Age-dependent onset of liver-specific IGF-I gene deficiency and its persistence in old age: implications for postnatal growth and insulin resistance in LID mice

Zhengyi Tang,1,2 Rong Yu,1 Yarong Lu,1 A. F. Parlow,2 and Jun-Li Liu1,2

1Fraser Laboratories for Diabetes Research, Department of Medicine, McGill University Health Centre, Montreal, Quebec, Canada; 2Endocrine and Metabolic Division, E-Institutes of Shanghai Universities, Shanghai Clinical Center for Endocrine and Metabolic Diseases, Shanghai, China; and 3National Hormone and Peptide Program, Harbor-University of California Los Angeles Medical Center, Torrance, California

Submitted 19 October 2004; accepted in final form 10 March 2005

Tang, Zhengyi, Rong Yu, Yarong Lu, A. F. Parlow, and Jun-Li Liu. Age-dependent onset of liver-specific IGF-I gene deficiency and its persistence in old age: implications for postnatal growth and insulin resistance in LID mice. Am J Physiol Endocrinol Metab 289: E288–E295, 2005. First published March 15, 2005; doi:10.1152/ajpendo.00494.2004.—To explore the limitations of the liver-specific IGF-I gene deficiency and its persistence in old age, we have studied these mice during the prepubertal period (from birth to 3 wk of age) and when they are 2 yr old. During the first 2 wk of life, IGF-I gene deficiency and the resulting reduction in serum IGF-I levels in LID mice did not reach sufficiently low levels when mice experience the most rapid and growth hormone (GH)-independent growth. It suggests that the role of liver-derived IGF-I in prepubertal, GH-independent postnatal growth cannot be established. From our previous studies, liver IGF-I mRNA level was abolished in adult LID mice, which causes elevated GH level, insulin resistance, pancreatic islet enlargement, and hyperinsulinemia. Interestingly in 2-yr-old LID mice, although liver IGF-I mRNA and serum IGF-I levels were still suppressed, serum insulin and GH levels had returned to normal. Compared with same-sex control littersmates, aged male LID mice had significantly reduced body weight and fat mass and exhibited normal insulin sensitivity. On the other hand, aged female LID mice exhibited normal weight and marginal resistance to insulin actions. The pancreatic islet percentage (reflecting islet cell mass) was also restored to normal levels in aged LID mice. Thus, although the IGF-I gene deficiency is well maintained into old age, the insulin sensitivity, islet enlargement, and hyperinsulinemia that occurred in young adult mice have been mostly restored to normal levels, further supporting the age-dependent and sexual dimorphic features of the LID mice.

Cre recombinase; sexual dimorphism; pancreatic islets; insulin-like growth factor I; liver-specific insulin-like growth factor I gene deficiency

IGF-I Belongs to a Family of polypeptides that have evolved from a common ancestor into IGF-I, IGF-II, and proinsulin (13, 20, 25). At the target cell membrane, the IGFs bind a distinct receptor (IGF-I receptor) with much higher affinity than insulin, whereas the latter has similar selectivity for its cognate receptor. IGF-I is expressed in many cells and tissues during embryonic and postnatal development and in adult animals and promotes postnatal growth in bone, muscle, fat, and many other tissues. Specific gene targeting and other experiments have demonstrated that IGF-I is essential for normal cell proliferation and differentiation, intrauterine development, and postnatal growth (13, 20, 25). The primary source of circulating IGF-I is the liver, although it is also synthesized by extrahepatic tissues (13, 38). Using Cre/loxP-mediated, liver-specific IGF-I gene-deficient (LID) mice, we have previously suggested that the liver-derived IGF-I is not required for normal growth but is indispensable for maintaining normal carbohydrate homeostasis (37, 38, 40). Thus adult LID mice exhibited significant hyperinsulinemia because of pancreatic islet hyperplasia, growth hormone (GH) hypersecretion, and insulin resistance in skeletal muscle and liver despite normal blood glucose concentration.

