Effects of cell size and animal age on glucose metabolism in pig adipose tissue[1]

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Abstract Adipose tissue slices were prepared from middle subcutaneous or perirenal adipose tissue excised from pigs of different ages (and obesity) and incubated with [U-^{14}C]glucose. After incubation, the slices were fixed with osmium tetroxide and separated into diameter ranges of 20–63, 63–102, and 102–153 μm, respectively. Following determination of cell size and number, the fixed adipocytes were decolorized with H_2O_2 prior to quantification of glucose conversion to total lipid, glyceride fatty acids, glyceride-glycerol, and CO_2. Glucose conversion to total lipid or CO_2 was unaffected by the presence of purified porcine insulin (0, 10, 100, 1000, and 100,000 μU/ml). Within animals, adipocytes of different sizes were not different with regard to insulin sensitivity. Within a weight (age) group, conversion of glucose to total lipid (insulin present) or to glyceride fatty acids and glyceride-glycerol (insulin absent) per cell was significantly greater in large adipocytes compared to small adipocytes, regardless of the group examined. With increasing weight or age, there was a markedly decreased conversion of glucose to total lipid and glyceride fatty acids among adipocytes of similar size within a cell-size fraction. The diminution in glucose metabolism was greater (as a percentage) in 20–63 μm adipocytes than for 63–102 or 102–153 μm adipocytes. However, for all cell-size fractions there was a marked decrease in glucose conversion to fatty acids. Glyceride-glycerol synthesis was impaired in adipocytes from older pigs, but the decrease was less than observed for glyceride fatty acid synthesis.—**Etherton, T. D., E. D. Aberle, E. H. Thompson, and C. E. Allen.** Effects of cell size and animal age on glucose metabolism in pig adipose tissue. *J. Lipid Res.* 1981. **22:** 72–80.

Supplementary key words obesity · cellularity · lipogenesis · age

During normal postnatal growth, accretion of adipose tissue in the pig is quite rapid and extensive. For example, the carcass of the new-born pig contains 1–2% lipid; at 2–3 weeks of age, 12–15% lipid is present; and by 6 months of age the carcass may contain 30–48% lipid (1). The preeminent site for storage of triacylglycerol in the pig is the subcutaneous adipose tissue.

Little information is available about glucose metabolism of the pig adipocyte and the changes which occur with advancing animal age or adipocyte enlargement. Enzymatic (1) or radiolabeled glucose experiments (2) have shown that conversion of glucose carbon to total lipid or CO_2 reaches a maximum at 4–5 months of age and thereafter declines. However, these data were expressed on a tissue weight basis. Data expressed on a tissue weight basis among animals of different age and obesity are confounded by the considerable variations in cell size and, therefore, are more equivocal than data expressed per cell.

The effects of animal age and obesity have been extensively examined in studies conducted with rat and human adipocytes. The ability of insulin to stimulate glucose oxidation and fatty acid synthesis in larger adipocytes from older, more obese rats is markedly impaired when comparisons are made with adipocytes from younger, leaner animals (3–6). Glycerol synthesis, however, is relatively unaffected and, in some instances, it has been shown to be greater in large adipocytes from older, more obese rats (4, 6). However, these studies are complicated by the unknown contribution of animal age and the degree of obesity.

Studies examining size and metabolic activity of human adipocytes are less extensive than rat studies and have been severely limited by a number of variables that are difficult to control. In one report (7), increasing adipocyte size was associated with unchanged rates of glucose oxidation, but was associated with an enhanced incorporation of glucose into

Abbreviations: GFA, glyceride fatty acids; GG, glyceride-glycerol; MSQ, middle subcutaneous; P, perirenal; KRB, Krebs-Ringer bicarbonate.

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glyceride-glycerol in the absence of insulin. Other studies (8, 9), have not noted any differences in lipid or CO_2 production from glucose which were related to increases in cell size. Davidson (10) reported that glucose oxidation (basal and insulin-stimulated) was higher in adipose tissue from obese subjects. Thus, it does not appear that any uniform conclusions can be drawn from these reports on human adipocytes and their metabolism of glucose.

There have been no reports in the literature which have examined the effects of age and obesity in the pig upon adipocyte metabolism with particular emphasis on comparisons of adipocytes of similar size from pigs of different age or obesity. It would be desirable to compare the metabolic activity of adipocytes of different size from a discrete anatomical region, as well as that among adipocytes of similar size from animals of dissimilar age or obesity. This would segregate the previously confounded effects of age or obesity on the metabolic alterations associated with changes in adipocyte size. A recently developed technique (11) permits the separation of adipocytes (with negligible cellular rupture) into different size fractions. With the advent of this technique, it is possible to compare the metabolic capacity of similar or dissimilar size adipocytes to utilize glucose. Using this technique, the conversion of glucose carbon into CO_2, glyceride fatty acids, and glyceride-glycerol by pig adipocytes was measured.

