Cellular and enzymatic changes in porcine adipose tissue during growth

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Abstract Experiments were designed to define some of the cellular and metabolic changes in various areas of porcine adipose tissue during growth and to establish a relationship between these changes and the accumulation of fat in the domestic pig. 35 male castrate pigs were killed at various ages from late fetal to 6.5 months. The following determinations were made on each animal: (*1*) total carcass fat, (*2*) adipose cell size and number by fixation of adipose tissue with osmium tetroxide, and (*3*) the activities of acetyl CoA carboxylase, citrate cleavage enzyme, glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, and malic enzyme from perirenal adipose tissue and each of the three layers of subcutaneous backfat. Carcass adipose tissue expanded by a combination of adipocyte hyperplasia and hypertrophy up to 5 months, after which adipose expansion was accomplished by cellular hypertrophy only, with no significant increase in cell number. The activities of the selected lipogenic enzymes (expressed on an adipose cell basis) increased markedly at weaning and again during the rapid increase in percentage of body fat between 3.5 and 5 months. Enzyme activities reached a peak at 5 months, after which activities decreased to values approaching mature levels.

Supplementary key words acetyl CoA carboxylase · citrate cleavage enzyme · malic enzyme · glucose-6-phosphate dehydrogenase · 6-phosphogluconate dehydrogenase · adipocyte size · adipocyte number · fat · growth

PREVIOUS STUDIES concerning the developmental changes of hepatic lipogenesis in the rat (1–4) and mouse (5) have shown that the rate of lipogenesis (in vitro incorporation of radioactive acetate and glucose into fatty acids) is high in the fetal animal, diminishes to a low level at birth and during the suckling period, and increases dramatically at weaning to a maximum level followed by a gradual decrease to maturity. The activities of citrate cleavage enzyme, malic enzyme, glucose-6-phosphate dehydrogenase, and acetyl CoA carboxylase followed a pattern similar to that shown for lipogenesis except that malic enzyme and acetyl CoA carboxylase activities were low in the fetal liver.

The rate of lipogenesis in adipose tissue has also been found to decrease during aging (6–8). This decreased lipogenesis that accompanies maturity is of particular interest because it has been noted for many years that fat deposition increases with age (9). Thus, an apparent contradiction exists: fat accumulation is increasing when the rate of lipogenesis is decreasing. Hirsch and his coworkers (10, 11) have explained some of this apparently contradictory data by emphasizing the importance of expressing metabolic data from adipose tissue on a cellular basis rather than on the conventional tissue weight basis. By expressing the data on a per adipose cell basis, Greenwood, Johnson, and Hirsch (11) have shown that the rate of lipogenesis in adipose tissue of old rats was not significantly different from that of young rats. Another important consideration is the balance between synthesis and degradation. A net increase in the accumulation of fat could result even during a period of decreased fat synthesis if there is simultaneously an even greater decrease in the turnover and catabolism of adipose tissue fatty acids.

The pig, as well as other domestic animals, reaches a point in its growth at which muscle growth decreases concomitantly with an increase in fat deposition (12). Adipose tissue is the primary site of fatty acid synthesis

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in the pig (13); therefore, it was of interest to determine the metabolic and cellular changes that were taking place in adipose tissue of the pig throughout development and particularly at the time of rapid fat deposition. Experiments were designed to determine: (*1*) if the increase in adipose tissue during growth was due to an increase in adipose cell proliferation (hyperplasia) or an increase in adipose cell size (hypertrophy) or a combination of the two, and (*2*) if the activities of lipogenic enzymes expressed on an adipose cell basis change with advancing age, particularly at the time of rapid fat accretion.

EXPERIMENTAL

Materials

Chemicals were obtained from the following sources: glucose-6-phosphate (Na^+), 6-phosphogluconate (Na^+), L-malic acid (Na^+), reduced glutathione, coenzyme A, bovine serum albumin, reduced nicotinamide adenine dinucleotide, nicotinamide adenine dinucleotide phosphate, tris(hydroxymethyl)aminomethane (Tris), and collidine (2,4,6-trimethylpyridine), Sigma Chemical Co., St. Louis, Mo.; adenosine triphosphate and acetyl coenzyme A, P-L Biochemicals, Inc., Milwaukee, Wis.; dithiothreitol, Calbiochem, Los Angeles, Calif.; malate dehydrogenase, Boehringer Mannheim Corp., New York; Folin phenol reagent, Fisher Scientific, Chicago, Ill.; ^{14}C-labeled sodium bicarbonate, New England Nuclear, Boston, Mass.; other chemicals, Mallinckrodt Chemicals, St. Louis, Mo., or J. T. Baker Chemicals, Phillipsburg, N.J. Nylon screen for the separation of fixed adipose cells was purchased from Handler, Tobler, Ernst, and Traber through Kressilk Products, Des Plaines, Ill., and Nalgene filter units (without the usual fine membrane) were purchased from Wilkens-Anderson Co., Chicago, Ill.

