Adrenalectomy Improves Diabetes in A-ZIP/F-1 Lipoatrophic Mice by Increasing Both Liver and Muscle Insulin Sensitivity

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The virtually fatless A-ZIP/F-1 mouse is profoundly insulin resistant, diabetic, and a good model for humans with severe generalized lipoatrophy. Like a number of other mouse models of diabetes, the A-ZIP/F-1 mouse has elevated serum corticosterone levels. Leptin infusion lowers the corticosterone levels, suggesting that leptin deficiency contributes to the hypercorticosteronemic state. To test the hypothesis that the increased glucocorticoids contribute to the diabetes and insulin resistance, we examined the effect of adrenalectomy on A-ZIP/F-1 mice. Adrenalectomy significantly decreased the blood glucose, serum insulin, and glycated hemoglobin levels. Hyperinsulinemic-euglycemic clamps were performed to characterize the changes in whole-body and tissue insulin sensitivity. The adrenalectomized A-ZIP/F-1 mice displayed a marked improvement in insulin-induced suppression of endogenous glucose production, indicating increased hepatic insulin sensitivity. Adrenalectomy also increased muscle glucose uptake and glycogen synthesis. These results suggest that the chronically increased serum corticosterone levels contribute to the diabetes of the A-ZIP/F-1 mice and that removal of the glucocorticoid excess improves the insulin sensitivity in both muscle and liver. *Diabetes* 51: 2113–2118, 2002

To understand the physiological roles of adipose tissue, we studied the A-ZIP/F-1 transgenic mouse, which has virtually no white adipose tissue and a significantly reduced amount of brown adipose tissue (1). These mice express a dominant-negative protein in adipose tissue that heterodimerizes with and inactivates members of the CCAAT/enhancer binding protein (C/EBP) and Jun families of basic leucine zipper transcription factors. The A-ZIP/F-1 phenotype closely resembles that of humans with severe lipoatrophic diabetes (2,3). Features of the phenotype include hyperglycemia, hyperinsulinemia, hypoleptinemia, hepatic ste-

atosis, and increased serum triglyceride, fatty acid, and corticosterone levels (1,4,5). Reconstitution of adipose tissue by transplantation demonstrated that the lack of adipose tissue causes the metabolic phenotype (6). Leptin can increase insulin sensitivity (7,8), and leptin deficiency is a significant contributor to the etiology of the phenotype (9,10; C. Colombo and M.L.R., unpublished observations).

Glucocorticoids are crucial factors in the regulation of food intake and body adiposity (11,12), affecting energy metabolism at both the central and peripheral levels (13,14). In the central nervous system, glucocorticoids increase food intake and energy expenditure through multiple mechanisms, including stimulation of the hypothalamic neuropeptide Y pathway (15,16) and inhibition of corticotrophin-releasing factor/urocortin and norepinephrine pathways (17,18). In the periphery, glucocorticoids have many actions on lipid and carbohydrate metabolic pathways, usually antagonizing insulin effects. They stimulate hepatic gluconeogenesis by increasing activities of the crucial enzymes phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (19). In addition, glucocorticoids provide gluconeogenic substrates by increasing the release of amino acids from muscle and glycerol from fat and they also have a permissive action on glucagon- and epinephrine-stimulated gluconeogenesis (20). Glucocorticoids directly inhibit insulin secretion from pancreatic β-cells (21) and blunt glucose uptake in peripheral tissues through glucose transporter translocation from the plasma membrane to intracellular sites (22).

Increased glucocorticoid levels contribute to hyperphagia and insulin resistance in some rodent models of obesity, including leptin-deficient *ob/ob*, leptin receptor–deficient *db/db*, and agouti-overexpressing A^y/a mice (23–26). In these models, adrenalectomy reduces food intake and blood glucose and insulin levels, ameliorating the obesity and insulin resistance.

To clarify the contribution of the increased corticosterone levels to the A-ZIP/F-1 phenotype, we examined the effect of adrenalectomy. We demonstrate here that adrenalectomy significantly attenuates the diabetes and insulin resistance of the A-ZIP/F-1 mice, and that this occurs by increasing insulin sensitivity in both liver and skeletal muscle.

RESEARCH DESIGN AND METHODS

Animals. A-ZIP/F-1 mice hemizygous for the transgene on the FVB/N background were studied along with wild-type littermates. The mice were main-

tained on a 12-h light/dark cycle (lights on 0600 to 1800). Pellet diet (NIH-07, 12.9 kcal % fat) and drinking water were provided ad libitum. After adrenalectomy or sham operation, mice received 0.9% NaCl instead of water. Animal experiments were approved by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) Animal Care and Use Committee.

Surgery and animal handling. Operations were carried out under ketamine (100 mg/kg; Fort Dodge Animal Health, Fort Dodge, IA) and xylazine (10 mg/kg; Phoenix Scientific, St Joseph, MO) anesthesia. Adrenalectomy was performed by bilateral flank incision. Control animals underwent sham operations, in which the adrenal glands were grasped but not removed. Catheter insertion (procedure adapted from MacLeod and Shapiro [27]) was carried out 7–9 days after the first (adrenalectomy or sham) operation. Adrenalectomized mice were given one dose of hydrocortisone (4 mg/kg body wt i.p. in 0.9% saline; Sigma, St. Louis, MO) 2 h before anesthesia for catheter insertion. The silastic catheter (inner diameter 0.30 mm, outer diameter 0.64 mm, no. 508-001; Dow Corning, Midland, MI), filled with heparin solution (100 USP units/ml in 0.9% NaCl), was inserted via a right lateral neck incision, advanced into the superior vena cava via the right internal jugular vein, and sutured in place. The distal end of the catheter was knotted, tunneled subcutaneously, exteriorized first at the dorsal cervical midline, and then further tunneled subcutaneously and exteriorized in the dorsal midline, 2 cm above the tail. A silk suture was fastened around the catheter at the neck site. On the day of the clamp, the catheter was externalized by pulling the suture through the dorsal cervical incision site.

