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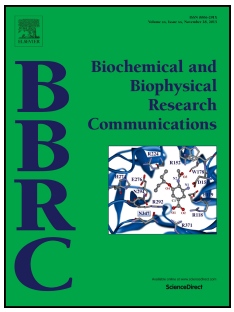

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IGF-1 Receptor Haploinsufficiency Leads to Age-dependent Development of Metabolic Syndrome

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Abstract

Individuals born small for gestational age (SGA) are at a higher risk of developing the metabolic syndrome later in life. IGF-1 resistance has been reported in placentae from SGA births and mutations in the *Igf1* receptor gene have been reported in several cohorts of SGA subjects. We have used the *Igf1r* heterozygous (*Igf1r*$^{+/-}$) male mouse as a model to investigate the mechanisms by which *Igf1r* haploinsufficiency leads to insulin resistance. Despite exhibiting IGF-1 resistance, insulin signaling is enhanced in young *Igf1r*$^{+/-}$ mice but is attenuated in the muscle of old *Igf1r*$^{+/-}$ mice. Although smaller than WT (wild type) mice, old-aged *Igf1r*$^{+/-}$ had increased adiposity and exhibit increased lipogenesis. We hypothesize that IGF-1 resistance initially causes a transient increase in insulin signaling thereby promoting a lipogenic phenotype, which subsequently leads to insulin resistance.

Keywords: IGF-1, IGF-1R, *Igf1r*$^{+/-}$, haploinsufficiency, SGA, insulin, Type 2 diabetes, metabolic syndrome, lipogenesis

1. Introduction

Metabolic homeostasis is largely controlled by insulin, which promotes glucose uptake, inhibits hepatic glucose production, and promotes glycogen, lipid (1), and protein synthesis (2). Insulin fulfills these functions by binding to the insulin receptor (IR), which results in the activation of the Phosphotidylinositol 3-Kinase (PI3K)/Akt and the Mitogen Activated Protein Kinase (MAPK) pathways (1). In contrast, insulin-like growth factor-I (IGF-I) is well established as a potent mitogen and survival factor, mediating many of the growth-promoting effects of growth hormone (GH) (3). However, IGF-1 was initially discovered as an “insulin-like” peptide, shares structural homology with proinsulin, and can lower blood glucose levels in humans and other animals, although on a molar basis it is less potent than insulin (4). Cellular actions of IGF-1 are mediated by its specific cognate receptor, the IGF-1 receptor (IGF-1R), which upon ligand binding becomes tyrosine phosphorylated leading to activation of similar pathways as the IR (5).

Of the three peripheral metabolic insulin target tissues, namely the skeletal muscle, liver and adipose, IGF-1R is abundant only in the skeletal muscle (4). Adult liver and adipose tissue contain negligible number of IGF-1R and are relatively refractory to IGF-1, although it has been suggested that brain IGF-1R can modulate liver metabolic effects of IGF-1 (6). Maintenance of euglycemia, by IGF-1R mediated muscle glucose uptake, was demonstrated in IR knockout mice as evidence for direct metabolic effects of IGF-1 through its own receptor in muscle (7). Expression of a dominant negative mutant IGF-1R in skeletal muscle of MKR mice impairs both IGF-1 and insulin signaling leading to severe insulin resistance and diabetes at a fairly young age in these mice (8).

Over the last decade it has been recognized that the IGF-1R axis has a critical role in mediating fetal and postnatal growth. Recently, *Igf1r* mutations resulting in reduced function of the IGF-1R have been described in subjects exhibiting fetal and post-natal growth retardation, classic examples of which are small for gestational age (SGA) children (9,10). Some case studies have now demonstrated that *Igf1r* mutations are associated with impaired carbohydrate metabolism in adult subjects who were born SGA (11-14). We have previously shown that mice heterozygous for the *Igf1r* (*Igf1r*$^{+/-}$) exhibit reduced postnatal growth and develop age-dependent insulin resistance. Aged male (22-24 month old), but not female *Igf1r*$^{+/-}$ mice, were glucose intolerant and both genders developed insulin resistance as they aged (15). Feeding middle-aged (14-15 month old) *Igf1r*$^{+/-}$ mice a high fat diet exacerbated insulin resistance, particularly in the female *Igf1r*$^{+/-}$ mice, suggesting that genetic factors can interact with confounding environmental factors to promote metabolic dysfunction (16). In the present study we sought to investigate the mechanisms by which reduced IGF-1 action leads to the development of insulin resistance by utilizing the *Igf1r*$^{+/-}$ mouse as a model of IGF-1 resistance.

2. Methods

2.1. Ethics

All procedures involving mice were approved by the Institutional Laboratory Animal Care and Use Committee of the University of Texas Health Science Center at San Antonio, TX.

2.2. Animals

The *Igf1r*$^{+/-}$ mice on the C57BL/6 genetic background were kindly provided by Dr. Argiris Efstradiatis (Columbia University College of Physicians and Surgeons, New York). The generation of *Igf1r*$^{+/-}$ mice has been described previously (15,17). Only male mice were used in this study. Both *Igf1r*$^{+/-}$ and WT mice were fed *ad libitum* a standard lab chow procured from Harlan, USA (Catalog No. Harlan Teklad LM-485 Mouse/Rat Sterilizable Diet 11.5 kcal% fat) and maintained in micro-isolator cages, 5 to a cage, on a 12-h dark/light cycle.