Animal growth is age-dependent, with the fastest acceleration in weight and length occurring in the early postnatal days and the peripubertal period. Previous studies demonstrating normal postnatal growth in LID mice had not carefully examined changes in liver IGF-I production earlier than 6 wk of age (30, 38). To reevaluate the role of liver IGF-I in normal growth and development, we thought to correlate Cre-mediated IGF-I gene deficiency with the normal growth curve. We have measured liver IGF-I mRNA and serum IGF-I levels in young mice aged 3 to 30 days after birth and correlated those changes with the growth rate. Our results revealed an insufficient liver IGF-I knockout within the first 2 wk of life, a crucial phase for mouse growth; this suggested that the LID mice model is inadequate in addressing the role of liver IGF-I in early (GH-independent) postnatal growth. It is well known that the development of insulin resistance, a state of reduced responsiveness to normal levels of insulin, is also age-dependent and often develops after middle age (27). It causes hyperinsulinemia before the onset of full-blown diabetes. In this study, we further extended our investigation of LID mice into old age to determine whether Cre-induced gene deletion was maintained and, if so, whether the phenotype of insulin resistance and islet enlargement was affected.

MATERIAL AND METHODS

Animal procedures. LID mice on a mixed C57BL/6, FVB/N, and 129Sv background were generated using the Cre/loxP system, as previously reported (38). Mice homozygous for IGF-I/loxP carrying the albumin-Cre transgene (L/L) were crossed with L/L mice aged 3 to 30 days after birth and correlated those changes with the growth rate. Our results revealed an insufficient liver IGF-I knockout within the first 2 wk of life, a crucial phase for mouse growth; this suggested that the LID mice model is inadequate in addressing the role of liver IGF-I in early (GH-independent) postnatal growth. It is well known that the development of insulin resistance, a state of reduced responsiveness to normal levels of insulin, is also age-dependent and often develops after middle age (27). It causes hyperinsulinemia before the onset of full-blown diabetes. In this study, we further extended our investigation of LID mice into old age to determine whether Cre-induced gene deletion was maintained and, if so, whether the phenotype of insulin resistance and islet enlargement was affected.

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
albumin-Cre transgene), and their offspring were used in experiments. To determine the presence of the Cre transgene (i.e., liver-specific IGFI gene knockout), genomic DNA was isolated from tail clips using standard methods, and primers Cre-5’ and Cre-3’ were used in PCR reactions, which yielded a 0.6-kb band for the Cre transgene, as previously reported (18). To detect gene recombination, primers ES-1 and ID-3 were used; these generate a 0.2-kb band after Cre-induced IGFI gene deletion (18). The animals were kept in 12:12-h dark-light cycles at room temperature with free access to food and water. All animal-handling procedures have been approved by the McGill University Animal Care Committee.

At the desired age [from postnatal day (P) 2, to P60], the mice were killed by decapitation; blood was collected for serum preparation; and liver, fat, and pancreatic tissues were removed immediately for biochemical or histological analysis. To correlate the levels of IGFI gene expression and gene deletion to postnatal growth rate, young pups were identified by toe clips at 7 days after birth. Their body weight was measured daily for the next 6 wk.

To study changes in old age, LID mice and their control littersmates of both sexes were kept until they reached 2 yr of age. An insulin tolerance test was performed in all mice 3 wk before death. Animals were injected with human insulin (0.75 IU/kg ip; Roche), and blood glucose levels were measured at 0, 20, 40, and 60 min after the injection. After death of the mice, we collected their serum, pancreas (for islet histology and total RNA isolation), and liver (for total RNA and genomic DNA) and measured the body weight and wet weight of retroperitoneal fat.

Quantification of mRNA levels. Total RNA was isolated from fresh tissues by acid guanidinium isothiocyanate-phenol-chloroform extraction (4). RNA concentration was determined by spectrophotometry at 260/280 nm. For Northern blot analysis, 5–30 μg of total RNA were electrophoresed on 1.5% agarose formaldehyde gels and transferred to Nytran membranes (Schleicher & Schuell, Keene, NH), and the RNA blots were hybridized for 18 h at 60°C with 50% formamide. 32P-labeled antisense RNA probes were transcribed from a mouse insulin I cDNA (Rsal-EcoRI, 478–725 bp; see Ref. 34), a rat prepro-IGF-I cDNA (a 0.5-kb PvuII/Aval fragment; see Ref. 22), and pTRI-β-actin-mouse (Ambion, Austin, TX). The blots were exposed to X-ray film for 1–2 days. For RNA protection assay, 5–20 μg of total RNA were hybridized to 32P-labeled antisense RNA probes transcribed from pMI-4 (encoding exon 4 of mouse IGFI gene; see Ref. 18), pTRI-β-actin-mouse, and pTRI-cyclophilin-mouse (Ambion). The reaction mixture was treated with RNase A, RNase T1, proteinase K, and phenol-chloroform and precipitated. Protected probes were denatured, electrophoresed on a 6–8% polyacrylamide gel, and exposed to X-ray film (36). The intensity of the hybridization signals on the autoradiogram was analyzed using a FluorChem 8900 imaging system (Alpha Innotech) and OptiQuant image analysis software (Packard BioScience).

