Preparation, characterization, and insulin sensitivity of isolated swine adipocytes: comparison with adipose tissue slices[1]

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Abstract The technique of Rodbell (*J. Biol. Chem.* **239:** 375) was modified considerably in order to isolate swine adipocytes without rupturing large cells. Cell size and diameter distributions were the same for adipocytes fixed with OsO_4 following isolation with collagenase and adipocytes liberated from OsO_4-fixed adipose tissue slices. Lipogenic rates were greater for isolated adipocytes compared with thin adipose tissue slices at low (0.5 mM) and high (10 mM) glucose concentrations (cells = 307 and 1100; slices = 139 and 744 nMoles glucose → lipid/10^6 cells/hr for 0.5 and 10 mM glucose, respectively, $P < 0.001$). Similar differences were found for glucose oxidation. Sensitivity to insulin was determined by measuring the stimulation of lipogenesis and glucose oxidation in the presence of 0, 1, 5, 25, and 100 ng/ml of purified porcine insulin at low (0.5 mM) and high (10 mM) glucose concentrations. Relative to basal incubations, the addition of insulin caused similar increases in glucose oxidation and lipogenesis for isolated adipocytes and adipose tissue slices when glucose concentration was 10 mM. These results indicate *1*) that isolated swine adipocytes can be prepared without alterations in cell size or diameter distribution, and *2*) that isolated adipocytes have higher rates of glucose oxidation and lipogenesis from glucose even though they retain a similar in vitro sensitivity to insulin.—**Etherton, T. D., and C. S. Chung.** Preparation, characterization, and insulin sensitivity of isolated swine adipocytes: comparison with adipose tissue slices. *J. Lipid Res.* 1981. **22:** 1053–1059.

Supplementary key words lipogenesis · glucose oxidation

Studies examining lipid metabolism and the endocrine regulation of cellular processes have benefited greatly from the use of isolated adipocytes incubated in vitro. Many of these studies have employed the technique pioneered by Rodbell (1) using collagenase to isolate adipocytes from the connective tissue matrix. As conducted in our laboratory and others, the isolation of rat adipocytes is straightforward and the cells maintain their ability to metabolize glucose and respond to a wide variety of hormones (1, 2). However, we have not attained suitable results when this technique has been used to isolate adipocytes from adipose tissue of larger animals such as swine (3).[3] The primary problem is one of extensive cellular rupture as evidenced by a large quantity of lipid droplets and an adipocyte diameter distribution that is shifted to the left.[3] This causes a significant decrease in average cell size when comparisons are made with cell distributions from tissue slices that are fixed with osmium tetroxide (OsO_4).[3] These observations indicate that larger swine adipocytes rupture to a greater extent than smaller adipocytes during the isolation procedure. As a result, isolated swine adipocytes would not represent the size distribution present in vivo and, therefore, would not reflect the metabolic activity of the adipocyte population in vivo.

Although adipocytes have been previously isolated from swine (4, 5), no quantitative assessment of the size distribution has been conducted. Whether the cells that remained intact after isolation retained their normal metabolic activity is questionable. This is supported by Mersmann et al. (5) who found that on a per cell basis lipogenic activity was always greater in slices than in isolated swine adipocytes.

The use of isolated adipocytes for in vitro incubations simplifies the problem of whether the specific activity of substrates in the medium reflects that of the interstitial spaces of the tissue slice (6). The objectives of this investigation were *1*) to develop a technique for isolation of adipocytes from swine that had a comparable cell size distribution to that of the original tissue; *2*) to determine if the ability of isolated cells

Abbreviations: OSQ, outer subcutaneous; MSQ, middle subcutaneous; KRB, Krebs-Ringer bicarbonate; BSA, bovine serum albumin; TCA, trichloroacetic acid.

[1] This paper was authorized for publication as paper number 6184 in the journal series of the Pennsylvania Agricultural Experiment Station. A portion of this work was presented (Abstract NO. 3729) at the 65th annual meeting of the Federation of American Societies for Experimental Biology, Atlanta, GA, 1981.

