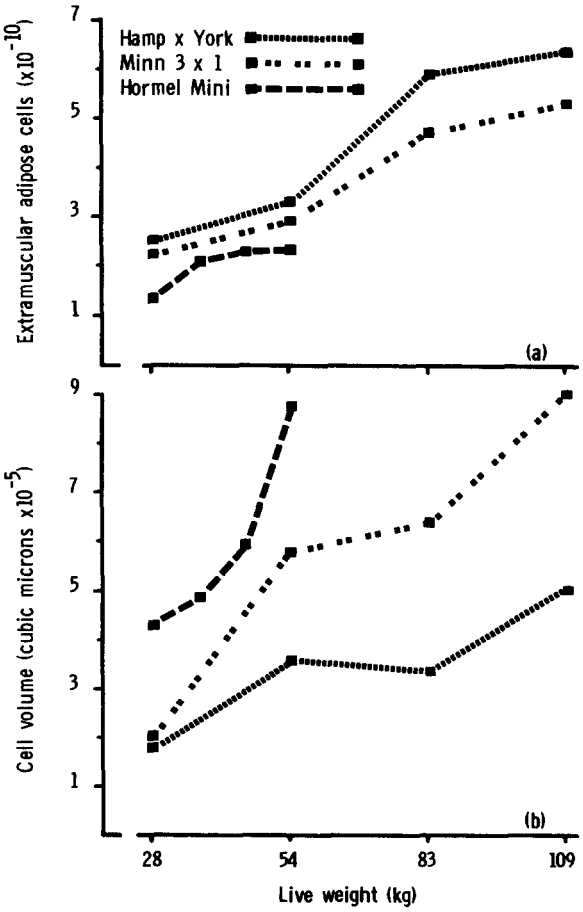

Fig. 2. Increase in the number and volume of extramuscular adipose cells during the growth of swine from three breeding groups.

TABLE 3. Comparisons among breeding groups of the ratio of extramuscular and perirenal adipose cells to true body size at similar percentages of extramuscular and perirenal depot fat

Trait	H × Y		Minn 3 × 1		HM	
	Extramuscular	Perirenal	Extramuscular	Perirenal	Extramuscular	Perirenal
Fat (%)	30.6	2.3	31.7	2.1	31.5	2.2
Live wt. (kg)	109.0	109.0	54.0	83.0	37.0	28.0
Fat-free carcass (kg)	54.7	54.7	24.0	32.7	16.9	12.2
Total number of adipose cells ($\times 10^{-9}$)	64.5	4.4	29.7	2.6	20.0	0.9
Number of adipose cells /kg fat-free carcass	1.18×10^9	8.0×10^7	1.24×10^9	7.9×10^7	1.18×10^9	7.4×10^7

fore at 109 kg live weight, the leaner H × Y pigs (30.6% extramuscular carcass fat) contained a larger ($P < 0.05$) number of adipose cells per extramuscular adipose tissue than the fatter (46.6% extramuscular carcass fat) Minn 3 × 1 pigs (Table 1). This observation indicates that differences in adiposity during normal growth between these two groups of pigs were due solely to adipose cell hypertrophy and not to adipose cell hyperplasia. For the 109 kg live weight groups, correlation coefficients of 0.46 (N = 6) and 0.44 (N = 6) were obtained between percent extramuscular fat and adipose cell volume for the H × Y and Minn 3 × 1 pigs, respectively. Correlations of less than 0.15 were obtained between percent extramuscular fat and cell number per total extramuscular fat for pigs in the 109 kg weight group, indicating that within a breed, adiposity was also due primarily to adipose cell size rather than cell number. Perusal of the cellularity data in **Table 4** will show that the fat content of littermates at constant weight was more closely related to adipose cell size rather than to the number of cells in the total extramuscular fat.

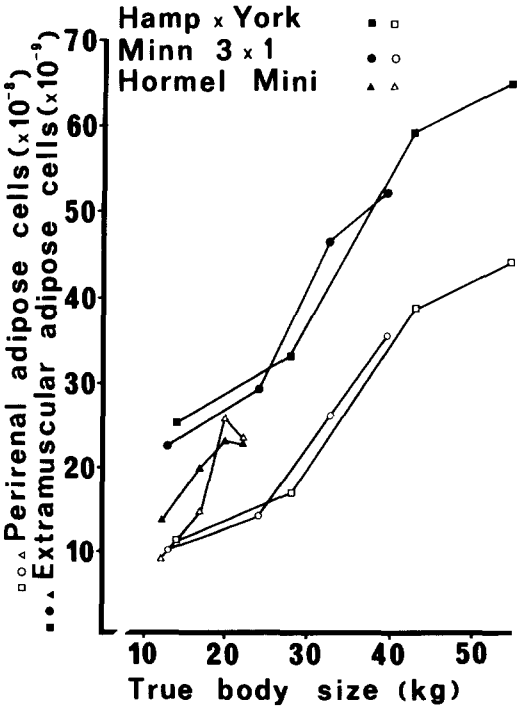

Fig. 3. Relationship between true body size and the number of extramuscular (closed symbols) and perirenal (open symbols) adipose cells.

