

# Liver-Derived IGF-I is of Importance for Normal Carbohydrate and Lipid Metabolism

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**IGF-I is important for postnatal body growth and exhibits insulin-like effects on carbohydrate metabolism. The function of liver-derived IGF-I is still not established, although we previously demonstrated that liver-derived IGF-I is not required for postnatal body growth. Mice whose *IGF-I* gene in the liver was inactivated at 24 days of age were used to investigate the long-term role of liver-derived IGF-I for carbohydrate and lipid metabolism. Serum levels of leptin in these mice were increased by >100% at 3 months of age, whereas the fat mass of the mice was decreased by 25% at 13 months of age. The mice became markedly hyperinsulinemic and yet normoglycemic, indicating an adequately compensated insulin resistance. Furthermore, they had increased serum levels of cholesterol. We conclude that liver-derived IGF-I is of importance for carbohydrate and lipid metabolism. *Diabetes* 50:1539–1545, 2001**

**I**GF-I promotes growth and differentiation in a variety of tissues (1). IGF-I also mimics some of the metabolic actions of insulin, including stimulation of glucose and amino acid uptake and inhibition of gluconeogenesis (2–4). These effects are largely mediated by the IGF-I receptor, although IGF-I can also bind to the insulin receptor with low affinity (5,6). Both receptors are widely distributed, with some tissues expressing hybrid receptors that combine insulin and IGF-I receptor subunits (7,8).

Recently, we developed a transgenic mouse model with the *IGF-I* gene inactivated specifically in the hepatocytes of the liver (LI-IGF-I<sup>-/-</sup> mice). Using the CRE/loxP system, we induced inactivation just before the pubertal

growth spurt and demonstrated that liver-derived IGF-I is the principal source of IGF-I in blood but is not required for postnatal body growth (9,10). One may speculate that the primary role of liver-derived IGF-I is the regulation of carbohydrate and lipid metabolism, rather than growth promotion. Clinical studies of short-term metabolic effects of recombinant human IGF-I (rhIGF-I) in healthy adults have shown that IGF-I is 6% as potent as insulin on a molar basis in the production of hypoglycemia (2). A patient with severe IGF-I deficiency (secondary to a homozygous deletion of a part of the *IGF-I* gene) had insulin resistance that was normalized by treatment with rhIGF-I (11). Furthermore, mice with a null mutation in the *IGF-I* gene had increased serum levels of insulin accompanied by decreased serum levels of glucose (12). However, the involvement of liver-derived IGF-I in the regulation of carbohydrate and lipid metabolism is not established. Therefore, the aim of the present study was to investigate the role of liver-derived IGF-I for carbohydrate and lipid metabolism by using mice with liver-specific *IGF-I* gene inactivation.

## RESEARCH DESIGN AND METHODS

**Animals.** The Mx-Cre 31 strain was generated by injection into C57BL/6x(CBA)F<sub>2</sub> eggs as previously described (13). The offspring were then backcrossed with C57BL/6 mice. Mice with exon 4 of the *IGF-I* gene flanked with loxP sites were generated in embryonic stem (ES) cells derived from 129sv mice, as previously described (14). The ES cells were injected into C57BL/6 blastocysts. Male chimeric offspring were backcrossed with C57BL/6 female mice. Mx-Cre 31 mice were crossed with mice with exon 4 of the *IGF-I* gene flanked with loxP sites. The different genotypes of mice were identified by polymerase chain reaction (PCR) analysis of DNA from tail biopsy specimens obtained 2 weeks after birth. Mice homozygous for loxP and heterozygous for Mx-Cre were given interferon (IFN) (IFN- $\alpha_2/\alpha_1$ ,  $4 \times 10^8$  U  $\cdot$  kg<sup>-1</sup>  $\cdot$  body wt<sup>-1</sup>) in three intraperitoneal injections on 24, 26, and 28 days of age. As controls, we used IFN-treated littermates homozygous for loxP but lacking Mx-Cre. We used the same IFN dose and injection schedule for controls as for LI-IGF-I<sup>-/-</sup> mice.

The animals had free access to fresh water and food pellets (B&K Universal AB, Sollentuna, Sweden) consisting of cereal products (76.9% barley, wheat feed, and wheat and maize germ), vegetable proteins (14.0% hipro soya), and vegetable oil (0.8% soya oil). The animal procedure was approved by the ethical committee at the University of Göteborg and Lund.

**Dual X-ray absorptiometry.** Previously, we developed and evaluated a combined dual X-ray absorptiometry (DXA) image analysis procedure for the in vivo measurement of fat content in mice (K.S., M.B.Y., C.O., N. Hellberg, L. Savendahl, I. Bosaeus, unpublished observations). The DXA measurements were performed with the Norland pDEXA Sabre (Fort Atkinson, WI) and the Sabre Research software (version 3.9.2). Three mice were analyzed in each scan. One mouse was killed at the beginning of the experiment and was included in all scans as an internal standard to avoid interscan variations. The software percent-fat procedure was used together with a setting that made areas with >50% fat appear white in the image. The accuracy of this setting was checked daily with a standard consisting of a gradient with 0–100% fat. The image was then printed, scanned, and imported to the software Scion