Procedure

30 Chester White male castrate pigs of common genetic origin were randomly assigned to six groups to be slaughtered at birth, 1, 2, 3.5, 5, and 6.5 months (castrated at 2 wk of age). In addition, one litter of fetal pigs was taken by Caesarean section approximately four days prepartum. After weaning at 6 wk of age, the animals were fed ad lib. The fat content of the diet was maintained at approximately 4%, and the protein content was changed to meet the animals' changing requirements with advancing age (14). The diet consisted of corn supplemented with minerals and vitamins and a 2:1 mix of soybean meal and meat scraps. The protein and fat content of the diet is given in Table 1. Animals were subjected to seasonal variations in temperature and photoperiod. To circumvent possible problems of changing enzyme circadian rhythms due to variations in eating pattern from season to season (15), animals were switched to a once-per-day feeding schedule 1 wk prior to sampling and were killed 2 hr after feeding at approximately 10 AM. O'Hea and Leveille (16) have shown that meal feeding has no significant influence on the lipogenic rate or the activities of lipogenic enzymes (malic enzyme, glucose-6-phosphate dehydrogenase, and 6-phosphogluconate dehydrogenase) in porcine adipose tissue as compared with animals fed ad lib.

Immediately after exsanguination, samples were obtained from perirenal adipose tissue and from three layers of subcutaneous backfat (outer, middle, and inner), as described previously (17). Each sample was divided into three portions, one (100 mg) for the adipose cell size and number determination by the osmium tetroxide fixation procedure of Hirsch and Gallian (18), a second (5 g) for ether-extractable lipid content, and a third for lipogenic enzyme analysis and soluble protein determination. This third portion (3 g) was homogenized for 20 sec with a Polytron PT-10 homogenizer in 9 ml of 0.15 M KCl in 50 mM Tris (Cl^-), pH 7.4. The homogenate was centrifuged at 100,000 *g* for 1 hr at 1°C. All enzyme activity determinations were made on the decanted supernatant fluid. Protein concentration of the supernate was determined by the method of Lowry et al. (19). All determinations except enzyme assays were made in duplicate.

Enzyme activity determinations were made immediately after centrifugation consecutively as they are listed below. Acetyl CoA carboxylase (EC 6.4.1.2) activity was measured by the [^{14}C]bicarbonate fixation method of Dakshinamurti and Desjardins (20); citrate cleavage enzyme (EC 4.1.3.8) by the method of Kornacker and Ball (21); glucose-6-phosphate dehydrogenase (EC 1.1.1.49) and 6-phosphogluconate dehydrogenase (EC 1.1.1.44) by the double-substrate method of Glock and McLean (22); and malic enzyme (EC 1.1.1.40) by the method of Ochoa (23), with special attention given to the purity of malic acid (24).

TABLE 1. Estimated protein and fat content of diet

Period	Percentage of Diet	
	Protein	Fat
Birth–1.5 mo (milk)	28[a]	46[a]
1.5–2.5 mo	18	4
2.5–4 mo	16	4
4–5.5 mo	14	4
5.5–6.5 mo	12	4

[a] Dry weight basis, from Perrin (40).

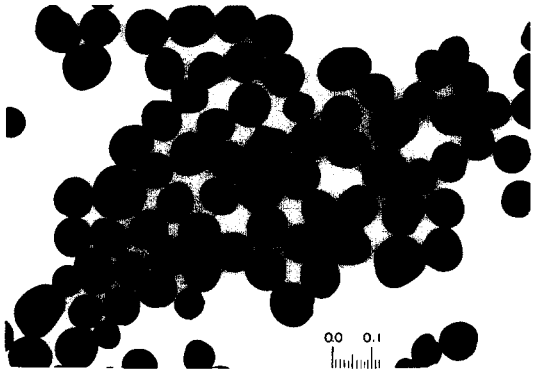

FIG. 1. Example of osmium tetroxide-fixed adipose cells which have been prepared for counting on a Coulter electronic counter by the method of Hirsch and Gallian (18). Inset scale is in millimeters.