Pancreatic histochemistry. The pancreata from 2-yr-old control and LID mice, four each from both males and females, were removed, fixed, and embedded in paraffin, after which 5-μm sections were cut. They were stained immunohistochemically for insulin and glucagon (rabbit polyclonal antibody) using the avidin-biotin-peroxidase complex technique, which resulted in a red immunoreactive signal with a nuclear counterstain using methyl green (11, 21). Images of all pancreatic islets were captured with a Retiga 1300 digital camera (Q imaging; Burnaby, BC, Canada) at magnifications of ×25, ×100, or ×40. The whole pancreatic sections were digitally recorded by multiple ×25 microscopic fields. The areas of the whole pancreatic tissue and of the insulin-stained islets were measured separately using Northern Eclipse computer software, version 6.0 (Empix imaging, Mississauga, ON, Canada). Islet percentage is derived from total islet area divided by total pancreatic tissue area from each animal. To reflect individual islet cell growth, average cell size was calculated in hematoxylin- and eosin-stained ×400 images using total islet area divided by the number of cell nuclei. For this purpose, a minimum of 10 mature islets was chosen from each group of control and LID mice, both male and female.

Blood chemistry. Serum concentrations of insulin (Linco Research, St. Charles, MO) and total IGF-I (using acid-ethanol extraction; rat IGFI DSL-2900; Diagnostic Systems Laboratories, Webster, TX) were determined using RIA kits. Serum GH was measured using RIA at the National Hormone and Peptide Program, Harbor-University of California Los Angeles Medical Center (Torrance, CA). Blood glucose levels were measured using the OneTouch blood glucose meter (LifeScan Canada, Burnaby, BC).

Statistical analysis. Data are presented as means ± SE. Student’s t-test and one-way ANOVA were performed using the software InStat version 3 (GraphPad Software, San Diego, CA).

RESULTS

Age-dependent onset of liver-specific IGFI gene inactivation. The albumin promoter is specifically expressed in hepatocytes at low levels in fetal development and increased gradually until maximum levels were reached at 1–2 wk postnatally (28, 33, 38). The age-dependent expression pattern of the albumin-Cre transgene, and the onset of Cre-mediated liver-specific IGFI gene recombination have not been well characterized. We determined liver IGFI mRNA levels in LID mice and their control littersmates from age P2 to P60 using RNAse protection assay. The mRNA levels were corrected by either the levels of β-actin or cyclophilin mRNAs. As shown in Fig. 1A, up to P11, the level of IGFI gene expression in LID mice, although significantly reduced, was maintained at >72% of the control level (P < 0.01 vs. controls). Thereafter, it was decreased sharply to 36% of the control level at P16 and 30% at P23. Liver expression of the IGFI gene in LID mice further decreased to 13% at P30 and 1% at P60, representing an almost complete gene inactivation by adult age. Thus a significant albumin-Cre-mediated IGFI gene recombination was initiated at around P17 and reached a major decrease in IGFI mRNA level between P16 and P23 before reaching near completion at P30 and after.

Because liver expression of the IGFI gene was diminished with age in LID mice, there was a concurrent decrease in the relative level of circulating IGFI (Fig. 1B). In control littersmates, there was a steady increase in serum IGFI-I levels from P16 to P50 in parallel to increasing levels of liver IGFI gene expression (Fig. 1C). In comparison, LID mice exhibited an age-dependent decrease of relative serum IGFI-I levels from 78, 63, 44, to 40% (P < 0.001 vs. control littersmates; Fig. 1B). As reported previously, by 2 mo of age, serum IGFI-I level decreases to ~25% of control level (37). Thus a major reduction in serum IGFI-I was only achieved by around P30.