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AIR, acute first phase insulin secretion response; AUC<sub>ins</sub>, area under the curve for insulin; DXA, dual X-ray absorptiometry; ES, embryonic stem; FFA, free fatty acid; GDI, global disposition index; GH, growth hormone; GHD, GH-deficient; IGF-BP, IGF-binding protein; IFN, interferon; K<sub>G</sub>, glucose tolerance index; LDLr, LDL receptor; PCR, polymerase chain reaction; rhIGF-I, recombinant human IGF-I; RPA, RNase protection assay; S<sub>G</sub>, glucose effectiveness; S<sub>I</sub>, insulin sensitivity index

Image (Scion, Frederick, MD). The imported image was set to a threshold of 50 arbitrary units, making lean mass and bone black, whereas the fat area appeared as white holes. Thereafter, the "analyze particle" procedure was performed, first with the white areas of the mice included ( $A1 = \text{total mouse area}$ ) and then without the white area included ( $A2 = \text{lean area} + \text{bone area}$ ). The percent fat area was then calculated as  $([A1 - A2]/A1) \times 100$ . The interassay CV for the measurements of percent fat area was  $<3\%$ .

**BMI and dissection of fat.** The animals were weighed before they were killed. The crown-rump length was measured on the same DXA scan as the body fat, and it was defined as the distance between the crown of the skull and a point located in the middle of a line between the two caput femora. The BMI was then calculated as the body weight in grams/crown-rump length in millimeters<sup>2</sup>. The retroperitoneal and gonadal fat depots were dissected and weighed (wet weight).

**Lipoprotein characterization.** The cholesterol distribution profiles were measured using a size exclusion high performance liquid chromatography system (SMART) with column Superose 6 PC 3.2/30 (Amersham Pharmacia Biotech, Uppsala, Sweden). The chromatographic system was linked to an air-segmented continuous flow system for online postderivatization analysis of total cholesterol using enzymatic colorimetric reagents.

The SMART system was connected to a sample injector (Gina 50; GynkoteK, Germering, Germany). Elution buffer consisted of 0.01 mol/l Tris and 0.03 mol/l NaCl, pH 7.40, with a flow rate of 35  $\mu\text{l}/\text{min}$ . The online flow system was equipped with a peristaltic pump (flow rate 0.7 ml/min) and an incubation coil for 8 min at 37°C. The absorbance was measured at 500 nm with a UV-vis detector, (Jasco UV-970; Jasco International, Japan). Data were integrated with the Chromeleon chromatography data system (GynkoteK). The distribution of lipoproteins was continuously measured as total cholesterol using enzymatic colorimetric reagent, reconstituted in water in double volume, according to the manufacturer instructions. The commercial kits (cholesterol, [CHOD-PAP, 1489437] and triglyceride [450032]) were purchased from Roche Diagnostics, Germany. The separation was performed within 60 min on a 10- $\mu\text{l}$  sample. The integrated area of the fractions was expressed in molar concentration. The various peaks in the profiles are designated VLDL, LDL, and HDL for simplicity, although it is clear that the separation is determined primarily by the size of the lipoproteins.

**Intravenous glucose tolerance test.** An intravenous glucose tolerance test was performed in young mice (4 months) and repeated when the mice were older (9 months). The animals were anesthetized with an intraperitoneal injection of midazolam (0.4 mg/mouse) (Dormicum; Hoffman-La-Roche, Basel, Switzerland) and a combination of fuanison (0.9 mg/mouse) and fentanyl (0.02 mg/mouse) (Hypnorm; Janssen, Beerse, Belgium). Thereafter, a blood sample was taken from the retrobulbar, intraorbital, and capillary plexus, after which the animals were given an intravenous injection of D-glucose (1 g/kg) (British Drug Houses, Poole, U.K.). The volume load was 10  $\mu\text{l} \cdot \text{g}^{-1} \cdot \text{body wt}^{-1}$ . New blood was sampled after 1, 5, 10, 20, 30, and 50 min (75  $\mu\text{l}$  per sample). The samples for glucose and insulin were collected in heparinized tubes, and after immediate centrifugation, plasma was separated and stored at  $-20^\circ\text{C}$  or  $-80^\circ\text{C}$  until analysis.

**Data analysis.** The insulin and glucose data from the seven-sample intravenous glucose tolerance test provide indexes of glucose tolerance ( $K_G$ ) and insulin secretion.  $K_G$  is calculated as the slope of the logarithm of glucose concentration over time in the interval of 5–30 min. Insulin secretion is assessed by the acute first phase insulin secretion (AIR), calculated as the mean of suprabasal 1- and 5-min insulin levels, and by the area under the curve for insulin ( $\text{AUC}_{\text{ins}}$ ), calculated from suprabasal values with the trapezoidal rule. Analysis with the minimal model technique (15) provides insulin sensitivity index ( $S_i$ ), which is defined as the ability of insulin to enhance glucose disappearance and to inhibit glucose production (16), and glucose effectiveness ( $S_G$ ), which represents glucose disappearance per se from plasma without any change in dynamic insulin (17). Finally, a unitless index called the global disposition index (GDI) can be calculated by multiplying  $S_i$  by  $\text{AUC}_{\text{ins}}$ . This index describes the global effect of insulin because it includes both insulin secretion and insulin sensitivity. The model has been applied for use in mice by obtaining seven samples (18,19).