Hyperinsulinemic-euglycemic clamp. The clamps were performed as described by Kim et al. (28) using [3-³H]glucose and 2-deoxy-D-[1-¹⁴C] glucose (both from NEN Life Science Products, Boston, MA) for the estimation of whole-body glucose fluxes and tissue glucose uptake, respectively.

In vivo glucose flux analysis. The determination of plasma [3-³H]glucose and 2-deoxy-D-[1-¹⁴C] glucose concentrations and tissue 2-deoxy-D-[1-¹⁴C] glucose-6-phosphate was performed as described previously (28).

Calculations. Basal endogenous glucose production was calculated as the ratio of the preclamp [3-³H]glucose infusion rate (dpm/min) to the specific activity of the plasma glucose (mean of the 90- to 120-min values in dpm/μmol). Clamp whole-body glucose uptake was calculated as the ratio of the [3-³H]glucose infusion rate (dpm/min) to the specific activity of plasma glucose (dpm/μmol) during the last 20 min of the clamp (mean of the 100- to 120-min samples). Whole-body glycolysis was determined from the rate of increase in plasma ³H₂O determined by linear regression using the 80- to 120-min points. Plasma ³H₂O concentrations were measured from the difference between nondried versus dried plasma ³H counts. Clamp endogenous glucose production was determined by subtracting the average glucose infusion rate in the last 20 min of clamp from the whole-body glucose uptake. Whole-body glycogen and lipid synthesis were estimated by subtracting the whole-body glycolysis from the whole-body glucose uptake, which assumes that glycolysis and glycogen/lipid synthesis account for the majority of insulin-stimulated glucose uptake (29). Muscle glucose uptake was calculated from the plasma 2-deoxy-D-[1-¹⁴C] glucose concentration profile (using plasma ¹⁴C counts at 80–120 min, the area under the curve was calculated by trapezoidal approximation) and tissue 2-deoxy-D-[1-¹⁴C] glucose-6-phosphate content as described previously (30). Skeletal muscle glycogen synthesis was calculated from the ³H incorporation to muscle glycogen as described (30). Skeletal muscle glycolysis was estimated by subtracting the skeletal muscle glycogen synthesis from the skeletal muscle glucose uptake.

Biochemical and hormonal assays. Glucose was measured using a Glucometer Elite (Bayer, Elkhart, IN). Insulin (no. SRI-13K, Linco Research, St Charles, MO), corticosterone (no. 07-120102; ICN Biomedicals, Costa Mesa, CA), triglycerides (no. 337-B; Sigma), nonesterified fatty acids (no. 13831175; Roche Molecular Biochemicals), and glycated hemoglobin (no. 442-B; Sigma) were measured with the indicated kits. Liver triglycerides were measured by solvent extraction followed by a colorimetric assay (31).

RNA analysis. Total RNA extraction and Northern blots were done as previously described, using probes excised from plasmids (32,33).

Statistical analysis. Data are expressed as means ± SE. Statistical significance between the groups was determined with SigmaStat (SPSS, Chicago, IL) using Student's t test or two-way ANOVA.

RESULTS

Leptin treatment reverses the hypercorticosteronemia of fed A-ZIP/F-1 mice. A-ZIP/F-1 mice have increased corticosterone levels as compared with wild-type littermates (4). The average corticosterone level in fed A-ZIP/F-1 mice was sevenfold higher than in wild-type controls (Fig. 1). In fasted mice, the difference between
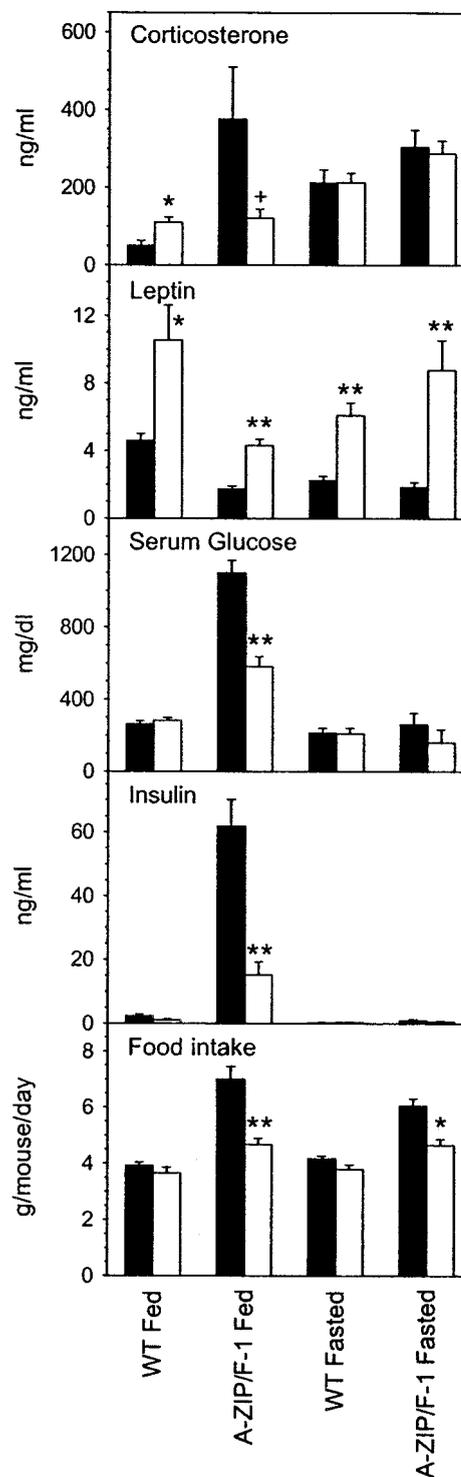