Correlation to early postnatal growth. Interestingly, the ontogeny of liver-specific IGFI-I gene deletion occurred as liver expression of IGFI-I gene exhibiting an age-dependent, steady elevation and expanded two phases of postnatal growth in mice (Fig. 1, C–E; see Ref. 16). In phase 1 (from birth to 3 wk, when pups were weaned), there was a peak in the body weight gain (>17%/day; Fig. 1E) that was accompanied by a minor peak in liver IGFI-I gene expression (on P11) in control mice (Fig. 1C). This phase enabled 46% of postnatal growth, judging by adult body weight (Fig. 1D), and yet LID mice were mostly unaffected in their liver IGFI-I gene expression and particularly in circulating IGFI-I levels (Fig. 1, A and B). After a pause of several days (P14–P20; before weaning), phase 2 of postnatal
Fig. 1. Age-dependent hepatic IGF-I gene expression/inactivation in control and liver-specific IGF-I gene-deficient (LID) mice. Liver IGF-I mRNA levels were determined by RNase protection assay and corrected with that of β-actin and cyclophilin by densitometry. A: onset of liver-specific IGF-I gene deficiency caused by albumin-Cre expression. Liver IGF-I mRNA levels are expressed as percentage of that of control littermates, after correction with β-actin mRNA levels; n = 5–7 mice. **P < 0.01 and ***P < 0.001 vs. control littermates. B: age-dependent deficiency in circulating IGF-I. LID mice of both sexes and their control littermates were killed at postnatal days (P) 16, 23, 30, and 50. Total IGF-I concentrations were determined by RIA; n = 8–13. ***P < 0.001 vs. control littermates. C: age-dependent IGF-I mRNA expression in the liver of control mice. mRNA levels are expressed in relative units as per β-actin or cyclophilin mRNAs. Vertical bars represent SE (n > 6). D: postnatal growth curve of male wild-type mice. Body weight was measured every day in mice from 1 to 7 wk old. The mean weight, from 4 animals, was plotted against the age in days. E: change in growth rate from 1 to 7 wk. The rate of weight gain (%body weight gain vs. the previous day) was plotted against age. The dashed line indicates zero growth.
growth was activated at around P21, with a peak growth rate of
8% weight gain/day around P26 (Fig. 1E). During this phase,
derived IGF-I production was virtually abolished, and
serum IGF-I level was decreased by 60–75% in LID mice (Fig.
1, A and B) without affecting growth rate (38). The age-
dependent onset of IGF-I gene inactivation in LID mice pro-
vides new evidence to the debate on the role of liver-derived
IGF-I on postnatal growth.

Sustained liver-specific inactivation of the IGF-I gene in
aged LID mice. In young adult LID mice, ~95% of hepatocytes exhibit Cre-induced IGF-I gene recombination (38). In
aged mice, this ratio might change because of constant regen-
eration of “normal (IGF-I intact)” hepatocytes or declined albumin promoter activity. To study possible changes in the
extent of liver gene deficiency, insulin resistance, and islet
growth compensation, we further studied LID mice that were
maintained for two years after birth.

The genotype of aged mice was reconfirmed by a double
PCR strategy, i.e., primers Cre-5’ and Cre-3’ to detect Cre
transgene (0.6-kb band) and primers ES-1 and ID-3 to detect
gene deletion product (0.2-kb band), as previously reported
and shown in Fig. 2A (18). Having reconfirmed their genotype, we
have identified a total of 16 control (9 male, 7 female) and 13
LID (7 male, 6 female) mice. Not included were two female
LID mice that had developed gross hepatic tumors revealed
during postmortem dissection.

To confirm liver-specific IGF-I gene deficiency, we hybrid-
ized liver RNA to a full-length rat IGF-I cDNA in Northern
blots. As shown in Fig. 2B, intact IGF-I mRNA in control mice
exhibits a major form of 0.7 kb. In LID mice, the mRNA
abundance was unaffected, except for a size shift to ~0.5 kb,
reflecting deletion of the 182-bp exon 4. The intact IGF-I
mRNA (0.7 kb) was undetectable in LID mice, indicating
complete gene deletion. Thus the mRNA detected is the result
of transcription from the residue IGF-I gene after Cre-mediated
deletion of exon 4 in hepatocytes of the LID mice. The IGF-I
gene deletion was also reconfirmed using RNase protection (Fig.
2C). Serum IGF-I level was significantly reduced in LID
mice to 30–40% of that of their control littermates of same sex
(Fig. 2D), levels similar to that obtained from young mice of
P50 (Fig. 1B) but higher than the 25% reported in young adults
using a different assay (38).