**Serum analysis.** Serum leptin levels were measured by a radioimmunoassay (Chrysal Chem) with an intra- and interassay CV of 5.4 and 6.9%, respectively. Free fatty acids (FFAs) were measured by an enzymatic colorimetric method (acyl-CoA synthetase-acyl-CoA oxidase; Wako Chemicals, VA) with an intra-assay CV of  $<3\%$ . Serum corticosterone levels were measured by radioimmunoassay (ICN Biomedicals), with intra-assay and interassay CVs of 6.5 and 4.4%, respectively. Serum IGF-I levels were measured by double-antibody IGF binding protein-blocked radioimmunoassay (20). Plasma insulin was determined radioimmunochemically with the use of a guinea pig anti-rat insulin antibody, <sup>125</sup>I-labeled human insulin as tracer, and rat insulin as standard (Linco Research, St Charles, MO). The sensitivity of the assay was 12 pmol/l,

and the intra-assay CV was  $<3\%$ . Plasma glucagon from freely fed animals was determined radioimmunochemically with the use of a guinea pig antiglucagon antibody specific for pancreatic glucagon, <sup>125</sup>I-labeled-glucagon as tracer, and glucagon as standard (Linco). The sensitivity of the assay was 7.5 pg/ml, and the intra-assay CV was  $<9\%$ . Plasma glucose was determined with the glucose oxidase method (21). Plasma was assayed for levels of triglycerides (catalogue no. 450032, Triglycerides/GB; Roche Diagnostics) and cholesterol (catalogue no. 2016630, Cholesterol, CHOD-PAP; Boehringer Mannheim) using commercially available kits and following the manufacturers instructions.

**RNase protection assay.** RNase protection assay (RPA) was done according to the manufacturers instructions (RPA kit; Ambion, Austin, TX). The 354-bp probe used in the RPA was generated with PCR and corresponds to exon 4 and part of exon 3 of the LDL receptor (LDLr) gene. cDNA was synthesized from total mouse liver RNA, and then PCR was run using the following primers: 5'CGGGCTGGCGGTAGACTG3' and 5'CGATTGCCCCCGTTGACA3'. The PCR fragment was isolated and T/A-cloned into the PCR-II vector (Invitrogen, Leek, the Netherlands), and the insert was verified by sequencing. The vector was linearized with *Xho*I before in vitro transcription with SP6 polymerase in the presence of  $\alpha$ -<sup>32</sup>P-UTP. Bands were visualized and quantified using a PhosphorImager (Molecular Dynamics, Sunnyvale, CA). LDLr mRNA levels determined by RNase protection assay were expressed as a ratio between the density of the band corresponding to the LDLr transcript and the density of the band corresponding to the internal standard (18 S).

## RESULTS

**Mice lacking liver-produced IGF-I are leaner.** At 3 weeks of age, the mice were given IFN to induce recombination of the *IGF-I* gene in liver, as previously described (9). Serum IGF-I levels were decreased by 85% ( $P < 0.01$ ) in the LI-IGF-I<sup>-/-</sup> mice 1 week after IFN induction and stayed low throughout the experiment (350 days) (Fig. 1A). As in our previous study (9), no significant difference in body weight was seen during the first 50 days after IFN induction. Later on, we saw a significant weight difference between the LI-IGF-I<sup>-/-</sup> mice and controls, which became more pronounced with age (13 months of age: females  $-20.0\%$ ,  $P < 0.05$ ; males  $-22.5\%$ ,  $P < 0.01$ ) (Figs. 1B and C).

To investigate whether this weight difference was caused by an altered body composition, the mice were followed by repeated in vivo measurements using DXA. At 8 months of age, there was a nonsignificant tendency toward decreased fat mass in the LI-IGF-I<sup>-/-</sup> mice ( $-9.4\%$ , data not shown). When the mice were killed at 13 months of age, the amount of fat was decreased by 26.5% ( $P < 0.05$ ) (Fig. 2), and BMI was decreased by 14% ( $P < 0.05$ , Student's *t* test, data not shown). Dissection of fat depots confirmed this finding. Both females and males had significantly reduced retroperitoneal fat pads (females  $-23.7\%$  and males  $-25.5\%$ ) (Fig. 3A), and the parametrial/epididymal fat pads were also reduced, although the difference was only significant in males (females  $-26.9\%$  and males  $-26.9\%$ ) (Fig. 3B). Leptin levels have been shown to correlate with the amount of fat in humans and rodents (22,23). Surprisingly, we found increased serum leptin levels in the young adult LI-IGF-I<sup>-/-</sup> mice by 3 months of age (females 183.8% and males 103.5% over control) (Fig. 3C). At 13 months of age, serum levels of leptin had increased in both groups, and the difference between LI-IGF-I<sup>-/-</sup> mice and controls disappeared (Fig. 3C).

