Proposed Mechanism for Increased Insulin-mediated Glucose Transport in Adipose Cells from Young, Obese Zucker Rats

LARGE INTRACELLULAR POOL OF GLUCOSE TRANSPORTERS

Michele Guerre-Millo, Marcelle Lavau, J. Scott Horne, and Lawrence J. Wardzala

From the Unité de Recherches sur la Physiopathologie de la Nutrition, Institut National de la Santé et de la Recherche Médicale U.177, Institut Biomédical des Cordeliers, 15 Rue de l’Ecole de Médecine, 75006 Paris, France

The mechanism for hyperresponsive insulin-mediated glucose transport in adipose cells from 30-day-old obese Zucker rats was examined. Glucose transport was assayed by measuring 3-O-methylglucose transport, and the concentration of glucose transporters was estimated by measuring specific d-glucose-inhibitable cytochalasin B binding. Insulin increased glucose transport activity by approximately 17 pmol/cell/min in cells from obese rats compared to 3 pmol/cell/min in lean littersmates. Insulin increased the concentration of glucose transporters in the plasma membrane fraction by about 15 pmol/mg of membrane protein in both groups. The insulin-mediated decrease in the concentration of transporters in the low-density microsomal fraction was 30 pmol/mg of membrane protein for the obese rats compared to 15 pmol/mg of membrane protein for the lean controls. An estimated number of glucose transporters was calculated using membrane protein and enzyme recoveries for each group. In addition, insulin decreased the number of transporters/cell in the intracellular membrane pool by approximately 4 x 10^6 sites/cell for the obese rats and 0.9 x 10^6 sites/cells for the lean rats. The total number of transporters/cell was about 7 x 10^6 sites/cell for the obese animals and 1.6 x 10^6 sites/cells for the lean controls. In the basal state, more than 80% of these transporters were located in the intracellular pool for both the lean and obese rats. Thus, the marked hyperresponsive insulin-mediated glucose transport observed in adipose cells from 30-day-old obese Zucker rats may be the consequence of a marked increase in the number of glucose transporters in the intracellular pool.

The marked hyperinsulinemia in vivo and marked insulin resistance both in vivo and in vitro in the adult Zucker rat model of obesity are well established (1, 2). However, Cushman et al. (3) and Czech et al. (4) have demonstrated a marked increase in insulin’s stimulatory effect on glucose transport activity and transport-limited metabolism in isolated epididymal adipose cells from 6- and 10-week-old Zucker rats, respectively. Indeed, preliminary studies from this laboratory have shown an even greater stimulation of glucose transport by insulin in isolated inguinal adipose cells from 30-day-old obese Zucker rats as compared to their lean littermates (5), despite the presence of marked hyperinsulinemia in these young animals.

Recent reports by Cushman and co-workers (6, 7) and Kono and co-worker (8) have demonstrated that insulin stimulates glucose transport in the rat adipocyte cell through a rapid, reversible, and insulin concentration-dependent translocation of glucose transporters from a large intracellular pool, associated with the cell’s low density microsomes, to the plasma membrane.

More recently, a series of studies in isolated adipose cells from aged, obese rats (9), rats fed a high fat/low carbohydrate diet (10), and the streptozotocin diabetic rat (11) have suggested that the markedly reduced stimulatory action of insulin on glucose transport in these experimental animal models of insulin resistance is the consequence of a relative depletion of glucose transporters in the intracellular pool.

The present study was, therefore, undertaken to determine if an enriched intracellular pool and increased translocation of glucose transporters to the plasma membrane might explain the hyperresponsiveness of insulin-mediated glucose transport in adipose cells of the 30-day-old obese Zucker rat.

MATERIALS AND METHODS

The Zucker rats used in this study were bred in this laboratory from pairs originally provided by the Harriet G. Bird Laboratory (Stowe, MA). Known lean heterozygous (Fa/fa) females and obese homozygous (Fa/FA) males were mated. From this mating, 50% of the litter is expected to be obese (fa/fa) and 50% lean (FA/FA). For each study, approximately eight litters were selected with about 10 pups of both sexes in each. Pups were given free access to the dam’s diet and were separated from their mother at 28 days of age. Two days later, the pups were killed by cervical dislocation between 8 and 10 a.m.

