Lipogenic enzyme activities and cellularity of porcine adipose tissue from various anatomical locations

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Abstract The activities of acetyl CoA carboxylase, citrate cleavage enzyme, malic enzyme, glucose-6-phosphate dehydrogenase, and 6-phosphogluconate dehydrogenase were determined in porcine adipose tissue samples taken from seven anatomical locations, including three layers of backfat, intermuscular, perirenal, mesenteric, and leg subcutaneous adipose tissues. Adipocyte size and number, as well as lipid and soluble protein content, were also measured in order to establish some of the differences that exist between different areas of porcine adipose tissue. It was found that adipose tissue from areas where fat is deposited very readily (particularly the perirenal region) had higher enzyme activities, larger adipose cells, a lesser amount of stromal tissue, a greater amount of ether-extractable lipid, and a lower concentration of adipose cells per gram of tissue than samples from areas where fat is deposited only sparsely (leg subcutaneous).

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

The domestic pig (*Sus domesticus*), like other animals, deposits fat heavily in some areas and only sparsely in others. Locational differences in fat deposition have been recognized for many years in both animals and man (1). Benjamin et al. (2) found that there were significant differences in the metabolic activity (incorporation of [^{14}C]acetate into mixed lipids) of adipose tissue samples from various anatomical locations in the rat.

It was the purpose of this experiment to determine if porcine adipose tissue samples from different anatomical locations possessed different levels of activity of selected lipogenic enzymes and, if differences did exist, to determine whether they were due to differences at the level of the adipose cell or if they were due, rather, to differences in the degree of cellularity at various adipose tissue locations; that is, to determine if the low enzyme activity of a given adipose tissue sample was due to a low enzyme content of the adipose cells or to a lesser number of adipose cells in that sample.

A comparison of the lipogenic capacity of adipose cells obtained from areas where fat is deposited very readily relative to those obtained in areas where fat is deposited only sparsely was of primary interest. In addition, comparisons of outer and middle layers of subcutaneous backfat were of special interest to determine the feasibility of using a simple needle biopsy technique (3) for metabolic studies of porcine subcutaneous fat. It is difficult to separate samples of outer and middle subcutaneous adipose tissue taken by this biopsy procedure; therefore, the technique can be used only if the two layers are found to have similar metabolic activity. The fatty acid composition of the two layers has been shown to be distinctly different, the outer layer having a higher proportion of unsaturated fatty acid than the middle layer (4, 5). Metabolic differences between the two areas have not been reported.

Differences in adipose cell size were also determined because it has been suggested by a number of workers that some aspects of adipose tissue metabolism, particularly the rate of lipogenesis and insulin responsiveness, are closely associated with adipocyte size (6–9). In addition, the amounts of lipid and soluble protein in adipose tissue samples were determined so that enzyme activities could be expressed in four different ways for comparative purposes: per gram of wet tissue, per 10^6 adipose cells, per milligram of soluble protein, and per gram of lipid.

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Similar results were obtained when activities were expressed on a soluble protein basis as when expressed on an adipose cell basis.

EXPERIMENTAL

Materials

The following chemicals were obtained from the designated sources: adenosine triphosphate (ATP) and acetyl coenzyme A, P-L Biochemicals, Inc., Milwaukee, Wis.; dithiothreitol, Calbiochem, Los Angeles, Calif.; reduced glutathione (GSH), bovine serum albumin (BSA), tris(hydroxymethyl)aminomethane (Tris), reduced nicotinamide adenine dinucleotide (NADH), nicotinamide adenine dinucleotide phosphate (NADP), glucose-6-phosphate (Na^+) (G-6-P), 6-phosphogluconate (Na^+) (6-PG), L-malic acid (Na^+), coenzyme A (CoA), and collidine (2,4,6-trimethylpyridine), Sigma Chemical Co., St. Louis, Mo.; malate dehydrogenase (MDH), Boehringer Mannheim Corp., New York; Folin phenol reagent, Fischer Scientific, Chicago, Ill.; sodium [^{14}C]-bicarbonate, New England Nuclear, Boston, Mass.; other chemicals, of analytical grade, Mallinckrodt Chemical Works, St. Louis, Mo., or J. T. Baker Chemical Co., Phillipsburg, N.J. Nylon screen, Nitex H. C. 250 (pore size 250 μm) and Nitex H. C. 25 (pore size 25 μm), was purchased from Handler, Tobler, Ernst, and Traber through Kressilk Products, Des Plaines, Ill. Nalgene filter units (without the usual fine membrane) were purchased from Wilkens-Anderson Co., Chicago, Ill.