**Mice lacking liver-produced IGF-I are insulin resistant.** Young adult LI-IGF-I<sup>-/-</sup> mice had elevated basal levels of insulin compared with controls (4 months of age: females 149.9% and males 163.5% over control) (Fig. 4A). Both LI-IGF-I<sup>-/-</sup> and control mice exhibited a significant increase in basal insulin levels with age (Fig. 4A). At 9

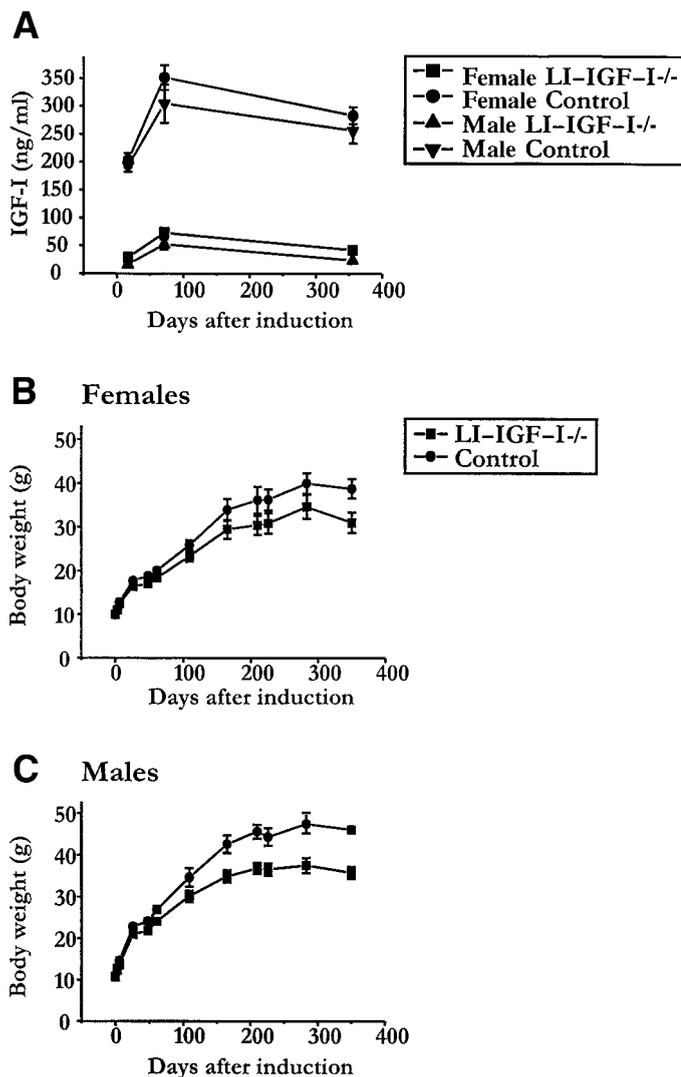


FIG. 1. Mice were given IFN at 24–28 days of age to induce recombination of the *IGF-I* gene in liver. Serum IGF-I levels (A) and body weight in females (B) and males (C) were monitored up to 12 months after the IFN induction. Data are given as means  $\pm$  SEM. The number of observations (*n*) in each group is 4–7.

months of age, there was no significant difference in basal insulin levels between LI-IGF-I<sup>-/-</sup> and control mice (Fig. 4A). When compiling all animals together from each individual experiment at that age, the LI-IGF-I<sup>-/-</sup> mice displayed a tendency to increase in basal insulin levels compared with control mice ( $49\% \pm 21$  over control,  $P = 0.065$ , Student's *t* test; LI-IGF-I<sup>-/-</sup>  $n = 36$  and control  $n = 33$ ). Despite the increased levels of basal insulin, serum glucose levels were unchanged in LI-IGF-I<sup>-/-</sup> mice compared with control mice at both 4 and 9 months of age (data not shown).

To test whether the LI-IGF-I<sup>-/-</sup> mice had an altered glucose tolerance, an intravenous injection of glucose (1 g/kg) was given. The insulin response was more pronounced in the LI-IGF-I<sup>-/-</sup> mice than in the controls (Figs. 4B and C). After 1 min, the plasma insulin had gone from basal levels to  $2,510 \pm 299$  pmol/l in the female LI-IGF-I<sup>-/-</sup> mice and to only  $1,087 \pm 505$  pmol/l in the controls (Fig. 4B). Similar results were seen in male mice (Fig. 4C). After the initial increase, plasma insulin rapidly returned

toward baseline values in both groups. In addition, plasma glucose levels peaked after 1 min, but there was no significant difference in the glucose levels between the LI-IGF-I<sup>-/-</sup> and control mice during the entire experiment. Thus, despite the more marked increase in plasma insulin after glucose administration, the glucose elimination was not altered in the LI-IGF-I<sup>-/-</sup> mice (Figs. 4D and E).

Metabolic parameters from the minimal model analysis as well as those directly calculated from the intravenous glucose test are shown in Table 1. Insulin, both in terms of peak (AIR) and total amount (AUC<sub>ins</sub>) was markedly increased in LI-IGF-I<sup>-/-</sup> mice, whereas  $S_I$  was decreased by 50%. Hypersecretion of insulin adequately compensated for reduced sensitivity; in fact, both  $K_G$  and GDI did not change from control animals. No significant difference was observed in the  $S_G$  or in the levels of glucagon (LI-IGF-I<sup>-/-</sup>  $48.4 \pm 8.5$  and controls  $36.9 \pm 10.6$  ng/l).