Isolated adipose cells were obtained by enzymatic digestion of the inguinal fat pads from approximately 40 lean and 40 obese rats (12), and adipose cell size was determined microscopically (13). All incubations were carried out in Krebs-Ringer bicarbonate/Hepes buffer, pH 7.4, 37°C, containing 10 mg of bovine serum albumin (Sigma) per ml (6). Cells were incubated for 3 min in the absence or presence of 2.5 nM insulin. Samples of cells were removed to assess the rate of 3-O-methylglucose transport and the intracellular water space (steady-state uptake levels) using 0.5 mM substrate (7). Plasma and low-density microsomal membrane fractions were then prepared from the remaining cells by differential ultracentrifugation, equilibrium d-glucose-inhibitable cytochalasin B binding was measured, and the concentrations of glucose transporters were calculated (6). The specific 5’-nucleotidase and galactosyltransferase activities of each homogenate were determined (6). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

The abbreviations used are: Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.
mogenate and membrane fraction were assayed (6-8). Protein was determined by the method of Bradford (14). Plasma glucose and insulin concentrations were measured using the glucose oxidase method and with a radioimmunooassay kit provided by Sorin, Commission à l'Énergie Atomique, Gif-sur-Yvette, France, using a rat insulin standard (Novo Laboratories, Copenhagen, Denmark), respectively. Comparisons were made using a t test of statistical significance, and differences were accepted as significant at the p ≤ 0.05 level.

RESULTS

Some of the general characteristics of the experimental animals are presented in Table I. The 30-day-old obese Zucker rats were markedly hyperinsulinemic but eucregic and weighed somewhat more than their lean littermates. Adipose cell size and water space were increased 4-5-fold in the obese animals.

Basal and maximally insulin-stimulated 3-O-methylglucose transport activity in the adipose cells from 30-day-old lean and obese Zucker rats are illustrated in Fig. 1. Both basal and insulin-stimulated transport were approximately 6-fold greater in the cells from obese rats compared to the lean rats when expressed per cell (Fig. 1A) and approximately 2-fold greater when expressed per unit cellular surface area (Fig. 1B). However, the latter differences were not significantly different. The corresponding distribution of glucose transporters between the plasma and low-density microsomal membrane fractions from the same cells is illustrated in Fig. 2. In the basal state, the number of glucose transporters/mg of membrane protein in the plasma membrane fraction was approximately 2-fold greater in the cells from the obese rats compared to the lean rats (p ≤ 0.05). However, the insulin-mediated increase in the concentration of glucose transporters in the plasma membrane fraction was similar in both groups (Fig. 2A).

The numbers of glucose transporters/mg of membrane protein in the low-density microsomal membranes prepared from the basal cells of the obese rats was also increased by approximately 70% compared to the lean rats. In contrast to what was observed in the plasma membranes, insulin induced a decrease in the number of glucose transporters in this membrane fraction in both groups of rats. The insulin-mediated decrease in the concentration of glucose transporters in the low-density microsomal membranes was 2-fold greater in the cells from the obese rats than the lean rats. Thus, the numbers of glucose Transporters remaining to the low-density microsomal membranes after insulin stimulation were about the same between the two groups.

Table I also demonstrates the recoveries of protein in the original homogenates and each of the two membrane fractions examined here. In parallel with the increased intracellular water space, more protein was recovered in the homogenate and both membrane fractions from the adipose cells from the obese animals than from the lean animals, regardless of expressing the results per cell or per unit cellular surface area. In addition, the ratio of homogenate protein to intracellular water space and the per cent recoveries of both plasma and low-density microsomal membrane protein from the original homogenate were similar between the cells from the two groups of animals.

Table II demonstrates the relative enrichments (Table II, part A) and per cent recoveries (Table II, part B) of two marker enzymes, one each for the plasma and low-density microsomal membranes. Despite a marked reduction in the 5'-nucleotidase-specific activity in the plasma membrane fraction from the adipose cells from the obese rats compared to the lean rats, the plasma membrane fractions from the cells from both groups of animals were relatively enriched in this plasma membrane marker enzyme activity when compared to the corresponding low-density microsomal membrane fractions (Table II, part A). The relative contamination of the low-density microsomal membrane fraction from the cells from the obese rats with 5'-nucleotidase activity was, however, approximately half that from the cells from the lean animals. In contrast, only the low-density microsomal membrane fraction from the cells from the lean animals was relatively enriched in galactosyltransferase activity, a Golgi marker enzyme (Table II, part A). Indeed, the galactosyltransferase-specific activity was slightly greater in the plasma membrane fraction than the low-density microsomal membrane fraction from the cells from the obese rats. The per cent recoveries of 5'-nucleotidase activity from the original homogenate in both membrane fractions were similar between the cells from both groups of animals. However, the per cent recoveries of galactosyltransferase activity in both membrane fractions were consistently about 4-5-fold greater for cells from the obese rats compared to the lean rats (Table IIb, part B).^2