FIG. 1. Effect of leptin treatment in 6-week-old male mice. Corticosterone, leptin, glucose, and insulin were measured in serum from nonfasting (Fed) or 24-h fasted (Fasted) mice, obtained at 0900–1100 h. Leptin treatment is indicated by open bars and control saline infusions are shown in closed bars. Leptin (R&D Systems), 30 μg/day for 6 days, was delivered by continuous osmotic pump (Alzet) infusion. Food intake was measured over treatment days 1–5. Some previously reported data are included for comparison (4,10). Values are means ± SE; $n = 6$ per group. $+P = 0.06$, $*P \leq 0.01$, $**P \leq 0.001$ vs. respective saline-treated group.

A-ZIP/F-1 and wild-type mice was only 1.4-fold, largely due to an increase in corticosterone levels in wild-type mice with fasting. Leptin regulates the hypothalamic-pituitary-

TABLE 1
Effect of adrenalectomy in female mice.

	Wild type		A-ZIP/F-1	
	Sham	Adrenalectomy	Sham	Adrenalectomy
Body weight, 8 weeks (g)	22.9 ± 0.8	23.4 ± 0.6	25.7 ± 0.7†	26.9 ± 0.8†
Body weight, 10 weeks (g)	23.4 ± 0.2	24.2 ± 0.3	26.3 ± 0.3†	28.3 ± 0.3*†
Blood glucose (mg/dl)	149 ± 7	128 ± 4*	624 ± 35†	263 ± 9*†
Insulin (ng/ml)	1.13 ± 0.04	0.84 ± 0.03*	172 ± 15†	75 ± 11*†
Glycated hemoglobin (%)	7.4 ± 2.7	6.6 ± 2.6	12.8 ± 0.4†	8.9 ± 0.2*
Triglycerides (mg/dl)	137 ± 6	147 ± 4	774 ± 66†	476 ± 24†
Fatty acids (µmol/l)	210 ± 8	221 ± 19	682 ± 24†	605 ± 15†
Corticosterone (ng/ml)	113 ± 12	34 ± 2*	340 ± 55†	20 ± 5*†
Liver weight (g)	1.21 ± 0.04	1.33 ± 0.03	2.92 ± 0.11†	2.82 ± 0.08†
Liver triglycerides (µmol/g tissue)	2.43 ± 0.39	2.96 ± 0.38	11.9 ± 3.0†	10.9 ± 2.1†
Food intake (g/mouse/day)	3.50 ± 0.40	3.37 ± 0.14	5.31 ± 0.18†	3.65 ± 0.12*

Adrenalectomy was performed at 8 weeks and mice were studied at 10 weeks. Mice were bled in the morning in the nonfasted state. Food intake was measured in individually housed mice 7–11 days after adrenalectomy. Data are means ± SE; $n = 6$ per group. *$P < 0.05$ vs. sham group of the same genotype; †$P < 0.05$ vs. wild-type sham.

adrenal axis, so the effects of leptin on the corticosterone levels were studied. Leptin treatment for 6 days reduced the corticosterone levels of fed A-ZIP/F-1 mice ($P = 0.06$), but did not change the levels in fasting mice. Efficacy of leptin treatment was established by the increase in serum leptin levels and, in A-ZIP/F-1 mice, reduction in food intake, glucose, and insulin levels (Fig. 1). These results suggest that leptin deficiency contributes to the hypercorticosteronemia of the A-ZIP/F-1 mice.

Adrenalectomy attenuates the diabetes of A-ZIP/F-1 mice. Adrenalectomy was used to study the contribution of the hypercorticosteronemia to the phenotype of the A-ZIP/F-1 mice. In the first study, the blood chemistry and anatomy of female mice 10 weeks old and 2 weeks postadrenalectomy were examined in the nonfasted state. The sham-operated A-ZIP/F-1 had elevated blood glucose (4-fold), insulin (170-fold), glycated hemoglobin (2-fold), triglyceride (5-fold), fatty acid (3-fold), and corticosterone (3-fold) levels, as compared with sham-operated wild-type littermates (Table 1), confirming previous data (1). The weight and triglyceride content of A-ZIP/F-1 livers were also markedly increased, as was body weight, albeit to a lesser degree (Table 1). Adrenalectomy of the A-ZIP/F-1 mice significantly lowered blood glucose, serum insulin, and glycated hemoglobin levels as compared with the sham-operated controls (Table 1). However, these parameters remained higher than in wild-type mice. A tendency (which did not reach statistical significance) toward lower serum triglycerides and fatty acid levels was also observed in adrenalectomized A-ZIP/F-1 mice. Adrenalectomy increased body weight of A-ZIP/F-1 mice as compared with sham-operated A-ZIP/F-1 group despite decreased food intake. A-ZIP/F-1 liver weight was not changed by adrenalectomy.