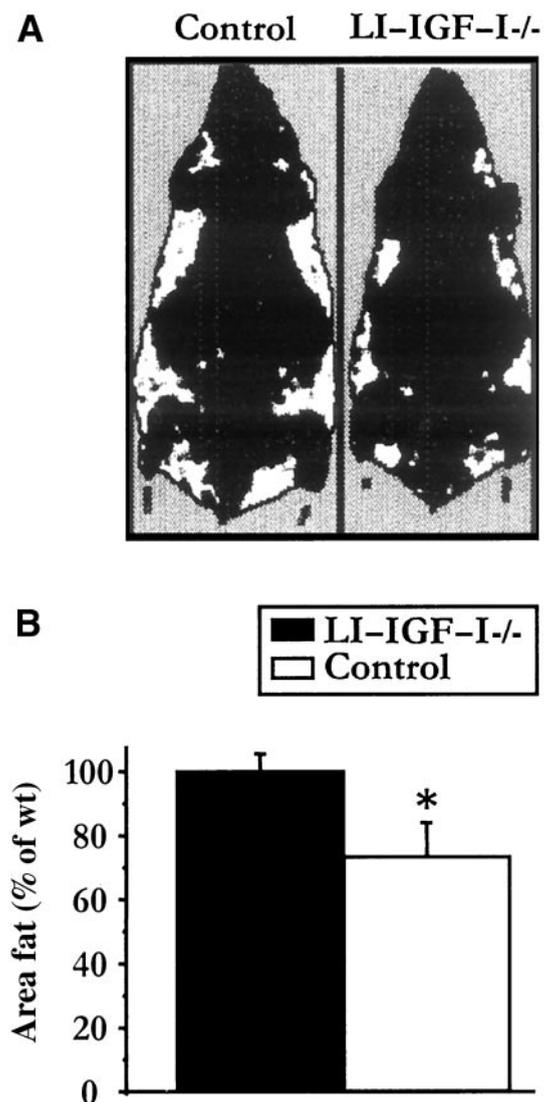


FIG. 2. Total body fat, as measured using DXA, in 13-month-old pooled male and female LI-IGF-I<sup>-/-</sup> and control mice. A: DXA/Image analysis of fat content in representative LI-IGF-I<sup>-/-</sup> and control mice. Areas with >50% fat are shown as white areas, whereas areas with lean mass and bone are shown as black areas. B: Data are expressed as percent of control and given as means  $\pm$  SEM ( $n = 11$ ). \*Probability level of random difference between groups,  $P < 0.05$ , Student's *t* test for LI-IGF-I<sup>-/-</sup> versus control.

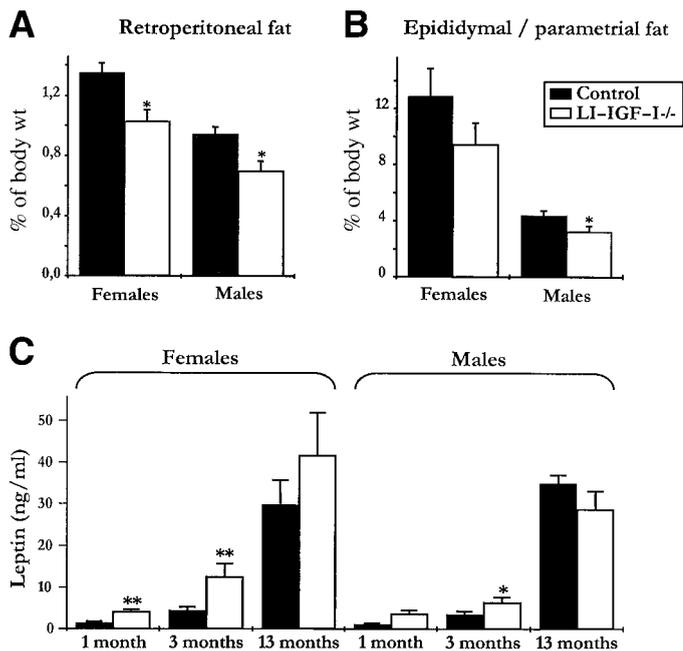


FIG. 3. The amount of dissected retroperitoneal fat (A) and epididymal/parametrial fat (B) in 13-month-old male and female mice is expressed as percent of body weight. Serum leptin levels (C) were measured at 1, 3, and 13 months of age. Data are given as means  $\pm$  SEM. Probability level of random difference between groups: \* $P < 0.05$ ; \*\* $P < 0.01$ ; Student's  $t$  test for LI-IGF1<sup>-/-</sup> versus control,  $n = 5-7$ .

**Mice lacking liver-produced IGF-I have increased serum cholesterol.** To further investigate the metabolic consequences of reduced serum IGF-I, we measured factors involved in fat metabolism. Serum cholesterol was elevated in young male and female LI-IGF1<sup>-/-</sup> mice compared with controls (Table 2). Lipoprotein profiles were characterized at 3 and 8 months of age, demonstrating that the increase in cholesterol was mainly seen in the HDL and LDL fraction (Table 3 and data not shown, respectively). The differences in serum cholesterol and lipoprotein profile were no longer present in 13-month-old mice (Table 2 and data not shown, respectively).