2.3. Body Composition

Whole body composition analysis was conducted using quantitative magnetic resonance (qMR) machine (Echo Medical System, Houston, TX, USA) as described by Tinsley et al 2004 (18). This machine uses nuclear magnetic resonance to calculate fat mass, lean mass and free water. Live mice, without anesthesia, were used for this procedure.

2.4. Serum insulin, NEFA and triglycerides

Commercially available kits were used to measure serum insulin (Ultra-Sensitive Rat ELISA Kit; Crystal Chem, Downers Grove, IL, USA), free fatty acids (NEFA) (Wako; Richmond, VA, USA) and triglycerides (Triglyceride Assay Kit; Cayman Chemical Company, Ann Arbor, MI, USA).

2.5. IGF-1 Tolerance Test (IGF-1TT)

IGF-1TT was performed by fasting mice for 4 hrs followed by i.p. injection of 0.5 mg/kg body weight of rhIGF1 (Austral Biologicals, San Ramon, CA, USA). Blood glucose was measured using an Accucheck glucometer (Roche Diagnostics, Indianapolis, IN, USA) at 0, 30, 60 and 90 min.

2.6. Real-time PCR

Mice were fasted overnight and tissues were removed and frozen in liquid nitrogen. Total RNA was isolated using RNA STAT-60 (Tel-test, Friendswood, TX, USA). Real-time PCR reaction was performed using TaqMan Universal PCR Master Mix (P/N 4324018) and TaqMan-MGB probes for *Acc1* (Mm01304257_m1), *Fas* (Mm00662319_m1), *Srebp1c* (Mm00550338_m1), *Pparγ* (Mm01184322_m1), *Cd36* (Mm00432403_m1), *Cpt1* (Mm00487200_m1), *Igfbp1* (Mm00515154_m1), *Igfbp2* (Mm00492632_m1), *Igfbp3* (Mm00515156_m1) and *B2m* (Mm00437762_m1) all of which were purchased from ABI. All samples were run in duplicate and quantitated in an ABI 7500 thermal cycler.

2.7. Glucose uptake

Isolated soleus muscles were preincubated in Krebs-Ringer bicarbonate (KRB) buffer containing 2 mM pyruvate at 37°C for 40 min. The muscles were then incubated at 37°C for 30 min in buffer with or without rhIGF1 (7.5nM). The concentration was determined on the basis of preliminary dose-response experiments (data not shown). Muscles were then incubated in KRB containing 1 mM 3-*O*-methyl-D-[3H]glucose (1.5 mCi/ml) and 1 mM D-[14C]mannitol (0.45 mCi/ml) at 30°C for 10 min, and then immediately frozen in liquid nitrogen. rhIGF1 was also added to the KRB if it had been present during the previous incubation period. Muscles were weighed and processed by incubating in 300 ul of 1 mol/1 NaOH at 80°C for 10 min. Digests were neutralized with 300 ul of 1 mol/1 HC1, and particulates were precipitated by centrifuging at 14,000g for 5 min. Radioactivity of the digested protein was determined by liquid scintillation counting for dual labels, and the extracellular and intracellular spaces were calculated.

2.8. Tissue Triglycerides

Liver and muscle tissues were homogenized in 1 ml of 2:1 chloroform–methanol and solubilized overnight at 4 °C with shaking. 0.5 ml 0.6% NaCl was added followed by centrifugation at 800*g* for 20 min at 4 °C. The organic phase was transferred to a new tube and the samples were dried under nitrogen gas and reconstituted with 100 µl 1% Triton X-100/PBS. Triglyceride concentrations were measured using a Triglyceride Assay Kit (Cayman Chemical Company, Ann Arbor, MI).

2.9. Western Blotting

For in vivo insulin signaling experiments, mice were fasted overnight followed by an i.p. injection of 5 mU/g body weight of insulin (Novolin, Novo Nordisk, Princeton, NJ, USA) or an equivalent volume of sterile saline. After 5 min, liver and muscle tissue were harvested and frozen in liquid nitrogen. For basal signaling, liver and skeletal muscle were harvested from ad libitum fed mice and frozen in liquid nitrogen. For immunoblotting, primary antibodies directed against phospho-Akt (anti-pAkt Ser473), total Akt (anti-Akt), total Acc (anti-Acc), total Fas (anti-Fas), phospho-mTOR (anti-pmTOR Ser2448), total mTOR (anti-mTOR), phospho-p70 S6 kinase (anti-p-p70 S6 kinase Thr389) and total p70 S6 kinase (anti-p70 S6 kinase) were purchased from Cell Signaling Technologies (Danvers, MA, USA) and primary antibodies directed against IR (anti-IR) and GAPDH (anti-GAPDH) and HRP-linked secondary antibody were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

2.10. Statistics

Results were analyzed by unpaired t-test or t-test with Bonferroni's post-hoc test, or by two-way ANOVA. Prism 5 software (GraphPad, San Diego, CA, USA) was used for all statistical analysis.