Data presented in Fig. 2a indicate that Minn 3 × 1 pigs contained fewer extramuscular adipose cells than H × Y pigs of a similar live weight. However, when the number of extramuscular adipose cells was plotted against true body size, that is, the weight of the fat-free carcass, curves (**Fig. 3**) for pigs of both conventional breeding groups were nearly coincident. A similar relationship was apparent when the number of adipose cells per adipose organ (e.g., perirenal depot) was plotted against true body size. Similar data plotted for the more mature HM pigs indicates that the curves (Fig. 3) were somewhat similar in shape but different in magnitude from those for the two conventional groups of pigs.

A HM, a Minn 3 × 1 and a H × Y pig at 37, 54 and 109 kg, had similar quantities of extramuscular fat (**Table 3**). At these live weights, cell size (Table 2) and consequently the number of cells per g of MSQ adipose tissue (2.46×10^6, 2.30×10^6, and 2.31×10^6, respectively) were very similar for pigs of these three diverse groups. At equivalent levels of body fat, the number of cells in the extramuscular and perirenal carcass fat was noticeably different (Table 3), indicating once more that cell size and not cell number was the more important factor determining adiposity in the pig. In the HM pigs at 45 and 54 kg live weight, the number of extramuscular adipose cells was constant at about 23×10^9. However, the extramuscular adipose tissue mass increased by 43% between 45 and 54 kg live weight, which was due to a sharp increase in adipose cell volume (Fig. 2b).

Fig. 4 illustrates the relationship between MSQ

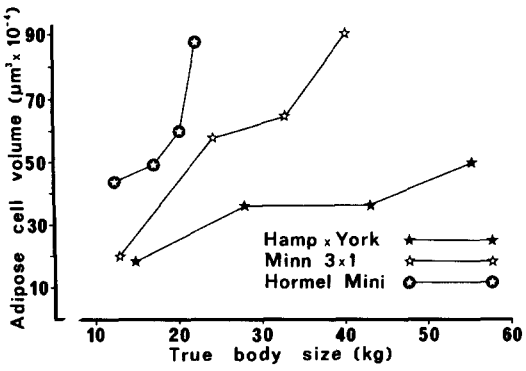

Fig. 4. Relationship between fat-free carcass weight and adipose cell volume in three types of swine.

adipose cell volume and true body size for the HM, Minn 3 × 1, and H × Y barrows. The rate of increase in adipose cell volume was the most accelerated in the HM barrows, intermediate in the Minn 3 × 1 barrows, and lowest in the H × Y barrows. These differences in adipose cell hypertrophy appear to be related to fat-free carcass weight. For example, the final adipose cell size in the HM and Minn 3 × 1 barrows was similar, but the rate of increase in the Minn 3 × 1 barrows was lower than in the HM barrows (Fig. 4). This is possibly due to a larger potential for growth of the fat-free carcass in the Minn 3 × 1 barrows. Among breeding groups of the same fat-free carcass weight, adipose cell volume was largest in groups with the lowest potential for increases in fat-free carcass weight. In other words, adipose cell volume appears to be a reasonable index to predict fatness of the animal, the stage in growth maturity and the potential of a pig to produce a heavy fat-free carcass weight.

Cellularity of individual pigs

The results shown in **Table 4** were obtained from two sets of littermate H × Y pigs studied at either 83 or 109 kg live weight. Individual differences in the total number of cells were considerable within the H × Y groups, but differences between littermates were small (Table 4). Data depicted in Fig. 2a indicate a rapid increase in adipose cell number between 54 and 83 kg live weight, with no significant increase beyond 83 kg live weight. When individual littermates were compared at 83 and 109 kg, no further change in the number of adipose cells per extramuscular fat was observed for the lean H × Y pigs beyond 83 kg. Replicate littermates were not available for the Minn 3 × 1 pigs and only one pig from each litter was allotted to each weight group.

Considering the two weight groups, namely 28 and 54 kg, where adipose tissue growth and development were due to both hypertrophy and hyperplasia, the effect of age on adipose cell number can be observed (**Fig. 5**). At a constant weight, extramuscular adipose tissue from pigs with a rapid growth rate contained more adipose cells than that from slower growing pigs. This relationship was not present at live weights of 83 and 109 kg, indicating that, although growth rate influences the number of adipose cells in the young immature pig, the final number of cells was independent of growth rate. The effects of breeding and weight groups were statistically removed by pooling the sums of squares and cross products for each breeding and weight group.