Sexual dimorphic insulin resistance in aged LID mice. As
the animals age, some of the 2-yr-old control mice accumulate
significant body fat compared with young adults. As shown in
Fig. 3A, male LID mice are significantly leaner than control, a
phenomenon not seen in female mice, thus partially consistent
with an early report (of lean phenotype in both male and female
mice of 13 mo old) using a similar model of liver IGF-I gene
deficiency (31). To investigate whether this reflects changes in
fat mass, we removed retroperitoneal fat from all animals and
measured the wet weight. As shown in Fig. 3B, male LID mice
indeed exhibited significantly reduced fat weight, which at
least partially explains their lean phenotype. On the other hand,
female LID mice were normal in both fat and body weights.

As we have reported previously, young adult LID mice are
significantly resistant to insulin action on skeletal muscles,
primarily because of elevated GH levels (37, 40). In 2-yr-old
LID mice, the change in insulin resistance becomes sexually
dimorphic. Aged male LID mice had normal insulin sensitivity,
determined by insulin tolerance test in Fig. 4A, since both
control and LID mice responded to insulin injection by signif-
cant reductions in blood glucose level. On the contrary, aged
female LID mice still exhibited significant insulin resistance
(Fig. 4B), with a mere 20% reduction in blood glucose level vs.
a 50% reduction in control mice.
Also different from young adult mice that exhibited fourfold increased serum insulin levels, aged LID mice were no longer hyperinsulinemic (37). The slight elevation, particularly in male LID mice was not statistically significant (Table 1). Consistent with unchanged serum insulin level, there was no change in pancreatic insulin mRNA levels between 2-yr-old control and LID mice (data not shown). Finally, the serum GH levels were no longer elevated in either male or female LID mice compared with their control littermates of same sex. Thus, compared with their control littermates, male LID mice were lean and insulin sensitive; female LID mice had normal weight but largely maintained the phenotype of insulin resistance; both groups, however, exhibited normal insulin and GH levels.

**Restored pancreatic islet percentage in aged LID mice.** As we have reported previously, in addition to insulin resistance and hyperinsulinemia, young adult LID mice exhibit significantly enlarged pancreatic islets (37, 40). To verify whether the islet phenotype is maintained in the face of altered insulin sensitivity, we studied pancreatic histology in aged LID mice. The islet percentage was calculated from insulin-stained sections as reported (Table 1 and Ref. 17). As expected from the lack of insulin resistance, there was no longer an increase in islet percentage (reflecting islet cell mass). The average islet sizes were also normal (data not shown). To further exclude islet cell hypertrophy, we measured the average islet cell size in hematoxylin- and eosin-stained sections and found no evidence of cellular hypertrophy in either male or female LID mice (data not shown). Thus, unlike their younger counterparts, aged LID mice seemed to have restored their islet cell mass to normal.

**Discussion**

Using Cre/loxP-mediated liver-specific gene targeting, we have previously demonstrated that liver-derived IGF-I, although accounting for 75% of total serum level, is not required for normal growth and development in mice (30, 38). It was a

---

**Fig. 3.** Sexual dimorphic, male-specific lean phenotype in 2-yr-old LID mice. A: changes in total body weight of LID mice vs. same-sex, age-matched control mice. B: changes in retroperitoneal fat weight [in % body weight (BW)] in aged LID mice. Values are expressed as means ± SE. Nos. of animals are 9 male control, 7 male LID, 7 female control, and 6 female LID. *P < 0.05 and **P < 0.01 vs. same-sex control mice.