3. Results

3.1. Igf1r haploinsufficiency results in resistance to the hypoglycemic effects of IGF-1

We have previously shown that the male *Igf1r*$^{+/-}$ mice develop glucose intolerance and insulin resistance with age, as assessed by glucose tolerance test (GTT) and insulin tolerance test (ITT) respectively (15). We now show that both young (Fig. 1a) and old (Fig. 1b) *Igf1r*$^{+/-}$ mice are resistant to the hypoglycemic effects of IGF-1 during IGF-1TT. Blood glucose levels were significantly higher in young and old *Igf1r*$^{+/-}$ mice, specifically at time 60 and 120min after IGF-1 injection, when compared to WT controls. To determine if the resistance to hypoglycemic effects of IGF-1 in vivo reflected reduction in IGF-1-stumulated muscle glucose uptake, we measured IGF-1 mediated glucose uptake ex vivo in soleus muscle of young (Fig. 1c) and old mice (Fig. 1d). IGF-1 mediated glucose uptake was significantly reduced in

young *Igf1r*$^{+/-}$ and had a trend towards decrease in old *Igf1r*$^{+/-}$ mice consistent with the in vivo IGF-1TT in *Igf1r*$^{+/-}$ mice.

3.2. Igf1r$^{+/-}$ mice demonstrate increased age-related whole body adiposity

We have previously reported that *Igf1r*$^{+/-}$ mice have reduced postnatal body weight (15). Although *Igf1r*$^{+/-}$ mice were smaller than WT controls throughout lifespan, whole body adiposity increased in an age-dependent manner. Young *Igf1r*$^{+/-}$ (Fig. 2a) mice did not show any difference in fat mass when compared to WT controls, while both middle (Fig. 2b) and old (Fig. 2c) aged *Igf1r*$^{+/-}$ mice had significantly increased fat mass, when normalized to body weight, relative to WT controls. Increased adiposity in old *Igf1r*$^{+/-}$ mice led us to measure the triglyceride content in the liver and muscle tissues of *Igf1r*$^{+/-}$ mice. We found that triglyceride content was higher in the liver and muscle of old *Igf1r*$^{+/-}$ mice when compared to their respective WT tissues (Fig. 2d).

3.3. Effects of *Igf1r* deficiency on serum insulin, NEFA and triglycerides and expression of IGFBPs.

Consistent with our published report that aged *Igf1r* $^{+/-}$ mice are insulin resistant, we observed that fasting serum insulin levels were higher in middle and old aged *Igf1r*$^{+/-}$ mice as compared to WT controls (Table 1a) while there was no change in insulin levels of young *Igf1r*$^{+/-}$ mice. Serum NEFAs were not altered in either young or old *Igf1r*$^{+/-}$ mice (Table 1a). No difference was detected in the fasting triglyceride levels between *Igf1r*$^{+/-}$ and WT mice of either young or old age (Table 1a). Postprandial serum triglycerides did not change in the young *Igf1r*$^{+/-}$ mice but were higher in the old *Igf1r*$^{+/-}$ mice when compared to their corresponding WT controls (Table 1a).

Serum IGFBP1 and 2 are regulated by insulin (19,20) and thus we expected that since the *Igf1r*$^{+/-}$ mice became hyperinsulinemic, IGFBP1 and 2 would also be altered. There was no change in the gene expression of *Igfbp2* or 3 in the liver of young male *Igf1r*$^{+/-}$ mice when compared to WT controls (Table 1b). The expression of *Igfbp1* in the young (Table 1b) and old male *Igf1r*$^{+/-}$ (Table 1b) mice exhibited a trend towards decreased expression compared to WT controls, but the effect was not statistically

196 significant. However, expression of *Igfbp2* in old *Igf1r*$^{+/-}$ liver was significantly reduced and that of
197 *Igfbp3* was significantly increased when compared to WT controls (Table 1b).
198
199 **3.4. Expression of genes regulating lipid metabolism**
200 Increased levels of serum and tissue triglycerides led us to investigate changes in the expression of genes
201 regulating lipogenesis, fatty acid uptake and fatty acid oxidation in the liver and muscle tissues of young
202 and old WT and *Igf1r*$^{+/-}$ mice. The mRNA and protein expression of the lipogenic genes acetyl CoA
203 carboxylase 1 (*Acc1*) and fatty acid synthase (*Fas*) did not change in the liver (Fig. 3a and e) and muscle
204 (Fig. 3b and f) of the young male *Igf1r*$^{+/-}$ mice compared to WT. However, the old *Igf1r*$^{+/-}$ mice had
205 significantly elevated mRNA and protein expression of *Acc1* and *Fas* in both liver (Fig. 3c and g) and
206 muscle (Fig. 3d and h). Expression of sterol regulatory element binding protein 1c (*Srebp1c*), the
207 transcription factor regulating expression of *Acc1* and *Fas* (21), also did not change in the liver and
208 muscle tissues of young *Igf1r*$^{+/-}$ mice (Fig. 3a and b) whereas in the old *Igf1r*$^{+/-}$ mice only the liver
209 demonstrated increased expression of *Srebp1c* (Fig. 3c). Expression of peroxisome proliferator-activated
210 receptor γ (*Pparγ*), another transcription factor regulating lipogenesis (22), was interestingly found
211 elevated in the liver (Fig. 3a) but not in the muscle (Fig. 3b) of young *Igf1r*$^{+/-}$ mice. Consistent with
212 increased expression of *Acc1*, *Fas* and *Srebp1c*, the expression of *Pparγ* was found to be higher in the
213 liver of old *Igf1r*$^{+/-}$ mice (Fig. 3c). Although, *Srebp1c* expression did not change in the muscle of old
214 *Igf1r*$^{+/-}$ mice, *Pparγ* expression was found to be higher (Fig. 3d). Expression of cluster of differentiation
215 (*Cd36*), a downstream target of *Pparγ* (22), were increased in the liver of both young and old *Igf1r*$^{+/-}$ mice
216 (Fig. 3a and c). The expression of *Cpt1*, whose gene product regulates the rate of beta oxidation of fatty
217 acids, was reduced in the liver of old *Igf1r*$^{+/-}$ mice (Fig. 3c).
218
219 **3.6. In vivo analysis of the insulin signaling pathway in liver and muscle**
220 To determine the molecular mechanism of insulin resistance in the *Igf1r*$^{+/-}$ mice, we compared signaling
221 events in insulin stimulated liver and muscle from young and old *Igf1r*$^{+/-}$ mice with that of WT controls.