TABLE 4. Adipose tissue cellularity and carcass traits within littermates of the Hampshire × Yorkshire group

Littermates[a]	Live wt.	Age	Fat[b]	Number of Cells[b]	MSQ Adipose Cell Volume[c]
	kg	*days*	%	$\times 10^{-9}$	
A220	83	144	24.9	67.1	26.7
A223	83	144	27.2	68.2	32.8
A224	83	152	26.5	62.4	29.8
A221	109	172	26.5	70.7	38.7
A222	109	166	32.7	70.5	49.2
A225	109	164	29.8	63.5	48.2
B231	83	130	24.5	54.3	33.2
B232	83	126	28.5	55.3	41.4
B230	109	168	28.4	54.5	57.6
B230	109	168	28.4	54.5	57.6
B233	109	152	32.3	55.7	60.6

[a] Pigs with the same letter belong to the same litter.
[b] Total extramuscular carcass fat.
[c] Middle subcutaneous adipose cell volume (cubic microns $\times 10^{-9}$).

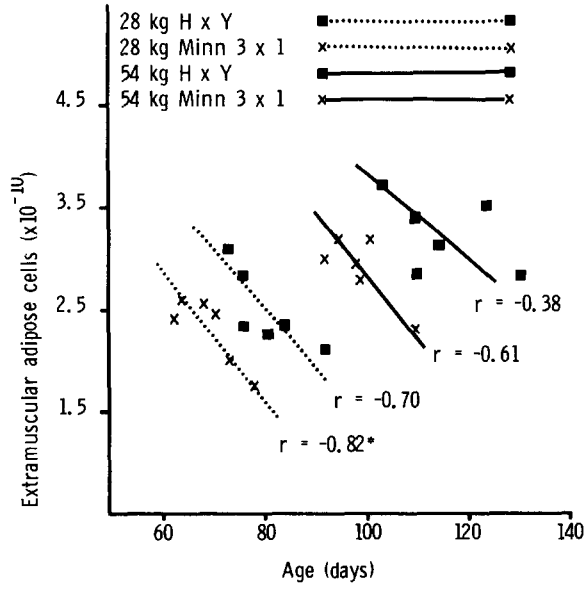

Fig. 5. The effect of breeding group and age at a given live weight on the number of extramuscular adipose cells.

When calculated in this manner, there was a significant ($P < 0.01$) pooled correlation of -0.59 between age at a constant weight (growth rate) and number of extramuscular adipose cells in the young, growing pig.

A significant ($P < 0.01$) pooled correlation coefficient of -0.72 was obtained between these same two parameters when only the 28 kg pigs of the two groups were combined. Using the same data, age and the number of extramuscular adipose cells were positively and significantly ($P < 0.01$) correlated ($r = 0.73$) when the effects of live weight and breeding group were disregarded, indicating a general increase in cell number with age. This emphasizes how the interpretation of one set of data can lead to correlation coefficients with completely different meanings. Caution should be observed in the calculation and interpretation of correlation coefficients determined from variables in grouped data.

DISCUSSION

Adipose tissue cellularity of pig adipose tissue has been studied by several other groups (5, 11, 17) who also report that adipose cell size increases with animal weight and proliferation of the adipose tissue depots. The growth and development of adipose tissue in the pig is similar to that reported in the human (8) and the rat (8, 18). In agreement with these studies both perirenal and extramuscular adipose tissue growth were accompanied by an increase in both adipose cell number and adipose cell size. By approximately 18–20 weeks of life, hyperplasia was complete in the conventional pig. Any further increase in adipose tissue mass was due to cellular hypertrophy, that is, the deposition of lipid in existing adipose cells. Anderson and Kauffman (5) have also reported that between 5 and 6.5 months essentially all of the increase in adipose tissue mass was due to increase in cell size. Hirsch and Han (1) concluded that both adipose cell size and number increased up to the 15th week of life in the rat and that further increase was achieved largely through cellular hypertrophy. In adequately fed rats, this finding was confirmed by Hubbard and Matthew (4) who reported that epididymal adipocyte hyperplasia was complete between 9 and 14 weeks of life.