MATERIALS AND METHODS

Animals

In the first experiment, the incorporation of glucose into total lipid in various cell-size fractions prepared from middle subcutaneous (MSQ) and perirenal (P) adipose tissue from different age pigs was examined. For this experiment three male castrate pigs of Hampshire × Yorkshire breeding from each of six litters were randomly allocated to three experimental groups. Average weight at this time was approximately 16 kg. The second experiment was conducted to measure the conversion of glucose to GFA, GG, and CO_2 in adipocytes of similar and dissimilar size from MSQ adipose tissue. Twenty-four male castrate pigs of similar breeding from eight litters were randomly allocated to three experimental groups at approximately 8 weeks of age (average weight was 16 kg). Pigs were fed ad libitum a corn and soybean meal diet with appropriate mineral and vitamin supplementation. Protein content of the diet was 16% until the pigs reached 34 kg of body weight and 14% thereafter.[4] Pigs in Group I, II, and III of Experiment 1 were studied at 45, 91, and 136 kg, respectively, whereas, pigs in Experiment 2 were studied at similar weights of 56, 94, and 124 kg. Animal age, weight, and backfat thickness are presented in **Table 1.** Four male castrate pigs (90 kg) were used for preliminary studies to examine the in vitro responsiveness of pig adipose tissue to insulin. All pigs were fed ad libitum until 1 hour before death.

TABLE 1. Age, weight, and carcass data of experimental animals[a]

	Age[b]	Weight	Backfat Thickness[c]
	(days)	*(kg)*	*(cm)*
Experiment I			
Group I	107 ± 1	45 ± 0.9	2.74 ± 0.10
Group II	171 ± 4	91 ± 0.5	4.01 ± 0.20
Group III	233 ± 5	138 ± 2.5	5.28 ± 0.25
Experiment II			
Group I	111 ± 1	57 ± 0.8	3.0 ± 0.2
Group II	155 ± 1	94 ± 1.3	4.4 ± 0.1
Group III	201 ± 1	124 ± 2.4	5.2 ± 0.3

[a] Values are means ± SEM for six (Experiment I) or eight (Experiment II) pigs within an age group.
[b] Animal age at day of killing.
[c] Backfat thickness dorsal to the first rib.

Tissue preparation

Perirenal and middle subcutaneous adipose tissue samples (excised dorsal to the first rib) were obtained following exsanguination and placed in 37°C Tris-sucrose buffer (30 mM Tris-HCl, 0.3 M sucrose, 1 mM EDTA, 1 mM glutathione; pH 7.4 at 37°C).

Slices weighing 50–140 mg of 200–300 μm in thickness were cut, rinsed twice in 37°C Tris-sucrose buffer without EDTA and placed in 25-ml Erlenmeyer flasks for subsequent incubation. Elapsed time from sampling to commencement of the last incubation was routinely no longer than 10 min. Conversion of glucose to total lipid increased linearly when slices weighing 50–140 mg were incubated (data not shown).

Tissue incubation

Duplicate adipose tissue slices were incubated in 25-ml Erlenmeyer flasks which contained 3 ml of

[4] Composition of diets as a percentage: corn, 77.8 (16% protein diet); corn 83.3 (14% protein diet); soybean oil meal, 20 (16% protein diet); soybean oil meal, 11.5 (14% protein diet); dehydrated alfalfa meal, 3; dicalcium phosphate, 1; ground limestone, 0.75; salt, 0.50; mineral mix, vitamin-antibiotic mix. Calculated % fat, 3 for both diets. Composition of mineral mixture provided the following (mg/kg diet): Zn, 100; Fe, 50; Cu, 1.38; Co, 0.5; Mn, 27.5; I, 0.075. Composition of vitamin-antibiotic mixture provided the following (per kg diet): riboflavin, 1.1 mg; calcium pantothenate, 4.4 mg; niacin, 11 mg; vitamin A, 2200 IU; vitamin B_{12}, 11 μg; antibiotic (either oxytetracycline or chlorotetracycline), 22 mg.