Increases in serum cholesterol can be caused by changes in lipoprotein receptors on the surfaces of cells that bind circulating lipoproteins and mediate the internalization of lipoprotein cholesterol by cells. We measured the mRNA levels of one of these receptors, the LDL receptor, in liver, but there were no statistical differences between LI-IGF1<sup>-/-</sup> and control mice ( $86.3 \pm 10.6\%$  of control). Triglycerides were decreased in females but unchanged in males, and there was no difference in FFAs (Table 2). Corticosterone is a hormone often associated with insulin resistance and disturbed fat metabolism. We found that young male LI-IGF1<sup>-/-</sup> mice had elevated levels of corticosterone (51.3% over control,  $P < 0.05$ ), and a similar tendency was seen in young females (53.3% over control, NS) (Table 2). However, no effect on levels of corticosterone was seen in the 13-month-old LI-IGF1<sup>-/-</sup> mice (Table 2).

**DISCUSSION**

The results presented here clearly demonstrate that liver-derived IGF-I has a metabolic function. The mouse model we used in the present study made it possible to induce a

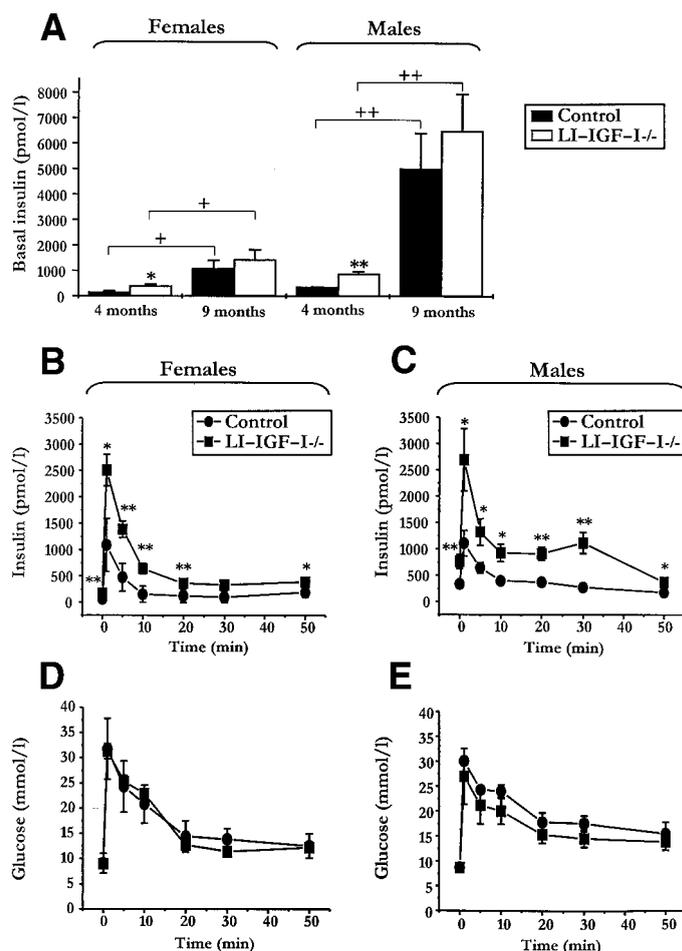


FIG. 4. Basal insulin levels were measured in anesthetized mice at 4 and 9 months of age (A). Plasma insulin in females (B) and males (C) and glucose in females (D) and males (E) was measured immediately before and 1, 5, 10, 20, 30, and 50 min after intravenous injection of glucose (1 g/kg) in 4-month-old anesthetized mice. Data are expressed as means  $\pm$  SEM. Probability level of random difference between groups: \* $P < 0.05$ ; \*\* $P < 0.01$ , LI-IGF1<sup>-/-</sup> versus control; + $P < 0.05$ ; ++ $P < 0.01$  for 9- vs. 4-month-old mice; Student's  $t$  test,  $n = 4-7$ . The glucose tolerance test was repeated twice with similar results.

complete recombination of the *IGF-I* gene in hepatocytes. Inactivation was induced at 3 weeks of age, resulting in a decrease in serum IGF-I levels by 85%. This decrease was still present when the mice were 13 months old, indicating

TABLE 1  
Parameters calculated from the seven-sample intravenous glucose test and estimated by the minimal model

Parameter	Controls	LI-IGF-I <sup>-/-</sup>
$n$	10	11
$K_G$ (% min <sup>-1</sup> )	2.11 $\pm$ 0.27	2.87 $\pm$ 0.32
AIR (nmol/l)	0.63 $\pm$ 0.11	1.67 $\pm$ 0.17*
AUC <sub>ins</sub> (nmol/l in 50 min)	7.1 $\pm$ 2.4	18.8 $\pm$ 3.1**
$S_I$ (10 <sup>-4</sup> · min <sup>-1</sup> · pmol/l <sup>-1</sup> )	1.25 $\pm$ 0.25	0.64 $\pm$ 0.15**
$S_G$ (min <sup>-1</sup> )	0.052 $\pm$ 0.013	0.063 $\pm$ 0.011
GDI	16.1 $\pm$ 0.9	13.9 $\pm$ 1.3

Data are means  $\pm$  SEM. The samples were obtained from pooled male and female mice at 4 months of age. Statistical evaluation was performed by Student's  $t$  test. Asterisks indicate the probability level of random difference between the groups (\* $P < 0.01$ , \*\* $P < 0.05$ ). AUC<sub>ins</sub>, area under the 50-min insulin curve.