In wild-type mice, adrenalectomy also decreased insulin and glucose levels, but the changes were modest in magnitude. No effect of adrenalectomy on glycated hemoglobin, triglycerides, fatty acids, food intake, and body or liver weight was observed in wild-type mice (Table 1). Adrenalectomy efficacy was confirmed by the reduction in serum corticosterone levels. Taken together, these data demonstrate that reversal of the hypercorticosteronism by adrenalectomy greatly improves the diabetes and tends to improve the hyperlipidemia of the A-ZIP/F-1 mice.

Adrenalectomized mice have decreased plasma glucose and basal endogenous glucose production after 12 h of fasting. We next performed hyperinsulinemic-euglycemic clamps to elucidate the mechanisms by which adrenalectomy improved the diabetes of A-ZIP/F-1 mice. At the beginning of the clamps (after 12 h of fasting) both wild-type and A-ZIP/F-1 adrenalectomized groups had lower plasma glucose and insulin levels than the sham-operated controls (Table 2). In addition, some adrenalectomized mice (both wild type and A-ZIP/F-1) had decreased body temperature after 12 h of fasting. Basal endogenous glucose production was greatly reduced in both adrenalectomized groups as compared with sham-operated animals (Fig. 2), suggesting that decreased basal endogenous glucose production contributes to the lower basal glucose values.

TABLE 2
Metabolic parameters during basal (12-h fasted) period and hyperinsulinemic-euglycemic clamp in the sham-operated and adrenalectomized wild-type and A-ZIP/F-1 female mice.

	Wild type		A-ZIP/F-1	
	Sham	Adrenalectomy	Sham	Adrenalectomy
Body weight (g)	21.6 ± 0.6	22.8 ± 0.8	21.2 ± 1.1	22.0 ± 0.6
Basal plasma glucose (mg/dl)	137 ± 13	76 ± 12*	243 ± 12†	64 ± 18*†
Basal plasma insulin (ng/ml)	0.96 ± 0.09	0.47 ± 0.11*	1.22 ± 0.27	0.83 ± 0.16*
Clamp plasma glucose (mg/dl)	110 ± 7	114 ± 3	110 ± 3	105 ± 3
Clamp plasma insulin (ng/ml)	3.6 ± 0.4	3.5 ± 0.7	4.0 ± 0.4	4.0 ± 1.3
Corticosterone (ng/ml)	101 ± 22	15 ± 5*	251 ± 68†	5 ± 2*†

Values are means ± SE; $n = 6$ per group. *$P < 0.05$ vs. sham group of the same genotype; †$P < 0.05$ vs. wild-type sham.
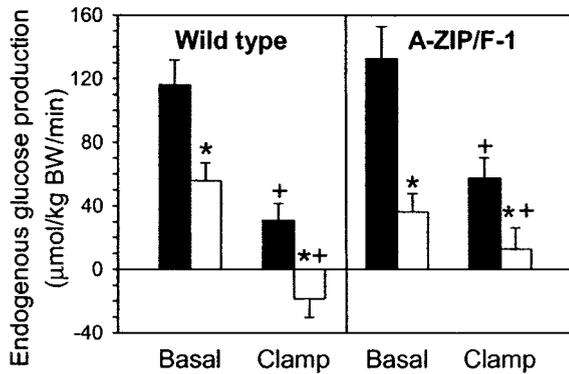
FIG. 2. Basal and clamp endogenous glucose production in female sham-operated (black bars) and adrenalectomized (white bars) wild-type and A-ZIP/F-1 mice. Values are means ± SE; $n = 6$ per group. *$P < 0.05$ vs. respective sham group; +$P < 0.05$ vs. basal endogenous glucose production of the same group.
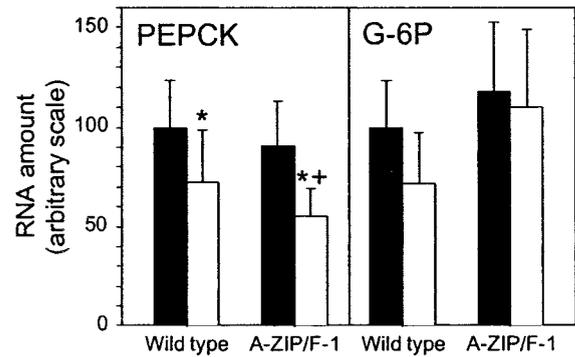

FIG. 4. Liver PEPCK and glucose-6-phosphatase (G-6P) expression in female sham-operated (black bars) and adrenalectomized (white bars) wild-type and A-ZIP/F-1 mice. Data are expressed as a percentage of wild-type control and are means ± SE; $n = 6$ per group. *$P < 0.05$ vs. sham group of the same genotype; +$P < 0.05$ for A-ZIP/F-1 groups vs. wild-type sham group.

Under the hyperinsulinemic clamp conditions, the sham-operated A-ZIP/F-1 mice had decreased whole-body glucose uptake and whole-body glycolysis as compared with wild-type controls (Fig. 3), confirming previous results (5). The difference in whole-body glycogen and lipid synthesis between sham-operated A-ZIP/F-1 and wild-type group was of borderline statistical significance ($P = 0.054$). Whole-body glucose uptake and whole-body glycolysis were higher in adrenalectomized as compared with sham-operated A-ZIP/F-1 mice while no difference was found between adrenalectomized versus sham-operated wild-type mice (Fig. 3). The reduction in serum corticosterone levels confirmed the technical adequacy of the adrenalectomies (Table 2).

