Mechanism of Insulin Resistance in A-ZIP/F-1 Fatless Mice*

(Received for publication, December 18, 1999, and in revised form, January 20, 2000)

Jason K. Kim‡§, Oksana Gavrilova¶, Yan Chen‡, Marc L. Reitman‡, and Gerald I. Shulman‡‡

From the ¶Howard Hughes Medical Institute and the Department of Internal Medicine, Yale University School of Medicine, New Haven, Connecticut 06536 and the §Diabetes Branch, NIDDK, National Institutes of Health, Bethesda, Maryland 20892

Insulin resistance is a major factor in the pathogenesis of type 2 diabetes and may be related to alterations in fat metabolism. Fatless mice have been created using dominant-negative protein (A-ZIP/F-1) targeted gene expression in the adipocyte and shown to develop diabetes. To understand the mechanism responsible for the insulin resistance in these mice, we conducted hyperinsulinemic-euglycemic clamps in awake fatless and wild type littermates before the development of diabetes and examined insulin action and signaling in muscle and liver. We found the fatless mice to be severely insulin-resistant, which could be attributed to defects in insulin action in muscle and liver. Both of these abnormalities were associated with defects in insulin activation of insulin receptor substrate-1 and -2-associated phosphatidylinositol 3-kinase activity and a 2-fold increase in muscle and liver triglyceride content. We also show that upon transplantation of fat tissue into these mice, triglyceride content in muscle and liver returned to normal as does insulin signaling and action. In conclusion, these results suggest that the development of insulin resistance in type 2 diabetes may be due to alterations in the partitioning of fat between the adipocyte and muscle/liver leading to accumulation of triglyceride in the latter tissues with subsequent impairment of insulin signaling and action.

Insulin resistance plays an essential role in the development of type 2 diabetes (1) and may be related to alterations in fat metabolism (2–6). Using aP2 enhancer/promoter to target adipocyte-specific transgene expression of a dominant-negative protein termed A-ZIP/F-1, which inhibits the DNA binding and function of B-ZIP proteins in both the C/EBP and AP-1 families of transcription factors, Moitra et al. (7) have developed transgenic mice with virtually no white fat tissue and dramatically reduced amounts of inactive brown fat tissue (hence called “fatless”). Despite the virtual absence of fat tissue in the body, the fatless mice develop a type 2 diabetes phenotype as they are hyperinsulinemic and hyperglycaemic at 1 and 4 weeks of age, respectively (7).

To determine the mechanisms by which the fatless mice become insulin-resistant, insulin action and signaling were examined in the liver and muscle of awake fatless and wild type mice during a hyperinsulinemic-euglycemic clamp. In order to prevent effects due to glucose toxicity experiments were performed in young, normoglycemic, fatless, and wild type littermates.

EXPERIMENTAL PROCEDURES

Animals

Male fatless (A-ZIP/F-1; n = 19) and wild type (n = 25) littermates were studied at 3 weeks of age (9–13 g of body weight) at least 7 days after arrival. The mice were kept with their mothers until the day of experiment. Animals were housed under controlled temperature (23 °C) and lighting (12-h light, 0600–1800 h; 12-h dark, 1800–0600 h) with free access to water and standard mouse chow. All procedures were approved by the Yale University Animal Care and Use Committee.

Surgery and Animal Handling

At least 4 days before experiments, mice were anesthetized with Avertin (0.5 g of tribromoethanol and 0.25 g of tert-amyl alcohol in 39.5 ml of water; 0.02 ml/g of body weight), and an indwelling catheter was inserted in the left internal jugular vein and externalized after an incision in a skin flap behind the head.

Experimental Protocol

Two studies were conducted, starting at 1000 h, after an overnight fast (mice were removed from their mothers at 1700 h on the day before the experiment).