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to metabolize glucose was comparable to that of cells in thin adipose tissue slices; and *3*) to compare effects of porcine insulin on glucose oxidation and lipogenesis in isolated adipocytes and thin adipose tissue slices.

MATERIALS AND METHODS

Adipose tissue samples were excised from Yorkshire and Duroc swine (80–110 kg body weight) immediately after death. The animals were fed ad libitum until 30 min before slaughter. The tissue used was subcutaneous adipose tissue excised dorsal to the first rib. Both outer subcutaneous (OSQ) and middle subcutaneous (MSQ) adipose tissue were used. Immediately after extirpation, the tissue sample was placed in 37°C buffer for transport to the laboratory. The Krebs-Ringer bicarbonate buffer contained 118 mM NaCl, 4.8 mM KCl, 1.3 mM $CaCl_2$, 1.2 mM KH_2PO_4, 1.3 mM $MgSO_4$, 10 mM $NaHCO_3$, and 10 mM N-2-hydroxyethylpiperazine-N^1-2-ethanesulfonic acid (HEPES). The pH was adjusted to 7.4 with NaOH after the buffer had been equilibrated with 95% O_2, 5% CO_2. The KRB-HEPES buffer used for transport contained 5 mM glucose.

The techniques used for preparing swine adipose tissue slices and subsequent incubation were similar to those previously described (3, 7) with the following modifications. Slices of 50–140 mg were rinsed in 37°C KRB-HEPES containing 5 mM glucose and then incubated in polyethylene scintillation vials rather than 25-ml Erlenmeyer flasks. Vials were gassed with 95% O_2, 5% CO_2 and incubated at 37°C in 3 ml of KRB-HEPES that contained 3% bovine serum albumin (BSA), 1.0 μCi of [U-^{14}C]glucose (New England Nuclear), and various concentrations of glucose and porcine insulin (see figure legends and table footnotes). Incubations were stopped by the addition of 0.25 ml of 1 N H_2SO_4. Carbon dioxide was collected on filter paper saturated with hyamine hydroxide that was placed in a suspended plastic well. Lipid extraction of adipose tissue slices was conducted as previously described (3, 7).

Isolation of swine adipocytes

Numerous preliminary experiments were conducted to isolate swine adipocytes and the technique that ultimately proved acceptable is described. The most important consideration in the success of this technique was the composition of the tubes, flasks, and screens. The use of polypropylene containers resulted in much less cellular rupture than did polyethylene, polystyrene, and siliconized glass containers. The anatomical location of adipose tissue selected affected results. OSQ and MSQ proved to be acceptable; however, unsatisfactory results were obtained when perirenal adipose tissue was used.

Samples of adipose tissue weighing approximately 1 g were sliced with a razor blade into slices approximately 200 μm in thickness. The slices were placed in 3 ml of KRB-HEPES buffer with 5 mM glucose, 3% BSA, and 4.3 mg/ml of collagenase (Type I; Worthington Biochemical Corp., Freehold, NJ) in a 30-ml polypropylene beaker. The slices were minced thoroughly with a pair of scissors and the mixture was poured into a 25-ml polypropylene Erlenmeyer flask, capped, and incubated with gentle swirling for 60 min at 37°C in a New Brunswick gyratory water bath/shaker (New Brunswick Scientific Co., Edison, NJ). After 60 min, the isolated adipocytes and remaining tissue fragments were poured through polypropylene mesh (Small Parts, Inc., Miami, FL) with a pore size of 1000 μm into a 30-ml polypropylene beaker and then transferred to a polypropylene test tube held at 37°C. Although swine adipocytes range in size from 20 to 200 μm in diameter (3, 7), the 1000 μm pore size was used to reduce cellular rupture that occurred with a pore size of 250 μm. While this resulted in some small tissue fragments passing through the screen, these were removed by the washing technique used.