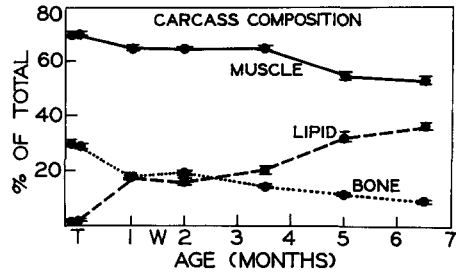

FIG. 2. Changes in the proportion of carcass components with growth plotted as percentage of the total carcass ± SEM. Muscle percentage is expressed on a lipid-free basis. *T* and *W* refer to term and weaning.

The modifications of enzyme procedure and the order of enzyme assays have been discussed previously (17).

Adipose cells were prepared for counting as follows. Uniform slices of adipose tissue 0.5 mm in thickness, weighing approximately 100 mg, were blotted dry, weighed, and transferred to a 20-ml vial containing 10 ml of isotonic 2% osmium tetroxide in 50 mM collidine (Cl^-), pH 7.4. A Stadie-Riggs microtome was used to insure uniformity of the slices. After 48–72 hr of fixation at room temperature, the fixed cells were separated from the surrounding stromal tissue by filtration through a 250-μm mesh nylon screen. Adipose cells from the screening were caught on a 25-μm mesh nylon screen where they were washed with copious amounts of distilled water. The fixed cells were then transferred to a 400-ml round-bottomed beaker, taken up to a known volume in 0.15 M NaCl, and counted using a Coulter electronic counter (18). The diameters of osmium-fixed adipose cells were determined by using an eyepiece micrometer on a light microscope. The volume of each of 120 cells/sample was calculated directly from diameter measurements ($^1/_6 \pi d^3$) by assuming that all fat cells were spherical. Diameter was measured in only one plane. Therefore, cells that were slightly oblong were randomly measured through either the short or long axis. Nonspherical cells were more frequently large in size. An example of the fixed cells is shown in Fig. 1.

To determine carcass composition, all muscle and adipose tissue (including perirenal adipose tissue) from the entire carcass was dissected from the skeleton, ground together, and mixed thoroughly. A random 200-g sample of the mixture was frozen in liquid nitrogen and powdered in a Waring blender. Two 5-g portions of the powder were dried for 24 hr in a vacuum oven at 70°C and extracted for 24 hr with diethyl ether in a Soxhlet extractor to estimate total ether-extractable carcass fat. Thicknesses of the outer, middle, and inner subcutaneous backfat layers were measured from the midpoint of the longissimus perpendicular to the surface at the 10–11th costal area before the carcass was dissected. Duncan's new multiple range test was used for statistical comparisons (25).

RESULTS

Live animal and carcass measurements

Table 2 includes the live weights and subcutaneous backfat thicknesses at the various sampling periods. The three layers of backfat increased in thickness at different

TABLE 2. Live weight and backfat thickness at 10th thoracic vertebra

Period	Live Weight	Subcutaneous Backfat Thickness		
		Inner	Middle	Outer
	kg		*cm*	
Fetal	1.2 ± 0.1	—[a]	—	—
Birth	1.2 ± 0.2	—	—	—
1 mo	7.7 ± 0.3	0.04 ± 0.03	0.29 ± 0.03	0.44 ± 0.03
2 mo	16.5 ± 1.7	0.04 ± 0.03	0.42 ± 0.04	0.44 ± 0.04
3.5 mo	43.1 ± 2.9	0.10 ± 0.03	0.68 ± 0.08	0.71 ± 0.03
5 mo	72.6 ± 4.2	0.58 ± 0.04	1.24 ± 0.17	1.04 ± 0.10
6.5 mo	106.3 ± 6.0	0.94 ± 0.04	1.48 ± 0.12	1.07 ± 0.06

Values are means ± SEM.
[a] Not measured; total of all three areas was less than 0.1 cm.

