THEMATIC REVIEW

40 YEARS of IGF1
Understanding the tissue-specific roles of IGF1/IGF1R in regulating metabolism using the Cre/loxP system

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Abstract

It is clear that insulin-like growth factor-1 (IGF1) is important in supporting growth and regulating metabolism. The IGF1 found in the circulation is primarily produced by the liver hepatocytes, but healthy mature hepatocytes do not express appreciable levels of the IGF1 receptor (IGF1R). Therefore, the metabolic actions of IGF1 are thought to be mediated via extra-hepatocyte actions. Given the structural and functional homology between IGF1/IGF1R and insulin receptor (INSR) signaling, and the fact that IGF1, IGF1R and INSR are expressed in most tissues of the body, it is difficult to separate out the tissue-specific contributions of IGF1/IGF1R in maintaining whole body metabolic function. To circumvent this problem, over the last 20 years, investigators have taken advantage of the Cre/loxP system to manipulate IGF1/IGF1R in a tissue-dependent, and more recently, an age-dependent fashion. These studies have revealed that IGF1/IGF1R can alter extra-hepatocyte function to regulate hormonal inputs to the liver and/or alter tissue-specific carbohydrate and lipid metabolism to alter nutrient flux to liver, where these actions are not mutually exclusive, but serve to integrate the function of all tissues to support the metabolic needs of the organism.

Introduction

Although many tissues produce insulin-like growth factor-1 (IGF1), it is now recognized that the hepatocyte is the primary source of circulating IGF1 (Sjogren et al. 1999, Yakar et al. 1999, LeRoith 2008). Despite the fact that the hepatocyte is the major contributor to circulating IGF1, mature hepatocytes do not express appreciable levels of the IGF1 receptor (IGF1R). However, IGF1R is expressed during early hepatocyte development (Waraky et al. 2016) and in hepatocellular carcinomas (Scharf & Braulke 2003, Liu et al. 2011), a topic beyond the scope of this review. The majority of IGF1 circulates bound to IGF1-binding proteins (IGFBP3 or IGFBP5) and acid labile subunit (ALS) (Hjortebjerg et al. 2014). These ternary complexes serve to extend the half-life of IGF1 and regulate its bioavailability (Yee 2018). In the healthy liver, growth hormone (GH) signaling through the GH receptor (GHR)/JAK2/STAT5B
pathway is required to maintain hepatocyte expression of IGF1, ALS and IGFBP3 (Rotwein 2012, Feigerlova et al. 2013). Both IGF1 and GH work together to promote longitudinal growth (Wit & Camacho-Hubner 2011), as well as modulate metabolic function in adults, as previously reviewed in detail (Moller & Jorgensen 2009, Clemmons 2012). In brief, IGF1 works in conjunction with insulin to inform cells of adequate nutrient stores to support protein accretion, cell growth and proliferation. Circulating IGF1 levels are reduced under fasting conditions and type 1 diabetes, associated with elevated GH levels. Given the healthy hepatocyte does not express appreciable levels of the IGF1R, the metabolic actions of IGF1 are thought to be due to extra-hepatocyte actions.

Although a metabolic role for IGF1 is well accepted, there are several challenges in regard to understanding how IGF1 mediates these effects. Although the hepatocyte is the primary contributor to circulating IGF1, it is also locally produced by the majority of cells in the body, where most (except the mature hepatocyte) express the IGF1R. Therefore, the relative role of circulating vs locally produced IGF1 has been difficult to differentiate. In addition, as the name implies, IGF1 shares high homology to insulin, and in fact, in most non-vertebrate species, only a single insulin-like molecule is found. It is thought that the insulin and IGF1 genes arose from duplication of the common ancestral gene (McRory & Sherwood 1997). In mammals, it is commonly accepted that the primary action of IGF1 is to promote structural growth, while the primary action of insulin is to regulate nutrient utilization in response to a meal. Despite these primary actions, IGF1 and insulin demonstrate important crossover effects on growth and metabolism (Messina 2010). Some of these effects may be due to the high structural and functional homology of the IGF1R and insulin receptor (INSR) (Siddle 2011). However, this interaction may only be relevant when IGF1 or insulin levels are at the supraphysiologic levels or in the context of in vitro experiments, where the levels of bioactive IGF1 applied can be many fold that observed in vivo, when binding proteins are absent. In vitro, it has also been shown that the IGF1R and INSR can interact by forming hybrid receptors or by crosstalk of downstream intracellular signaling pathways, thereby modifying the response to their respective ligands (Belfiore et al. 2009, Belfiore et al. 2017). In vivo, the relative expression levels of IGF1R and INSR vary between tissues and across development stages, and these changes serve to dictate the relative tissue-specific role of IGF1 vs insulin in regulating metabolic function.