Krebs-Ringer bicarbonate (KRB) buffer, pH 7.4, (12). The KRB buffer contained one-half the recommended Ca^{2+} concentration with 30 mg of defatted and purified bovine serum albumin present per ml. Bovine serum albumin (Sigma Chemical Company, St. Louis, MO) was treated with activated charcoal at pH 3 to remove any endogenous fatty acids (13). After the removal of fatty acids, the albumin was dialyzed against 20 volumes of distilled water for 3 days at 4°C (14). After dialysis the albumin was lyophilized and stored at −20°C until used. Unless indicated otherwise in table or figure footnotes, the KRB buffer contained 10 μmoles of glucose (V_{max} for substrate concentration) and 0.3 μCi of [U-^{14}C]glucose (New England Nuclear) per ml. In Experiment I, incubations contained 0.1 IU insulin[5] per ml. Insulin was omitted in Experiment II, since no effect could be demonstrated. After the tissue slice was added to the flask, a 10 × 34 mm glass vial containing a strip of filter paper was added for subsequent $^{14}CO_2$ collection. Each flask was flushed with O_2–CO_2 95:5, sealed with a rubber serum cap, and incubated at 37°C (15) for 2 hr in a shaking water bath. Preliminary experiments documented that glucose conversion to total lipid or CO_2 increased in a linear fashion during a 2-hr incubation. The reaction was stopped by injection of 0.25 ml of 1 N H_2SO_4 into the buffer after 0.1 ml of 25% KOH had been injected into the 10 × 34 mm glass vial. After the addition of H_2SO_4, the flasks were returned to the water bath and shaking was continued for 1 hr to collect the $^{14}CO_2$. Acid-volatile ^{14}C collected from parallel incubations in the absence of any tissue was measured and subtracted from the $^{14}CO_2$ trapped.

Adipocyte sizing and counting

Following $^{14}CO_2$ collection, tissue slices were rinsed three times in 37°C 0.154 M NaCl, blotted carefully, and weighed. The procedure for fixation and isolation of OsO_4-fixed adipocytes was previously described by Etherton, Thompson, and Allen (11).

Osmium-fixed adipocytes are not susceptible to breakage which greatly facilitates separation of fixed adipocytes into fractions of different size. Because of the difficulties in quantifying adipocytes less than 20 μm in diameter with a Coulter Counter, the smallest adipocytes counted were equal to or greater than 20 μm. Adipocytes were screened into three diameter fractions: 20–63 μm, 63–102 μm, and 102–153 μm. Very few adipocytes greater than 153 μm in diameter were observed in Experiment I. A small number of adipocytes greater than 153 μm in diameter was observed in Experiment II; however, the small quantity precluded quantification of substrate incorporation. Separation of cells into diameter fractions was achieved by filtering the adipocyte suspension through serial nylon mesh screens with mesh opening of 253, 153, 102, 63, and 20 μm, respectively. The 253-μm screen was utilized to remove any clumps or debris.

Adipocytes in each size fraction were suspended in saline and were counted with a Model B Coulter Counter (Experiment I) or a Model ZB Coulter Counter equipped with a Coulter Channelyzer, Log Amplifier and a X-Y Recorder II plotter (Experiment II). Adipocytes in all size fractions were counted and sized with a 400-μm aperture. Diameter distributions were determined at 10-μm intervals from volume measurements. Diameter distributions were then calculated by pooling cell number in each diameter range for all three fractions. Total adipocyte number and the percentage of adipocytes in each 10 μm interval were determined and the distribution was plotted as a histogram. All adipocyte number determinations were corrected for coincidence of counting.

Average adipocyte diameters for respective cell-size fractions and depot sites were calculated. Separation of cell-size fractions was quite satisfactory with less than 10% of the adipocytes within a size fraction outside the designated diameter ranges. For MSQ adipocytes, the mean diameter of adipocytes for both experiments across age groups was 44, 86, and 111 μm for the 20–63, 63–102, and 102–153 μm cell-size fractions, respectively. These mean diameters were very similar to those for the P adipocytes studied in Experiment I. Within a cell-size fraction across age groups, adipocyte size was similar (data not shown).

Quantification of ^{14}C incorporation

Decolorization of fixed adipocytes and measurement of glucose conversion to total lipids or fatty acids and glyceride-glycerol in each cell-size fraction was conducted as described (11) with the following modification. After evaporation of $CHCl_3$ from the total lipid fraction, 6 ml of hexane was added. In those experiments where glucose conversion to total lipid was measured, a 2-ml portion of the hexane extract was transferred directly to a scintillation vial and the hexane was evaporated under N_2. Ten ml of toluene scintillation fluid was added and the samples were counted in a liquid scintillation counter. In Experiment II, a second 2-ml aliquot of the hexane extract was transferred to a test tube and washed

[5] Purified crystalline porcine insulin was generously supplied by Dr. Ronald Chance of Eli Lilly Company, Indianapolis, IN.

three times with one volume of freshly prepared 50 mM $NaHCO_3$ in ethanol–water 1:1 (v/v) to remove any ^{14}C-labeled unesterified fatty acids (16). Measurement of ^{14}C incorporation into GFA or GG was then performed as described previously (11). The glyceride-glycerol samples were counted in toluene-Triton scintillation fluid (2 volumes toluene/1 volume Triton X-100; 6 g 2,5-diphenyloxazole and 75 mg 1,4-bis-2-(4-methyl-5-phenyloxazoyl per liter). Corrections for quenching were conducted using the external standard ratio procedure. Appropriate background samples were run in parallel with each experiment.