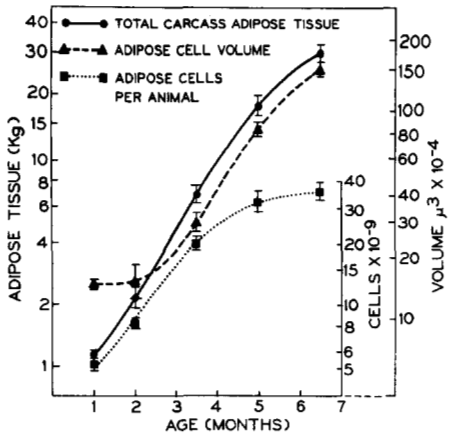

FIG. 3. Changes in the accumulated weight of carcass adipose tissue (solid line), the volume (μ = microns [micrometers]) of adipose cells from the middle subcutaneous backfat region (dashed line), and extrapolated total adipose cell number (dotted line) as determined by Coulter counter measurements of the osmium-fixed cells.

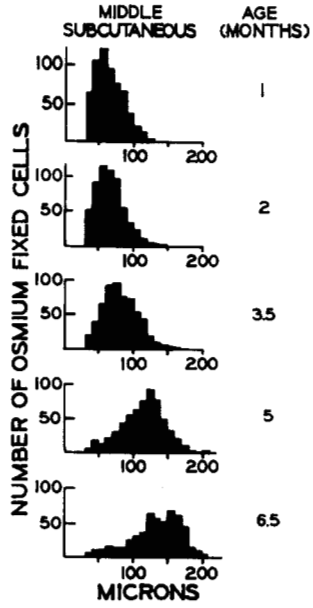

FIG. 4. Diameter distribution of osmium-fixed cells from middle subcutaneous backfat (microns = micrometers). Diameter measurements were made microscopically by the use of an eyepiece micrometer. 600 fixed cells were measured at each sample period (120/animal).

rates. At 1, 2, and 3.5 months, outer subcutaneous was the thickest layer; after 3.5 months, however, the middle and especially the inner layer began to develop more rapidly.

Fig. 2 summarizes the changes in carcass composition with advancing age. The most substantial increases in the percentage of carcass fat were made between birth and 1 month and between 3.5 and 5 months of age. These data are consistent with the finding of other workers showing that as a percentage of the total carcass weight, muscle and bone both decrease with maturity as fat increases (9, 26, 27).

Cellularity measurements

The rates of change of total carcass adipose tissue, adipose cell size, and adipose cell number are depicted on the semilog plot in Fig. 3. Total adipose cell number was extrapolated from the average cells per gram of outer subcutaneous and middle subcutaneous tissues since these two depots make up the majority of all carcass fat in the pig (28). The data indicate that increased carcass adipose tissue of young pigs (between 1 and 2 months) was due primarily to increases in the number of adipose cells. Between 2 and 5 months, changes were due to a combination of hypertrophy and hyperplasia. After 5 months there were no significant increases in adipose cell number; consequently, adipose mass increased solely by the process of cell enlargement.

The number of cells recorded at the younger ages, however, may be an underestimate of the actual number of cells present. A limitation of the osmium fixation technique, as discussed by Hirsch and Knittle (29), is that the procedure accounts for only those cells which are larger than 25 μm in diameter. The lower limit is

TABLE 3. Ether-extractable lipid, soluble supernatant protein, and adipose cell number and volume of middle subcutaneous adipose tissue

Period	Lipid	Soluble Protein (mg)	Adipocytes × 10^{-6}	Cell Volume	
	g	*mg*		*μg lipid/cell*	*μm³ × 10⁻⁴*
Fetal	0.051 ± 0.002	18.6 ± 1.2	—[a]	—	—
Term	0.040 ± 0.009	13.7 ± 1.5	—	—	—
1 mo	0.754 ± 0.022	7.6 ± 0.7	4.75 ± 0.40	0.161	15.0 ± 1.0
2 mo	0.750 ± 0.027	9.7 ± 1.2	4.58 ± 0.37	0.163	15.5 ± 2.9
3.5 mo	0.831 ± 0.009	7.4 ± 0.9	3.55 ± 0.39	0.234	29.8 ± 3.5
5 mo	0.897 ± 0.023	5.5 ± 0.3	1.67 ± 0.26	0.537	83.8 ± 5.8
6.5 mo	0.935 ± 0.007	3.8 ± 0.2	1.04 ± 0.08	0.899	151.5 ± 9.9

Amounts are per gram wet tissue (means ± SEM).
[a] Not determined.