Adrenalectomy increases liver insulin sensitivity in both A-ZIP/F-1 and wild-type mice. In A-ZIP/F-1 mice, the clamp glucose infusion rate was significantly higher in adrenalectomized as compared with sham-operated controls (Fig. 3). Thus, clamp endogenous glucose production (the difference between whole-body glucose uptake) was lower in adrenalectomized mice (Fig. 2). As noted above, the basal endogenous glucose production was reduced in

adrenalectomized A-ZIP/F-1 mice, but this was further reduced during the clamp (Fig. 2). Thus the pronounced effect of adrenalectomy to sensitize endogenous glucose production to suppression by insulin was observed in both wild-type and A-ZIP/F-1 mice.

As glucocorticoids are important stimulators of gluconeogenesis, the mRNA levels of two gluconeogenic enzymes, PEPCK and glucose-6-phosphatase, were measured. Liver PEPCK and glucose-6-phosphatase mRNA levels did not differ between sham-operated wild-type and A-ZIP/F-1 mice (Fig. 4). However, the PEPCK mRNA expression in adrenalectomized A-ZIP/F-1 and wild-type mice was significantly lower, while the changes in glucose-6-phosphatase mRNA did not reach statistical significance (Fig. 4).

Adrenalectomy increases muscle insulin sensitivity in A-ZIP/F-1 mice. Gastrocnemius muscle glucose uptake was higher in adrenalectomized A-ZIP/F-1 mice than in sham-operated A-ZIP/F-1 mice, but was still lower than in sham-operated wild-type mice (Fig. 5). The rate of gastrocnemius glycogen synthesis was completely normalized in adrenalectomized A-ZIP/F-1 mice. No significant difference
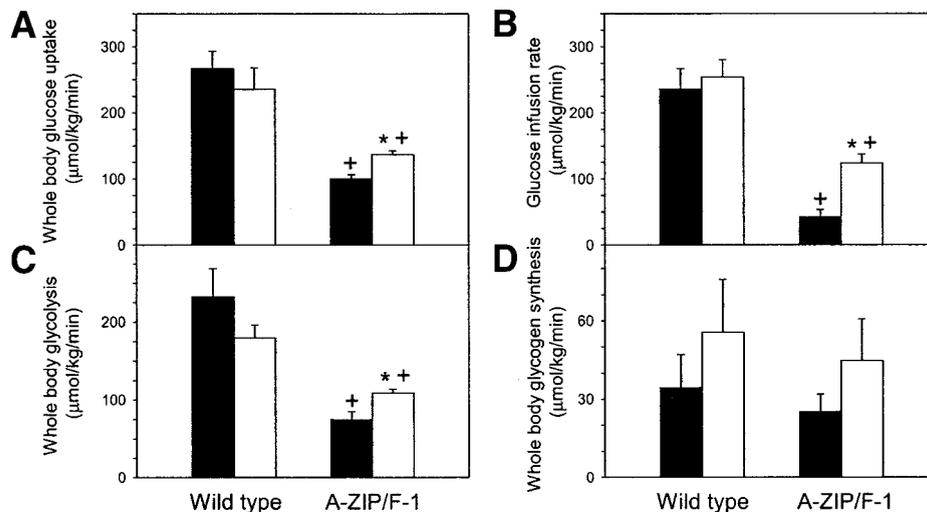

FIG. 3. Whole-body glucose fluxes during hyperinsulinemic-euglycemic clamps in female sham-operated (black bars) and adrenalectomized (white bars) wild-type and A-ZIP/F-1 mice. *A*: whole body glucose uptake. *B*: glucose infusion rate. *C*: whole-body glycolysis. *D*: whole-body glycogen/lipid synthesis. Values are means ± SE; $n = 6$ per group. *$P < 0.05$ vs. sham group of the same genotype; +$P < 0.05$ for A-ZIP/F-1 groups vs. wild-type sham group.
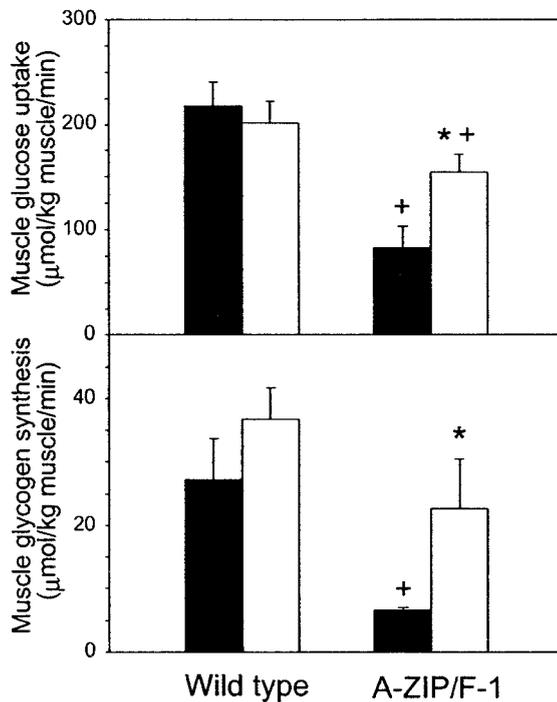
FIG. 5. Muscle (gastrocnemius) glucose uptake and muscle glycogen synthesis in female sham-operated (black bars) and adrenalectomized (white bars) wild-type and A-ZIP/F-1 mice. Values are means ± SE; $n = 6$/group. $*P < 0.05$ vs. sham group of the same genotype; $+P < 0.05$ for A-ZIP/F-1 groups vs. wild-type sham group.

between adrenalectomized wild-type and sham-operated wild-type mice in muscle glucose uptake or muscle glycogen synthesis was found (Fig. 5).

