

Insulin Resistance in the Liver-Specific IGF-1 Gene-Deleted Mouse Is Abrogated by Deletion of the Acid-Labile Subunit of the IGF-Binding Protein-3 Complex

Relative Roles of Growth Hormone and IGF-1 in Insulin Resistance

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Liver IGF-1 deficient (LID) mice demonstrate a 75% reduction in circulating IGF-1 levels and a corresponding fourfold increase in growth hormone (GH) levels. At 16 weeks of age, LID mice demonstrate, using the hyperinsulinemic-euglycemic clamp, insulin insensitivity in muscle, liver, and fat tissues. In contrast, mice with a gene deletion of the acid-labile subunit (ALSKO) demonstrate a 65% reduction in circulating IGF-1 levels, with normal GH levels and no signs of insulin resistance. To further clarify the relative roles of increased GH and decreased IGF-1 levels in the development of insulin resistance, we crossed the two mouse lines and created a double knockout mouse (LID+ALSKO). LID+ALSKO mice demonstrate a further reduction in circulating IGF-1 levels (85%) and a concomitant 10-fold increase in GH levels. Insulin tolerance tests showed an improvement in insulin responsiveness in the LID+ALSKO mice compared with controls; LID mice were very insulin insensitive. Surprisingly, insulin sensitivity, while improved in white adipose tissue and in muscle, was unchanged in the liver. The lack of improvement in liver insulin sensitivity may reflect the absence of IGF-1 receptors or increased triglyceride levels in the liver. The present study suggests that whereas GH plays a major role in inducing insulin resistance, IGF-1 may have a direct modulatory role. *Diabetes* 52:2483–2489, 2003

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ALS, acid-labile subunit; GH, growth hormone; IGFBP, IGF binding protein. © 2003 by the American Diabetes Association.

Carbohydrate homeostasis depends on appropriate insulin secretion from the pancreatic islets and normal response of peripheral tissues to insulin. The primary insulin responsive tissues include liver, muscle, and fat. In the liver, insulin controls hepatic glucose production, thereby preventing unnecessary elevations in fasting plasma glucose levels, and, similarly, after a meal when glucose is absorbed from the gastrointestinal tract, insulin inhibits endogenous (hepatic) glucose production (1). In fat cells, whereas glucose uptake is under the control of insulin via the GLUT4 mobilization to the plasma membrane (2,3), the major effect of insulin is to inhibit lipolysis (4). Finally, muscle is the major organ responsible for insulin-induced glucose uptake and accounts for ~80% of whole-body glucose disposal after the meal by facilitated glucose transport, again via GLUT4 (3). All of the above events are initiated by the interaction of insulin with the cell-surface insulin receptors followed by a varied number of postreceptor signaling cascades, eventuating in the appropriate biological responses. One of the major common phenomena seen in obesity, the metabolic syndrome, and type 2 diabetes is insulin resistance (5), with insulin unable to regulate hepatic glucose production, lipolysis in fat cells, and whole-body (muscle) glucose disposal. In the case of obesity and the metabolic syndrome, normoglycemia is maintained at the expense of excessive β -cell insulin secretion and hyperinsulinemia (6), whereas in type 2 diabetes, the β -cells fail to compensate for this excessive demand (5).

The effect of the growth hormone (GH)–IGF-1 axis on carbohydrate homeostasis has become of increasing interest. IGF-1 circulates in molar amounts (of 200–250 ng/ml, depending on the mouse strain), and under normal conditions the majority of IGF-1 (~80%) is present in a ternary complex comprised of one IGF-1 molecule, IGF binding protein (IGFBP)-3, and the acid-labile subunit (ALS). The ALS stabilizes the complex and increases its half-life; the