**Fig. 4.** Sexual dimorphic, female-specific insulin resistance in 2-yr-old LID mice. Insulin tolerance test was performed by injecting insulin (0.75 IU/kg ip) to random-fed mice and measuring their blood glucose level at 0, 20, 40, and 60 min. Values (in % of 0 min) are expressed as means ± SE. A: male mice. B: female mice. Two sets of statistics were performed. ANOVA was used to determine whether the changes in blood glucose levels within a single group at different time points are significant, i.e., whether the animals responded to insulin injection. P values are indicated besides the curves that reached statistical significance. Student’s t-tests were performed for each time point to determine whether the decrease in glucose level was significant from the time 0 value of the same group. *P < 0.05, **P < 0.01, and ***P < 0.001 vs. time 0. There was no point-to-point test among different groups of mice.
Table 1. Changes in serum insulin and GH levels and pancreatic islet percentage in 2-yr-old LID mice

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>LID</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum insulin level, ng/ml</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>3.1±0.6 (9)</td>
<td>4.5±0.4 (7)</td>
<td>0.08</td>
</tr>
<tr>
<td>Female</td>
<td>1.7±0.3 (7)</td>
<td>2.3±0.6 (6)</td>
<td>0.37</td>
</tr>
<tr>
<td>Serum GH level, ng/ml</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>8.4±3.2 (9)</td>
<td>8.9±3.0 (7)</td>
<td>0.92</td>
</tr>
<tr>
<td>Female</td>
<td>3.0±0.8 (7)</td>
<td>5.2±1.6 (6)</td>
<td>0.22</td>
</tr>
<tr>
<td>Pancreatic islet, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>0.65</td>
<td>0.64</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>0.33</td>
<td>0.32</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE; no. of mice in parentheses. GH, growth hormone; LID, liver-specific IGF-I gene deficient. Islet histochemistry using insulin antibody and hematoxylin-eosin staining was performed in four mice from each genotype/sex group and analyzed using Northern Eclipse software. Islet percentage was derived from analyzing all 16 mice, which results in 200 fields of insulin-stained, ×100 images.

e challenge to the classic somatomedin hypothesis and raised a question on the physiological relevance of liver-derived endocrine IGF-I. Subsequent studies have demonstrated that liver-derived IGF-I is crucial for regulating GH secretion at the pituitary level (30, 35, 37). Furthermore, lack of endocrine IGF-I caused insulin resistance in skeletal muscles, pancreatic islet hyperplasia and hyperinsulinemia, and decreased fat mass resulting from elevated GH levels (31, 37). It seemed that liver-derived IGF-I regulates GH secretion and fuel metabolism. In this study, we have reexamined the LID model in an age-dependent manner and revealed an incomplete IGF-I deficiency (in mRNA and serum levels) in the first 3 wk after birth, which prompts a reevaluation on the role of liver-derived IGF-I in early postnatal growth. In addition, we have demonstrated sustained liver-specific IGF-I gene deficiency in 2-yr-old LID mice. Compared with their younger counterparts, these aged mice exhibited diminished and sexual dimorphic insulin resistance and restored pancreatic islet cell mass.

Mouse postnatal growth can be clearly divided into two phases, before and after weaning (at P21). From birth up to ~3 wk of age, there is a very rapid acceleration in body weight gain, judged either by daily weight measurements or percentage body weight gains per day, which accounts for ~46% of total weight gain. The growth in the first phase is mostly unaffected by GH receptor gene deficiency and cannot be further stimulated by exogenous GH administration, clearly indicating a GH-independent mechanism (16, 41). In the meantime, liver expression of IGF-I gene is activated and rises to adult levels (Fig. 1C; see Ref. 29). The somatic growth in this phase is affected by a global IGF-I gene deficiency in that the surviving IGF-1−/− dwarfs weighed only ~40% at 3 wk after being born with 65% of the wild type weight, i.e., a worsened growth retardation from 35% at birth to 60% by 3 wk of age (16). Clearly, IGF-I (a major portion of it produced from the liver) is involved in promoting growth in the first phase. Because of delayed activation of the Cre transgene or a possible need of accumulative effect of gene recombination, LID mice exhibited insufficient deletion to liver IGF-I gene up to P11 (when control mice have a first peak in liver IGF-I mRNA level; Fig. 1, A and C). The gene deficiency only became sufficient after P16, before reaching a near complete inactivation on P30 (Fig. 1B). Serum IGF-I levels reflected a similar although further delayed response, achieving a 60% reduction only by P30. Correlation of these curves indicates that the normal somatic growth in LID mice during the first postnatal phase might have been maintained by the presence of a sufficient amount of liver-produced IGF-I. In other words, liver-produced IGF-I could still be required for early postnatal growth, but this cannot be validated in LID mice and has to be reexamined in a model with an earlier-onset, highly efficient Cre expression.