Insulin stimulated signaling, as depicted by pAkt levels, was higher in both liver (Fig. 4a) and muscle (Fig. 4b) from young *Igf1r*$^{+/-}$ mice compared to WT controls. In the old mice, insulin stimulated pAkt levels were higher in the liver (Fig. 4c) from *Igf1r*$^{+/-}$ mice but were significantly reduced in the muscle tissue (Fig. 4d) from *Igf1r*$^{+/-}$ mice, compared to WT. We also measured basal levels of phosphorylation of signaling molecules downstream of Akt, namely the mammalian target of rapamycin (mTOR). Basal levels of pmTOR were higher in the liver and muscle (Fig. 4e and 4f) from young as well as old (Fig. 4g and 4h) *Igf1r*$^{+/-}$ mice compared to WT controls.

4. Discussion

Disruptions in the IGF-1 signaling pathway resulting in IGF-1 resistance, as seen in SGA subjects, have been associated with impaired carbohydrate metabolism but the mechanism(s) responsible for impaired insulin action is unknown (11-14). Here we have utilized the *Igf1r*$^{+/-}$ mouse as a model for the understudied mechanisms of how reduced IGF-1 action leads to the development of insulin resistance as seen later in life in SGA subjects. Inactivation of one of the *Igf1r* alleles resulted in an age dependent insulin resistant phenotype (15), which we propose is driven by aberrant deposition of lipids.

We previously demonstrated that *Igf1r*$^{+/-}$ mice exhibited IGF-1 resistance, with no compensatory increases in serum IGF-1 or tissue *Igf1* mRNA levels, and developed age-related insulin resistance (15). IGF-1 resistance was further confirmed in the present study by reduced sensitivity of both young and old *Igf1r*$^{+/-}$ mice to the hypoglycemic effect of IGF-1. Moreover, reduced IGF-1 stimulated glucose uptake in isolated soleus muscle from young *Igf1r*$^{+/-}$ mice suggested that IGF-1 resistance preceded insulin resistance.

Alterations in body composition can occur independently of body weight changes (23) and thus we speculated that even though body weight of *Igf1r*$^{+/-}$ mice was reduced, age-derived increase in fat stores could influence insulin action. Increased BMI and adiposity have been observed in SGA children when compared to those born appropriate for gestational age (AGA) (24) and indeed we observed that whole

248 body adiposity was increased in middle aged and old *Igf1r*$^{+/-}$ mice. Hyperinsulinemia has been implicated
249 as a causative factor in lipid accum.ulation, consistent with observations in rodents and humans that
250 hyperinsulinemia precedes development of insulin resistance (21,25,26). Indeed, *Igf1r* haploinsufficiency
251 resulted in fasting hyperinsulinemia, but only in middle and old aged *Igf1r*$^{+/-}$ mice. In conjunction with
252 hyperinsulinemia, old *Igf1r*$^{+/-}$ mice also demonstrated postprandial hypertriglyceridemia and increased
253 lipid accrual in the liver and muscle tissue. In order to determine mechanisms for the enhanced lipid
254 accrual, we assessed the expression of critical genes involved in lipid metabolism. While in the young
255 *Igf1r*$^{+/-}$ mice only liver exhibited increased expression of *Pppar*γ and *Cd36*, in the old *Igf1r*$^{+/-}$ mice gene
256 expression and protein levels of lipogenic markers were higher in both liver and muscle suggesting
257 increased lipogenesis in these tissues. Increased lipogenic gene expression may favor the insulin resistant
258 phenotype of the old *Igf1r*$^{+/-}$ mice consistent with studies showing that accumulation of toxic lipid
259 intermediates results in impaired insulin signaling and thus insulin resistance (27).

260 SGA infants have been shown to be hypoglycemic (28) and hypoinsulinemic (29) and it has been
261 suggested that an early and transient phase of increased insulin sensitivity might precede the development
262 of insulin resistance (24). An evaluation of the insulin signaling pathway showed enhanced insulin-
263 stimulated pAkt in the liver tissue of both young and old *Igf1r*$^{+/-}$ mice, despite the fact that IGF-1R is
264 negligible in the liver tissue. Whether the metabolic effects of IGF-1 in liver are regulated by brain IGF-
265 1R, is yet to be determined. Interestingly while insulin-stimulated pAkt in the muscle of young *Igf1r*$^{+/-}$
266 mice were high, the muscle tissue of old *Igf1r*$^{+/-}$ mice was found to be insulin resistant. Thus, our data
267 suggests a tissue-specific insulin resistance phenotype in face of *Igf1r* haploinsufficiency. We posit that
268 liver of *Igf1r*$^{+/-}$ mice remains insulin sensitive whereas the muscle exhibits insulin resistance with age but
269 remains insulin sensitive with respect to lipid accretion.