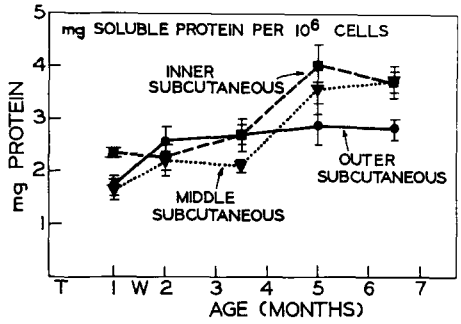

FIG. 5. Soluble protein content (± SEM) of outer subcutaneous (circles), middle subcutaneous (triangles), and inner subcutaneous backfat (squares). Soluble protein is expressed on a cellular basis (mg soluble supernatant protein/g tissue divided by adipose cells $\times 10^{-6}$/g tissue). *T*, term; *W*, weaning.

employed to eliminate the counting of large quantities of extraneous matter (bits of connective tissue, etc.). This limitation is particularly a problem when determining cell number in younger animals because there is a greater possibility that a large portion of the adipose cells are less than 25 μm in diameter and would not be counted. This possibility is demonstrated in Fig. 4. At 1 and 2 months the cell diameter distribution shows a sharp cutoff below 25 μm diameter, indicating that there may have been a significant number of cells below 25 μm that were not counted simply because they were too small to be measured by the technique employed.

Some of the developmental changes in middle subcutaneous adipose tissue are recorded in Table 3. The changes were the expected ones: lipid content increased, soluble protein decreased, and adipose cell number per gram of tissue decreased during growth due to increasing cell size. The lipid content of the fetal and term tissue was extremely low. It was difficult to distinguish adipose tissue from fibrous connective tissue at this age.

Table 3 also shows cell volume computations by the method of Hirsch and Gallian (18) as compared with direct measurements of the osmium-fixed cells under a light microscope. The former method obtains cell size by dividing lipid per gram of tissue by cells per gram of tissue to obtain a value of micrograms of lipid per cell as a measure of cell volume. In this experiment, cell diameter was measured on 120 fixed cells per adipose sample. The volume of each cell was calculated and then averaged for each age group. This direct method of cell measurement was used since it is independent of cell number. By the method of Hirsch and Gallian (18), cell size is dependent on the accuracy of the cell number determination. Either method, however, would result in the same conclusion in this particular experiment.

Soluble protein on an adipose cell basis was calculated (soluble supernatant protein per gram of tissue divided by cells $\times 10^{-6}$ per gram of tissue) since Anderson,