Study 1. Insulin-stimulated Whole Body and Skeletal Muscle Glucose Flux

Hyperinsulinemic-Euglycemic Clamp—A 120-min hyperinsulinemic-euglycemic clamp was conducted with a prime continuous infusion of human insulin (Humulin, Lilly) at a rate of 15 pmol/kg/min to raise plasma insulin concentration to ~850 pmol/l. Blood samples (20 μl) were collected at 30-min intervals for the immediate measurement of plasma glucose concentration, and 20% glucose was infused at variable rates to maintain plasma glucose at ~6.3 mm. Insulin-stimulated whole body glucose flux was estimated using a prime continuous infusion of high pressure liquid chromatography-purified [3-3H]glucose (10–μCi bolus, 0.1 μCi/ml; NEN Life Science Products) throughout the clamps. To estimate insulin-stimulated glucose transport activity and metabolism in skeletal muscle, 2-deoxy-D-[1-14C]glucose (2-[14C]DG; NEN Life Science, Boston, MA) was administered as a bolus (10 μCi) at 45 min before the end of clamps. Blood samples (20 μl) were taken at 77, 80, 85, 90, 100, 110, and 120 min after the start of clamps for the determination of plasma [3H]glucose, 2-[14C]DG, and H2O concentrations. Additional blood samples (10 μl) were collected before the start and at the end of clamps for measurement of plasma insulin concentrations. All infusions were done using microdialysis pumps (CMA/Microdialysis, Acton, MA). At the end of clamps, animals were anesthetized with sodium pentobarbital injection. Within 5 min, four muscles (soleus, gastrocnemius, tibialis anterior, and quadriceps) from both hindlimbs, visceral adipose tissue (in the wild type mice), and liver were taken. Each tissue, once exposed, was dissected out within 2 s, frozen immediately using liquid N2-cooled aluminum blocks, and stored at ~−70 °C for later analysis. In separate experiments, the basal rates of glucose turnover were measured.
Alteration of Distribution of Body Fat Leads to Insulin Resistance

8457

TABLE I
Metabolic parameters during basal and hyperinsulinemic-euglycemic clamp periods in the wild type and fatless mice, wild type, sham-operated fatless, and fat-transplanted fatless mice

| n  | Age (weeks) | Basal period | Clamp period |
|----|-------------|--------------|--------------|
|    |             | Plasma glucose (μmol/kg/min) | Plasma insulin (μmol/kg/min) | Plasma glucose (μmol/kg/min) | Plasma insulin (μmol/kg/min) |
| Wild type | 6 | 3 ± 1 | 11 ± 2 | ND | 6.6 ± 0.2 | 108 ± 4 | 6.2 ± 0.1 | 805 ± 50 |
| Fatless | 6 | 3 ± 1 | 12 ± 2 | ND | 7.3 ± 0.5 | 181 ± 11 | 6.4 ± 0.3 | 882 ± 37 |
| Wild type | 4 | 10 ± 1 | 24 ± 1 | 4.1 ± 0.1 | 7.0 ± 0.2 | 87 ± 11 | 6.3 ± 0.1 | 838 ± 68 |
| Sham-operated fatless | 3 | 10 ± 1 | 24 ± 1 | 6.4 ± 0.5 | 11.1 ± 1.0 | 328 ± 69 | 7.4 ± 0.6 | 941 ± 103 |
| Fat-transplanted fatless | 3 | 10 ± 1 | 24 ± 1 | 3.6 ± 0.0 | 7.7 ± 0.4 | 128 ± 31 | 6.0 ± 0.3 | 885 ± 72 |

a ND, not determined.

b p < 0.05 versus wild type group by unpaired Student’s t test.

c p < 0.05 for fat-transplanted fatless group versus sham-operated fatless group by unpaired Student’s t test.

Results

In Vivo Glucose Flux Analysis—Plasma glucose during clamps was analyzed using 10 μl of plasma by a glucose oxidase method on a Beckman glucose analyzer II (Beckman, Fullerton, CA), and plasma insulin was measured by radioimmunoassay using kits from Linco Research (St. Charles, MO). For the determination of plasma [3-14C]-glucose and 2-[14C]DG concentrations, plasma was deproteinized with ZnSO4 and Ba(OH)2, dried to remove 3H2O, resuspended in water, and counted in scintillation fluid (Ultima Gold, Packard, Meriden, CT) on dual channels for separation of 4H and 14C. The glucose concentration of 3H2O was determined by the difference between 3H counts without and with drying. For the determination of muscle 2-[14C]DG-6-phosphate (2-DG-6-P) content, muscle samples were homogenized, and the supernatants were subjected to an ion-exchange column to separate 2-DG-6-P from 2-DG, as described previously (8). The radioactivity of 3H in muscle glycogen was determined by digesting muscle samples in KOH and precipitating glycogen with ethanol as described previously (9). Muscle glycogen synthesis was measured using 1-C14DP-glucose, and triglyceride contents in muscle and liver were determined using a method adapted from Storlien et al. (10).