270 Further evidence for a role for hyperinsulinemia in promoting metabolic alterations in *Igf1r*$^{+/-}$ mice comes
271 from our data on the expression of *Igfbp*s which are produced by the liver (30) and regulated by insulin
272 status (19,20). Expression of *Igfbp1* is rapidly transcriptionally downregulated by insulin (19) and

reduced IGFBP1 synthesis and circulating levels are observed in states of insulin resistance (31). Expression of *Igfbp1* had a trend towards reduced levels in both young and old *Igf1r*$^{+/-}$ liver. Less studied is the regulation of IGFBP2 by insulin which is affected by chronically high insulin levels (19) and the expression of which was significantly reduced in the old *Igf1r*$^{+/-}$ livers, relevant with our finding of chronic hyperinsulinemia in the middle and old aged *Igf1r*$^{+/-}$ mice. Consistent with reports of overexpression of *Igfbp3* being associated with glucose intolerance (32), we found that old *Igf1r*$^{+/-}$ liver showed higher expression of *Igfbp3*.

An important regulator of lipogenesis which has shown to play a seminal role in promoting lipotoxicity dependent development of insulin resistance is mTOR (33). Insulin stimulated mTOR pathway in liver remains paradoxically sensitive to insulin even during insulin resistance, reflecting the state of mixed insulin sensitivity/insulin resistance (33). We observed that basal phosphorylation of mTOR was higher in the liver and muscle tissue of *Igf1r*$^{+/-}$ mice throughout lifespan suggesting that the mTOR pathway is stimulating the lipogenic phenotype. Consistent with this observation, our laboratory has previously published that knockdown of PI3K catalytic subunit isoforms resulted in increased basal and IGF-I-stimulated phosphorylation of mTOR and p70S6K (34).

Our results thus suggest that development of insulin resistance in old male *Igf1r*$^{+/-}$ mice cannot be attributed to *Igf1r* haploinsufficiency alone since young *Igf1r*$^{+/-}$ mice do not exhibit insulin resistance. Aging, by itself, is an independent causative factor contributing to the pathology of the metabolic syndrome (35-37) which is in line with the observation that SGA individuals develop metabolic syndrome in the fifth to sixth decade of their life (38). It has been suggested that in the elderly population insulin resistance is mostly confined to the skeletal muscle (37), consistent with our observation of impaired insulin signaling specifically in the muscle of the old *Igf1r*$^{+/-}$ mice. Based on our results we propose that IGF-1 resistance initially increases insulin sensitivity, but with age leads to adiposity and eventually confers insulin resistance. Adiposity and hyperinsulinemia constitute a vicious positive feed forward loop and it remains a conundrum as to what appears first. An alternative hypothesis could be that *Igf1r*

haploinsufficiency directly augments adipogenesis, through an as yet unidentified mechanism, which could then lead to insulin resistance. Since adiposity in *Igf1r*$^{+/-}$ mice increases from middle age, an intensive characterization of *Igf1r*$^{+/-}$ mice and study of insulin signaling pathways needs to be conducted at regular intervals of age to determine the onset of the lipogenic phenotype. In summary, we have identified the *Igf1r*$^{+/-}$ mouse as a clinically relevant model to study the genetic basis of age acquired insulin resistance and metabolic syndrome as seen in the SGA subjects.

Table 1

a.

Serum parameters		**Young**		**Old**	
		WT	**Igf1r+/-**	**WT**	**Igf1r+/-**
Insulin (ng/mL)	**fast**	0.29±0.06	0.37±0.07	0.55±0.2	1.01±0.2*
NEFA (mEq/L)	**fast**	1.3±0.1	1.3±0.1	1.2±0.1	1.5±0.1
	fed	0.9±0.1	1.0±0.1	0.9±0.2	1.1±0.1
Triglycerides (mg/dL)	**fast**	60.1±7.3	49.3±4.1	70.25±9.5	89.1±8.5
	fed	67.2±5.4	73.5±7.9	71.6±6.2	102.9±7.0*

Middle-aged mice		**WT**	**Igf1r+/-**
Insulin (ng/ml)	**fast**	0.31±0.01	0.83±0.21*

b.

Relative mRNA levels of *IGFBP* in liver	Young		Old	
	WT	Igf1r+/-	WT	Igf1r+/-
IGFBP1	0.66±0.16	0.39±0.07	0.95±0.23	0.52±0.08
IGFBP2	0.84±0.09	1.61±0.2	1.61±0.13	1.13±0.14*
IGFBP3	1.8±0.37	2.25±0.4	1.02±0.1	1.76±0.25*

Figure and Table Legends

Fig. 1 IGF-1TT and glucose uptake. IGF-1TTs (0.5mg/kg body wt, i.p.) were performed in young (4-5 month old) **(a)** and old (22-24 month old) **(b)** mice after 4 hr fast, and blood glucose was recorded at times indicated. Left panel shows the IGF-TT time course experiment and right panel shows the AUC. Glucose uptake was measured in isolated soleus muscle from young (4-5 month old) **(c)** and old (22-24 month old) **(d)** mice with or without rhIGF-1. Black lines with solid circles represent WT mice (n=7-8) and black lines with solid squares represent *Igf1r*$^{+/-}$ mice (n=7) for IGF-1TTs; white bars represent WT mice (n=6) and shaded bars represent *Igf1r*$^{+/-}$ mice (n=6) for glucose uptake. The mean and SEM are shown, * indicates difference between *Igf1r*$^{+/-}$ and WT, and $^{\#}$ indicates difference between control and treated, where $p<0.05$. The Student's t-test was used for the comparisons. Individual time points were also compared in the same manner and corrected for multiple comparisons.