The isolated adipocytes and small tissue fragments were allowed to float to the surface of the buffer prior to washing of the cell layer. No slow-speed centrifugation steps were employed to expedite this process because centrifugation caused cell rupture. After the samples had sat for about 2 min it was observed that the tissue fragments had a density such that they floated immediately beneath the layer of isolated cells. A syringe with a siliconized needle was used to gently aspirate the infranatant and cell fragments. The cell layer was washed with 37°C KRB-HEPES buffer containing 3% BSA and glucose (levels varied depending upon glucose concentration in subsequent incubation). Aspiration of the infranatant and washing with warm buffer (without collagenase) was repeated three times. The addition of warm buffer to the cells resulted in some cellular rupture if the rinse buffer was poured directly into the tube. Because of this, a Pasteur pipet was used to add the rinse buffer to the wall of the tube. After the addition of each successive aliquot of rinse buffer, the tube was rotated gently by hand to ensure mixing of the cells with the buffer.

Incubation of isolated swine adipocytes

Following washing of adipocytes, cells from several tubes were pooled in a 50-ml polypropylene Erlenmeyer flask that contained 37°C KRB-HEPES buffer with 3% BSA. Glucose concentration was either 0.5,

5.0, or 10 mM depending upon the subsequent incubation conditions. After gently swirling the flask, samples of the cell suspension were aliquoted with an adjustable pipet fitted with a polypropylene tip. Using this technique it was possible to aliquot cells rapidly with uniform accuracy and repeatability. Incubation of isolated adipocytes was conducted in the same buffer used for tissue slices. Cell aliquots (100–500 μl) were incubated in 17 × 100 polypropylene test tubes in a total incubation volume of 1 ml.

After addition of the cell preparation, the tubes were gassed with 95% O_2, 5% CO_2 and sealed with a serum stopper that had a suspended plastic center well (Kontes, Vineland, NJ) containing a piece of filter paper. The tubes were placed in a gyratory water bath and swirled gently at 37°C for the desired incubation time. Reactions were terminated and CO_2 was trapped as described previously (3, 7). For determination of ^{14}C-labeled lipids, 5 ml of Dole's extraction mixture was added directly to the cell solution in the test tube and extracted as described by Dole (8). Portions of the upper phase were analyzed for total lipid radioactivity as described (3, 7). The quantity of glucose carbon converted to CO_2 and total lipid was calculated from the initial specific activity of glucose in the medium and the quantity of radioactivity in the products. All data are expressed as a function of adipocyte number.

Purified porcine insulin was a gift of Eli Lilly (courtesy of Dr. Ron Chance). It was dissolved in 0.01 N HCl (1 mg/l ml) and diluted in KRB-HEPES buffer before aliquoting to respective incubation vessels. The concentration of insulin added to the incubation vessels was checked by radioimmunoassay (9). ^{125}I-labeled insulin was prepared at a specific activity of 100–150 μCi/μg (10) and purified over Sepadex G-50 (fine). ^{125}I-labeled insulin was 98% precipitable in 10% trichloroacetic acid and 94% precipitable in the presence of excess antibody. Degradation of insulin in the incubation buffer was determined by removing 50-μl aliquots of medium to which approximately 0.2 ng of ^{125}I-labeled insulin/ml had been added and adding the aliquot to 1 ml of 4°C KRB-HEPES buffer that contained 3% BSA. This was followed by the addition of 1 ml of cold 10% TCA. The pellet was sedimented by low speed centrifugation and the radioactivity that was TCA-soluble was considered to represent the proportion of insulin degraded.

Determination of adipocyte size and number

Adipocyte number and diameter distributions of adipose tissue slices were determined with a Model ZB Coulter Counter as described (3, 7). The use of 8 M urea to extract the connective tissue matrix of the fixed slice was essential to eliminate any debris so that accurate comparisons could be made with adipocytes isolated by collagenase treatment (3). Adipocytes that had been isolated with collagenase were added directly to a glass scintillation vial that contained collidine buffer and OsO_4. All subsequent steps for determination of cell number and diameter distributions were the same as described previously (3, 7) with the omission of 8 M urea treatment. Treatment differences were determined by a two-way analysis of variance using pig × insulin level as the error term to test for differences among insulin levels (11). The mean separation procedure used was a Waller-Duncan K-Ratio T test.