ternary complex crosses the capillary barrier poorly and thus acts as a relatively stable reservoir of circulating IGF-1 (7,8). A smaller proportion (~15%) of circulating IGF is found in a binary complex composed of other serum IGF-BPs, and <5% of the IGF-1 is present in a free (unbound) form (9). The IGF-1 receptor is closely related to the insulin receptor (10,11); both are members of the subclass of transmembrane tyrosine kinase receptors and often use identical signaling cascades, even though the IGFs (IGF-1 and IGF-2) are primarily growth factors. Muscle, in particular, expresses a large concentration of IGF-1 receptors that have been shown to activate the GLUT4 translocation process and enhance muscle glucose uptake (12). The expression of both insulin and IGF-1 receptors by skeletal muscle leads to the presence of hybrid receptors, as an oligomer with one $\alpha\beta$ -subunit of the IGF-1 receptor and one $\alpha\beta$ of the insulin receptor. Hybrid receptors demonstrate higher affinity for IGF-1 than insulin and therefore may represent the mechanism whereby IGF-1 induces glucose uptake in muscle; activation of the hybrid receptor by IGF-1 may induce autophosphorylation of the $\alpha\beta$ insulin receptor half and induce the cascade responsible for glucose transport.

With the advent of recombinant DNA technology, recombinant human IGF-1 has become available for use in animals and humans. Use in type 2 diabetic patients for example has demonstrated a marked improvement in insulin resistance (13). Investigators have concluded that this improvement in insulin resistance is most probably via muscle IGF-1 receptors enhancing glucose uptake (13). More recent studies have used a binary complex of recombinant human IGF-1 with IGF-BP-3 (14) to increase the half-life of the injected IGF-1. GH, on the other hand, has been shown to induce insulin resistance (15). Chronic GH excess, whether due to a GH-secreting tumor or chronic GH administration, causes insulin resistance in different target tissues, including liver, fat, and muscle (16). Although this effect is seen at the early signaling events in the insulin receptor and postreceptor cascades, the exact mechanism(s) are as yet undefined.

We have previously created a tissue-specific gene-deleted mouse model where IGF-1 gene expression from the liver was abrogated (LID mice) (17), resulting in reduced circulating IGF-1 and elevated circulating GH levels. The LID mice developed insulin resistance, which we attributed to excess GH (18), but the role of IGF-1 could not be totally excluded. In contrast, gene deletion of the ALS (ALSKO) in mice resulted in a marked reduction in circulating IGF-1 with no increase in GH secretion. Crossing of the LID mice with ALSKO mice revealed a mouse line that has a further reduction in circulating IGF-1 and a more extreme elevation in circulating GH levels (19).

In the present study, we have used the LID, ALSKO, and LID+ALSKO mice and studied their glucose homeostasis using the hyperinsulinemic-euglycemic clamp.

RESEARCH DESIGN AND METHODS

Animal husbandry and genotyping. The generation and genotyping of LID mice (17) (with a mixed genetic background of FVB/N, C57BL, and 129Sv) and ALSKO mice (20) (with a mixed genetic background of BALB/c and 129Sv) has been described previously. LID and ALSKO mice were crossed to create a double gene disruption (LID+ALSKO) of both liver *Igf-1* gene and the *Als* gene as described previously (19). In the present study, we analyzed control (which express exon 4 of the IGF-1 flanked by two lox-P sites), LID, ALSKO,

and LID+ALSKO mice. All the mice analyzed were littermates and have the same genetic background.

All procedures were approved by the Animal Care and Use Committee of the National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health.

Biochemical and hormonal assays. Blood was collected from the retro-orbital sinus of 16-week-old male mice in the nonfasted state (between 9:00 A.M. and 12:00 noon). Glucose was measured using a Glucometer Elite (Bayer, Elkhart, IN). Commercial kits were used for measurements of serum triglycerides (#337-B; Sigma) and nonesterified fatty acids (#13831175; Roche Molecular Biochemicals). Liver triglycerides were measured by solvent extraction followed by a radiometric assay for glycerol as previously described (21). Serum insulin and adiponectin levels were measured using sensitive rat insulin and mouse adiponectin RIA kits (#SRI-13K and #MADP-60HK, respectively; Linco Research, St. Charles, MO).

Glucose tolerance tests. The 16-week-old male mice were fasted overnight. The 20% glucose (2 g/kg) was injected intraperitoneally, and blood glucose was measured using a Glucometer Elite at 0, 15, 30, 60, and 120 min.