Expression of results

In preliminary studies, the data were expressed per cell or per gram of tissue. In the latter instance, the pigs were of similar age and obesity and the adipocyte number per gram of tissue did not differ. For all subsequent experiments, conversion of glucose to total lipids or GFA and GG was expressed in three different ways. In the first, incorporation of glucose was expressed as nmoles $\times 10^{-6}$ per cell within a given cell-size fraction. Data were also expressed as nmoles glucose incorporated/fraction per 10^6 adipocytes in the total cell distribution, computed as follows. The percentage of adipocytes within a given cell-size fraction was determined relative to the total distribution by dividing the number of adipocytes in a respective cell-size fraction by the total number of adipocytes. The resulting percentage was then adjusted to represent the absolute number of adipocytes present within a cell-size fraction if 10^6 adipocytes over the entire distribution had been counted. This value was then multiplied by the incorporation per cell within each fraction (nmol $\times 10^{-6}$/cell). The last method of data expression was glucose incorporation per unit cell surface area, which was derived from volume measurements.

Statistical analyses

Simple linear regression was performed according to procedures outlined by Steel and Torrie (17). Statistical differences within a cell-size fraction were determined by first computing a one-way analysis of variance, and then if the F value for treatment effects was significant ($P < 0.05$), a Least Significant Difference (LSD) procedure for means was used (17).

Tests of statistical significance among cell-size fractions within an age group were performed by analysis of repeated measurements. Because the different cell-size fractions from an animal originated from the same tissue slice, the metabolic rates among the fractions should be correlated. To account for this correlation among animals, Hotelling's T^2 statistic was calculated and converted to an F value to discern differences among cell-size fractions within an age group. This multicomparison method is an extension of the Scheffe technique for repeated measurement designs (18).

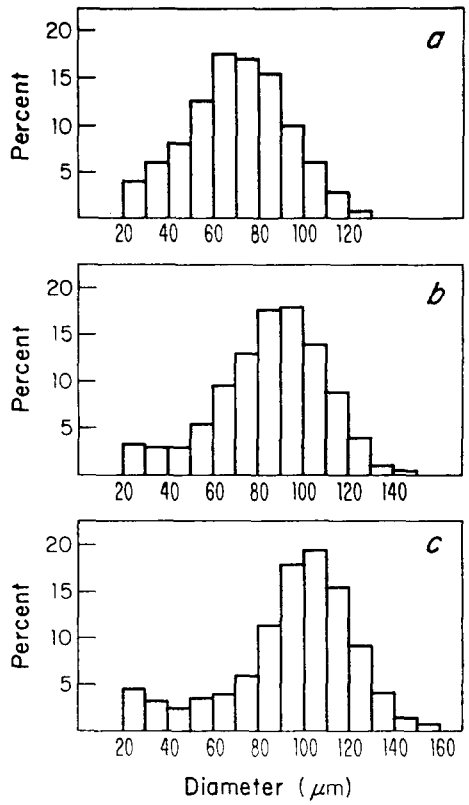

Fig. 1. Middle subcutaneous adipocyte diameter distributions from pigs of different age and obesity in Experiment II. Histogram *a* represents the adipocyte distribution for 57-kg pigs ($\bar{x}$ diameter is 71 μm). Histogram *b* represents the adipocyte distribution for 94-kg pigs ($\bar{x}$ diameter is 85 μm). Histogram *c* represents the adipocyte distribution for 124-kg pigs ($\bar{x}$ diameter is 95 μm).

RESULTS

During growth, prolific accretion of subcutaneous adipose tissue (backfat) occurred in both experiments (Table 1). This rapid development of adipose tissue was paralleled by an increase in average adipocyte size (**Fig. 1**). If the overall MSQ average diameters are expressed on a volume basis, the adipocytes from Group III pigs in Experiments I and II (average diameter was 92 μm) were approximately 2.4-fold larger than adipocytes from pigs in Group I (average diameter was 69 μm). The trends in adipocyte diameter distributions for MSQ and P adipose tissue (data not shown) of pigs in Experiment I were similar to those presented for Experiment II in Fig. 1.