DISCUSSION

The results of the present investigation demonstrate that both basal and maximally insulin-stimulated rates of glucose transport activity in the inguinal adipose cells from 30-day-old obese Zucker rats are increased by approximately 6-fold compared to the cells from the same fat depot of their lean littermates (Fig. 1A). However, when the increased size of the cells from the obese animals is taken into account by expressing the glucose transport activities/unit cellular surface area, the difference between the cells from the two groups of animals is markedly reduced although a tendency toward greater glucose transport activities in cells from the obese animals remains (Fig. 1B). Moreover, the remaining tendencies toward greater basal and insulin-stimulated glucose transport activities/unit cellular surface area in cells from the obese rats than from lean rats correlate well with the corresponding small increases in the concentrations of glucose transporters/mg of membrane protein in the plasma membranes obtained from the cells from the obese animals (Fig. 2A).

The disproportionately greater basal concentration of glucose transporters in the plasma membranes from the adipose cells from the obese rats than from lean rats (Fig. 2A) appears to be accounted for by an increased contamination with glucose transporters from the low-density microsomes. An approximately 60% increase in the basal concentration of glucose transporters in the low-density microsomal membrane fraction from cells from the obese rats compared to the lean rats (Fig. 2B) and an approximately 60% increase in the relative enrichment of the plasma membrane fraction from the cells from the obese animals compared to lean animals with galactosyltransferase activity (Table II, part A), namely a marker enzyme of the Golgi-enriched low-density microsomal membranes, could potentially increase the concentration of glucose transporters in the plasma membrane fraction by 2.5-fold, a factor nearly identical to the difference actually observed. A similar increase is observed in the membrane protein

^2 The recoveries of 5'-nucleotidase activities from adipose cells from lean and obese Zucker rats are similar to previously published data for recoveries of this enzyme from small and large adipose cells from Sprague-Dawley rats (9). The recovery of galactosyltransferase activity from adipose cells from lean Zucker rats is lower than that from cells from obese Zucker rats and lean and obese Sprague-Dawley rats (9). This may be due to the difficulty in homogenizing these very small cells. However, this lower recovery is taken into account when calculating the numbers of glucose transporters/cell (Table III).
Increased Glucose Transporters in fa/fa Rat Adipocytes

TABLE I

General characteristics of 30-day-old lean and obese Zucker rats

| Parameter                        | Lean (mg)       | Obese (mg)      |
|---------------------------------|-----------------|-----------------|
| Body weight (g)                 | 58 ± 1 (20)     | 63 ± 1 (18)     |
| Plasma glucose (mM)             | 9.16 ± 0.38 (20)| 8.65 ± 0.15 (18)*|
| Plasma insulin (nM)             | 0.28 ± 0.03 (20)| 0.74 ± 0.13 (18) |
| Adipose cell size (ng lipid/cell)| 38 ± 4 (4)      | 179 ± 7 (4)     |

**Table I**

Lean and obese Zucker rats were littermates. Isolated adipose cells were prepared and cell size, intracellular water, and the content of cellular proteins were measured as described under "Materials and Methods." Results are the means ± S.E. of the indicated numbers of observations.

| Parameter                        | Lean (mg)       | Obese (mg)      |
|---------------------------------|-----------------|-----------------|
| Adipose intracellular water     | 0.41 ± 0.04 (4) | 70.5 ± 6.9      |
| Adipose cell protein contents   | Per cell        | Per unit cell   |
| Homogenate (mg/cell)            | 261 ± 16 (4)    | 44.1 ± 3.0      |
| Plasma membrane fraction        | 9.1 ± 1.3 (4)   | 1.5 ± 0.2       |
| Low-density microsomal membrane fraction | 7.5 ± 1.8 (4) | 1.3 ± 0.3 |

*Difference between lean and obese rats is not statistically significant at the p ≤ 0.05.