DISCUSSION

Glucocorticoids play a pivotal role in the regulation of whole-body fuel homeostasis. Chronic glucocorticoid excess causes increased food intake, visceral obesity, and insulin resistance, while the lack of glucocorticoids decreases food intake, body adiposity, and circulating glucose and insulin levels. The effects of glucocorticoids are documented; however, to our knowledge, no in vivo glucose clamp studies directly exploring the influence of adrenalectomy on glucose homeostasis have been performed previously. We demonstrate that adrenalectomy in wild-type mice produces a profound drop in basal endogenous glucose production and greatly sensitizes the liver to insulin-induced suppression of endogenous glucose production. Although glucocorticoid excess is known to induce insulin resistance and adrenalectomy might be expected to increase muscle insulin sensitivity, no changes in muscle glucose uptake were observed in adrenalectomized wild-type mice.

We showed previously that suppression of endogenous glucose production during a hyperinsulinemic clamp is significantly blunted in A-ZIP/F-1 mice, indicating liver insulin resistance (5). Glucocorticoids contribute importantly to regulation of hepatic glucose production by stimulating gluconeogenic enzymes PEPCK and glucose-6-phosphatase. Adrenalectomy markedly decreased endogenous glucose production in both A-ZIP/F-1 and wild-type mice in part by reduced liver PEPCK expression. The improvement caused by adrenalectomy may be due to reversal of the hypercorticosteronemic state, to creation of a hypocorticosteronemic state, or both.

It should be noted that the beneficial effect of adrenalectomy in A-ZIP/F-1 mice might not reflect a selective reversal of lipoatrophic pathology, but rather a second insult that nonspecifically ameliorates their diabetes.

Glucocorticoid excess contributes to muscle insulin resistance in A-ZIP/F-1 mice. In humans, cortisol excess decreases whole-body (chiefly muscle) glucose uptake (35,36). Glucocorticoids reduce muscle glucose uptake by decreasing GLUT4 translocation to the cell surface (22,37,38). Insulin-stimulated muscle glucose uptake in adrenalectomized A-ZIP/F-1 mice was nearly doubled as compared with A-ZIP/F-1 controls, but was still less than that in wild-type mice. Muscle glycogen synthesis in A-ZIP/F-1 mice was normalized by adrenalectomy. No such changes were observed in adrenalectomized wild-type mice. Possibly, the effects of glucocorticoids on muscle glucose uptake can be observed only by comparing elevated with normal or low levels and not by comparing normal with low glucocorticoids levels.

Adrenalectomy reduced food intake in the A-ZIP/F-1 mice. However, the improvement in insulin resistance is not simply due to reduced food intake, since pair feeding A-ZIP/F-1 mice or aP2-SREBP-1c lipoatrophic mice to wild-type levels does not significantly reduce the severity of the diabetes (39,9; O.G. and M.L.R., unpublished observations). Tissue-specific ablation of the glucocorticoid receptor gene (40) should allow identification of the relevant sites of glucocorticoid action. It should also be noted that some obese models (such as melanocortin receptor-4 knockout [41] and syndecan-1–overexpressing [42] mice) and most models of human obesity do not appear to have high systemic glucocorticoid levels. It was recently demonstrated that increased local corticosterone production in adipose tissue by transgenic overexpression of 11β-hydroxysteroid dehydrogenase type-1 in fat induces visceral obesity with insulin resistance and diabetes without changing systemic corticosterone levels (43).

What causes the chronic hypercorticosteronemia of the A-ZIP/F-1 mice? One contributor is leptin deficiency, which increases signaling by hypothalamic CRH (corticotropin releasing hormone) neurons, stimulating pituitary ACTH (adrenocorticotropic hormone) release, and thus increasing adrenal glucocorticoid production (44–46). This mechanism could explain the hypothalamic-pituitary-adrenocortical axis response to starvation. Leptin treatment of A-ZIP/F-1 mice normalized fed but not fasting corticosterone levels, suggesting that the high-fed corticosterone levels are the result of severe leptin deficiency, while the fasting levels have multiple causes. Reduction of corticosterone levels may be one of the mechanisms by which leptin treatment improves glucose and insulin levels in A-ZIP/F-1 mice.

In conclusion, the chronically increased corticosterone levels contribute to the diabetes and insulin resistance of the A-ZIP/F-1 mice. Adrenalectomy relieves the glucocorticoid excess and improves insulin sensitivity in liver and skeletal muscle.