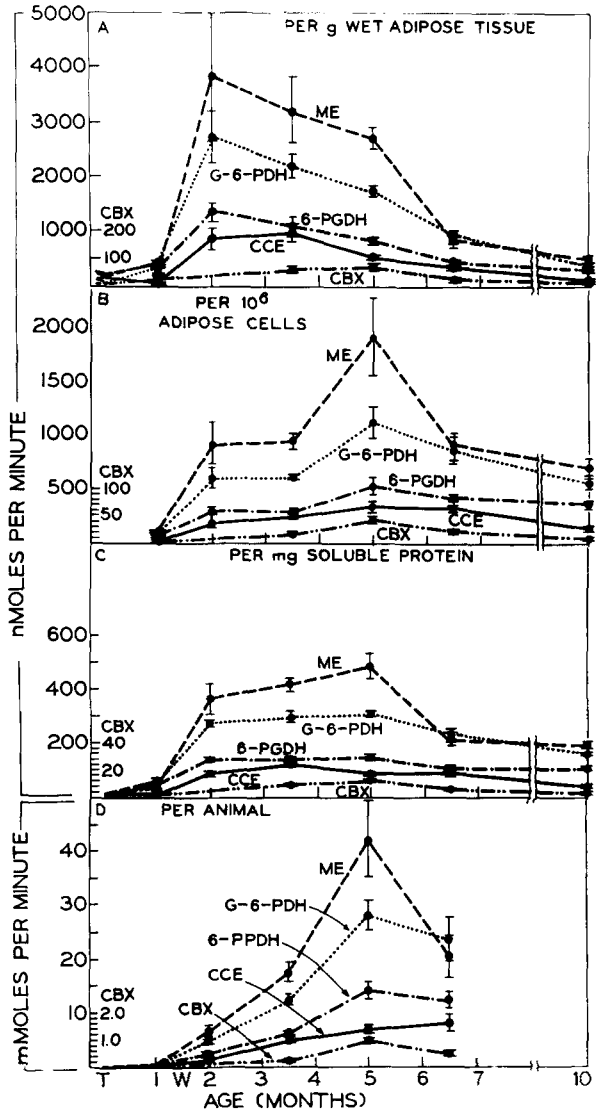

FIG. 6. Enzyme activity of middle subcutaneous backfat. Activity is expressed (*A*) per gram of wet adipose tissue, (*B*) per 10^6 adipose cells, (*C*) per milligram of soluble supernatant protein, and (*D*) per total adipose tissue mass. Activities measured were malic enzyme (ME), glucose-6-phosphate dehydrogenase (G-6-PDH), 6-phosphogluconate dehydrogenase (6-PGDH), citrate cleavage enzyme (CCE), and acetyl CoA carboxylase (CBX). CBX activity was not determined at 2 months and is plotted according to the smaller scale on the ordinate of each graph. The 10-month values are taken from Anderson et al. (17) to indicate approximate values for animals approaching maturity. Each value is the mean ± SEM of five animals. *T*, term; *W*, weaning.

Kauffman, and Kastenschmidt (17) have shown that this quantity is similar for seven areas of adipose tissue in the 10-month-old pig. The results in Fig. 5 show that this quantity changes with advancing age and specific area of adipose tissue sampled. Soluble protein in the outer subcutaneous tissue increased from 1.7 to 2.6 mg/10^6 cells at 2 months and remained constant throughout the remainder of the experimental periods. In contrast, the

inner and middle subcutaneous areas showed a marked increase between 3.5 and 5 months. This increase in soluble protein between 3.5 and 5 months coincided with the increased rate of fat deposition in the middle and inner backfat layers during that same period, as shown in Table 2.

Enzyme activities

The activities of malic enzyme (ME), glucose-6-phosphate dehydrogenase (G-6-PDH), 6-phosphogluconate dehydrogenase (6-PGDH), citrate cleavage enzyme (CCE) and acetyl CoA carboxylase (CBX) for middle subcutaneous adipose tissue are given in Fig. 6. The relationship of these enzymes to lipogenesis in pig adipose tissue has been demonstrated by O'Hea and Leveille (30). The 10-month values are from a previous experiment in this laboratory in which the animals were fed a diet identical with that fed the animals in this experiment (17). These values are included to indicate approximate values of animals approaching maturity.

The growth patterns of enzyme activity changes are dependent on the method of expressing the activities. The activities on a wet weight basis (Fig. 6*A*) increased dramatically after weaning between 1 and 2 months; thereafter, the activities of ME, G-6-PDH, and 6-PGDH decreased over the remaining portion of the experiment. CCE activity remained high until 3.5 months and then decreased. CBX was not measured at 2 months but remained at a high level until 5 months and then decreased. The pattern of enzyme activities on a wet weight basis was similar for all anatomical sites except that CBX activity decreased after 3.5 months for outer and inner subcutaneous and perirenal adipose tissues. If activities are expressed on a dry tissue basis or on an ether-extractable lipid basis, the pattern of activities is the same as that when recorded on a wet tissue basis except that the fetal and term activities are higher due to the high water content and low lipid content at this age (Table 3).

A different pattern was observed when the enzyme activities were expressed on a cellular basis (Fig. 6*B*). Again there was a marked increase in the activities of all enzymes measured between 1 and 2 months (CBX was not determined at 2 months). This activity was maintained until 3.5 months, and at 5 months there was approximately a two-fold increase in all enzyme activities except CCE. After 5 months, enzyme activities decreased to levels approaching mature values. When the activities were expressed on a soluble supernatant protein basis (Fig. 6*C*), a similar pattern was obtained except that there was no general increase between 3.5 and 5 months.

Enzyme activities were also calculated for the entire adipose tissue mass (Fig. 6*D*), using the average activities per gram of tissue from the outer and middle subcutaneous multiplied by the total grams of adipose tissue. The greatest total activity for all enzymes except CCE occurred at 5 months. CCE activity continued to increase until the termination of the experiment at 6.5 months.