Congenital whole-body knockout of IGF1 results in severe growth retardation and, depending on the genetic background, mice die shortly after birth, while others can survive into adulthood (Liu et al. 1993, Powell-Braxton et al. 1993). Congenital whole-body knockout of IGF1R is embryonically lethal (Powell-Braxton et al. 1993). Therefore, in order to understand the role IGF1/IGF1R play in regulating metabolism and to separate the action of IGF1/IGF1R from that of insulin/INSR, over the last two decades, multiple laboratories have taken advantage of the Cre recombinase/loxP system (Van Duyne 2015) to manipulate IGF1 and IGF1R in a tissue-specific fashion. This review will focus on those studies, as they relate to how IGF1/IGF1R may regulate tissues to alter the production of metabolic hormones and/or alter insulin sensitivity to ultimately control substrate utilization. It is important to note that the phenotype observed in the mouse models described below may not only be due to loss of function of IGF1/IGF1R actions, but also due to the model of the floxed mouse used, including background strain and the position of the loxP sites (Liu et al. 1998, Dietrich et al. 2000, Holzenberger et al. 2000, Kloting et al. 2008) and the promoter and method used to drive Cre expression (see below and Table 1 for model details). Where the later will dictate time (i.e. developmental stage) of gene knockdown and tissue (cell) specificity.

As summarized in the following sections, IGF1/IGF1R can alter extra-hepatocyte function to regulate hormonal inputs to the liver and/or alter tissue-specific carbohydrate and lipid metabolism to alter nutrient flux to liver, where these actions are not mutually exclusive, but serve to integrate the function of all tissues to support the metabolic needs of the organism, as illustrated in Fig. 1.

**IGF1 can indirectly regulate (hepatocyte) metabolism through modulation of hormonal inputs**

**Hepatocyte-specific reduction in IGF1 increases GH**

The first application of the Cre/loxP system to study IGF1/IGF1R was the work of LeRoith and colleagues who crossed breeding IGF1fl/fl mice with the hepatocyte-specific albumin promoter-Cre mice (expressed early in embryonic development), referred to as liver IGF1-deficient (LID) mice (Yakar et al. 1999, LeRoith 2008), or the interferon-inducible liver/lymphocyte-specific Mx-Cre mice (Sjogren et al. 1999, Wallenius et al. 2001). In both models, circulating IGF1 levels were less than 20% of controls, and GH levels were elevated, but structural growth was relatively normal. When LID mice were crossedbreed to ALSKO mice, circulating IGF1 levels were reduced further,
leading to reduced bone growth, but not to the extreme observed in mice with whole-body knockout of IGF1 (Liu et al. 1993, Powell-Braxton et al. 1993). These studies revealed that locally produced IGF1 is critical to support normal growth during development.

Despite relatively normal growth, adult LID mice exhibit systemic insulin resistance and impaired response of skeletal muscle to acute insulin challenge (Yakar et al. 2001). Under hyperinsulinemic/euglycemic clamp conditions, LID mice also exhibit impaired insulin-mediated hepatic glucose suppression (Yakar et al. 2004). Importantly, the alterations in systemic insulin resistance and hepatic glucose output were normalized in LID mice when treated with a GH-releasing hormone (GHRH) antagonist or when crossbred to transgenic mice expressing a GH antagonist (Yakar et al. 2001, 2004). Like LID mice, mouse models with congenital, liver-specific knockout of the GHR or its downstream effectors, JAK2 or STATS, have extremely low levels of IGF1 and high levels of GH, associated with systemic insulin resistance (Cui et al. 2007, Fan et al. 2009, Mueller et al. 2011).