Procedure

Five littermate Yorkshire × Poland China crossbred male castrate pigs were fed a 12% protein and 4% fat diet formulated from corn, soybean meal, and meat scraps plus mineral and vitamin supplementation. Animals were conditioned to meal eating for the 2 months preceding the experiment (excess feed for a period of 2 hr each day). Animals were slaughtered 2 hr after feeding (10 AM) at the approximate weight of 170 kg. The animals had a thick backfat (4.4 cm) and large quantities of perirenal fat.

Immediately after killing the animals, samples of adipose tissue were taken from each of seven different anatomical areas that included three layers of subcutaneous backfat located near the 10th–11th costal area: outer subcutaneous (OS), middle subcutaneous (MS), and inner subcutaneous (IS). Each layer is separated by a distinct sheet of fascia. OS and MS make up the majority of all body fat (10). Intermuscular adipose tissue (IM) was taken from the thoracic region between the serratus ventralis and pectorales profundus muscles; perirenal adipose tissue (PR) was taken laterally from the kidney; mesenteric adipose tissue (M) was taken from around the intestines; and subcutaneous adipose tissue was taken from the lower medial portion of the hind leg (L), a place where little fat is deposited.

A 3-g portion of the tissue from each adipose site was homogenized in 9 ml of 0.15 M KCl in 50 mM Tris–HCl, pH 7.4, with a Polytron PT-10 homogenizer for 20 sec. The homogenate was centrifuged at 40,000 *g* for 1 hr. The supernatant fluid was decanted and all enzyme determinations were made on it. A determination of protein content of the supernate was made by the method of Lowry et al. (11). Another portion of the tissue was dried in a vacuum oven and extracted with diethyl ether on a Goldfisch apparatus. To determine the number of adipose cells, a third portion was treated with 2% osmium tetroxide in 50 mM collidine buffer (pH 7.4) by the method of Hirsch and Gallian (12). The osmium tetroxide reacts with lipid material, thus fixing the entire adipose cell. The fixed adipose cells fall loose and are separated from the surrounding stromal tissue by filtering through a 250-μm mesh nylon screen. The cells were trapped on a 25-μm mesh screen and washed with distilled water to remove any small particles. The cells were transferred to a 400-ml round bottom beaker and counted on a Coulter electronic counter to determine the number of cells per gram of tissue.

Enzyme assays

All assays were run consecutively immediately after centrifugation in the order of their decreasing lability (Fig. 1): acetyl CoA carboxylase (CBX), citrate cleavage enzyme (CCE), 6-phosphogluconate dehydrogenase (6-PGDH), glucose-6-phosphate dehydrogenase (G-6-PDH), and malic enzyme (ME).

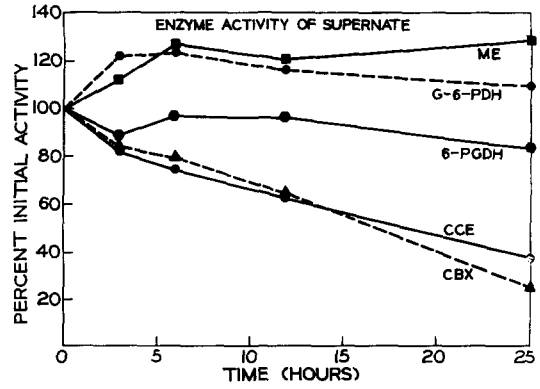

FIG. 1. Changes in enzyme activities of adipose tissue supernatant fraction kept at 0°C. Activities of malic enzyme (ME), glucose-6-phosphate dehydrogenase (G-6-PDH), 6-phosphogluconate dehydrogenase (6-PGDH), citrate cleavage enzyme (CCE), and acetyl CoA carboxylase (CBX) are expressed as percentages of the initial activity obtained immediately after centrifugation. Each point represents the average of two samples.