**FIG. 1.** Glucose transport activity per cell (A) and per unit cellular surface area (B) in the intact adipose cell from lean (L) and obese (Ob) 30-day-old Zucker rats incubated in the presence of 0 or 2.5 nM insulin and sampled for measurements of 3-O-methylglucose transport as described under "Materials and Methods." Results are the mean ± S.E. of the individual mean values obtained from quadruplicate samples in four experiments.

**FIG. 2.** Concentration of glucose transporters in the plasma membrane (A) and low-density microsomal membrane (B) fractions of the adipose cells from 30-day-old lean (L) and obese (Ob) Zucker rats. Membrane fractions were prepared from the isolated cells described in the legend to Fig. 1, and the concentrations of glucose transporters were determined using D-glucose-inhibitable cytochalasin B binding as described under "Materials and Methods." Results are the mean ± S.E. of the individual values obtained in four experiments.

---

recovered in the plasma membrane fraction from the cells from the obese rats compared to lean rats when these recoveries are expressed per unit cellular surface area (Table I). Thus, the increases in basal and maximally insulin-stimulated glucose transport activity observed in the cells from the obese animals appear to be fully explained by the increased size of the cells and their corresponding expanded surface area. The concentrations of glucose transporters in the plasma membrane of cells from obese rats are essentially unchanged from that observed in the much smaller cells from the lean animals.

Insulin has been shown to stimulate glucose transport in the rat adipose cell through a translocation of glucose transporters from the intracellular pool associated with the low-density microsomal membrane fraction to the plasma membrane (6-8). Furthermore, the magnitude of the response to insulin has been shown to reflect the size of this intracellular pool in the basal state, at least in several insulin-resistant states (9-11). Thus, the increased basal concentration of glucose transporters in, and the loss of glucose transporters in response of insulin from, the low-density microsomal membrane fraction from the cells from the obese rats compared to the lean rats appear to be inconsistent with the similar increases in the concentration of glucose transporters in the plasma membranes induced by insulin in the cells from both groups of animals (Fig. 2). However, a tentative reconciliation
Increased Glucose Transporters in fa/FA Rat Adipocytes

Relative marker enzyme-specific activities among membrane fractions (A) and recovery of marker enzyme activities (B) from adipose cells from 30-day-old lean and obese Zucker rats

For A, marker enzyme activities were measured in the membrane fractions prepared in the experiments described in the legend to Fig. 2 using the procedures described under "Materials and Methods." Within each experiment, marker enzyme-specific activities were obtained for each membrane fraction prepared from basal and insulin-stimulated cells, the activities obtained for the basal and insulin-stimulated cells were averaged, and the average activities were expressed as a percentage of their respective highest activity observed. Results are the mean ± S.E. of the individual values obtained in each of the cell preparations studied (four for each group). For B, within each experiment, total marker enzyme activities were calculated for each homogenate and membrane fractions, prepared from basal and insulin-stimulated cells, the activities obtained for the basal and insulin-stimulated cells were summed, and the summed activities were expressed as a percentage of the summed activity for the homogenates. Results are the mean ± S.E. of the individual values obtained in each of the cell preparations studied (four for each group).

| Marker enzyme activity | Phenotype  | Membrane fraction | Plasma membranes | Low-density microsomal membranes |
|-----------------------|------------|-------------------|------------------|---------------------------------|
|                       |            |                   | mean ± S.E.      |                                 |
| A. 5'-Nucleotidase    | Lean       | 0.153 ± 0.012 (100%) | 0.064 ± 0.016 (118%) |
| (nmol/mg)             | Obese      | 0.001 ± 0.002 (100%) | 0.006 ± 0.001 (20%) |
| Galactosyltransferase | Lean       | 37.6 ± 5.4 (69.5%)   | 25.3 ± 3.6 (100%)  |
| (nmol/mg/2 h)         | Obese      | 43.6 ± 4.3 (113%)   | 38.6 ± 2.0 (100%)  |
| B. 5'-Nucleotidase    | Lean       | 15.2 ± 2.4          | 3.8 ± 0.8         |
|                       | Obese      | 19.6 ± 3.1          | 3.4 ± 0.5         |
| Galactosyltransferase | Lean       | 7.8 ± 1.7           | 6.7 ± 1.1         |
|                       | Obese      | 37.5 ± 9.3          | 28.0 ± 1.8        |

* Actual specific activity values set at 100%.