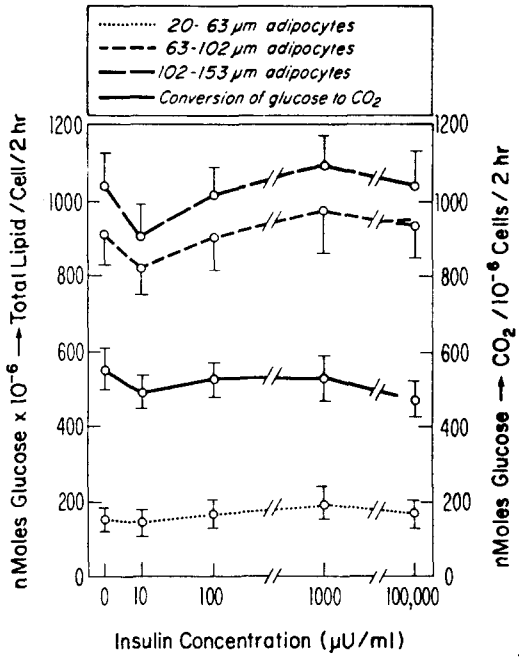

Fig. 2. Effect of insulin concentration on conversion of glucose to total lipid or CO_2 in pig middle subcutaneous adipose tissue. Conversion of glucose to total lipid is presented for three cell-size fractions. Data are from four fed pigs (122 kg); duplicate adipose tissue incubations per pig were conducted. CO_2 data are for the entire cell population rather than cells of a given fraction.

Glucose oxidation and lipid synthesis rates of different size pig adipocytes were unaffected by the presence of any concentration of porcine insulin (10 μU/ml to 100,000 μU/ml; **Fig. 2**). In the presence or absence of insulin, glucose oxidation in both the MSQ and P depots was greatest in Group II pigs (**Table 2**). Adipose tissue from pigs in Group III oxidized significantly less glucose than pigs in Group II.

Within an age (weight) group, lipid synthesis by perirenal adipose tissue (in the presence of insulin; **Table 3**) was significantly greater in larger cell-size fractions compared to the 20–63 μm fraction. However, within each of the three cell fractions where the effect of animal age (weight) could be examined, total lipid production from glucose decreased significantly with animal age (weight). Although not shown, the same trend was observed for the MSQ adipocytes, even though the conversion of glucose to total lipid was somewhat higher than for the perirenal adipocytes.

In Experiment II, insulin was not added and the conversion of glucose into GFA and GG was determined. These results (**Table 4**) support the initial conclusions deduced from data in Table 3 that the decrease in lipogenesis per cell with advancing age, weight, or obesity is a specific effect of age and not cell size. The significant age effect on the conversion rate of glucose to GFA and GG was the greatest in the smallest cell-size fraction (20–63 μm). In addition, this age effect was more dramatic for the conversion of glucose to GFA than to GG.

Data expressed per cell within a cell-size fraction does not reflect the contribution of a particular fraction to the total incorporation of all fractions (Tables 3 and 4). Compared with other cell fractions, adipocytes in the 63–102 μm fraction incorporated more glucose-carbon into GFA in Groups I and II (**Table 5**).

TABLE 2. Effect of animal age on glucose oxidation by adipocytes from pigs of different age

	Middle Subcutaneous[b]	Perirenal[b]
Experiment I[a]		
Group I[c]	432 ± 27[d]	338 ± 24[d]
Group II	667 ± 37[e]	400 ± 34[d]
Group III	347 ± 50[d]	115 ± 14[e]
Experiment II		
Group I[c]	589 ± 36[d]	
Group II	639 ± 51[d]	
Group III	392 ± 63[e]	

[a] Incubations in Experiment I contained 0.1 IU insulin/ml.
[b] Values are mean ± SEM nmoles glucose oxidized to $CO_2 \times 10^{-6}$/cell per 2 hr.
[c] Age and weight for pigs in each group are given in Table 1.
[d,e] Values with different superscripts within an experiment and an adipose tissue depot are significantly different ($P < 0.05$).

TABLE 3. Conversion of glucose to total lipid by perirenal adipose tissue (Experiment I)[a,b]

	Pig Perirenal Adipose Tissue			
	20–63 μm	63–102 μm	102–153 μm	>153 μm
Group I[c]	$574 \pm 46_w{}^d$	$1229 \pm 148_x{}^d$	$1700 \pm 200_x{}^d$	
Group II	$366 \pm 67_w{}^e$	$976 \pm 146_x{}^d$	$818 \pm 133_x{}^e$	
Group III	$37 \pm 13_w{}^f$	$381 \pm 62_x{}^e$	$281 \pm 52_x{}^f$	$305 \pm 73_x$

[a] Incubations contained 0.1 IU of insulin/ml.
[b] Values represent the mean ± SEM nmoles glucose $\times 10^{-6}$/cell per 2 hr incorporated.
[c] Age and weight for pigs in each group are given in Table 1.
[d,e,f] Different superscripts within a cell size fraction denote that the means are significantly different ($P < 0.05$) among pigs of different age.
[w,x] Different subscripts within an age group among cell-size fractions denote that the means are significantly different ($P < 0.05$).