Acetyl CoA carboxylase (EC 6.4.1.2) was assayed by the method of Dakshinamurti and Desjardins (13). Concentrations of reagents were 3 mM GSH, 8 mM $MgCl_2$, 0.1 mM EDTA, 5 mM citrate (K^+), 0.6 mg/ml BSA, 5 mM ATP, 0.2 mM acetyl CoA, and 10 mM $NaH^{14}CO_3$ (0.4 μCi/μmole), with 0.75 ml of supernate in a final volume of 1.00 ml buffered in 50 mM Tris–HCl at pH 7.5. Reaction was started by adding ATP, acetyl CoA, and $NaH^{14}CO_3$ after a 30-min preincubation with citrate. (Preincubation for 20–40 min gave maximal activation.) A blank was run with no ATP. Aliquots (0.2 ml) were removed at 1 and 2 min and delivered into scintillation vials containing 0.3 ml of 0.3 M HCl. Last traces of $H^{14}CO_3^-$ were removed by adding dry ice to the vials. 18 ml of scintillation solution (14) was added after neutralization with 0.2 ml of 1 M Tris. Activity was calculated as nanomoles of $H^{14}CO_3$ fixed per minute.

Citrate cleavage enzyme (EC 4.1.3.8) activity was measured spectrophotometrically at 340 nm by the method of Srere (15) as adapted to adipose tissue by Kornacker and Ball (16). The assay mixture contained 10 mM $MgCl_2$, 4 mM dithiothreitol, 1 mM KCN, 2.5 IU of MDH, 20 mM citrate (K^+), 0.4 mM CoA, 0.2 mM NADH, 5 mM ATP, and 1 mM Tris–HCl at pH 7.3. The addition of 0.50 ml of supernate gave a final volume of 1.00 ml. Blank activity was determined in the absence of ATP. Initial velocity was determined after a 5-min preincubation by adding ATP to start the reaction.

Glucose-6-phosphate dehydrogenase (EC 1.1.1.49) and 6-phosphogluconate dehydrogenase (EC 1.1.1.44) activities were assayed by the double-substrate procedure of Glock and McLean (17). Reagent concentrations for the determination of the combined activities of both enzymes were 20 mM $MgCl_2$, 0.15 mM NADP, 1.0 mM G-6-P, 1.0 mM 6-PG, and 50 mM Tris–HCl, pH 7.6. G-6-P was omitted to determine the activity of 6-phosphogluconate dehydrogenase. In each case, 0.10 ml of supernate was added to give a final volume of 1.00 ml. The glucose-6-phosphate dehydrogenase activity was calculated by difference.

Malic enzyme (EC 1.1.1.40) was measured by the method of Ochoa (18), but with increased malate concentration. Concentrations of reagents were 1.0 mM $MnCl_2$, 0.15 mM NADP, 1.5 mM L-malate (Na^+), and 50 mM Tris–HCl at pH 7.4. Again, 0.10 ml of supernate was used, with a final volume of 1.00 ml.

The pH of the reagent mixture for each enzyme assay was determined to authenticate that the reagents were buffered adequately at the pH stated for each assay.