Hyperinsulinemic-euglycemic clamp. This protocol was based on the clamp protocol developed by Jason Kim and Gerald Shulman (22). Mice were anesthetized with 100 mg/kg ketamine and 10 mg/kg xylazine. A catheter was inserted via a right lateral neck incision, advanced into the superior vena cava via the right internal jugular vein, and sutured in place, according to the protocol of MacLeod and Shapiro (23). Clamp studies were performed on male mice 16–18 weeks of age, between 4 and 6 days after catheter insertion as described previously (24). Clamp results were analyzed as described previously (24).

Statistical analysis. Data are expressed as means \pm SE. Statistical significance between the groups was determined with SigmaStat (SPSS, Chicago) using one-way ANOVA or the *t* test as appropriate.

RESULTS

At 16 weeks of age, the changes in circulating IGF-1 and GH were in agreement with previously published data (19). Both LID and ALSKO mice showed 75 and 65%, respectively, reduction in circulating total IGF-1 levels relative to controls. LID+ALSKO mice displayed a reduction of 85% in circulating IGF-1 levels (Fig. 1A). GH levels were increased in LID mice (when $n > 10$, there was an increase of 4.5-fold in GH levels compared with controls) (19) and even further increased in LID+ALSKO mice (when $n > 10$, there was an increase of 15-fold in GH levels compared with controls) (19), whereas in ALSKO mice, this parameter was similar to that of the control group (Fig. 1B). Because we observed a marked decrease in circulating IGF-BPs in the sera of LID, ALSKO, and LID+ALSKO mice, free IGF-1 levels were analyzed in all the groups. Free levels of IGF-1 in control, LID, and ALSKO mice ranged between 6 and 9 ng/ml, whereas in LID+ALSKO mice, we observed a marked increase in free levels of IGF-1 to 22.3 ± 3.2 ng/ml. Body weight and body length of LID+ALSKO mice were lower compared with controls and LID mice at 16 weeks of age (Table 1). ALSKO mice tended to have a lower body weight than controls, although it was not statistically significant.

Nonfasted blood glucose levels were significantly lower in the ALSKO group compared with the other groups and tended to be higher in LID mice (Fig. 1C). Insulin levels in the LID and LID+ALSKO mice increased approximately three- to fourfold compared with control and ALSKO mice (Fig. 1D). Circulating triglyceride levels significantly increased in LID mice compared with the other groups, whereas no differences in this parameter were found between control, ALSKO, and LID+ALSKO mice, respectively (Fig. 1E).

Liver weight increased in the LID mice compared with the other groups (Table 1, Fig. 2). When expressed as a percentage of body weight, there was an ~20% increase in