RESULTS

During the development of this technique it was routinely observed that swine adipose tissue fragments were not completely dissociated by collagenase as were fragments of rat adipose tissue. Before any studies were conducted to compare metabolic activity of isolated cells and tissue slices, it was essential to determine whether the diameter distribution of the adipocytes released as a result of collagenase was similar to that found in adipose tissue slices. The diameter distribution and average size of isolated OSQ adipocytes was not significantly different from adipocytes in tissue slices (**Fig. 1**). A similar response was observed for MSQ adipose tissue (data not shown). All attempts to isolate perirenal adipocytes were un-

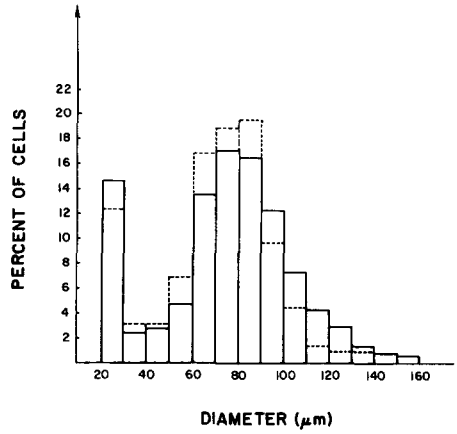

Fig. 1. Diameter distribution histograms of adipocytes isolated with collagenase (– – –) and from adipose tissue slices (——). Average diameter of isolated adipocytes and adipocytes from adipose tissue slices was 70.2 ± 0.9 and 73.1 ± 1.3 μm, respectively (means not significantly different, $P > 0.05$). Although not shown, no statistical differences were found between isolated adipocytes and adipocytes from tissue slices for any size range interval. Samples of OSQ adipose tissue were obtained from eight swine in triplicate.

successful. In all size range intervals for OSQ and MSQ histograms, the standard error of the mean (SEM) was less than 5% of the mean value. These data indicate that even though swine adipose tissue fragments were not completely dissociated by collagenase, the cells liberated did represent the distribution of adipocytes found in tissue slices. Furthermore, the absence of particles larger than 160 μm indicates that the small tissue fragments were essentially removed during the washing steps. Cell recovery was approximately 30–40% after 1 hr of incubation. This was determined by comparing the number of adipocytes in a known weight of tissue with the number of adipocytes that were isolated in a respective incubation.

Conversion of glucose to CO_2 and lipid increased linearly with incubation time for both isolated adipocytes and tissue slices (**Fig. 2**). However, the quantity of glucose that was converted to CO_2 and lipid was greater for isolated adipocytes at each respective time point. To determine whether adipocyte number had an influence on glucose metabolism, incubations were conducted with various cell numbers. As shown in **Fig. 3**, glucose oxidation and lipid synthesis from glucose increased as a linear function of adipocyte number over a wide range in cell number. We have previously shown that conversion of glucose to lipid

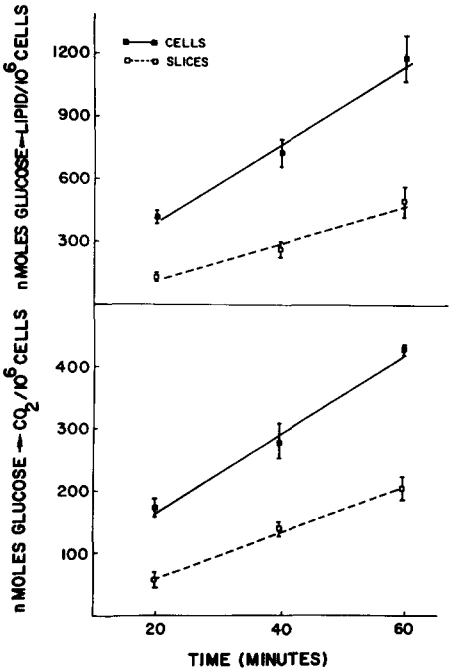

Fig. 2. Conversion of glucose to CO_2 and lipid by isolated OSQ adipocytes and tissue slices with time of incubation. Comparisons between cells and slices were significantly different ($P < 0.05$) at all time points for CO_2 and lipid data (n = 6). Incubations were conducted in KRB-HEPES containing 3% BSA and 5 mM glucose. No insulin was added. The bar associated with each point is the SEM.