RESULTS

Adipose tissue samples from various anatomical locations differed significantly in enzyme activity content

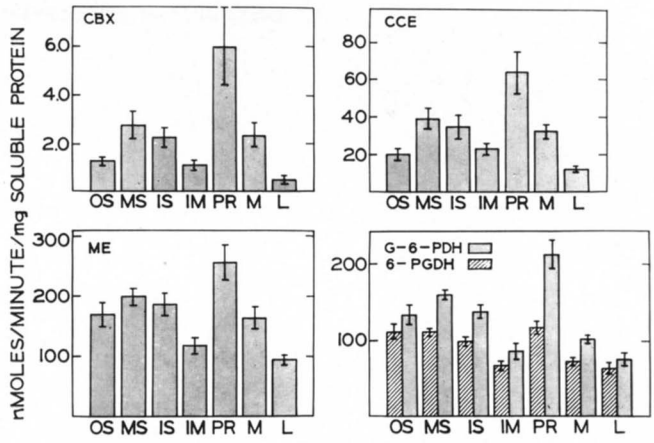

FIG. 2. Comparison of enzyme activities in adipose tissue from seven anatomical locations. The anatomical locations are: outer (OS), middle (MS), and inner (IS) subcutaneous backfat, intermuscular (IM), perirenal (PR), mesenteric (M), and leg subcutaneous (L) adipose tissue. Activities of acetyl CoA carboxylase (CBX), citrate cleavage enzyme (CCE), malic enzyme (ME), glucose-6-phosphate dehydrogenase (G-6-PDH), and 6-phosphogluconate dehydrogenase (6-PGDH) are recorded in nmoles/min/mg soluble supernatant protein ± SEM.

(Fig. 2). The differences between sites on a relative basis were most pronounced for acetyl CoA carboxylase (CBX) and citrate cleavage enzyme (CCE) activities. The relative differences were not as pronounced for malic enzyme (ME) and the dehydrogenases of the hexose monophosphate shunt, but the patterns of activities were similar to those observed with CBX and CCE.

For all enzymes, the activity for the subcutaneous adipose tissue of the lower leg (L) was lowest and that from the perirenal region (PR) was greatest. Activity differences between adipose tissue sites were compared statistically by Duncan's new multiple range test (19). Perirenal adipose tissue was significantly higher ($P < 0.01$) than all other sites in CBX, CCE, and ME activity. Glucose-6-phosphate dehydrogenase (G-6-PDH) and 6-phosphogluconate dehydrogenase (6-PGDH) activity of the perirenal region was significantly different from that of intermuscular, mesenteric, and leg subcutaneous regions, but was not different from that of outer, middle, or inner subcutaneous backfat.

The activities of the outer subcutaneous enzymes were generally lower than those from the middle layer. These differences were significant ($P < 0.05$) for CBX, CCE, and G-6-PDH. There was a similar trend for ME, but the difference was not significant at the 95% level of probability.

Differences among adipose tissue sites were affected by the method of expression of enzyme activity (Fig. 3). The activities of malic enzyme (3*A*) and citrate cleavage enzyme (3*B*) were chosen to demonstrate this effect. When activities were expressed on a soluble supernatant