Our observations are consistent with the findings in pancreatic endoplasmic reticulum eukaryotic initiation factor-2α kinase (PERK) gene-deficient mice, which exhibited neonatal growth retardation accompanied by a 75% reduction in liver production of IGF-I. Injection of IGF-I (but not GH) partially reversed the growth defect (14). It was proposed that PERK is required to regulate the expression of IGF-I in the liver during the neonatal period, when IGF-I expression is GH independent, and that the lack of this regulation results in severe neonatal growth retardation. Of course, one has to bear in mind that, during this first phase, young pups get most nutrients and perhaps some IGF-I from their mothers’ milk. The nutrient intake is not only essential for their rapid growth but also important for maintaining a normal IGF-I level. Moreover, milk itself contains a high level of IGF-I, and milk proteins protect IGF-I from rapid degradation in the intestine (2, 3, 24). Thus we cannot exclude completely the possibility that milk consumption partially compensates the growth rate of LID mice in the first phase.

As shown in Fig. 1, D and E, the second phase of postnatal growth was activated around P21 after a pause of several days. It represents a longer and slower buildup and contributes to more than one-half of the overall growth. Contrary to the first phase, the growth in this phase is mostly abolished by GH receptor gene deficiency and can be further enhanced by GH administration in wild-type mice (16, 41). According to this study, the liver expression of IGF-I gene in LID mice was effectively abolished early during this phase. Serum IGF-I levels were decreased to only 40–25% of the control level (37). The fact that they were completely normal in postnatal growth strongly supports the notion that liver-derived IGF-I is not required in this phase of GH-dependent postnatal growth (12, 38). Of course, one has to note that LID mice are able to maintain a normal level of free IGF-I in circulation through reduced IGF-binding protein levels and therefore this model alone cannot completely disqualify IGF-I as an endocrine somatomedin (37).

Most studies using Cre-mediated conditional gene targeting have so far used young adult mice. It is unclear whether the level of Cre expression and the efficiency of the recombination would decline as animals become old. This is a particular concern for hepatocytes because they undergo constant regeneration during which IGF-I-deficient cells might have a disadvantage in replication compared with normal hepatocytes that maintain intact IGF-I gene. Slowly, normal cells might take over and become a dominant population (23). In a recent report of ffrs2: C2 mice, target gene recombination was found to be age dependent. Islet β-cell-specific insulin receptor substrate-2 gene deletion occurred in mice 6 mo old or younger, but Cre expression quickly diminished and gene deletion was no longer detectable in 10-mo-old mice (15). The authors have proposed that gene-deleted β-cells have been taken over by cells that
never express Cre, through replication. In this study, the results of the PCR reactions using genomic DNA prepared from the liver, Northern blots, and RNase protection of liver RNA, as well as serum IGF-I determination, reassured the usefulness of LID mice in old age. Northern blot analysis of liver IGF-I mRNA from aged LID and control mice confirmed Cre-induced exon 4 recombination and, for the first time, demonstrated the presence and normal-level expression of an alternate spliced transcript in the liver of LID mice. The 70-amino-acid full-length IGF-I peptide consists of domains B (residues 1–29), C (residues 30–41), A (residues 42–62), and D (residues 63–70; see Refs. 7 and 8). As previously reported, we have targeted the entire exon 4 (182 bp) of the mouse gene that encodes for residues 26–70 of the peptide, including all three known residues (Tyr24, Tyr31, and Tyr60) that are critical for receptor binding (1, 10). Moreover, deletion of the entire exon would cause a frame shift in the remaining message should a peptide be translated. Thus we are confident of the gene deletion in this model. The steady expression of the void mRNA is interesting, since it suggests that lack of normal IGF-I production from the liver does not upregulate its promoter activity and the level of endogenous IGF-I mRNA synthesis, unlike many other negative feedback systems that exist in endocrine systems. It might be related to a normalized GH level in the aged LID mice.

Although liver-specific IGF-I gene deficiency is maintained, aged LID mice are quite different from their young counterparts in terms of insulin sensitivity, pancreatic islet hyperplasia, and hyperinsulinemia. Thus they exhibit normal islet cell mass and serum insulin levels. Except for some sexual dimorphic changes (see below), they are largely insulin sensitive. Insulin resistance in LID mice is caused by elevated GH release as a consequence of decreased liver IGF-I production. Elevated GH, together with insulin resistance, would cause pancreatic islet hyperplasia and hyperinsulinemia (37, 39, 40). For some reason, the serum GH level has been normalized, especially in male mice, in the course of growing older, which might explain normalized insulin sensitivity, islet growth, and insulinemia. As for female LID mice, there was still a tendency of GH elevation, which was insignificant because of large variations and limited samples (Table 1).