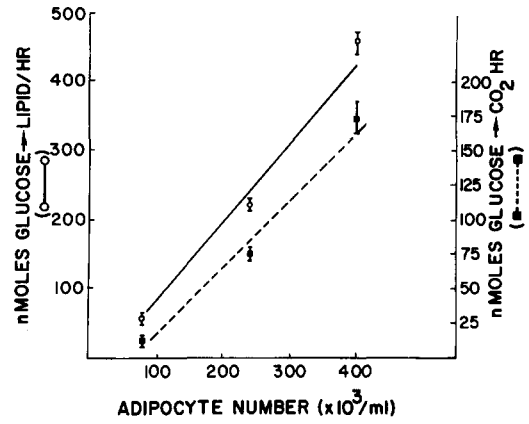

Fig. 3. Conversion of glucose to CO_2 and lipid by isolated OSQ adipocytes. Different volumes of isolated adipocyte suspensions were incubated for 60 min in 1 ml of KRB-HEPES containing 3% BSA and 5 mM glucose. No insulin was added. The bar associated with each point represents the SEM (n = 4).

increases linearly when adipose tissue slice weight increases from 50 to 140 mg (7).

Different concentrations of collagenase were used during the isolation procedure to determine if this affected glucose metabolism. There were no statistical differences in glucose oxidation (81 ± 24 versus 57 ± 13 nmoles glucose → $CO_2/10^6$ cells/hr) and lipid synthesis from glucose (186 ± 33 versus 225 ± 26 nmoles glucose → lipid/10^6 cells/hr) when 3 mg/ml or 4.3 mg/ml of collagenase were used (n = 4, medium glucose concentration was 0.5 mM). Thus, collagenase was used at a concentration of 4.3 mg/ml to increase cell yield (data not shown). To clarify the viability of isolated adipocytes further, the insulin sensitivity of cells and slices were compared. This was determined at a low medium glucose concentration (0.5 mM) where transport may be rate-limiting and at a glucose concentration (10 mM) that was saturating for oxidation and lipid synthesis where glucose transport is not rate-limiting. Glucose oxidation and lipid synthesis in isolated adipocytes and adipose tissue slices was not affected by insulin addition at low medium glucose concentrations (**Tables 1 and 2**). There was a stimulatory effect of insulin on lipid synthesis at high glucose concentrations for both isolated cells and tissue slices. Maximal stimulation of lipid synthesis by insulin was observed at insulin concentrations of 25 ng/ml (Tables 1 and 2). Similar trends were observed for glucose oxidation in the presence of different insulin concentrations. Degradation of ^{125}I-labeled insulin was less than 25% after 1 hr of incubation.

To establish further the fact that isolated adipocytes metabolized glucose at a greater velocity than adipose tissue slices, data were pooled across insulin treat-

TABLE 1. Influence of insulin on glucose metabolism in isolated swine adipocytes

Insulin	Buffer Glucose Concentration 0.5 mM	10 mM
ng/ml		
Glucose conversion to lipid[a]		
0	296[b]	940[b]
1	286[b]	1013[b,c]
5	294[b]	1112[b,c]
25	322[b]	1232[c]
100	337[b]	1210[b,c]
Glucose oxidation[d]		
0	90[b]	125[b]
1	76[b]	260[b]
5	91[b]	255[b]
25	89[b]	243[b]
100	104[b]	299[b]

[a] Values are average nmol of glucose → lipid/10^6 cells/1 hr for incubation conducted in triplicate from six swine. Isolated swine adipocytes were incubated in KRB with two glucose concentrations: 0.5 mM and 10 mM.