REFERENCES

1. Moitra J, Mason MM, Olive M, Krylov D, Gavrilova O, Marcus-Samuels B, Feigenbaum L, Lee E, Aoyama T, Eckhaus M, Reitman ML, Vinson C: Life without white fat: a transgenic mouse. *Genes Dev* 12:3168–3181, 1998
2. Foster DW: The lipodystrophies and other rare disorders of adipose tissue. In *Harrison's Principles of Internal Medicine.* 14th ed. Fauci AS, Braunwald E, Isselbacher KJ, Wilson JD, Martin JB, Kasper DL, Hauser SL, Longo DL, Eds. New York, McGraw-Hill, 1998, p. 2209–2214
3. Reitman ML, Arioglu E, Gavrilova O, Taylor SI: Lipoatrophy revisited. *Trends Endocrinol Metab* 11:410–416, 2000
4. Gavrilova O, Leon LR, Marcus-Samuels B, Mason MM, Castle AL, Refetoff S, Vinson C, Reitman ML: Torpor in mice is induced by both leptin-dependent and -independent mechanisms. *Proc Natl Acad Sci U S A* 96:14623–14628, 1999
5. Kim JK, Gavrilova O, Chen Y, Reitman ML, Shulman GI: Mechanism of insulin resistance in A-ZIP/F-1 fatless mice. *J Biol Chem* 275:8456–8460, 2000
6. Gavrilova O, Marcus-Samuels B, Graham D, Kim JK, Shulman GI, Castle AL, Vinson C, Eckhaus M, Reitman ML: Surgical implantation of adipose tissue reverses diabetes in lipoatrophic mice. *J Clin Invest* 105:271–278, 2000
7. Kamohara S, Burcelin R, Halaas JL, Friedman JM, Charron MJ: Acute stimulation of glucose metabolism in mice by leptin treatment. *Nature* 389:374–377, 1997
8. Rossetti L, Massillon D, Barzilai N, Vuguin P, Chen W, Hawkins M, Wu J, Wang J: Short term effects of leptin on hepatic gluconeogenesis and in vivo insulin action. *J Biol Chem* 272:27758–27763, 1997
9. Shimomura I, Hammer RE, Ikemoto S, Brown MS, Goldstein JL: Leptin reverses insulin resistance and diabetes mellitus in mice with congenital lipodystrophy. *Nature* 401:73–76, 1999
10. Gavrilova O, Marcus-Samuels B, Leon LR, Vinson C, Reitman ML: Leptin and diabetes in lipoatrophic mice. *Nature* 403:850, 2000
11. Schwartz MW, Figlewicz DP, Baskin DG, Woods SC, Porte D Jr: Insulin in the brain: a hormonal regulator of energy balance. *Endocr Rev* 13:387–414, 1992
12. Bray GA, Fisler J, York DA: Neuroendocrine control of the development of obesity: understanding gained from studies of experimental animal models. *Frontiers in Neuroendocrinology* 11:128–181, 1990
13. Wisialowski T, Parker R, Preston E, Sainsbury A, Kraegen E, Herzog H, Cooney G: Adrenalectomy reduces neuropeptide Y-induced insulin release and NPY receptor expression in the rat ventromedial hypothalamus. *J Clin Invest* 105:1253–1259, 2000
14. Mantha L, Deshaies Y: Energy intake-independent modulation of triglyceride metabolism by glucocorticoids in the rat. *Am J Physiol Regul Integr Comp Physiol* 278:R1424–R1432, 2000
15. Larsen PJ, Jessop DS, Chowdrey HS, Lightman SL, Mikkelsen JD: Chronic administration of glucocorticoids directly upregulates prepro-neuropeptide Y and Y1-receptor mRNA levels in the arcuate nucleus of the rat. *J Neuroendocrinol* 6:153–159, 1994
16. King BM: Glucocorticoids and hypothalamic obesity. *Neurosci Biobehav Rev* 12:29–37, 1988
17. Sawchenko PE: Adrenalectomy-induced enhancement of CRF and vasopressin immunoreactivity in parvocellular neurosecretory neurons: anatomic, peptide, and steroid specificity. *J Neurosci* 7:1093–1106, 1987
18. Pacak K, Kvetnansky R, Palkovits M, Fukuhara K, Yadid G, Kopin IJ, Goldstein DS: Adrenalectomy augments in vivo release of norepinephrine in the paraventricular nucleus during immobilization stress. *Endocrinology* 133:1404–1410, 1993
19. Pilkis SJ, Granner DK: Molecular physiology of the regulation of hepatic gluconeogenesis and glycolysis. *Annu Rev Physiol* 54:885–909, 1992
20. Exton JH: Mechanisms of hormonal regulation of hepatic glucose metabolism. *Diabete Metab Rev* 3:163–183, 1987
21. Lambillotte C, Gilon P, Henquin JC: Direct glucocorticoid inhibition of insulin secretion: an in vitro study of dexamethasone effects in mouse islets. *J Clin Invest* 99:414–423, 1997
22. Horner HC, Munck A, Lienhard GE: Dexamethasone causes translocation of glucose transporters from the plasma membrane to an intracellular site in human fibroblasts. *J Biol Chem* 262:17696–17702, 1987
23. Mayer J, Russel RE, Bates MW, Dickie MM: Metabolic, nutritional and endocrine studies of the hereditary obesity-diabetes syndrome of mice and mechanism of its development. *Metabolism* 2:9–21, 1953
24. Solomon J, Mayer J: The effect of adrenalectomy on the development of the obese-hyperglycemic syndrome in ob-ob mice. *Endocrinology* 93:510–512, 1973
25. York DA, Bray GA: Dependence of hypothalamic obesity on insulin, the pituitary and the adrenal gland. *Endocrinology* 90:885–894, 1972