TABLE 4. Incorporation of glucose into glyceride fatty acids and glyceride-glycerol (per cell) by middle subcutaneous adipocytes from pigs of different age (Experiment II)[a]

	Cell-Size Fraction			
	20–63 μm	63–102 μm	102–153 μm	Overall Fractions
Glyceride fatty acids				
Group I[b]	$511 \pm 40_w{}^c$	$1055 \pm 94_x{}^c$	$1337 \pm 80_y{}^c$	900 ± 80^c
Group II	$402 \pm 24_w{}^d$	$1019 \pm 51_x{}^c$	$1149 \pm 62_x{}^c$	940 ± 45^c
Group III	$70 \pm 11_w{}^e$	$380 \pm 50_x{}^d$	$369 \pm 57_x{}^d$	340 ± 48^d
Glyceride-glycerol				
Group I[b]	$58 \pm 4_w{}^c$	$94 \pm 8_x{}^c$	$141 \pm 12_y{}^c$	87 ± 7^c
Group II	$44 \pm 3_w{}^d$	$102 \pm 5_x{}^c$	$108 \pm 9_y{}^c$	96 ± 5^c
Group III	$17 \pm 4_w{}^e$	$65 \pm 8_x{}^d$	$72 \pm 10_x{}^d$	64 ± 8^d

[a] Values represent the mean (±SEM) nmoles of glucose × 10^{-6}/cell per 2 hr incorporated into glyceride fatty acids or glyceride-glycerol.

[b] Age and weight for pigs in each group are given in Table 1.

[c,d,e] Different superscripts within a cell-size fraction denote that the means are significantly different ($P < 0.05$) among pigs of different age.

[w,x,y] Different subscripts within an age group among cell-size fractions denote that the means are significantly different ($P < 0.05$).

This was due to a combination of high metabolic activity and a larger number of adipocytes present in this particular fraction than for the other two fractions. With an increase in the average size of the MSQ adipocyte population during animal growth (Fig. 1), the larger cell-size fractions became increasingly more important in converting glucose to GFA. Adipocytes 102–153 μm in diameter contributed 17% of the total GFA synthesized in Group I, whereas 54% of the GFA synthesized in Group III was accounted for by these large adipocytes (Table 5). Associated with this was a progressive decline in GFA synthesis by both the 20–63 and 63–102 μm fractions between Groups I and III.

TABLE 5. Incorporation of glucose into glyceride fatty acids and glyceride-glycerol by respective middle subcutaneous cell-size fractions (Experiment II)[a]

	Cell-Size Fraction			
	20–63 μm	63–102 μm	102–153 μm	Overall Fractions
Glyceride fatty acids				
Group I[b]	$173 \pm 12_x{}^c$ (19%)	$586 \pm 56_x{}^c$ (64%)	$160 \pm 25_w{}^c$ (17%)	900 ± 80^c
Group II	$74 \pm 6_w{}^d$ (8%)	$561 \pm 24_x{}^c$ (60%)	$305 \pm 39_y{}^d$ (32%)	940 ± 45^c
Group III	$10 \pm 2_w{}^e$ (3%)	$146 \pm 20_x{}^d$ (43%)	$184 \pm 33_x{}^c$ (54%)	340 ± 48^d
Glyceride-glycerol				
Group I[b]	$20 \pm 2_w{}^c$ (22%)	$51 \pm 4_x{}^c$ (58%)	$17 \pm 3_w{}^c$ (19%)	87 ± 7^c
Group II	$8 \pm 0.5_w{}^d$ (8%)	$58 \pm 3_x{}^c$ (60%)	$31 \pm 4_y{}^d$ (32%)	96 ± 5^c
Group III	$3 \pm 0.5_w{}^e$ (5%)	$25 \pm 3_x{}^d$ (40%)	$35 \pm 6_x{}^d$ (56%)	64 ± 8^d

[a] Values represent mean (±SEM) nmoles of glucose incorporated into glyceride fatty acids or glyceride-glycerol/fraction per 10^6 adipocytes for a 2-hr incubation. The cell number reflects the entire distribution of adipocytes, not within a fraction. The number within parentheses indicates contribution of fraction to overall glucose incorporation into GFA or GG.

[b] Age and weight for pigs in each group are given in Table 1.

[c,d,e] Means within a respective fraction among animals of different ages with dissimilar superscripts are significantly different ($P < 0.05$).