Table 1

<table>
<thead>
<tr>
<th>Gene</th>
<th>Tissue</th>
<th>Promoter-driven Cre</th>
<th>Phenotype</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Igf1&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>Whole-body knockout</td>
<td>–</td>
<td>– No IGF1/elevated GH</td>
<td>Liu et al. (1993), Powell-Braxton et al. (1993)</td>
</tr>
<tr>
<td>Igf1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Liver/lymphocyte</td>
<td>Interferon-inducible</td>
<td>– Very low IGF1/elevated GH</td>
<td>Sjogren et al. (1999)</td>
</tr>
<tr>
<td>Igf1r&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Somatotrope</td>
<td>rGHp-Cre</td>
<td>– Minimal growth defect</td>
<td>Luque et al. (2007), Gahe et al. (2013), Romero et al. (2010)</td>
</tr>
<tr>
<td>Igf1r&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Pancreatic β-cell</td>
<td>Insulin promoter 2</td>
<td>– Insulin resistance</td>
<td>Kulkarni et al. (2002), Xuan et al. (2002)</td>
</tr>
<tr>
<td>Igf1r&lt;sup&gt;e&lt;/sup&gt;</td>
<td>Ovarian granulosa cells</td>
<td>Esr2 + Cyp19</td>
<td>– Reduced relative brain weight</td>
<td>Laustsen et al. (2007)</td>
</tr>
<tr>
<td>Igf1r&lt;sup&gt;e&lt;/sup&gt;</td>
<td>Leydig cells and adrenal cells</td>
<td>SCC-Cre</td>
<td>– Reduced fat mass</td>
<td>Boucher et al. (2016)</td>
</tr>
<tr>
<td>Igf1r&lt;sup&gt;e&lt;/sup&gt;</td>
<td>Muscle</td>
<td>Actin</td>
<td>– Reduced fat synthesis</td>
<td>O’Neill et al. (2015)</td>
</tr>
<tr>
<td>Igf1r&lt;sup&gt;e&lt;/sup&gt;</td>
<td>Heart</td>
<td>Creatine kinase</td>
<td>– Reduced adipokines</td>
<td>O’Neill et al. (2015)</td>
</tr>
<tr>
<td>Igf1r&lt;sup&gt;e&lt;/sup&gt;</td>
<td>Adipose tissue</td>
<td>Adiponectin</td>
<td>– No change on glucose homeostasis</td>
<td>Palmiter et al. (2013)</td>
</tr>
<tr>
<td>Igf1r&lt;sup&gt;e&lt;/sup&gt;</td>
<td>Adipose tissue brain/adrenal medulla</td>
<td>aP2</td>
<td>– Reduced relative brain weight</td>
<td>Kloting et al. (2008)</td>
</tr>
<tr>
<td>Igf1r&lt;sup&gt;e&lt;/sup&gt;</td>
<td>Myeloid cells (macrophages)</td>
<td>Lys2</td>
<td>– Increased fat mass</td>
<td>Spadaro et al. (2017)</td>
</tr>
<tr>
<td>Igf1r&lt;sup&gt;e&lt;/sup&gt;</td>
<td>Thyrocyte</td>
<td>Thyroid-specific thyroglobulin</td>
<td>– Early death</td>
<td>Muller et al. (2011)</td>
</tr>
</tbody>
</table>

Superscripts a, b, c, d and e indicate the specific floxed model used: aLiu et al. (1998), Igf1 exon 4 flanked by loxP sites; bPowell-Braxton et al. (1993), Igf1 exon 3 flanked by loxP sites; cKloting et al. (2008), Igf1r exon 3 flanked by loxP sites; dHolzenberger et al. (2000), Igf1r exon 3 flanked by loxP sites; eDietrich et al. (2000), Igf1r exon 3 flanked by loxP sites.

GH, growth hormone; IGF1, insulin-like growth factor-1.
Figure 1
Summary of the information gained by use of the Cre/loxP system to assess the importance of hepatocyte-specific production of IGF1 and to determine the tissue-specific role of IGF1R on metabolic function. These studies have revealed IGF1/IGF1R can alter extra-hepatocyte function, which may regulate hormonal inputs to the liver and/or alter tissue-specific carbohydrate/lipid metabolism to alter nutrient flux to liver, where these actions would not be mutually exclusive, but serve to integrate the function of all tissues to support the metabolic needs of the organism (as depicted by the thick gray shaded arrows forming the circle). Solid green lines (with arrowheads) denote a positive action, while red lines with flat heads denote a negative action. Dashed lines indicate pathways not yet explored or questions remain requiring further investigation. *Asterisk denotes direct IGF1 signaling plays a minimal role in how muscle mediates whole-body metabolic function. GHR, growth hormone receptor; GHRH, growth hormone-releasing hormone; IGF1, insulin-like growth factor-1; IGF1R, IGF1 receptor; SST, somatostatin. 