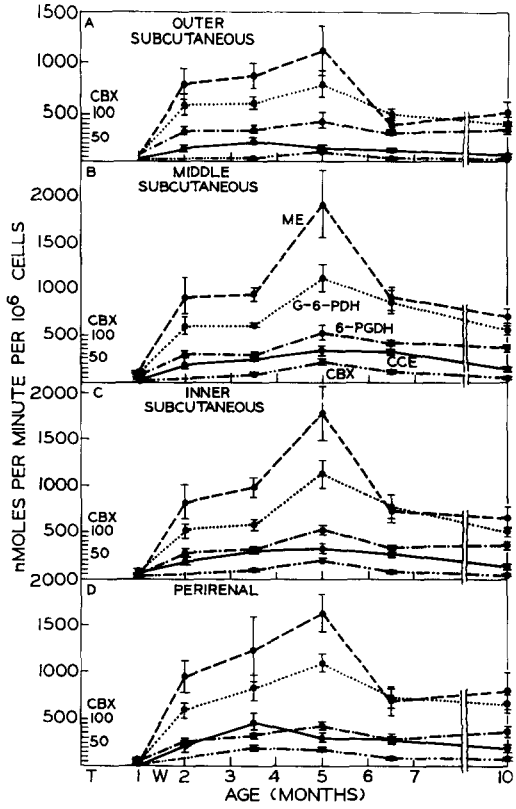

FIG. 7. Enzyme activity in pig adipose tissues. Activities are expressed on a cellular basis ± SEM for outer, middle, and inner subcutaneous backfat and perirenal adipose tissues. The 10-month values are taken from Anderson et al. (17) to indicate approximate values for animals approaching maturity. *T*, term; *W*, weaning.

Fig. 7 compares the enzyme activities of the four areas of adipose tissue that were sampled. The middle (MS) and inner (IS) subcutaneous CBX activity was significantly higher than outer (OS) subcutaneous at all ages ($P < 0.05$). At 1, 2, and 3.5 months the activities of the other enzymes were not significantly different in any of the backfat layers. However, at all sample periods after 3.5 months the ME, G-6-PDH, and CCE activities were significantly higher in the middle and inner subcutaneous ($P < 0.01$) as compared with outer subcutaneous. 6-PGDH activity tended to be higher but the differences were not significant. The enzyme activities of perirenal adipose tissue were higher than all subcutaneous areas at 3.5 months and thereafter were higher than OS but were not significantly different from MS or IS.

DISCUSSION

Cellular development

The cellular development of adipose tissue in rats and humans has been studied extensively in recent years largely because of the development of an accurate technique by Hirsch and Gallian (18) for the separation and counting of individual adipose cells from adipose tissue matrix (29, 31–36). The advantages and limitations of this osmium tetroxide fixation procedure compared with previous methods, which extrapolated cellular development from histological studies and adipose tissue DNA changes, have been discussed (18, 29, 33).

The cell fixation data of this experiment indicate that the cellular development of pig adipocytes is similar to that found for the rat (33, 35) and the mouse (36). At the younger ages (between 1 and 2 months), the increase in adipose tissue was due primarily to the introduction of new adipose cells (Fig. 3). Then, as the animal grew older, hyperplasia diminished in importance and the hypertrophy of existing cells assumed primary responsibility for the increase in adipose tissue mass. Between 5 and 6.5 months, essentially all of the adipose tissue increase was due to increase in cell size. The recent work of Hood and Allen[2] also shows that a plateau in adipose cell number is reached between the ages of 5 and 6 months in the normal miniature pig. It should be remembered, however, that cells smaller than 25 μm in diameter were not counted by this procedure. Thus, at the younger ages it is possible that a substantial number of adipocytes less than 25 μm could have raised the average cell size erroneously high and underestimated the true cell number. These considerations, however, do not alter the conclusion that at later stages of growth the increase in adipose tissue mass was primarily the result of adipose cell hypertrophy.

In the rat, adipose cell number plateaus at about 15 wk of age and then remains constant to adulthood or to a point at which a relatively stable body weight is reached (33). Whether or not the plateau in cell number at 5 months is the maximum attainable number of cells in the pig is uncertain. It seems possible that if the pig were to continue to grow and add large amounts of lipid to its body composition, at some point cell size would become so large that hyperplasia would again be necessary.

Enzyme activity measurements

On a wet tissue basis, the growth changes in lipogenic enzyme activities were similar to those reported for hepatic lipogenesis in the rat and mouse (1–3, 5). Enzyme activities increased markedly after weaning and thereafter decreased with advancing age. The dramatic increases in lipogenic enzyme activities and in vitro lipogenesis after weaning have been shown to be due to a change in diet from milk, which contains a high quantity of fat, to a standard rat or mouse chow, which is relatively low in fat and high in carbohydrate (3). Consequently, after weaning, the enzyme systems adjust to the synthesis of fat from excess dietary carbohydrate.