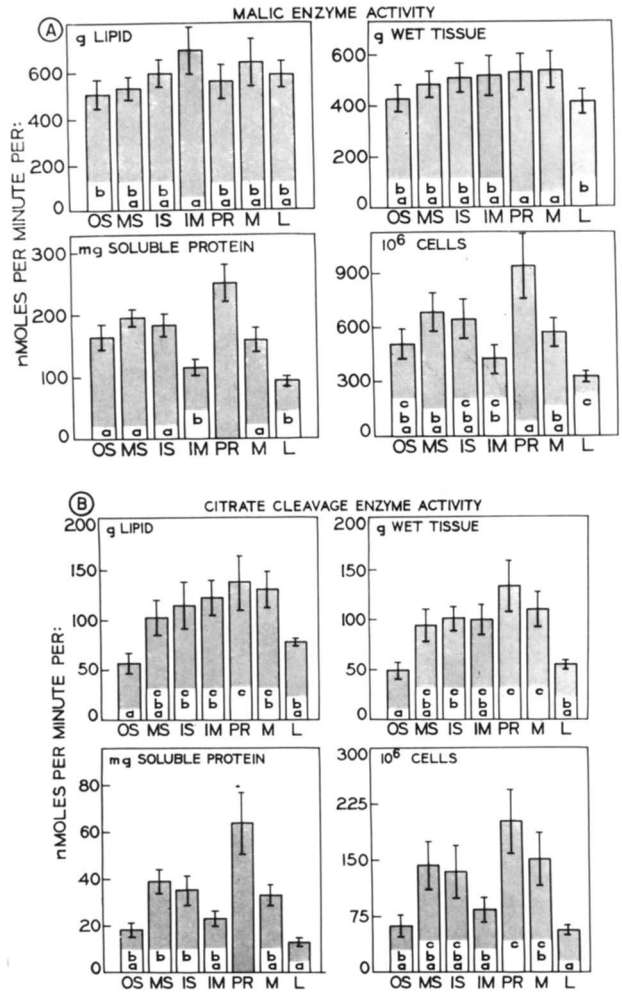

FIG. 3. Comparison of four methods for expressing malic enzyme activity (*A*) and citrate cleavage enzyme activity (*B*). Identical activity data are expressed in nmoles per min per: (*1*) g ether-extractable lipid, (*2*) g wet tissue, (*3*) mg soluble supernatant protein, and (*4*) 10^6 adipose cells. Each bar represents adipose tissue samples from a different anatomical location: outer (OS), middle (MS), and inner (IS) subcutaneous backfat, intermuscular (IM), perirenal (PR), mesenteric (M), and leg subcutaneous (L). Each value is the mean of five animals ± SEM. Activities in adipose tissue locations with the same letter within a graph are not significantly different ($P < 0.01$).

protein basis or on a cellular basis, differences among anatomical sites were most pronounced. If, however, the enzyme activity is expressed on an ether-extractable lipid basis or on a wet tissue basis, the differences are diminished and in some cases reversed. For example, intermuscular adipose ME activity was significantly higher than outer subcutaneous activity when expressed on a lipid basis but was significantly lower when expressed on a soluble protein basis. Further comparisons of the various methods for expressing enzyme activities are available for CBX, G-6-PDH, and 6-PGDH (20).

Soluble protein per gram of wet tissue and number of adipose cells per gram of wet tissue paralleled each other

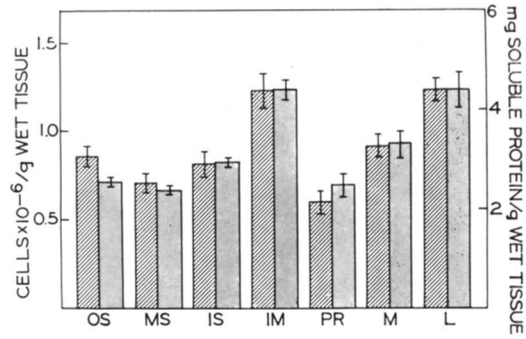

FIG. 4. Comparison of cellularity and soluble protein content of adipose tissue from seven anatomical locations. Soluble supernatant protein/g wet tissue (solid bar) is plotted against adipose cells × 10^{-6}/g wet tissue (striped bars) for each anatomical location: outer (OS), middle (MS), and inner (IS) subcutaneous backfat, intermuscular (IM), perirenal (PR), mesenteric (M), and leg subcutaneous (L). Simple correlation of individual values was 0.77. Each value represents the mean of five animals ± SEM.

quite closely at all adipose tissue locations (Fig. 4). The simple correlation of individual values was 0.77. That is, when the soluble protein per gram of tissue was low (e.g., perirenal), then the number of cells per gram of tissue was also low, and when the soluble protein was high (e.g., leg subcutaneous), cell number was also high. This finding confirms the recent work of Salans and Dougherty, who showed that the protein content of fat cells is relatively constant over a wide range of fat cell size (21).

Table 1 shows the differences in lipid content of porcine adipose tissue from different locations. Leg subcutaneous and intermuscular adipose tissues were lowest in lipid content, perirenal was the highest, and other areas were intermediate. Measurements of cell size followed a similar pattern (Table 2). Perirenal adipose tissue possessed the largest cells and leg subcutaneous and intermuscular the smallest; the other adipose locations were intermediate.