Fig. 2 Body composition and tissue triglycerides**.** Whole body fat mass was measured in young (4-5 month old) **(a)**, middle aged (14-15 month old) **(b)** and old (22-24 month old) **(c)** mice and normalized to body weight (gms). Triglyceride levels were measured in the liver and muscle tissues of old (24-26 months old) mice and normalized to the weight of the tissue **(d)**. White bars represent WT mice (n=9-15 for body composition and 3-9 for tissue triglycerides) and shaded bars represent *Igf1r*$^{+/-}$ mice (n=12-18

for body composition and 10-16 for tissue triglycerides); the mean and SEM are shown, * indicates difference between *Igf1r*$^{+/-}$ and WT where p<0.05. The Student's t-test was used for the comparisons.

Fig. 3. Expression of genes regulating lipid metabolism in the liver and muscle. The mRNA levels of lipogenic genes were measured in the liver (left panel) and muscle (right panel) of young (4-5 month old) **(a, b)** and old (24-26 month old) **(c, d)** mice. Protein levels of ACC and FAS were measured in the liver (left panel) and muscle (right panel) of young **(e, f)** and old **(g, h)** mice. White bars represent WT mice (n=4-8) and shaded bars represent *Igf1r*$^{+/-}$ mice (n=4-9); the mean and SEM are shown, * indicates difference between *Igf1r*$^{+/-}$ and WT where p<0.05. The Student's t-test was used for the comparisons.

Fig. 4 Insulin signaling *in vivo* in liver and muscle**.** Levels of phosphorylated Akt were measured in the liver and muscle of young (4-5 month old) **(a, b)** and old (24-26 month old) **(c, d)** mice following injection of saline or insulin (5U/kg body wt.). Levels of phosphorylated mTOR were measured in the liver and muscle of young (4-5 month old) **(e, f)** and old (24-26 month old) **(g, h)** mice. n=3-6 WT mice per group and 3-6 *Igf1r*$^{+/-}$ mice per group.

Table 1. Serum parameters and *Igfbp* expression in *Igf1r*$^{+/-}$ and WT mice. **(a)** Data were obtained from young (4-6month old, n=6-14), middle aged (14-15 month old, n=6-14) and old (24-26 month old, n=6-10) *Igf1r*$^{+/-}$ and WT mice, **(b)** The mRNA levels of *Igfbp1*, *bp2* and *bp3* were measured in the livers of young (4-5 month old, n=9-11) and old (24-26 month old, n=9-11) WT (n=9-11) and *Igf1r*$^{+/-}$ (n=10-13) mice. The mean and SEM are shown, * indicates difference between *Igf1r*$^{+/-}$ and WT where p<0.05. The Student's t-test was used for the comparisons.

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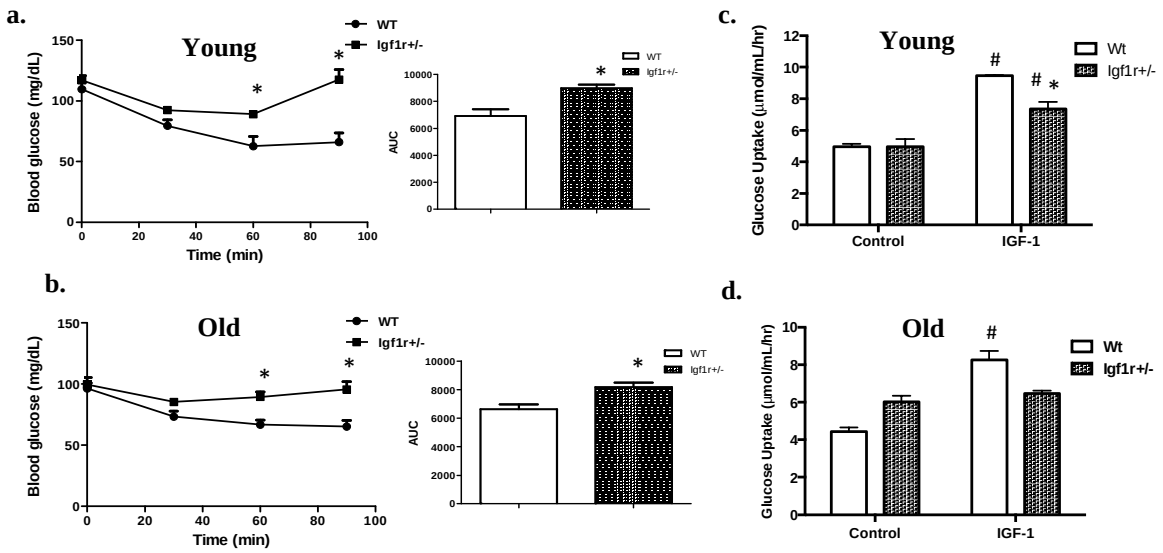
468

Fig. 1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Young
WT
Igf1r+/-
Blood glucose (mg/dL)
Time (min)
AUC
b.
Old
c.
Young
Wt
Igf1r+/-
Glucose Uptake (µmol/mL/hr)
Control
IGF-1
d.
Old