TABLE 2  
Metabolic serum parameters

	Age (months)	Female		Male	
		Control	LI-IGF-I <sup>-/-</sup>	Control	LI-IGF-I <sup>-/-</sup>
Cholesterol (mmol/l)	3	—	—	2.2 ± 0.2	4.0 ± 0.2*
	4	3.7 ± 0.2	4.7 ± 0.2*	3.6 ± 0.1	5.3 ± 0.3*
	13	4.1 ± 0.7	3.9 ± 0.5	3.9 ± 0.2	3.5 ± 0.3
Triglycerides (mmol/l)	4	1.1 ± 0.1	0.6 ± 0.1†	1.2 ± 0.2	1.1 ± 0.2
	3	2.0 ± 0.1	1.7 ± 0.1	1.7 ± 0.2	1.4 ± 0.05
FFA (mEq/l)	3	2.0 ± 0.1	1.7 ± 0.1	1.7 ± 0.2	1.4 ± 0.05
Corricosterone (ng/ml)	1	94.3 ± 18.5	144.6 ± 31.3	111.7 ± 10.1	169.0 ± 19.8**
	13	224 ± 30	228 ± 16	120 ± 22	132 ± 19

Data are means ± SEM. Asterisks indicate probability level of random difference between groups by Student's *t* test, *n* = 4–7 in each group. \**P* < 0.01, \*\**P* < 0.05 for LI-IGF-I<sup>-/-</sup> versus control.

a complete and sustained inactivation of the *IGF-I* gene in hepatocytes.

In contrast to the fluctuating levels of growth hormone (GH) and insulin, circulating IGF-I levels are relatively stable. This stability is caused by the fact that most circulating IGF-Is are bound to high-affinity IGF-binding proteins (IGF-BPs), which prolong the half-life and titrate the supply of this hormone to its receptor (24). Serum levels of IGF-BP2 and 3 are decreased in LI-IGF-I<sup>-/-</sup> mice (K.S., unpublished data), and it could be argued that the decrease in serum levels of IGF-I is not within the fraction of free and functional IGF-I. This would be consistent with the fact that there was no effect on postnatal body growth in LI-IGF-I<sup>-/-</sup> mice (9,10). Because of technical reasons, we have not yet been able to measure free serum IGF-I levels. The main reason for this is that the available assays require a large amount of serum to obtain valid results on free levels of serum IGF-I. However, the present study demonstrates that the carbohydrate and lipid metabolism is disturbed, indicating that the LI-IGF-I<sup>-/-</sup> mice have a decreased fraction of functional IGF-I in serum. Furthermore, we and others (9,10) have previously demonstrated that mice with liver-specific IGF-I inactivation have a decreased negative feedback on GH secretion.

In this study, we show that the LI-IGF-I<sup>-/-</sup> mice are at least partially protected against the obesity that normally develops in older mice. A lean phenotype is also found in mice overexpressing IGF-BP1 (25). The IGF-BPs limit the bioavailability of IGF-I, and the IGF-I-BP1 transgenic mice have a phenotype consistent with partial inhibition of IGF-I action in all tissues. The LI-IGF-I<sup>-/-</sup> mice, on the other hand, have a liver-specific inactivation of IGF-I but normal IGF-I production in peripheral, including adipose tissue (9). Thus, these results indicate that adipocyte-produced IGF-I is not sufficient for normal fat mass accumulation and that liver-produced IGF-I is required. Previous results from IGF-I-BP transgenic mice showed impaired preadipocyte proliferation and differentiation, which was interpreted as a role for IGF-I in adipocyte formation (25). However, from our results, it is impossible to conclude whether IGF-I has a direct or an indirect effect on adipose tissue. An indirect action may be exerted through GH, the levels of which are elevated in LI-IGF-I<sup>-/-</sup> mice. GH is known to increase lipolysis in adipose tissue, and GH-deficient (GHD) humans and rodents have increased fat mass, a condition that can be reversed by GH treatment. Because GH levels decrease with age, associated with an age-dependent increase in fat mass, one may

speculate that the LI-IGF-I<sup>-/-</sup> mice are partly protected against accumulation of fat mass via their increased GH levels.

The reduction in fat mass in LI-IGF-I<sup>-/-</sup> mice could also be secondary to their pronounced increased serum levels of leptin, a protein known to reduce adiposity (26). One of the most important regulators of leptin synthesis in rodents is insulin (27,28). IGF-I given to GHD humans decreases leptin (29). However, the effects of IGF-I on leptin synthesis is probably indirect via modulation of insulin levels because neither GH nor IGF-I has any direct effect on leptin expression in cultured adipocytes (30,31). These findings may indicate that the increased serum levels of insulin in LI-IGF-I<sup>-/-</sup> mice cause the increase in leptin.