A decline in the rate of lipogenesis (tissue weight basis) after weaning has been shown in this paper for porcine adipose tissue and has also been shown for adipose tissue of the rat (6–8). From a metabolic standpoint it did not seem logical that the rate of lipogenesis should decrease during the period when fat was being accumulated at a rapid rate. Part of the reason for this apparent contradiction is the method used to express metabolic activity. As pointed out by Salans, Knittle, and Hirsch (10) and by Greenwood et al. (11), the expression of metabolic activity on a cellular basis is much more meaningful than on a tissue weight basis, particularly for adipose tissue because of its variable lipid content. Therefore, in this experiment, the enzyme activities were expressed per adipose cell by dividing the activity per gram of tissue by the number of adipose cells per gram of tissue. A calculation of this type was considered to be a reliable expression because Rodbell (37) has demonstrated that the adipocytes are responsible for essentially all the lipogenic activity of adipose tissue. That is, the stromal portion of adipose tissue has virtually no lipogenic capacity.

On an adipose cell basis, the enzyme activities at 6.5 months were generally as high or higher than the activities immediately after weaning (2 months). In addition, there was a marked increase in enzyme activities between 3.5 and 5 months. These increases were particularly sharp in the middle and inner subcutaneous areas and corresponded to (*a*) a period of rapid growth of these two depots (Table 2); (*b*) a period when middle subcutaneous adipose cell size increased at its most rapid rate (Fig. 3); and (*c*) a period when total carcass adipose tissue relative to muscle mass made the largest increase of any period except during suckling (Fig. 2). The increases between 3.5 and 5 months were not detected when enzyme activities were expressed on a soluble supernatant protein basis.

When calculated for the entire adipose tissue mass, activities increased up to 5 months, after which all activities decreased except that of the citrate cleavage enzyme. This general decrease in enzyme activity between 5 and 6.5 months (total adipose tissue enzyme activity as well as activity per adipose cell) was accompanied by a continued rapid deposition of body fat

[2] Hood, R. L., and C. E. Allen. Unpublished results.

(11.9 kg between 5 and 6.5 months as compared with 10.9 kg between 3.5 and 5 months). If this decrease in enzyme activity is indicative of a decrease in the in vivo lipogenic capacity of the tissue, then these two results at first glance appear to be incompatible. One possible explanation, however, is that the decrease in enzyme activities may have been accompanied by a more pronounced decrease in turnover of fat. Thus, even though the capacity for lipogenesis has decreased, a concomitant decrease in fat catabolism could have allowed for a net accumulation of fat.

Benjamin et al. (6) have shown that the epinephrine-stimulated lipolysis decreases with age in rats, and Forn et al. (38) have offered even more convincing evidence that enzymes influencing lipolysis change during growth in the direction of decreased lipolysis and decreased turnover of adipose tissue triglyceride in older rats. Anderson, Kauffman, and Benevenga (39) have estimated that the turnover of fatty acids in pigs 8 to 12 months old is a very slow process ($t_{1/2}$ = 300 days). It is possible, therefore, that the catabolism of fatty acids in younger pigs is substantially faster.

It was also found that the accumulation of carcass fat in the pig was not a uniform process in all adipose tissue sites in the body. Simply by measuring the thickness of the three subcutaneous backfat layers (Table 2), it was noted that IS increased in thickness ninefold (0.84 cm) between 3.5 and 6.5 months, MS increased over twofold (0.80 cm) during the same period, but OS increased by only 50% (0.36 cm). These differences subjectively correlated with some of the biochemical and cellular measurements made on the tissues. For example, MS and IS had substantial increases in enzyme activities between 3.5 and 5 months that were not found in OS. Between 5 and 6.5 months, MS and IS activities remained significantly higher than OS activities. In addition, the soluble protein concentration when expressed on a cellular basis increased quite markedly between 3.5 and 5 months in the MS and IS but did not increase in the OS. Thus, the data indicate that not all areas of adipose tissue develop at the same rate and emphasize the importance of a thorough examination of adipose tissue variability in other species and a more definitive study of adipose tissue anatomy in the pig, particularly the proportions of outer subcutaneous to the more metabolically responsive middle and inner layers.

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