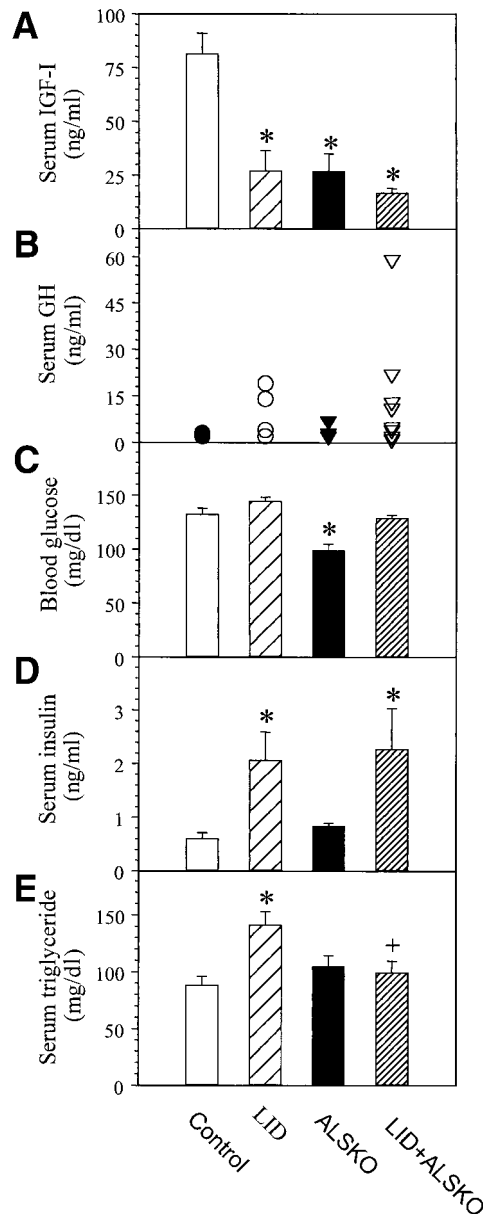


FIG. 1. Serum IGF-1 (A), GH (B), blood glucose (C), serum insulin (D), and serum triglycerides (E) of male control, LID, ALSKO, and LID+ALSKO mice at 16 weeks of age. Samples are from nonfasting mice bled between 9:00 A.M. and 12:00 noon. Data are means \pm SE with $n = 5-7$ /group. * $P < 0.05$ vs. control group; † $P < 0.05$ for LID+ALSKO vs. LID group.

TABLE 1

Metabolic parameters of 16-week-old control, LID, ALSKO, and LID+ALSKO male mice

	Control	LID	ALSKO	LID+ALSKO
Body weight (g)	24.3 \pm 0.5	24.4 \pm 0.7	22.6 \pm 1.36	19.1 \pm 1.3*†
Body length (cm)	8.7 \pm 0.1	8.7 \pm 0.1	8.5 \pm 0.1	8.0 \pm 0.1*†
Liver weight (g)	1.28 \pm 0.03	1.6 \pm 0.14*	1.2 \pm 0.11	1.25 \pm 0.11†
Liver triglycerides (μ mol/liver)	6.56 \pm 0.82	17.65 \pm 3.86*	10.16 \pm 2.63	15.03 \pm 1.56*
Inguinal fat pad weight (g)	0.33 \pm 0.02	0.43 \pm 0.03*	0.33 \pm 0.02	0.24 \pm 0.01*†
Gonadal fat pad weight (g)	0.38 \pm 0.02	0.64 \pm 0.06*	0.46 \pm 0.1	0.31 \pm 0.05*†
Brown adipose tissue (mg)	134 \pm 7	219 \pm 22*	126 \pm 23	148 \pm 15
Serum adiponectin (μ g/ml)	5.53 \pm 0.96	9.79 \pm 2.19	10.02 \pm 3.1	7.24 \pm 1.12
Serum free fatty acid (μ M)	358.5 \pm 46.6	421.5 \pm 67.1	404.5 \pm 31.5	388.5 \pm 45.0

Data are means \pm SE with $n = 5-7$ per group. Samples are from non fasting mice anesthetized with avertin and bled between 9:00 A.M. and 12:00 noon. * $P < 0.05$ vs. the control group; † $P < 0.05$ for the ALSKO/LID vs. LID group.