TABLE III
Estimated number (×10^6) of glucose transporters/cell in adipose cells from 30-day-old lean and obese Zucker rats

Numbers of glucose transporters/cell in the plasma membrane and intracellular pool were estimated from the mean concentration of glucose transporters/mg of membrane protein (Fig. 2), mean recovered membrane protein (Table I), and mean recovered 5'-nucleotide and galactosyltransferase activities (Table II, part B) in the plasma and low-density microsomal membrane fractions, respectively, prepared in the experiments described in the legend to Fig. 2. See text for detailed discussion of limitations of estimates.

| Phenotype | Insulin | Cellular Site | Total |
|-----------|---------|---------------|-------|
|           |         | Plasma membrane | Intracellular pool | |
| Lean      | −       | 0.22           | 1.57  | 1.79  |
|           | +       | 0.85           | 0.65  | 1.50  |
| Obese     | −       | 2.23           | 6.00  | 8.23  |
|           | +       | 5.03           | 1.90  | 6.93  |

The intact cells (Fig. 1A) strongly correlates with the number of plasma membrane glucose transporters in both the absence and presence of insulin. However, if the potential 2.5-fold contamination of plasma membrane glucose transporters by glucose transporters from the low-density microsomal membrane fraction in the basal cells from the obese rats is taken into consideration as described above, then the estimated number of basal plasma membrane glucose transporters in the intact cells from the obese animals is reduced from 2.23 × 10^6/cell to 0.86 × 10^6/cell, and glucose transporter activity correlates strongly with the number of plasma membrane glucose transporters over all conditions. 3) When 0.86 × 10^6 plasma membrane glucose transporters are assumed as the best estimate for the cells from the obese animals in the basal state, then the estimated total number of glucose transporters in the basal cells from the obese rats is taken into consideration as described above, then the estimated number of basal plasma membrane glucose transporters in the intact cells from the obese animals is reduced from 2.23 × 10^6/cell to 0.86 × 10^6/cell, a value similar to that obtained in the insulin-stimulated cells. In addition, the loss of intracellular glucose transporters becomes similar to the appearance of glucose transporters in the plasma membrane in response to insulin. As in the smaller cells from the lean animals, approximately 90% of the total glucose transporters in the enlarged cells from the obese animals are intracellular in the basal state. 4) The enlarged cells from the obese animals contain approximately 4-fold more total glucose transporters/cell than the smaller cells from the lean animals, a difference which is only slightly less than the difference in intracellular water space.

Previous studies from this laboratory have also demonstrated increased rates of glucose metabolism in adipose cells from 30-day-old obese Zucker rats (5). The exact mechanism for this increased metabolic activity is not known. However, since glucose transport activity is also increased as a result of an increased number of glucose transporters, it must certainly play a role in this increased metabolic response. Recently, it has been reported that both fatty acid synthetase (16) and lipoprotein lipase (17, 18) activities are enhanced in the young obese rats. It was proposed that these increases might be a
result of increased levels of glycolytic metabolites within the young obese rats. It was proposed that these increases might be a result of increased levels of glycolytic metabolites within the adipocytes through increased glucose uptake (16). Although glucose transport may be a primary controlling site for intracellular glucose metabolism, it must be emphasized that other possible enzymatic sites may also be important in controlling overall glucose utilization.

These present studies are in contrast with the idea that hyperinsulinemia and obesity are characterized by marked insulin resistance. Thirty-day-old obese Zucker rats do demonstrate marked systemic insulin resistance in that their plasma glucose concentrations are normal in the face of inappropriately elevated plasma insulin concentrations (Table I). However, in contrast to this systemic insulin resistance, the ability of insulin to stimulate glucose transport at the level of the adipose cell was not decreased. In addition, these present results are in contrast to previously published data concerning the insulin resistance accompanying adipose cell enlargement of the aging rat model of obesity (9). If adipose cells from 30-day-old obese Zucker rats are compared with data for cells of a similar size from a Sprague-Dawley rat, marked differences are observed. At this cell size, insulin’s ability to stimulate glucose transport and the number of glucose transporters translocated to the plasma membrane are already markedly reduced in cells from Sprague-Dawley rats. However, in cells from 30-day-old obese Zucker rats, the number of glucose transporters and insulin’s ability to stimulate glucose transport are greatly increased. In addition, there is a marked difference in the distribution of the total numbers of glucose transporters in adipose from young obese rats compared to the aged obese Sprague-Dawley rat. In the young, obese Zucker rat, 80–90% of the total number of glucose transporters are in the intracellular pool, whereas in the aged obese animal, most of the transporters are in the plasma membrane. This redistribution of transporters can account for the increased insulin stimulatory effect on transport in the young obese rats and the loss of insulin stimulation in the old obese rats. The cause for this altered distribution is presently not known. Some of these differences may be accounted for by the differences in the strains of the animals. However, the role of hyperinsulinemia observed as early as 17 days of age (16) in the development of the obesity syndrome may be very important in determining the number of glucose transporters and the activity of glucose transport in adipose cells (19, 20).