Fig. 2

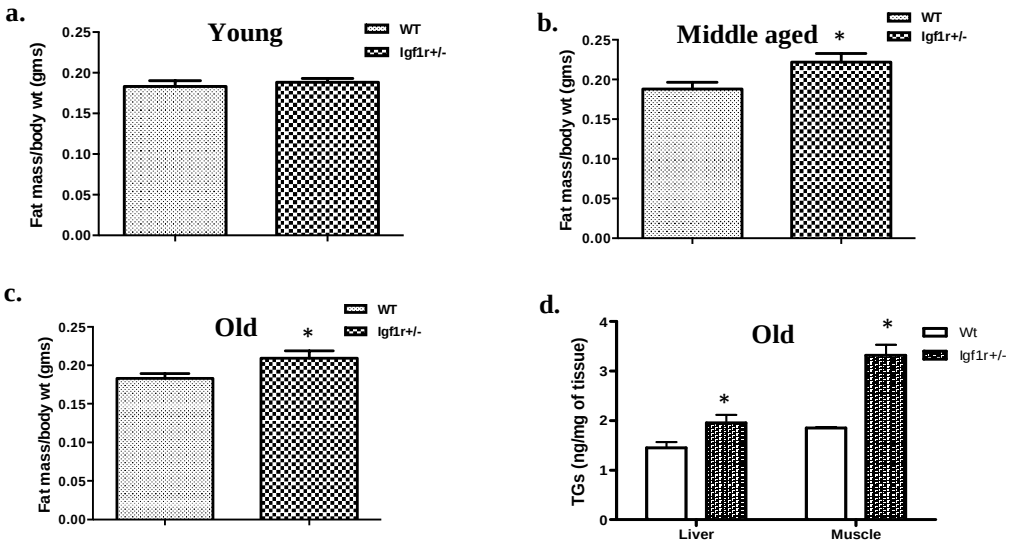

a.
Young
WT
Igf1r+/-
Fat mass/body wt (gms)
0.25
0.20
0.15
0.10
0.05
0.00
b.
Middle aged
*
WT
Igf1r+/-
Fat mass/body wt (gms)
0.25
0.20
0.15
0.10
0.05
0.00
c.
Old
*
WT
Igf1r+/-
Fat mass/body wt (gms)
0.25
0.20
0.15
0.10
0.05
0.00
d.
Old
*
*
Wt
Igf1r+/-
TGs (ng/mg of tissue)
4
3
2
1
0
Liver
Muscle