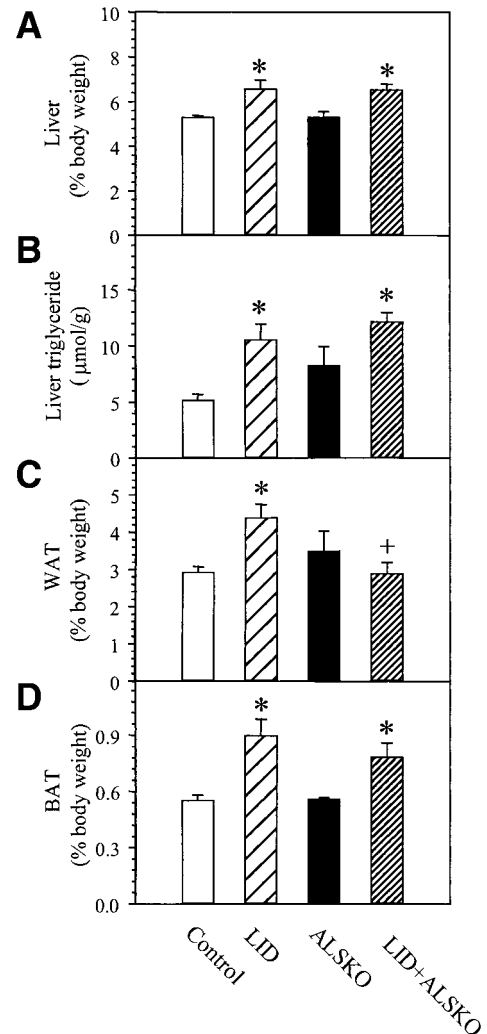


FIG. 2. Liver and adipose tissue weights of male control, LID, ALSKO, and LID+ALSKO mice at 16 weeks of age. A: Liver weight as percentage of body weight. B: Liver contents of triglycerides. C: White adipose tissue (WAT) weight as percentage of body weight. D: Brown adipose tissue (BAT) weight as percentage of body weight. * $P < 0.05$ vs. control group; † $P < 0.05$ for LID+ALSKO vs. LID group.

liver weight in both LID and LID+ALSKO mice compared with the control and ALSKO groups, respectively (Fig. 2A), probably reflecting the elevated serum GH levels. Liver triglyceride content increased significantly in both LID and LID+ALSKO mice compared with control mice (Table 1, Fig. 2B). However, triglyceride content in the livers of

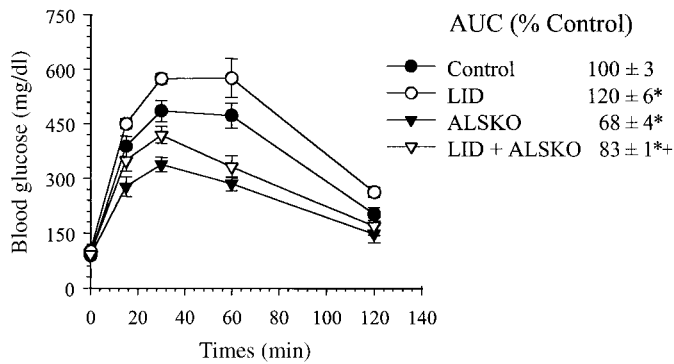


FIG. 3. Glucose tolerance test and the area under the curve in male control, LID, ALSKO, and LID+ALSKO mice at 16 weeks of age. Animals were fasted overnight. The 20% glucose (2 g/kg) was injected intraperitoneally, and blood glucose was measured using the Glucometer Elite at 0, 15, 30, 60, and 120 min. Data are means \pm SE with $n = 5-7$ /group. * $P < 0.05$ vs. control group; † $P < 0.05$ for LID+ALSKO vs. LID group.

ALSKO mice did not differ significantly from that of control mice. White adipose tissue (inguinal, gonadal) and brown adipose tissue were increased in the LID mice compared with the other groups (Fig. 2C); however, brown adipose tissue was increased in both LID and LID+ALSKO mice (Fig. 2D). No significant differences in serum free fatty acids and adiponectin levels were found between the groups (Table 1).

Glucose tolerance. As was previously shown, LID mice develop muscle-specific insulin resistance at the age of 8 weeks (18). In this study, at 16 weeks of age, insulin levels were elevated (approximately threefold) in LID and LID+ALSKO mice, indicating possible insulin insensitivity in both groups (Fig. 1D). A glucose tolerance test revealed that ALSKO mice cleared glucose significantly faster, with the area under the curve being $\sim 50\%$ smaller than that for control mice (Fig. 3). In contrast, LID mice showed a modest but statistically significant glucose intolerance relative to the control group. LID+ALSKO mice had faster glucose clearance than the LID group, and, interestingly, their glucose clearance was even faster than that of the control group, despite their increased insulin levels.

Changes in insulin sensitivity. A rapid glucose clearance during the glucose tolerance test can be a result of differences in insulin sensitivity and/or insulin secretion. To explore insulin sensitivity in control, ALSKO, LID, and LID+ALSKO mice, hyperinsulinemic-euglycemic clamps were performed on 16-week-old male mice.