Fat cell size was responsible for the increase in adipose tissue mass at moderate degrees of human obesity (7). However, with more severe obesity, fat cell number becomes increasingly important and dominates as a factor contributing to obesity (7, 8). The present study indicates that adiposity in the pig is due primarily to cellular hypertrophy, since leaner H × Y pigs (30.6% extramuscular fat) contained a larger number of adipose cells than the fatter Minn 3 × 1 pigs (46.6% extramuscular fat). Hypertrophy is the major factor in fattening as the pig nears its maximum or mature body size. Within a breeding group and at constant live weight, adiposity was positively correlated with average cell size and not with adipose cell number. The similarity in adipose cell number within pigs of a litter, while pigs of the same breed from another litter are quite different in adipose cell number, indicates the role of the genetic background of the animal in determination of the final adipose cell number. In studies with rats, experimental obesity, produced by destruction of the ventromedial hypothalamic nuclei at 7.5 weeks of life, could be accounted for by increases in adipose cell size and was not in any way related to cell number (1). In the genetically obese Zucker rat, characterized by extreme obesity recognizable by 3 weeks of life, Johnson et al. (2) reported that increases in cell number, accompanied by extreme cell enlargement, occurred until the 26th week in all adipose depots. These data indicate that the mechanism for adiposity is not the same for these two types of obesity. Naeye and Roode (19) showed in obese humans that increases in cell size and number occurred in the parenchymal cells of organs other than adipose tissue.

During the growth of pigs or other animals, protein accretion eventually plateaus and the deposition of fat exceeds deposition of lean tissue (20). Likewise, animals can grow to different sizes before they achieve a similar body composition. This size is primarily dependent on heredity. However, there is limited information available on the mechanisms responsible for this in either muscle or adipose tissue. Some insight into these mechanisms can be achieved from the relationships between either the number of adipose cells or the volume of the MSQ adipose cells and the true body size (fat-free carcass weight) of the H × Y (lean), Minn 3 × 1 (obese) and HM (miniature) barrows. At any live weight, the leaner H × Y barrows tended to have a larger number of extramuscular adipose cells than either the Minn 3 × 1 or HM barrows, both of which were fatter (Table 1). This indicates that a genetic difference existed among these breeding groups. However, Fig. 3 illustrates that when the number of extramuscular or perirenal adipose cells was plotted against true body size, the plots for both the H × Y and Minn 3 × 1 barrows were similar. The similarity in this relationship between two widely different groups of conventional-size pigs suggests that adipose cell number and true

body size have a physiological relationship. A similar relationship may be expected for other animals, including humans, and it is even possible that this relationship may hold across species.

When pigs of different phenotypes were studied at a similar carcass composition, those with a heavier fat-free carcass had a larger total number of adipose cells, but the ratio of density of adipose cells to weight of fat-free carcass was similar in all three phenotypes (Table 3). This would enable the pig with a larger fat-free carcass or heavier "mature weight" to eventually be able to achieve a similar degree of fatness as a pig with a smaller fat-free carcass or lighter "mature weight". This concept is supported by the data for both the extramuscular and perirenal adipose depots (Table 3).

Porcine adipose cell number increased until the adipose tissue reached physiological maturity, that is, when adipose tissue growth was due solely to cellular hypertrophy. A positive relationship existed between growth rate and number of extramuscular adipose cells for young, growing pigs, although growth rate had no bearing on the final number of extramuscular adipose cells. Hubbard and Matthew (4) reported that chronic underfeeding of young rats (80 g) delayed, but did not prevent, the attainment of a normal epididymal fat cell population. On the other hand, Knittle and Hirsch (21) demonstrated that early nutritional effects changed the adult adipose cell number. When rats were nutritionally deprived for 21 days before weaning, the adipose depots remained small, with a low cell number and a small size. Adipose tissue from rats maintained in the cold had a much larger number of adipose cells that were smaller in size when compared with cells from rats grown at ambient temperature (22). Rakow et al. (23) observed no difference in the number of fat cells in the epididymal fat pads of obese mice and their lean littermates. However, an increased level of total DNA in the fat pads of the obese mice was due to a higher cell number in the connective tissue. The increase in the number of mast cells in adipose tissue from obese mice (24) may account for the increased nitrogen content (25) and DNA content of adipose tissue from obese rodents.

Enzyme activities have been traditionally expressed on the basis of wet tissue weight, lipid, soluble protein, or DNA content of the adipose tissue, the latter two being the methods of choice in most instances. However, Rodbell (26) found only about one-half of the adipose tissue protein content was associated with the adipocytes and estimated that the DNA derived from other cells (fibroblasts, mast cells, macrophages) can exceed the DNA content from adipose cells. It follows that, since cell number and cell size of a given adipose depot influence its metabolic activity (27, 28), metabolic comparisons should be expressed per adipose cell, rather than the previously used parameters, unless the adipose cells are isolated before these parameters are determined. Quantitative adipose cell analyses are important, since without quantitative data on adipose cell number, metabolic effects cannot be clearly attributed to nutritional effects, breed differences, or even normal growth.

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