Fig. 3

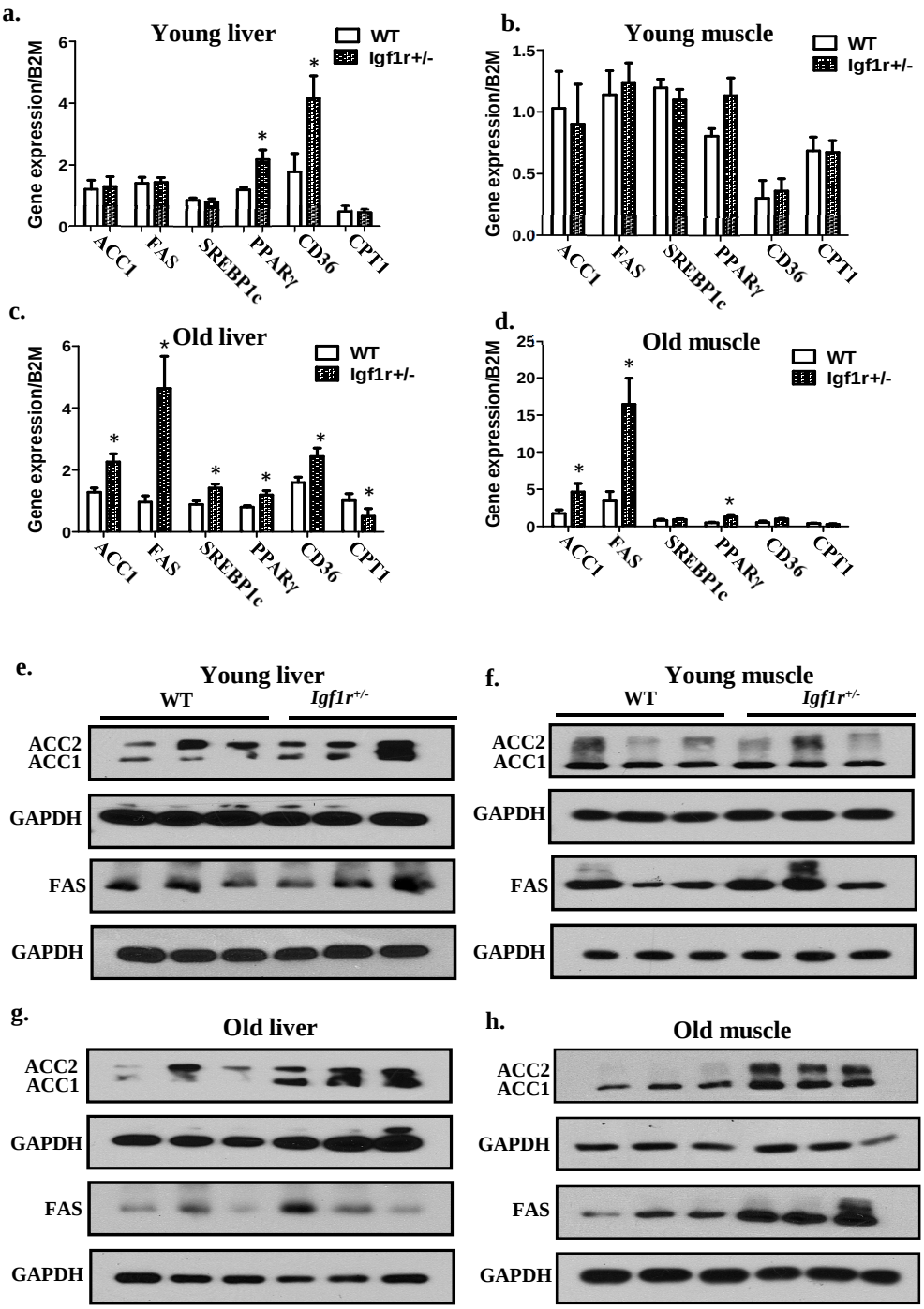
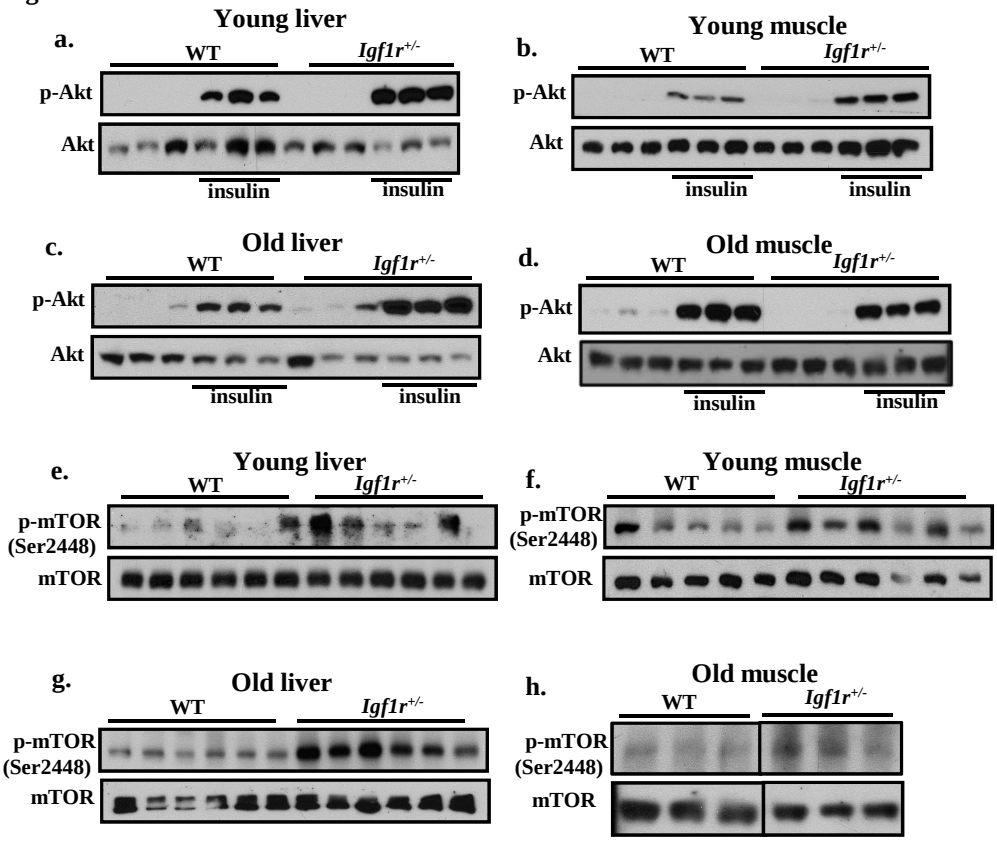

a.
Young liver
WT
Igf1r+/-
Gene expression/B2M
ACC1
FAS
SREBP1c
PPARγ
CD36
CPT1
b.
Young muscle
c.
Old liver
d.
Old muscle
e.
Young liver
WT
Igf1r+/-
ACC2
ACC1
GAPDH
FAS
f.
Young muscle
g.
Old liver
h.
Old muscle

Fig. 4


a.
Young liver
WT
Igf1r+/-
p-Akt
Akt
insulin
insulin
b.
Young muscle
WT
Igf1r+/-
p-Akt
Akt
insulin
insulin
c.
Old liver
WT
Igf1r+/-
p-Akt
Akt
insulin
insulin
d.
Old muscle
WT
Igf1r+/-
p-Akt
Akt
insulin
insulin
e.
Young liver
WT
Igf1r+/-
p-mTOR (Ser2448)
mTOR
f.
Young muscle
WT
Igf1r+/-
p-mTOR (Ser2448)
mTOR
g.
Old liver
WT
Igf1r+/-
p-mTOR (Ser2448)
mTOR
h.
Old muscle
WT
Igf1r+/-
p-mTOR (Ser2448)
mTOR

Research Highlights

- SGA infants are at a higher risk of developing the metabolic syndrome in adult life
- Reduced IGF-1 action is associated with low birth weight and post-natal growth
- IGF-1 resistance, by increasing insulin signaling, promotes lipogenesis
- Igf1r+/- mouse as a model to study the genetic basis of the SGA phenotype