[w,x,y] Means within an age group among cell-size fractions with different subscripts are significantly different ($P < 0.05$).

TABLE 6. Conversion of glucose to glyceride fatty acids and glyceride-glycerol in middle subcutaneous adipocytes from pigs of different age, expressed on a surface area basis (Experiment II)[a]

	Cell-Size Fraction			
	20–63 μm	63–102 μm	102–153 μm	Overall Fractions
Glyceride fatty acids				
Group I[b]	$6.79 \pm 0.48_w{}^c$	$5.27 \pm 0.39_x{}^c$	$3.90 \pm 0.21_y{}^c$	5.18 ± 0.33^c
Group II	$5.65 \pm 0.26_w{}^d$	$4.47 \pm 0.21_x{}^c$	$3.03 \pm 0.18_y{}^d$	3.96 ± 0.17^d
Group III	$1.29 \pm 0.20_{w,x}{}^e$	$1.47 \pm 0.20_w{}^d$	$0.91 \pm 0.14_x{}^e$	1.12 ± 0.15^e
Glyceride-glycerol				
Group I[b]	$0.77 \pm 0.05_w{}^c$	$0.47 \pm 0.03_x{}^c$	$0.40 \pm 0.03_x{}^c$	0.50 ± 0.03^c
Group II	$0.61 \pm 0.03_w{}^d$	$0.45 \pm 0.02_x{}^c$	$0.32 \pm 0.02_x{}^d$	0.40 ± 0.02^d
Group III	$0.32 \pm 0.07_w{}^e$	$0.25 \pm 0.03_{w,x}{}^d$	$0.19 \pm 0.03_x{}^e$	0.21 ± 0.03^e

[a] Data are the mean (±SEM) nmoles of glucose incorporated/cm² cell surface area per 2 hr into glyceride fatty acids.

[b] Age and weight for pigs in each group are given in Table 1.

[c,d,e] Means with different superscripts within a cell-size fraction are significantly different ($P < 0.05$).

[w,x,y] Different subscripts among cell-size fraction means within an age group indicate that the means are significantly different ($P < 0.05$).

Because of the large differences in volume between large and small cells, it was not unexpected that glucose conversion to GFA should be greater in the large cell-size fraction than that observed in the smaller fraction. In an effort to determine the relationship between surface area and glucose incorporation, these data were expressed per cm² of surface area (**Table 6**). Since pig adipocytes vary greatly in size during animal growth, comparisons based on cell size do not reflect equal size units, whereas data expressed per cm² of surface area reflect equivalent size units. The results in Table 6 indicate that smaller adipocytes incorporated more glucose carbon into GFA and GG per unit of surface area, and there was a progressive decline with increasing adipocyte size within age groups. Thus, when these data are expressed per unit of surface area, the largest adipocytes incorporated less glucose into GFA or GG. This result is the opposite from that found when the data were expressed on a per cell basis (Table 4). However, regardless of which way the data were expressed, there was a significant decrease in glucose conversion to GFA or GG within each cell fraction as animal age, body weight and adiposity increased.

DISCUSSION

O'Hea and Leveille (19) noted that the rate of glucose oxidation and fatty acid synthesis in subcutaneous adipose tissue from 40–60 kg pigs was not enhanced by the presence of insulin (0.1 IU/ml). However, rates of glucose metabolism were doubled in the presence of insulin when the adipose tissue samples were derived from 12-kg pigs. The magnitude of the latter response was considerably less than that observed for rat adipose tissue (6, 20). Our results agree with previous reports that in vitro glucose metabolism (19, 21) and protein synthesis (22) in adipose tissue from pigs weighing more than 30 kg are unresponsive to insulin.

It is possible that endogenous insulin was bound to a specific plasma membrane receptor prior to the commencement of the in vitro studies in sufficient quantities to elicit a maximal response. However this seems very unlikely, since preincubation of adipose tissue slices with anti-porcine insulin serum prior to subsequent incubations with insulin was ineffective in stimulating glucose oxidation or synthesis of lipid (19). Although we did not measure insulin degradation, the likelihood that degradation contributed to the observed response does not seem feasible. Degradation of insulin is relatively minor in isolated rat adipocyte preparations (23). Degradation of insulin would have had to occur at an extraordinarily high rate to diminish biological activity with the higher levels of insulin used in our preliminary experiments. Last, it seems unlikely that the saturating glucose concentration was the major reason for the lack of an observed insulin response, since insulin does not stimulate glucose utilization in pig adipose tissue when glucose concentrations range from 0.2 mM to 10 mM.[6] While we did not observe an in vitro response of porcine adipose tissue to added insulin, in vivo glucose metabolism of the pig is responsive to insulin. Alloxan-treated pigs become hyperglycemic

[6] Etherton, T. D., and C. S. Chung. Unpublished data.

and plasma glucose levels return to normal after insulin therapy (24). There is no information that indicates which tissues in the pig are involved in the insulin-dependent uptake of plasma glucose.