TABLE 2

Metabolic parameters during the basal (12-h fasted) period and hyperinsulinemic-euglycemic clamp in 16-week-old male control, LID, ALSKO, and LID+ALSKO mice

	Control	LID	ALSKO	LID+ALSKO
Body weight (g)	25.1 \pm 1.2	28.8 \pm 1.1*	20.2 \pm 0.8*	21.7 \pm 0.5*†
Basal plasma glucose (mg/dl)	160 \pm 19	165 \pm 14	120 \pm 11	131 \pm 10†
Basal plasma insulin (ng/ml)	0.58 \pm 0.12	1.13 \pm 0.09*	0.39 \pm 0.08	1.41 \pm 0.29*
Clamp plasma glucose (mg/dl)	120 \pm 2	116 \pm 3	118 \pm 2	118 \pm 3
Clamp plasma insulin (ng/ml)	2.1 \pm 0.08	3.22 \pm 0.42*	2.52 \pm 0.5	2.26 \pm 0.21
Basal endogenous glucose production ($\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$)	90.4 \pm 3.4	91.3 \pm 9.0	96.2 \pm 6.7	127.6 \pm 8.6*
Whole-body glycolysis ($\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$)	172.4 \pm 17.9	118.6 \pm 10.7	216.3 \pm 23.9	150.7 \pm 16.5
Whole-body glycogen synthesis ($\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$)	52.0 \pm 9.9	28.5 \pm 10.5	51.3 \pm 22.4	49.5 \pm 12.3

Data are means \pm SE with $n = 5-7$ per group. * $P < 0.05$ vs. control group; † $P < 0.05$ for LID + ALSKO vs. LID mice.

After overnight fasting, there were no significant differences in plasma glucose levels, whereas insulin concentrations were approximately twofold higher in LID mice and approximately threefold higher in LID+ALSKO mice, respectively, relative to the control and ALSKO groups (Table 2). Basal endogenous glucose production was significantly higher in LID+ALSKO mice compared with the other groups (Table 2).

Endogenous glucose production and its suppression by hyperinsulinemia during the clamp is a measure of liver insulin sensitivity. Clamp endogenous glucose production in both LID and LID+ALSKO mice was approximately threefold higher compared with the control group, indicating liver insulin resistance (Fig. 4A). Interestingly, ALSKO mice tended to have further decreased endogenous glucose production compared with control mice ($P = 0.07$) (Fig. 4A).

Whole-body glucose uptake under hyperinsulinemic conditions is predominantly a measure of muscle glucose uptake. This variable was reduced by 35% in the LID mice compared with the control mice, indicating muscle insulin resistance (Fig. 4B). ALSKO mice, on the contrary, tended to have higher whole-body glucose uptake than controls (267 \pm 18 vs. 224 \pm 17, $P = 0.06$). Whole-body glucose uptake in LID+ALSKO mice was 25% higher than that in LID mice ($P < 0.01$), suggesting improved muscle insulin sensitivity. Whole-body glycolysis and whole-body glycogen synthesis followed the same pattern as whole-body glucose uptake (Table 2).

Muscle glucose uptake was determined by measuring 2-deoxy-D-[1- ^{14}C]glucose content. Glucose uptake was reduced in muscle of LID mice and relatively improved in the LID+ALSKO mice ($P = 0.054$ vs. LID mice) (Fig. 4C). ALSKO mice tended to have higher muscle glucose uptake relative to the control group, but the difference did not reach statistical significance. White adipose tissue glucose uptake was reduced in LID mice compared with the rest of the groups. The white adipose tissue glucose uptake in LID+ALSKO mice did not differ significantly from the ALSKO and control groups, respectively, and tended to be higher than that in LID mice ($P = 0.07$) (Fig. 4D). Brown adipose tissue uptake was significantly lowered in LID mice and tended to be lower in LID+ALSKO mice compared with the control group ($P = 0.07$) (Fig. 4E).