TABLE 1. Content of ether-extractable lipid of adipose tissue from seven anatomical locations

Adipose Location	% Lipid
OS	88.8 ± 0.5
MS	93.4 ± 0.7
IS	87.7 ± 1.8
IM	76.3 ± 1.4
PR	96.1 ± 0.4
M	84.0 ± 3.7
L	70.9 ± 4.2

Adipose tissue locations: outer (OS), middle (MS), and inner (IS) subcutaneous backfat, intermuscular (IM), perirenal (PR), mesenteric (M), and leg subcutaneous (L). Values are means of five animals ± SEM.

TABLE 2. Average diameter, volume, and surface area of adipose cells from seven anatomical locations

Adipose Location	Cell Diameter	Cell Volume	(μg TG[a]/Cell)	Cell Surface Area
	μm	$\mu m^3 \times 10^{-4}$		$\mu m^2 \times 10^{-3}$
OS[b]	129 ± 3.3	125 ± 10.3	(1.04)	54.3 ± 2.9
MS	134 ± 1.1	144 ± 22.3	(1.31)	59.4 ± 0.8
IS	131 ± 3.5	131 ± 11.5	(1.08)	56.0 ± 3.2
IM	108 ± 4.5	76 ± 9.4	(0.62)	38.9 ± 3.2
PR	153 ± 6.8	221 ± 31.9	(1.60)	78.0 ± 7.3
M	123 ± 3.3	110 ± 8.7	(0.92)	50.0 ± 2.7
L	106 ± 2.9	71 ± 6.5	(0.58)	37.3 ± 2.2

Diameters of osmium-fixed cells were measured by an eyepiece micrometer on a light microscope. Volume ($^1/_6\pi d^3$) and surface area (πd^2) were calculated from the diameter of each cell. Determinations were made on 80 cells/animal. Values are means of five animals ± SEM. The micron measurements are approximately 5–10% high, caused by a small amount of adipose cell swelling during the osmium fixation. The volumes in parentheses (μg TG/cell) were calculated by the method of Hirsch and Gallian (12).

[a] TG, triglyceride.

[b] Abbreviations as in Table 1.

DISCUSSION

Compositional differences among adipose tissues at various anatomical locations have been well documented (2, 5, 22, 23). Fatty acids from adipose tissue of surface areas are more unsaturated than those from inner body areas. This phenomenon has been attributed to a temperature adaptation of the adipose tissue in an attempt to maintain the physical fluidity of fat at all body locations (24). Thompson and Allen (25) have shown that stearic acid desaturase activity is higher in outer subcutaneous than in perirenal adipose tissue and may account for the difference in fatty acid composition.

Structural differences in adipose tissues from various anatomical locations have also been reported (1). In this experiment it was found that the extent of the connective or stromal tissue framework varied at different locations. The leg subcutaneous was composed of more stromal tissue than any other area, especially perirenal adipose tissue, as measured by the subjective evaluation of the amount of connective tissue separated on the 250-μm screen during the preparation of the osmium-fixed cells. In addition, Table 1 indicates that the nonlipid portion of the leg subcutaneous adipose tissue was 29% compared with only 4% for the perirenal tissue. Further studies are needed, however, to establish the exact nature of this nonlipid portion.

Cellularity (adipose cells/gram of wet tissue) differences were also evident (Fig. 4), but they were opposite from what might be expected. It was found that leg subcutaneous tissue, an area where fat is deposited only sparsely, has the highest concentration of adipose cells, and perirenal tissue, an area where fat is deposited very readily, has the lowest concentration. It is obvious that if leg subcutaneous tissue has the largest amount of stromal tissue and also the highest concentration of adipose cells, the cells themselves must necessarily be smaller. This was in fact found to be true (Table 2). The volume of leg subcutaneous adipose cells is only one-third that of perirenal adipose cells. A similar heterogeneity in the cellularity of various rat adipose depots has recently been reported by Di Girolamo, Mendlinger, and Fertig (26).