Glucose metabolism in human adipocytes either responds poorly (7, 25), or not at all (26) to insulin. However, Ciaraldi et al. (27) have recently indicated that glucose transport across the plasma membrane of human adipocytes is readily stimulated by insulin. Differences observed in these studies between glucose utilization (oxidation and lipogenesis) and transport studies may be attributable to the ability of insulin to augment glucose transport, whereas metabolic steps distal to insulin binding may not be as responsive to insulin. In pig adipocytes, glucose-carbon flux is considerably greater than in human adipocytes because adipose tissue is the major tissue site of de novo synthesis of fatty acids (28), whereas in human adipose tissue negligible rates of fatty acid synthesis from glucose occur (29). Calculations based upon our results and in vitro rates of glucose utilization in human adipocytes by Patel et al. (29) and Shrago and Spennetta (30) indicate that in vitro glucose-carbon flux is 4–10 times greater in pig adipocytes. While these values are only estimates and do not consider the difference in relative physiological age, they do indicate that the lack of an insulin response by pig adipocytes is unique, especially when glucose flux is markedly greater.

The suggestion that larger adipocytes have a decreased ability to utilize glucose has been obscured by several confounding factors such as animal age, obesity, or diet. With the exception of data presented by Holm et al. (31) for the rat, all previous results have emanated from comparisons of larger cells obtained from older animals (4–6), larger cells obtained from different anatomical locations within an animal (3), or comparisons made on adipocytes which have undergone either an increase or decrease in size attributable to dietary changes (7, 23). Porcine adipocytes from different anatomical locations have widely varying rates of substrate incorporation (32) and therefore, any metabolic differences which are attributed to cell size differences are equivocal. Metabolic changes associated with alterations of adipocyte size due to dietary manipulation may be confounded by changes in circulating hormone levels, and the altered dietary carbohydrate-fat ratio used. Data presented in this paper indicate that: *1*) large pig adipocytes incorporate more substrate per cell and less per unit of surface area regardless of animal age; and *2*) among adipocytes within a cell-size fraction, cells from older, more obese pigs have a suppressed ability to incorporate glucose-carbon. Our results, and those of Holm et al. (31) with rats, clearly indicate that glucose metabolism decreases in a manner which is not associated with changes in adipocyte size, but rather appears to be a function of animal or cell age.

Regardless of the exact mechanism(s) involved in this age-associated decrease in adipocyte glucose metabolism, it is apparent that for adipose tissue accretion to continue (data not shown) either triacylglycerol synthesis from other carbon sources is not perturbed or the decrease in triacylglycerol turnover (lipolysis) is greater than the fall in synthesis. In the pig it does not appear that major alterations in lipolytic rates occur which would enable accretion of triacylglycerol to continue. Mersmann, Phinney, and Brown (33) reported that basal rates of lipolysis in the pig were essentially unchanged between 45 and 150 days of age. This infers that either other sources of carbon are being utilized for fatty acid synthesis or that, alternatively, plasma free fatty acids or lipoprotein triacylglycerol-fatty acids are being incorporated into adipocyte triacylglycerol to a greater extent in older, more obese pigs. There have not been any studies reported which have measured absolute rates of fatty acid synthesis in pig adipose tissue utilizing ^3H_2O. Whether absolute rates of fatty acid synthesis change in pig adipose tissue with growth when the relative contribution of glucose as a precursor is decreasing is not known.

Lee and Kauffman (34) have found that lipoprotein lipase activity in MSQ adipose tissue increased with increasing body weight in pigs over a body weight range which was similar to that in the present study. This suggests that exogenous fatty acids may play a role in enabling triacylglycerol accretion to continue even though adipocyte de novo fatty acid synthesis from glucose is decreasing. This is supported by the observation that large adipocytes from older rats have an enhanced rate of GG synthesis from glucose (6), which implies that triacylglycerol synthesis in older adipocytes may be relatively unaffected and more dependent upon extracellular fatty acids than fatty acids synthesized de novo. Our data indicated that there was a decrease in GG synthesis with increasing age; however, this decline was significantly less than the decrease in GFA synthesis. The reason for the slight decrease in GG synthesis at a time when triacylglycerol accretion is continuing is obscure. However, we have recently found that in vitro esterification of [1-^{14}C]palmitic acid by adipocytes increases in pigs with advancing age at a time when fatty acid synthesis from glucose is decreasing (35).

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