Calculations—Rates of whole body glucose uptake and basal glucose turnover were determined as the ratio of the [3H]-glucose infusion rate (disintegrations per min) to the specific activity of plasma glucose (disintegrations per min/μmol) during the final 30 min of respective experiments. Hepatic glucose production (HGP) during clamps was determined by subtracting the glucose infusion rate from the whole body glucose uptake. Whole body glucose was calculated from the rate of increase in plasma 2-[14C]DG-6-phosphate (2-DG-6-P) content, muscle samples were homogenized, and the supernatants were subjected to an ion-exchange column to separate 2-DG-6-P from 2-DG, as described previously (8). The radioactivity of 3H in muscle glycogen was determined by digesting muscle samples in KOH and precipitating glycogen with ethanol as described previously (9). Muscle glycogen synthesis was measured using 1-C14DP-glucose, and triglyceride contents in muscle and liver were determined using a method adapted from Storlien et al. (10).

Study 2. IRS-1- and IRS-2-associated PI 3-Kinase Activity in Muscle and Liver

A 30-min hyperinsulinemic-euglycemic clamp was conducted with a prime continuous infusion of insulin and a variable infusion of 20% glucose as described in Study 1 to assess IRS-1- and IRS-2-associated PI 3-kinase activity during the insulin-stimulated condition. For the basal level of IRS-1- and IRS-2-associated PI 3-kinase activity, saline was infused for 30 min. At the end of 30 min, muscle (gastrocnemius and quadriceps) and liver were rapidly taken and stored for the measurement of IRS-1- and IRS-2-associated PI 3-kinase activity, respectively. IRS-1-associated PI 3-kinase activity in muscle and IRS-2-associated PI 3-kinase activity in liver were measured using the antibodies to IRS-1 and IRS-2 (kindly provided by Dr. Morris White, Joslin Diabetes Center, Boston, MA), respectively, as described previously (13).

Effect of Fat Transplantation into Fatless Mice—At 5 weeks of age, ~900 mg of parametrial fat from wild type littermates were transplanted into dorsal subcutaneous tissue of fatless mice (fat-transplanted fatless mice; n = 3) as described previously (14). Additional fatless mice received a sham operation at the same age and served as control (sham-operated fatless mice; n = 3). At 10 weeks of age, a 120-min hyperinsulinemic-euglycemic clamp combined with [3-14C]-glucose infusion and 2-[14C]DG injection was conducted in age-matched wild type (n = 4), fat-transplanted fatless, and sham-operated fatless mice, as described in Study 1, to assess the metabolic effects of fat transplantation into the fatless mice.

Statistical Analysis—Data are expressed as means ± S.E. Statistical significance between the wild type versus fatless mice (Study 1) and fat-transplanted fatless versus sham-operated fatless mice (Study 2) was determined by unpaired Student’s t test.