Metabolic differences in the lipid synthesizing capacity of various adipose tissue depots have been reported for the rat and man (2, 27, 28). Benjamin et al. (2) and Gellhorn and Marks (27) have demonstrated differences in the lipogenic capacity of various depots in the rat and in man, respectively. However, the recent work of Goldrick and McLoughlin (28) showed that smaller human omental cells had virtually the same rate of fatty acid synthesis from glucose as the larger subcutaneous fat cells. Definite differences in the enzyme activities of various porcine adipose tissue locations were found in the present experiment. Enzyme activities were highest in perirenal and lowest in leg subcutaneous. The differences were most apparent for the enzymes directly involved in the conversion of glucose carbon into fatty acids, i.e., acetyl CoA carboxylase and citrate cleavage enzyme. The differences were not due to the number of adipose cells because leg subcutaneous tissue had a higher concentration of adipose cells than perirenal tissue. They appeared instead to be due to differences in activity at the level of the adipose cell. The enzymatic results are in agreement with the evidence suggesting that lipogenesis in adipose tissue is related to adipocyte size (8). The activities of the measured enzymes were, in general, proportional to the cell size.

The importance of choosing the most appropriate

method for expressing metabolic parameters in adipose tissue has been discussed adequately by others (7, 29) and is shown graphically in Figs. 3*A* and 3*B*. Expressing data on a tissue weight or lipid basis diminished the differences that existed among adipose tissue areas. Larger cells had greater enzyme activities but fewer cells and more lipid per unit weight of adipose tissue. Expressing data on a cell volume or cell surface area basis would also result in a similar normalization of all adipose tissue areas, since a cell's activity was in general proportional to its size. The authors feel that the most meaningful method of expressing enzyme activity of adipose tissue would be on a cellular basis. In addition, however, the results indicate that comparisons of adipose tissue areas on a soluble protein basis gave results similar to those made on a cellular basis.

Since it was found that the larger cells have higher enzyme activities, it is tempting to postulate that they deposit fat more rapidly and thus enlarge more rapidly than smaller cells due to an increased capacity to synthesize fatty acids. If this were in fact true, the enzyme activities in all areas of adipose tissue would normalize or equalize on a tissue weight basis as the animal grew older and deposited more fat. That is, as fat is deposited, the fat cells with higher activities may increase in size more rapidly and consequently result in fewer cells per unit area of adipose tissue. Thus, various adipose areas would have similar activities per unit weight even though the individual cells at different sites may have widely varying activities.

Di Girolamo et al. (26) have shown that fat cell populations become progressively more homogeneous in size with advancing development and increased cell diameter, suggesting that the fat cells may have a limit for cell expansion which may be dictated by intercellular or intertissue controlling mechanisms. The point at which the rates of lipogenesis in all areas equalize may exert an influence on cell expansion and also may have some implications in the study of food intake patterns, mature fat distribution, and other problems related to the subject of body weight. It must be emphasized, however, that lipogenic enzyme levels are not necessarily indicative of the extent of in vivo or in vitro lipogenesis from glucose. The role of substrate supply, cellular and subcellular permeability to substrates and intermediates, the regulation of existing enzymes by substrates, cofactors, activators and inhibitors, as well as other factors, must also be considered in the total analysis.

In conclusion, porcine adipose tissue from different anatomical areas varies significantly in enzyme activity and cellularity. Adipose tissue samples from areas where fat is deposited very readily (especially perirenal) have higher enzyme activities, larger adipose cells, a lesser amount of stromal tissue, a greater amount of ether-extractable lipid, and a lower concentration of adipose cells per gram of tissue than samples from areas where fat is deposited only sparsely (leg subcutaneous). In addition, the results showed that soluble protein per gram of adipose tissue is proportional to the number of adipose cells per gram of adipose tissue and that enzyme activity expressed on a soluble protein basis is a close approximation of activity on a cellular basis. However, the relationship between soluble protein and adipose cell concentration is not uniform at all ages.[1]

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