Young-adult LI-IGF-I<sup>-/-</sup> mice have elevated basal as well as glucose-induced insulin levels but normal glucose levels, which could be interpreted as insulin resistance. This conclusion is also supported by the results of the intravenous glucose tolerance test that showed a reduced S<sub>I</sub> in the LI-IGF-I<sup>-/-</sup> mice. In rodents, glucose disposal after intravenous glucose is dependent not only on insulin but also on an insulin-independent action of glucose (32). This is a process that is quantified by the S<sub>G</sub> (33). We show that S<sub>G</sub>, in contrast to S<sub>I</sub>, was not affected in the LI-IGF-I<sup>-/-</sup> mice, indicating that IGF-I plays a major role in the action of insulin to dispose glucose. This finding is in line with proposals that IGF-I might be a potential therapy for type I and type II diabetes because it has been shown to reduce insulin resistance (3,34) and that IGF-I transgenic mice have decreased levels of insulin (35). GH, on the other hand, has anti-insulin effects and causes insulin resistance, as seen in GH-transgenic mice (35). The insulin resistance in our model could either be a consequence of the decreased serum IGF-I or the elevated GH levels. Despite insulin resistance in LI-IGF-I<sup>-/-</sup> mice, the net glucose elimination after the glucose bolus was not different

TABLE 3  
Lipoprotein profile

	Control	LI-IGF-I <sup>-/-</sup>
VLDL (mmol/l)	0.10 ± 0.03	0.13 ± 0.03
LDL (mmol/l)	0.46 ± 0.06	0.66 ± 0.07*
HDL (mmol/l)	3.00 ± 0.08	4.23 ± 0.28†

Data are means ± SEM. Samples were obtained from pooled male and female 8-month-old mice. Asterisks indicate probability level of random difference between groups by Student's *t* test, *n* = 8 in each group. \**P* < 0.05, †*P* < 0.01 for LI-IGF-I<sup>-/-</sup> versus control.

from the controls. This is explained by the markedly elevated insulin secretory response to glucose that compensates for the reduced insulin sensitivity. Although not specifically studied, it seems most likely that both first and second phases of insulin secretion are exaggerated in LI-IGF-I<sup>-/-</sup> mice because both the 1- and 50-min insulin levels are significantly elevated. It is known that insulin sensitivity and insulin secretion are reciprocally related in a hyperbolic manner (36). This means that when insulin resistance evolves, an adaptive increase in insulin secretion develops to compensate. Our results show that this adaptation is adequate in the LI-IGF-I<sup>-/-</sup> mouse, which is evident not only by the normal glucose elimination ( $K_G$ ), but also by the normal disposition index (GDI) (36). Hence, our study demonstrates a normal islet function adequately responding to the peripheral resistance to insulin in LI-IGF-I<sup>-/-</sup> mice.

Our results indicate that part of the phenotype in the young LI-IGF-I<sup>-/-</sup> mice, including increased serum levels of insulin, cholesterol, corticosterone, and leptin, is reduced or lost in the older mice. LI-IGF-I<sup>-/-</sup> mice had significantly increased basal insulin levels compared with controls at 4 but not at 9 months of age. The reason for this might be the generally increased levels of basal insulin in 9-month-old mice compared with 4-month-old mice. This age-dependent increase in basal insulin levels has been well documented previously in mice and is probably caused by the age-dependent increase in fat mass resulting in insulin resistance (23,37,38).

Serum levels of cholesterol were increased in young LI-IGF-I<sup>-/-</sup> mice. In concordance with this finding, clinical studies have demonstrated that IGF-I treatment decreases cholesterol levels in serum (3,34). GH levels are increased in mice with liver-specific IGF-I inactivation, whereas they are decreased in subjects given IGF-I treatment. Thus, one may speculate that the mechanism may either be a direct effect of the altered serum IGF-I or an indirect effect via regulation of GH. A possible direct non-GH-dependent effect of IGF-I on serum cholesterol has been studied in patients with GH insensitivity syndrome (Laron Dwarfism). However, contradictory results have been presented, including both unchanged and decreased serum cholesterol after IGF-I treatment in these patients (39–41). Several observations indicate that GH is of importance for the regulation of plasma lipoprotein metabolism. GH deficiency is associated with increased cholesterol, which is normalized by GH substitution (42–45). In contrast, GH treatment increases cholesterol in young rats with normal GH secretion (46). The latter result is in concordance with the increased cholesterol and GH levels found in LI-IGF-I<sup>-/-</sup> mice. The molecular mechanism of action for the increased cholesterol in LI-IGF-I<sup>-/-</sup> mice remains to be determined. No effect on cholesterol was seen in the older LI-IGF-I<sup>-/-</sup> mice. There might be several possible explanations for this observation—a study comparing young and old rats has shown age-dependent effects of GH administration on serum cholesterol and lipoprotein metabolism. Whereas GH increased cholesterol in young rats, the opposite effect was observed in older rats (46). One may also speculate that the absence of effect in the older mice is caused by the general decrease in GH secretion that occurs with age (47).

Serum levels of corticosterone are elevated in young LI-IGF-I<sup>-/-</sup> mice. An increase in corticosterone is also found in GH-transgenic mice with elevated levels of IGF-I (48). These observations suggest that the increased levels of corticosterone in LI-IGF-I<sup>-/-</sup> mice are caused by the elevated serum levels of GH and not by the decreased serum levels of IGF-I (9,10). No effect on corticosterone was seen in the older LI-IGF-I<sup>-/-</sup> mice. Similarly, as discussed for cholesterol, one may speculate that the absence of effect in the older mice is caused by the decrease in GH secretion that occurs with age. The GH secretion in the older LI-IGF-I<sup>-/-</sup> and control mice remains to be determined.

In summary, inactivation of IGF-I in hepatocytes results in increased serum levels of insulin, leptin, and cholesterol, resulting in a state of insulin resistance and eventually a decrease in fat mass. We conclude that liver-derived IGF-I is not required for postnatal body growth but is of importance for normal carbohydrate and lipid metabolism.

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