In summary, the hyperinsulinemic-euglycemic clamp showed that at the age of 16 weeks, LID mice develop insulin resistance in the muscle, liver, and adipose tissue. Although muscle insulin sensitivity was partially improved

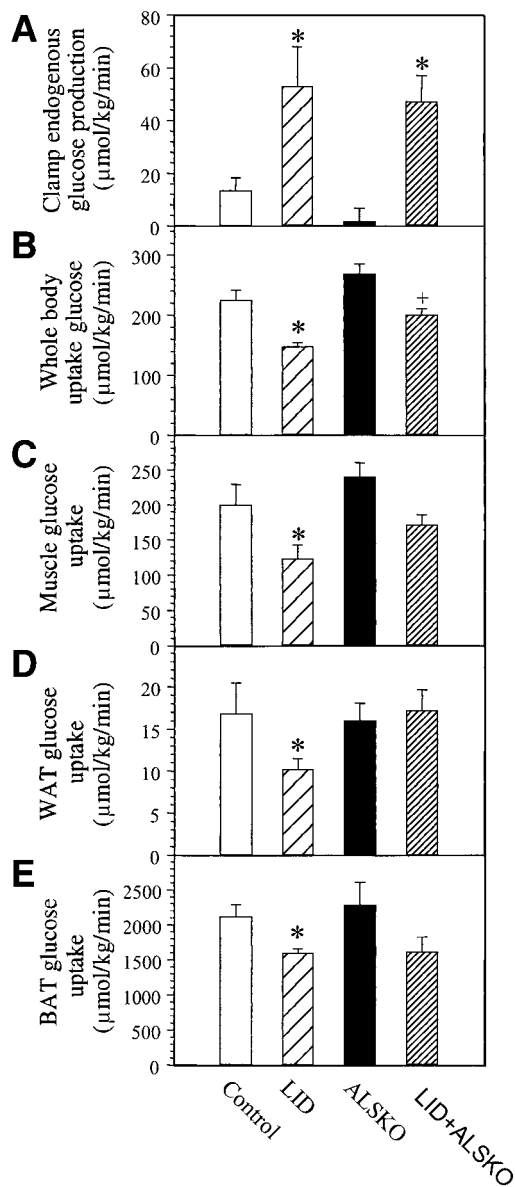


FIG. 4. Hyperinsulinemic-euglycemic clamp studies of male control, LID, ALSKO, and LID+ALSKO mice at 16 weeks of age. Animals were fasted overnight, and a hyperinsulinemic-euglycemic clamp was performed as described in RESEARCH DESIGN AND METHODS. Data are means \pm SE with $n = 5-7$ /group. * $P < 0.05$ vs. control group; + $P < 0.05$ for LID+ALSKO vs. LID mice. **A:** Clamp endogenous glucose production. **B:** Whole-body glucose uptake. **C:** Muscle glucose uptake. **D:** White adipose tissue (WAT) glucose uptake. **E:** Brown adipose tissue (BAT) glucose uptake.

when introducing the ALS null allele in the LID+ALSKO mice, liver insulin resistance remained virtually unchanged.

DISCUSSION

Chronically increased GH levels are known to induce insulin resistance by direct effects on insulin signal transduction and indirect effects on lipid and carbohydrate metabolism (25,26). We have previously demonstrated that LID mice develop muscle insulin resistance as early as 8 weeks of age (17). Treatment with either IGF-1 or a GH-releasing hormone antagonist improved muscle insulin sensitivity in LID mice, as demonstrated by more rapid

glucose clearance after insulin injection as well as by an enhanced tyrosine phosphorylation of the muscle insulin receptor and insulin receptor substrate 1 after insulin injection (18). In the present study, we have examined the impact of reduced circulating IGF-1 accompanied by increased GH levels on glucose homeostasis in four different mice groups: control, LID, ALSKO, and LID+ALSKO. Hyperinsulinemic-euglycemic clamp analyses show that LID mice, who have a 75% reduction in circulating IGF-1 and a fivefold increase in GH secretion, exhibit an extreme insulin resistance, as reflected by decreased fat and muscle glucose uptake and increased hepatic glucose production. However, the ALSKO mice, which have a similar 75% reduction in circulating IGF-1 levels, do not have increased GH secretion and demonstrate comparable insulin sensitivity as controls. Surprisingly, crossing of LID and ALSKO mice (LID+ALSKO), which represent the most profound changes in the GH/IGF-1 axis, having an 85% reduction of total circulating IGF-1 and a 15-fold increase in GH (19), showed an improved muscle and fat glucose uptake but did not show any improvement in hepatic glucose production compared with the LID mice. This unexpected result further underlines the complexity of the GH/IGF-1 system, indicating that total circulating IGF-1 does not necessarily correlate with its effects in particular tissues.