[b,c] Means with different superscripts for a given parameter and glucose concentration are significantly different ($P < 0.05$).

[d] Values are average nmol of glucose → CO_2/10^6 cells/1 hr.

ments for both isolated adipocytes and tissue slices. As shown in **Table 3,** glucose oxidation and lipid synthesis from glucose were higher in isolated adipocytes at both glucose concentrations. The difference in metabolic activity between isolated adipocytes and adipose tissue slices was greater at 0.5 mM than at 10 mM medium glucose. To determine if homogenates of isolated adipocytes oxidized glucose or synthesized lipid, aliquots of adipocyte suspensions were homogenized in a Potter-type glass homogenizer with a Teflon plunger. Aliquots of this suspension, that corresponded to the number of adipocytes incubated, were incubated for 1 hr. There was neither detectable oxidation of glucose nor lipid synthesis in three experiments. Thus, broken adipocyte preparations did not maintain any capacity for glucose oxidation or lipid synthesis.

DISCUSSION

The techniques described in this report are the first that allow swine adipocytes to be isolated from the connective tissue matrix that retain the diameter distribution of the adipocyte population present in a tissue slice. This finding is essential in that it indicates that rupture of larger cells was no more likely to occur than that of smaller adipocytes during the isolation process. Jamdar (12) recently found that the diameter distribution of isolated rat adipocytes was shifted to the left when compared with tissue fragments, indicating that larger rat adipocytes were more easily ruptured during isolation. Although swine adipocytes are considerably larger than rat adipocytes (3) and more friable, the observation that larger rat adipocytes are ruptured whereas larger swine adipocytes are not likely reflects important differences in techniques utilized to isolate adipocytes. This point is significant in view of the large number of studies that have compared hormone action and nutrient metabolism in isolated adipocytes from young, lean rats versus older, more obese rats. It should be emphasized that adipocytes are remarkably fragile (especially larger cells). Because of this, great care should be exercised to verify that isolated adipocytes retain the size distribution observed in the intact tissue.

The presence of adipocytes 20–40 μm in diameter in both distributions supports previous observations with swine adipose tissue that small cells are always present (3, 7). The proportion of these small adipocytes is greater in obese versus lean swine (13). Although their ability to metabolize glucose on a per cell basis is much less than larger cells (7), nothing is known about their insulin sensitivity relative to larger cells.

Although isolated adipocytes, primarily from the rat, have been used for innumerable experimental purposes, there is still controversy about their use in metabolic studies. Lipogenic rates have been found to be greater on a per cell basis in rat adipose tissue

TABLE 2. Influence of insulin on glucose metabolism in swine adipose tissue slices

Insulin	Buffer Glucose Concentration 0.5 mM	10 mM
ng/ml		
Glucose conversion to lipid[a]		
0	140[b]	680[b]
1	136[b]	689[b]
5	138[b]	745[b,c]
25	155[b]	838[c]
100	119[b]	770[b,c]
Glucose oxidation[d]		
0	23[b]	86[b]
1	25[b]	103[b,c]
5	26[b]	145[c]
25	27[b]	126[b,c]
100	20[b]	128[b,c]

[a] Values are average nmol of glucose → lipid/10^6 cells/1 hr for incubations conducted in triplicate from six swine. Isolated swine adipocytes were incubated in KRB with two glucose concentrations: 0.5 mM and 10 mM.

[b,c] Means with different superscripts for a given parameter and glucose concentration are significantly different ($P < 0.05$).

[d] Values are average nmol of glucose → CO_2/10^6 cells/1 hr.