RESULTS

Insulin action on glucose transport and metabolism was examined during a 2-h hyperinsulinemic-euglycemic clamp in awake wild type and fatless littermates at 3 weeks of age before the onset of hyperglycemia (7). Basal plasma insulin concentration was elevated by ~70% in the fatless mice compared with the wild type mice (p < 0.005) (Table I), suggesting that the fatless mice are insulin-resistant. During the clamps, plasma insulin concentration was raised to ~850 pm, whereas the plasma glucose concentration was maintained at ~63 mmol/l by a variable infusion of glucose in both groups (Table I). The glucose infusion rate required to maintain euglycemia increased rapidly in the wild type mice and reached a steady state within 90 min. In contrast, there was a markedly blunted insulin response during the hyperinsulinemic-euglycemic clamp studies in the fatless mice, as reflected by a much lower steady state glucose infusion rate in the fatless mice (18 ± 5 versus 192 ± 21 mmol/kg/min in the wild type mice; p < 0.001) (Fig. 1a). Insulin-stimulated whole body glucose uptake was decreased by 50% in the fatless mice (141 ± 7 versus 282 ± 11 μmol/kg/min in the wild type mice; p < 0.001) (Fig. 1c). Insulin-stimulated whole body glucose infusion and glycogen synthesis were decreased by 50% (84 ± 9 versus 190 ± 13 μmol/kg/min in the wild type mice; p < 0.001) and 38% (57 ± 8 versus 92 ± 16 μmol/kg/min in the wild type mice; p < 0.05), respectively, in the fatless mice (Fig. 1c). Rates of HGP during the basal state were similar in both groups (145 ± 7 and 153 ± 10 μmol/kg/min in the wild type and fatless mice, respectively; p > 0.05) (Fig. 1b). However, insulin’s ability to suppress HGP during the hyperinsulinemic-euglycemic clamp was severely impaired in the fatless mice (123 ± 8 versus 89 ± 11 μmol/kg/min in the wild type mice; p < 0.05) (Fig. 1b). The rate of insulin-stimulated glucose transport activity in skeletal muscle in vivo was estimated using 2-deoxy-D-[1-14C]glucose injection during hyperinsulinemic-euglycemic clamps in awake mice. Because 2-deoxyglucose is a glucose analog that is phosphorylated but not metabolized, insulin-stimulated glucose transport activity in skeletal muscle can be estimated by determining the muscle content of 2-deoxyglucose-6-phosphate. Insulin-stimulated glucose transport activity in skeletal muscle (gastrocnemius) was decreased by 55% in the fatless mice (118 ± 14 versus 264 ± 18 nmol/g of muscle/min in the wild type mice; p < 0.001) (Fig. 1d). Similar to the pattern...
of changes in whole body glucose flux and glucose transport activity, insulin-stimulated rates of glycolysis and glycogen synthesis in skeletal muscle were decreased by 58% (97 ± 14 versus 229 ± 20 nmol/g/min in the wild type mice; p < 0.001) and 38% (21 ± 2 versus 34 ± 4 nmol/g/min in the wild type mice; p < 0.05), respectively, in the fatless mice (Fig. 1d). In addition, insulin activation of glycogen synthase was also significantly decreased by 53% in the fatless mice (18 ± 4 versus 38 ± 6% activation in the wild type mice; p < 0.005). Taken together, these abnormalities in insulin activation of glucose transport and glycogen synthase activity suggest a common upstream defect in the insulin signaling cascade.

Recent studies in IRS-1 and IRS-2 gene-disrupted mice have suggested that IRS-1 is important in insulin activation of muscle glucose metabolism (i.e. insulin-stimulated glucose transport and glycogen synthase activity), whereas IRS-2 is more important in mediating insulin activation of hepatic glucose metabolism (i.e. insulin suppression of HGP) (15, 16). Basal IRS-2-associated PI 3-kinase activity in liver was similar in both groups (189 ± 10 and 243 ± 28 cpm × 10^3 in the wild type and fatless mice, respectively; p > 0.05) (Fig. 2a). In contrast, basal IRS-1-associated PI 3-kinase activity in muscle was decreased in the fatless mice (26 ± 7 versus 59 ± 8 cpm × 10^3 in the wild type mice; p < 0.01) (Fig. 2b). Insulin-stimulated IRS-2-associated PI 3-kinase activity in liver and IRS-1-associated PI 3-kinase activity in muscle were found to be decreased by 47% (350 ± 19 versus 677 ± 41 cpm × 10^3 in the wild type mice; p < 0.005) and 75% (47 ± 14 versus 111 ± 25 cpm × 10^3 in the wild type mice; p < 0.001), respectively, in the fatless mice following a 30-min hyperinsulinemic-euglycemic clamp (Fig. 2, a and b). These findings suggest that insulin resistance in these tissues in the fatless mice may be secondary to the observed defects in insulin signaling. Interestingly, triglyceride concentrations in liver and muscle were elevated by 2-fold in the fasted, prediabetic fatless mice (65.3 ± 6.6 versus 32.7 ± 5.0 μmol/g in liver of the wild type mice and 15.6 ± 3.0 μmol/g in muscle of the wild type mice; p < 0.05 for both) (Fig. 2, c and d), suggesting an important relationship between elevation of triglyceride and perturbation of insulin signaling in these tissues.