LID and ALSKO genotypes led to a marked decrease in circulating IGF-BPs, most likely because of degradation (17,19,20). The disequilibrium between IGF-1 and the IGF-BPs results in a different pool distribution of IGF-1. In the LID mice, most of the IGF-1 is most likely found in a ternary complex and is unable to regulate GH secretion by the pituitary. However, in the ALSKO mice, the majority of the IGF-1 is most likely found in a binary complex (composed of IGF-1 and IGF-BP-3), which probably crosses the capillary barrier and controls GH secretion as well as having an effect on peripheral tissues. In contrast, as was demonstrated in our previous study, the LID+ALSKO mice have a more pronounced decrease in total IGF-1 levels and circulating IGF-BP-3 levels and a threefold increase in free IGF-1 levels. Increased free IGF-1 levels are clearly not sufficient to suppress GH production appropriately nor can they ensure normal growth. This is most likely due to degradation of free IGF-1 once it leaves the circulation. The glucose metabolism in LID+ALSKO mice, however, differs from that in LID mice; there is improved muscle and white adipose tissue insulin sensitivity, whereas liver insulin resistance remains unchanged. Taken together, our and others' data suggest that chronic elevation in GH is the common causative factor of insulin resistance regardless of whether total IGF-1 levels are decreased, as in the LID mice, or increased, as in acromegalic patients with chronic endogenous GH overproduction (16).

Deletion of the *igf-1* gene has also been characterized in humans; similar to our LID mouse model, the patient had elevated GH and reduced IGF-1 levels (27). This patient also showed insulin resistance, which was reversed by IGF-1 replacement (27). IGF-1 administration has also been demonstrated to improve insulin sensitivity in both type 1 diabetic and type 2 diabetic patients (28,29). In another clinical study involving patients with acromegaly (30), combined treatment with GH receptor antagonist

plus IGF-1/IGFBP-3 was found to be more effective in improving insulin sensitivity than treatment with GH receptor antagonist itself, suggesting that the insulin-sensitizing effect of IGF-1 is partly independent of its ability to suppress GH secretion (30).

Liver insulin resistance is usually accompanied by increased deposition of triglycerides and/or other lipid metabolites in the liver (31,32). Indeed, liver triglycerides were significantly higher in both LID and LID+ALSKO mice that showed liver insulin resistance compared with control and ALSKO mice with normal liver triglycerides and normal liver insulin sensitivity. It is, however, uncertain whether this increase is the primary cause or rather the consequence of liver insulin resistance.

The results of the present study highlight the importance of GH and IGF-1 in the modulation of insulin action. We suggest that the overall metabolic phenotype of LID+ALSKO mice is the result of the counteracting effects of high GH levels and increased free IGF-1 levels. By acting through the type 1 IGF receptor, IGF-1 can directly activate the insulin receptor substrate 1–phosphatidylinositol 3-kinase cascade, resulting in GLUT4 translocation to the plasma membrane and thus stimulating glucose uptake in the muscle (33,34). Alternatively, IGF-1 may stimulate muscle glucose uptake via the hybrid receptors (described in detail in the introduction). It is also possible that simultaneous stimulation of this cascade through insulin and IGF-1 receptors can amplify insulin effects. The hypothesis of direct IGF-1 effects on insulin sensitivity in LID+ALSKO mice is further supported by tissue specificity of these changes. Although improvement occurs in the muscle that expresses many IGF-1 receptors, there is no change in the liver insulin sensitivity. IGF-1 may play a role in affecting insulin resistance. However, whether this effect on muscle in our present study (with lack of effect on liver) is via the increase in “unbound” IGF-1 or due to tissue-specific changes in triglycerides or insulin receptor levels remains to be established. It is important to note that adipose tissue in LID+ALSKO mice was significantly reduced compared with LID mice, and this may explain the improvement in muscle insulin sensitivity.

In summary, we have demonstrated that the changes in circulating IGF-1 and ALS levels could significantly affect glucose metabolism and insulin sensitivity in a tissue-specific manner.

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