TABLE 3. Comparison of glucose metabolism in isolated swine adipocytes and adipose tissue slices

	Buffer Glucose Concentration	
	0.5 mM	10 mM
Glucose conversion to lipid[a]		
Isolated adipocytes	307 ± 8	1100 ± 51
Adipose tissue slices	139 ± 5	744 ± 26
	($P < 0.0001$)[c]	($P < 0.05$)
Glucose oxidation[b]		
Isolated adipocytes	90 ± 4	236 ± 26
Adipose tissue slices	24 ± 1	118 ± 9
	($P < 0.006$)	($P < 0.006$)

[a,b] Values are average (± SEM) nmol of glucose → product/10^6 cells/1 hr. Two concentrations of glucose were used in the KRB: 0.5 mM and 10 mM.

[c] The α level for test of significance between isolated adipocytes and tissue slices.

slices than in isolated cells (12). Other groups, however, have reported that the in vitro metabolic activity of isolated rat adipocytes was equal to (1) or greater than (2) cells of a tissue slice. Human adipose tissue segments are metabolically more active than cells (2). The ability of isolated swine adipocytes to metabolize glucose at a greater rate than tissue slices as reported here differs from the results of Mersmann et al. (5). However, they did not compare adipocyte diameter distributions to ascertain whether any breakage of larger cells occurred. Based upon the results of the present study, it is unlikely that the isolation process caused any alteration in adipocyte integrity.

Other observations substantiate the fact that minimal cellular rupture occurred. Although it is difficult to quantify, observations were routinely made for lipid droplets in the isolated adipocyte preparations during washing and subsequent incubation. After pooling of adipocytes in a 50-ml polypropylene flask, some free lipid was observed on the surface of the buffer (presumably from mincing of tissue fragments). However, after gently swirling the flask to obtain a homogenous solution of cells, samples for subsequent incubation were obtained by placing the pipet tip beneath the surface of the buffer, thereby avoiding the floating free lipid. Very few lipid droplets were observed in any of these aliquots or in subsequent incubations. When polystyrene or polyethylene tubes were used, cellular rupture was more extensive as evidenced by an increase in free lipid droplets.

The connective tissue matrix of swine adipose tissue is considerably more extensive than that of the rat. Because of this, it was necessary to use a greater concentration of collagenase than we and other groups used for isolation of rat adipocytes. When collagenase concentrations ranged from 1 to 3 mg per ml of buffer, swine adipocytes were liberated; however, the number was much less than that observed with the use of 4.3 mg/ml of collagenase. Metabolism of glucose was not affected by different collagenase concentrations which shows that the enzyme did not impair metabolic activity. The observation that metabolic rates were appreciably greater in isolated adipocytes compared to tissue slices is similar to the results of Gries and Steinke (2) for rat adipocytes. Although the reasons for this are not clear, it can be speculated that diffusion of the buffer into the interstitial space of the tissue slice is impaired in vitro by the extensive connective tissue matrix in swine. Likewise, the egress of metabolic products from the cell may be impeded which, in turn, results in a reduced rate of glucose metabolism.

Although insulin has little influence on lipid synthesis in slices of swine adipose tissue during short-term (2-hr) in vitro incubations (7, 14, 15), we re-examined this in order to determine the responsiveness of isolated swine adipocytes. When glucose concentration was 10 mM, maximal stimulation of lipid synthesis was observed at 25 ng/ml of porcine insulin for both tissue slices and cells. The percentage increase in lipid synthesis above control incubations was 32% and 23% for cells and tissue slices, respectively. Although the absolute rates of lipid synthesis were different between isolated adipocytes and tissue slices, the similar increase as a percentage indicates that the isolation process had no effect on the in vitro response to insulin.

The techniques described allow adipocytes to be isolated from swine and cattle[4], both of which are species where the connective tissue matrix is quite different from the rat and the average adipocyte diameter is considerably greater. This method will be useful in studies examining hormone action, binding, and cellular metabolism in species where adipocytes have previously been difficult to isolate.

We thank Yildez Akin of Agricultural Data Processing Services for performing all statistical analyses. This project was supported in part by BRSG grant RR07082-14, awarded by Biomedical Research Support Grant Program, Division of Research Resources, National Institutes of Health.

Manuscript received 10 February 1981 and in revised form 27 April 1981.

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