26. Shimizu H, Shargill NS, Bray GA: Adrenalectomy and response to corticosterone and MSH in the genetically obese yellow mouse. *Am J Physiol* 256:R494–R500, 1989
27. MacLeod JN, Shapiro BH: Repetitive blood sampling in unrestrained and unstressed mice using a chronic indwelling right atrial catheterization apparatus. *Lab Anim Sci* 38:603–608, 1988
28. Kim JK, Michael MD, Previs SF, Peroni OD, Mauvais-Jarvis F, Neschen S, Kahn BB, Kahn CR, Shulman GI: Redistribution of substrates to adipose tissue promotes obesity in mice with selective insulin resistance in muscle. *J Clin Invest* 105:1791–1797, 2000
29. Rossetti L, Giaccari A: Relative contribution of glycogen synthesis and glycolysis to insulin-mediated glucose uptake: a dose-response euglycemic clamp study in normal and diabetic rats. *J Clin Invest* 85:1785–1792, 1990
30. Youn JH, Kim JK, Buchanan TA: Time courses of changes in hepatic and skeletal muscle insulin action and GLUT4 protein in skeletal muscle after STZ injection. *Diabetes* 43:564–571, 1994
31. Burant CF, Sreenan S, Hirano K, Tai TA, Lohmiller J, Lukens J, Davidson NO, Ross S, Graves RA: Troglitazone action is independent of adipose tissue. *J Clin Invest* 100:2900–2908, 1997
32. Yoo-Warren H, Cimbala MA, Felz K, Monahan JE, Leis JP, Hanson RW: Identification of a DNA clone to phosphoenolpyruvate carboxykinase (GTP) from rat cytosol: alterations in phosphoenolpyruvate carboxykinase RNA levels detectable by hybridization. *J Biol Chem* 256:10224–10227, 1981
33. Shelly LL, Lei KJ, Pan CJ, Sakata SF, Ruppert S, Schutz G, Chou JY: Isolation of the gene for murine glucose-6-phosphatase, the enzyme deficient in glycogen storage disease type 1A. *J Biol Chem* 268:21482–21485, 1993
34. Blair SC, Caterson ID, Cooney GJ: Effect of adrenalectomy on glucose tolerance and lipid metabolism in gold-thioglucose obese mice. *Am J Physiol* 266:E993–E1000, 1994
35. Rizza RA, Mandarino LJ, Gerich JE: Cortisol-induced insulin resistance in man: impaired suppression of glucose production and stimulation of glucose utilization due to a postreceptor detect of insulin action. *J Clin Endocrinol Metab* 54:131–138, 1982
36. Nosadini R, Del Prato S, Tiengo A, Valerio A, Muggeo M, Opocher G, Mantero F, Duner E, Marescotti C, Mollo F, Belloni F: Insulin resistance in Cushing's syndrome. *J Clin Endocrinol Metab* 57:529–536, 1983
37. Block NE, Buse MG: Effects of hypercortisolemia and diabetes on skeletal muscle insulin receptor function in vitro and in vivo. *Am J Physiol* 256:E39–E48, 1989
38. Dimitriadis G, Leighton B, Parry-Billings M, Sasson S, Young M, Krause U, Bevan S, Piva T, Wegener G, Newsholme EA: Effects of glucocorticoid excess on the sensitivity of glucose transport and metabolism to insulin in rat skeletal muscle. *Biochem J* 321:707–712, 1997
39. Ebihara K, Ogawa Y, Masuzaki H, Shintani M, Miyanaga F, Aizawa-Abe M, Hayashi T, Hosoda K, Inoue G, Yoshimasa Y, Gavrilova O, Reitman ML, Nakao K: Transgenic overexpression of leptin rescues insulin resistance and diabetes in a mouse model of lipoatrophic diabetes. *Diabetes* 50:1440–1448, 2001
40. Tronche F, Kellendonk C, Kretz O, Gass P, Anlag K, Orban PC, Bock R, Klein R, Schutz G: Disruption of the glucocorticoid receptor gene in the nervous system results in reduced anxiety. *Nat Genet* 23:99–103, 1999
41. Huszar D, Lynch CA, Fairchild-Huntress V, Dunmore JH, Fang Q, Berkemeier LR, Gu W, Kesterson RA, Boston BA, Cone RD, Smith FJ, Campfield LA, Burn P, Lee F: Targeted disruption of the melanocortin-4 receptor results in obesity in mice. *Cell* 88:131–141, 1997
42. Reizes O, Lincecum J, Wang Z, Goldberger O, Huang L, Kaksonen M, Ahima R, Hinkes MT, Barsh GS, Rauvala H, Bernfield M: Transgenic expression of syndecan-1 uncovers a physiological control of feeding behavior by syndecan-3. *Cell* 106:105–116, 2001
43. Masuzaki H, Paterson J, Shinyama H, Morton NM, Mullins JJ, Seckl JR, Flier JS: A transgenic model of visceral obesity and the metabolic syndrome. *Science* 294:2071–2074, 2001
44. Heiman ML, Ahima RS, Craft LS, Schoner B, Stephens TW, Flier JS: Leptin inhibition of the hypothalamic-pituitary-adrenal axis in response to stress. *Endocrinology* 138:3859–3863, 1997
45. Ahima RS, Saper CB, Flier JS, Elmquist JK: Leptin regulation of neuroendocrine systems. *Front Neuroendocrinol* 21:263–307, 2000
46. Schwartz MW, Seeley RJ, Campfield LA, Burn P, Baskin DG: Identification of targets of leptin action in rat hypothalamus. *J Clin Invest* 98:1101–1106, 1996