In previous reports, LID mice exhibited altered sexual dimorphism perhaps because of lack of serum IGF-I, elevated GH secretion, and enhanced liver growth. Male LID mice were resistant to exogenous GH in postnatal growth, whereas females had a normal response (19). The males also showed feminized liver gene expression patterns, i.e., decreased major urinary protein and increased prolactin receptor gene expression, whereas female LID mice had no change (35). Along those lines, in this study, we further demonstrate that, in old age, male LID mice become lean and have normalized insulin sensitivity while the females have normal weights and are still slightly resistant to insulin action. It further confirms both sexual dimorphic and age-dependent features of the LID mice. The sexual dimorphic patterns of GH release play a major role in sexual dimorphic responses in human and rodents. In conscious adult male rats (assuming mice as well), the GH secretion pattern is highly organized into a series of episodic bursts. These occur at intervals of 3–3.5 h, producing very high peaks of serum GH levels interspersed with trough levels that are undetectable (32). The pattern in females was found to be more complex, with a high continuous irregular pattern observed during the day and a much more rapid pulsatile pattern during darkness (5, 6, 9). In male LID mice, feminized GH secretory pattern (although difficult to measure) is expect to have caused the male-specific GH resistance and feminized liver gene expression (19, 35). Similarly, the body fat reduction and normalization in insulin responsiveness in male LID mice of old age might be related to restoration of the male GH pattern, a phenomenon that has yet to be confirmed. Because of the nature of the GH-releasing pattern, random measurements of static serum GH level may not be sufficient. Changes in fat deposition are intriguing, since IGF-binding protein 1 transgenic and 13-mo-old liver IGF-I-deficient mice (a different model) were both lean (26, 31). In future studies, when more aged mice become available, these changes need to be reconfirmed.

In summary, results from this study revealed an incomplete IGF-I gene inactivation in LID mice in the first 3 wk after birth, thus indicating that this model is not suitable to evaluate the role of liver-derived IGF-I in early (GH-independent, first-phase) postnatal growth. A better model using an early onset, highly efficient promoter to drive Cre expression in hepatocytes would be required. From 3 wk on, a clear deficiency in liver-produced IGF-I has been documented. The fact of normal growth suggests that liver-derived IGF-I is not required for GH-stimulated postnatal growth in the 2nd phase. LID mice maintained complete deficiency in liver IGF-I production well into old age, when significant sexual dimorphism was revealed in body fat deposition and insulin sensitivity. Thus male LID mice in old age become lean and have restored insulin sensitivity, whereas females have normal body weight and maintained insulin resistance. Concurrently, these 2-yr-old LID mice no longer exhibit elevated GH and insulin levels. This experiment provides evidence that, as animals age, lack of liver-produced IGF-I causes sexual dimorphic changes in insulin sensitivity and body fat deposition.

ACKNOWLEDGMENTS

We acknowledge contributions made by D. L. Chong and C. Santamaria of McGill University. Drs. Derek LeRoith and Shoshana Yakar of the National Institutes of Health provided the LID mice and helpful discussion. Dr. Shimon Efrat of Tel Aviv University, Israel, provided mouse insulin 1 cDNA probe.

GRANTS

This work was supported by a Career Development Award (2-2000-507) from the Juvenile Diabetes Research Foundation International (New York, NY), an operating grant (MOP-53206) from the Canadian Institutes of Health Research, and the Shanghai Education Commission (China). R. Yu was partially supported by the China Scholarship Council.

REFERENCES

   The roles of tyrosines 24, 31, and 60 in the high affinity binding of
   insulin-like growth factor-I to the type 1 insulin-like growth factor recep-
2. Burrin DG. Is milk-borne insulin-like growth factor-I essential for neo-
3. Burrin DG, Davis TA, Fiorotto ML, and Reeds PJ. Role of milk-borne
   vs endogenous insulin-like growth factor I in neonatal growth. J Anin Sci
   acid guanidium thiocyanate-phenol-chloroform extraction. Anal Bio-