To further examine the relationship between the distribution of body fat and insulin action, the parametrical fat from littermates was transplanted into the fatless mice (14), and the effect of restoring fat tissue in the fatless mice on insulin action was examined 5 weeks later. Sham-operated fatless mice had increased daily amount of food intake compared with the wild type mice, possibly because of a decreased leptin concentration, and fat transplantation normalized the food intake (Table I). Fat transplantation prevented the development of the type 2 diabetes phenotype (i.e. hyperglycemia and hyperinsulinemia) in the fatless mice (Table I). Fat transplantation increased insulin-stimulated whole body glucose uptake by 2.5-fold in the fatless mice (274 ± 45 versus 111 ± 21 μmol/kg/min in the sham-operated fatless mice; p < 0.05) (Fig. 3a), and this rate was similar to the wild type mice (261 ± 19 μmol/kg/min in the age-matched wild type littermates). Normalization of whole body glucose disposal could be attributed to a significant increase in insulin-stimulated muscle glucose transport activity in the fat-transplanted fatless mice (312 ± 19 versus 126 ± 17 nmol/g/min in the sham-operated fatless mice; p < 0.005) (Fig. 3b), and the rate was comparable with the wild type mice (291 ± 19 nmol/g/min). The ability of insulin to suppress HGP also normalized in the fatless mice following fat transplantation (9 ± 2 versus 106 ± 22 μmol/kg/min in the sham-operated fatless mice; p < 0.05) (Fig. 3b). In addition, fat transplantation in the fatless mice normalized insulin activation of IRS-1-associated PI 3-kinase activity in muscle (167 ± 16 versus 31 ± 18 cpm × 10^3 in the sham-operated fatless mice; p < 0.05) and IRS-2-associated PI 3-kinase activity in liver (368 ± 33 versus 253 ± 13 cpm × 10^3 in the sham-operated fatless mice; p < 0.05) (Fig. 3c). This improvement in insulin signaling and action in muscle and liver was associated with a 67% (7.0 ± 1.5 versus 20.7 ± 1.9 μmol/g) in the sham-operated fatless mice; p < 0.005) and 55% (39.1 ± 9.8 versus 87.5 ± 11.7 μmol/g in the sham-operated fatless mice; p < 0.05) reduction in muscle and liver triglyceride concentrations in liver and muscle.
Our findings support this concept in that the defect in insulin action in muscle and liver of the fatless mice was paralleled by increases in triglyceride concentration in these tissues, suggesting an important relationship between triglyceride content and insulin action. Similar inverse relationships between triglyceride content in skeletal muscle and insulin action have recently been demonstrated in normal and prediabetic humans (21–23). Pan et al. (24) found a significant inverse relationship between skeletal muscle triglyceride content and insulin action in non-diabetic Pima Indians. Similarly, Perseghin et al. (25) demonstrated that nondiabetic offspring of diabetic parents were characterized by insulin resistance and increased intramyocellular triglyceride content. These findings, together with our results in the fatless mice, suggest that increased intracellular triglyceride content in the skeletal muscle may be related to the defects in insulin action, which then lead to the development of diabetes.

Fat transplantation significantly lowered intramuscular triglyceride content and restored insulin's ability to stimulate IRS-1-associated PI 3-kinase activity as well as glucose transport activity in skeletal muscle of fatless mice. Fat transplantation also caused a significant decrease in intrahepatic triglyceride content, which was associated with a significant improvement in insulin activation of IRS-2-associated PI 3-kinase activity and suppression of HGP. One possible mechanism for this remarkable normalization of insulin action in skeletal muscle and liver is that the fat transplantation caused a redistribution of triglyceride away from skeletal muscle/liver and into the transplanted fat. Additionally, the normalization of insulin action could be due to increased plasma leptin concentration in the fat-transplanted fatless mice as suggested by Shimomura et al. (24). However, we find that leptin is only minimally effective at reversing the diabetes of the A-ZIP/F-1 mice (25).