Recently, an experimental model of hyperinsulinemia has suggested similar findings to the present investigation (19). When experimental chronic hyperinsulinemia is induced through the use of osmotic minipumps or insulin injections, similar, although smaller, increases in the rates of glucose transport are observed. Preliminary results have indicated that the mechanism of this increased glucose transport in this model of hyperinsulinemia is an increase in the number of glucose transporters (20).

In conclusion, this present study demonstrates that the increased rates of glucose transport activity observed in adipose cells from 30-day-old obese Zucker rats as compared to their lean littermates can be accounted for by a 4–5-fold increase in the number of glucose transporters, most of which are localized in an intracellular pool. Whether this may be accounted for by the genetic lesion of these obese Zucker rats or by the accompanying hyperinsulinemia remains to be determined.

Acknowledgments—We wish to thank Colette Guichard for her technical assistance, Jean Girard and Roger Assan for their encouragement of this work, Samuel W. Cushman for helpful suggestions in presenting these results, and Valérie Resve for typing this manuscript.

REFERENCES

1. Bray, G. A., and York, D. A. (1979) Physiol. Rev. 59, 719–809
2. Zucker, L. M., and Antoniades, H. N. (1972) Endocrinology 90, 1320–1330
3. Cushman, S. W., Zarnowski, M. J., Franzussoff, A. J., and Salans, L. B. (1978) Metab. Clin. Exp. 27, 1930–1940
4. Czech, M. P., Richardson, D. K., Becker, S. G., Walters, C. G., Gitomer, N., and Heinrich, J. (1978) Metab. Clin. Exp. 27, 1967–1981
5. Lavau, M., Guerre-Millo, M., and Guichard, C. (1983) Diabetologia 25, 175A
6. Cushman, S. W., and Wardzala, L. J. (1980) J. Biol. Chem. 255, 4758–4762
7. Karnieli, E., Zarnowski, M. J., Hissin, P. J., Simpson, I. A., Salans, L. B., and Cushman, S. W. (1981) J. Biol. Chem. 256, 4772–4777
8. Suzuki, K., and Kono, T. (1980) Proc. Natl. Acad. Sci. U. S. A. 77, 2542–2545
9. Hissin, P. J., Foley, J. E., Wardzala, L. J., Karnieli, E., Simpson, I. A., Salans, L. B., and Cushman, S. W. (1982) J. Clin. Invest. 70, 780–790
10. Hissin, P. J., Karnieli, E., Simpson, I. A., Salans, L. B., and Cushman, S. W. (1982) Diabetes 31, 589–592
11. Karnieli, E., Hissin, P. J., Simpson, I. A., Salans, L. B., and Cushman, S. W. (1981) J. Clin. Invest. 68, 811–814
12. Rodbell, M. (1964) J. Biol. Chem. 239, 375–380
13. Lavau, M., Susini, C., Knittle, J., Blanchet-Hirsch, S., and Greenwood, M. R. C. (1977) Proc. Soc. Exp. Biol. Med. 156, 251–256
14. Bradford, M. M. (1976) Anal. Biochem. 72, 248–254
15. Simpson, I. A., Yver, D. R., Hissin, P. J., Wardzala, L. J., Karnieli, E., Salans, L. B., and Cushman, S. W. (1983) Biochim. Biophys. Acta 763, 393–407
16. Bazin, R., and Lavau, M. (1982) J. Lipid Res. 23, 839–849
17. Boulangel, A., Planche, E., and de Gasquet, P. (1979) J. Lipid Res. 20, 857–864
18. Gruen, R., Hietanen, E., and Greenwood, M. R. C. (1978) Metab. Clin. Exp. 27, 1955–1966
19. Horton, E. S., Wardzala, L., Hirshman, M., Pofcher, E., and Horton, E. D. (1983) Diabetes 32, 25A
20. Kahn, B. B., Horton, E. S., and Cushman, S. W. (1984) Clin. Res. 32, 399A