More than 30 years ago, Randle et al. (4) introduced the concept of substrate competition between glucose and free fatty acids (i.e. glucose-fatty acid cycle) and postulated that free fatty acids cause insulin resistance through an increase in the NADH/NAD and acetyl-CoA/CoA ratios leading to the inhibition of pyruvate dehydrogenase activity (26). This event also causes an accumulation of citrate, which leads to the inhibition of phosphofructokinase activity (27). Subsequently, glucose 6-phosphate concentration increases, which leads to the inhibition of hexokinase activity and glucose uptake (28). Recent studies by our group suggest a different mechanism for free fatty acid-induced insulin resistance in humans (6, 29). We found that acute elevations in plasma free fatty acids in humans result in decreased glucose transport activity in skeletal muscle, as reflected by decreased concentrations of intracellular glucose and glucose 6-phosphate, which was associated with a reduction in IRS-1-associated PI 3-kinase activity (6). Our observation of parallel defects in insulin’s ability to stimulate glucose transport and glycogen synthase activity in the fatless mice further suggests a common upstream defect in the insulin signaling cascade. In this regard, insulin stimulation of both glucose transport and glycogen synthase activity has been associated with activation of IRS-1-associated PI 3-kinase activity (6). Our observations of parallel defects in insulin’s ability to stimulate glucose transport and glycogen synthase activity in the fatless mice further suggests a common upstream defect in the insulin signaling cascade. In this regard, insulin stimulation of both glucose transport and glycogen synthase activity has been associated with activation of IRS-1-associated PI 3-kinase activity (6). Our observations of parallel defects in insulin’s ability to stimulate glucose transport and glycogen synthase activity in the fatless mice further suggests a common upstream defect in the insulin signaling cascade. In this regard, insulin stimulation of both glucose transport and glycogen synthase activity has been associated with activation of IRS-1-associated PI 3-kinase activity (6).
of protein kinase C\(\theta\). In rats, we have shown that an acute elevation of plasma free fatty acids for 5 h results in activation of protein kinase C\(\theta\), a serine kinase, which is associated with decreased tyrosine phosphorylation of IRS-1 (13) and increased serine phosphorylation.\(^2\) This serine phosphorylation of IRS-1 in diacylglycerol, a known potent activator of protein kinase C, would in turn reduce the ability of IRS-1 to activate PI 3-kinase and this increase in acyl-CoA might lead to an increase in diacylglycerol, a known potent activator of protein kinase C\(\theta\) (32).

In summary, the results of the present study demonstrate that primary alterations in the adipocyte lead to alterations in the distribution of triglyceride between the adipocyte and liver/muscle and accumulation of triglyceride in liver/muscle, which subsequently leads to an impairment of insulin signaling and action in these tissues. These data also suggest a common mechanism for the development of insulin resistance in patients with lipodystrophy and patients with type 2 diabetes.

Acknowledgments—We are grateful to Dr. Pat McNulty, Ying Zhu, Veronika Walton, and Aida Groszmann for technical assistance.

REFERENCES
1. DeFronzo, R. A. (1988) Diabetes 37, 667–687
2. McGarry, J. D. (1992) Science 258, 766–770
3. Boden, G. (1997) Diabetes 46, 3–19
4. Randle, P. J., Garland, P. B., Hales, C. N., and Newsholme, E. A. (1963) Lancet 1, 785–789
5. Beaven, G. M., Hollenbeck, C. M., Jeng, C.-Y., Wu, M. S., and Chen, Y.-D. (1988) Diabetes 37, 1020–1024
6. Dresner, A., Laurent, D., Marcucci, M., Griffin, M. E., Dufour, S., Cline, G. W., Slezak, L. A., Andersen, D. K., Hundal, R. H., Rothman, D. L., Petersen, K. F., and Shulman, G. I. (1999) J. Clin. Invest. 103, 253–259
7. Maitra, J., Mason, M. M., Olive, M., Krylov, D., Gavrilova, O., Marcus-Samuels, B., Feigenbaum, L., Lee, E., Aoyama, T., Eckhaus, M., Reitman, M. L., and Vinson, C. (1998) Genes Dev. 12, 3168–3181
8. Ohshima, K., Shargill, N. S., Chan, T. M., and Bray, G. A. (1984) Am. J. Physiol. 246, E193–E197
9. Kim, J. K., Wi, J. K., and Youn, J. H. (1996) Diabetes 45, 446–453
10. Storlien, L. H., Jenkins, A. B., Chisholm, D. J., Pascoe, W. S., Khouri, S., and Kraegen, E. W. (1993) Diabetes 42, 280–289
11. Rossetti, L., and Giaccari, A. (1999) J. Clin. Invest. 85, 1758–1792
12. Youn, J. H., Kim, J. K., and Buchanan, T. A. (1994) Diabetes 43, 564–571
13. Griffin, M. E., Marcucci, M. J., Cline, G. W., Bell, K., Barucci, N., Lee, D., Goodyear, L. J., Kraegen, E. W., White, M. F., and Shulman, G. I. (1999) Diabetes 48, 1270–1274
14. Gavrilova, O., Marcus-Samuels, B., Graham, D., Kim, J. K., Shulman, G. I., Castle, A. L., Vinsen, C., Eckhaus, M., and Reitman, M. L. (2000) J. Clin. Invest. 105, 271–278
15. Yamauchi, T., Tobe, K., Tamemoto, H., Ueki, K., Kaburagi, Y., Yamamoto-Honda, R., Takahashi, Y., Yoshizawa, F., Aizawa, S., Akanuma, Y., Sonenberg, N., Yagaki, Y., and Radkowski, T. (1996) Mol. Cell. Biol. 16, 3074–3084
16. Previs, S. F., Ren, J.-M., White, M. F., and Shulman, G. I. (1999) Diabetes 48, A51
17. Shimomura, I., Hammer, R. E., Richardson, J. A., Ikemoto, S., Bashmakov, Y., Goldstein, J. L., and Brown, M. S. (1998) Genes Dev. 12, 3182–3194
18. Cline, G. W., Petersen, K. F., Krssak, M., Shen, J., Hundal, R. S., Zlatko, T., Inzucchi, S., Dresner, A., Rothman, D. L., and Shulman, G. I. (1999) N. Engl. J. Med. 341, 240–246
19. Petersen, K. F., Hendler, R., Price, T., Perseghin, G., Rothman, D. L., Held, N., Amatruda, J. M., and Shulman, G. I. (1998) Diabetes 47, 381–386
20. Rothman, D. L., Magnusson, I., Cline, G., Gerard, D., Kahn, C. R., Shulman, R. G., and Shulman, G. I. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 983–987
21. Pan, D. A., Lilinola, S., Kriketos, A. D., Minler, M. R., Baur, L. A., Bogardus, C., Jenkins, A. B., and Storlien, L. H. (1997) Diabetes 46, 985–988
22. Perseghin, G., Seifo, P., De Cobelli, F., Pagniti, E., Battezzati, A., Arelloni, C., Vanzulli, A., Testolin, G., Pozza, G., Del Maschio, A., and Luzi, L. (1999) Diabetes 48, 1600–1606
23. Krssak, M., Petersen, K. F., Dresner, A., DiPietro, L., Vogel, S. M., Rothman, D. L., Roden, M., and Shulman, G. I. (1999) Diabetologia 42, 113–116
24. Shimomura, I., Hammer, R. E., Ikemoto, S., Brown, M. S., and Goldstein, J. L. (1999) Nature 401, 73–76
25. Gavrilova, O., Marcus-Samuels, B., Leon, L. R., Vinson, C., and Reitman, M. L. (2000) Nature 403, 850
26. Kelley, D. E., Mokan, M., Simoneau, J.-A., and Mandarino, L. J. (1993) J. Clin. Invest. 92, 91–98
27. Zarrono, A., Balon, T. W., Brady, L. J., Rivera, P., Garetto, L. P., Young, J. C., Goodman, M. N., and Ruderman, N. B. (1985) Biochem. J. 232, 585–591
28. Vaag, A., Skott, P., Richter, E. A., and Beck-Nielsen, H. (1994) Eur. J. Endocrinol. 130, 70–79
29. Roden, M., Price, T. B., Perseghin, G., Petersen, K. F., Rothman, D. L., Cline, G. W., and Shulman, G. I. (1996) J. Clin. Invest. 97, 2859–2865
30. Kahn, C. R. (1994) Diabetes 43, 1066–1084
31. Chalkley, S. M., Hettiarachchi, M., Chisholm, D. J., and Kraegen, E. W. (1998) Metabolism 47, 1121–1126
32. Schmits-Peiffer, C., Browne, C. L., Oakes, N. D.,Watkinson, A., Chisholm, D. J., Kraegen, E. W., and Biden, T. J. (1997) Diabetes 46, 169–178

\(^2\) J. K. Kim and G. I. Shulman, unpublished observation.