et al. 2011; Sos et al. 2011, List et al. 2014). When congenital, hepatocyte-specific GHR-knockout mice are crossbred to mice expressing an IGF1 transgene in the liver, GH levels are reduced and systemic insulin resistance was normalized (Liu et al. 2016). Taken together, these studies indicate that liver-derived, circulating IGF1 mediates some of its metabolic effects indirectly by controlling GH secretion, as discussed below in more detail.

Although systemic insulin resistance develops in congenital liver-specific IGF1-knockout mice, as well as mice with liver-specific knockout of GHR signaling, the liver phenotype is dramatically different. LID mice show little change in hepatic lipid content, while mice with defects in hepatocyte GH signaling develop extreme fatty liver. More recently, it has been shown that adult-onset loss of hepatocyte GH signaling increases de novo lipogenesis and promotes fatty liver (Cordoba-Chacon et al. 2015), where this action maybe, in part, independent of reduced IGF1 (Liu et al. 2016). Therefore, the lack of hepatic fat accumulation in the LID mouse, compared to models with hepatocyte defects in GH signaling, may be due to high levels of circulating GH acting through intact, hepatocyte GHR to suppress de novo lipogenesis.

The metabolic phenotype of mice with hepatocyte IGF1 deficiency is consistent with an increase in GH actions, where GH antagonizes the actions of insulin and enhances lipid oxidation (Moller & Jorgensen 2009). Also, GH can promote white adipose tissue (WAT) lipolysis under physiologic conditions when relatively unopposed by insulin, or at pathophysiologic levels, as in acromegaly (Rabinowitz & Zierler 1963, Sakharova et al. 2008, Moller & Jorgensen 2009). In the context of normal physiology, these actions of GH are important under fasting conditions, where insulin and IGF1 levels are low and GH levels are elevated, helping to shift nutrient utilization toward lipids, to maintain circulating glucose levels to support neuronal function.

**IGF1 works through IGF1R to suppress somatotrope function**

The rise in circulating GH levels in the LID mice and in mice with defects in hepatocyte IGF1 signaling is due to loss of negative feedback inhibition of circulating IGF1 on hypothalamic/pituitary regulation of GH secretion. Specifically, GH is produced by a subpopulation of anterior pituitary cells, called somatotropes. Regulation of somatotrope function is dependent on the hypothalamic neuropeptides, GHRH and somatostatin (SST), which stimulate and inhibit GH secretion, respectively. GH is released into the systemic circulation in a pulsatile fashion, where the pattern of GH release dictates function. GH interacts with multiple target tissues, including the hepatocyte, to stimulate the synthesis of IGF1. Comprehensive reviews of the literature related to GH/IGF1 axis regulation and function, through 1999, are available online (http://www.comprehensivephysiology.com/WileyCDA/Section/id-420628.html). GH output is tightly controlled by negative feedback loops, where GH and IGF1 feedback at the level of the hypothalamus to reciprocally regulate SST and GHRH input to the pituitary somatotrope, to control GH secretion. It remains to be determined what neuronal cell types dictate these changes in GHRH and SST, and if IGF1 working directly through IGF1R is involved. However, IGF1 does act directly at the level of the somatotrope to suppress GH gene transcription in primary pituitary cell cultures in all species tested, including primates (Luque et al. 2006). It should be noted that pituitary somatotropes express...
both IGF1R and INSR, but in vitro IGF1 exclusively works through the IGF1R, not INSR, to suppress somatotrope function (Gahete et al. 2013).

The importance of the direct inhibitory action of IGF1 in regulating somatotrope function in vivo has been confirmed using the Cre/loxP system. Specifically, Igf1rfl/fl mice were crossed with mice that express Cre recombinase only in the GH-producing cells of the anterior pituitary, rGHp-Cre mice (Luque et al. 2007), leading to somatotrope-specific knockout of the IGF1R (SIGFRKO; (Romero et al. 2010, Gahete et al. 2013)). The gross morphology of the SIGFRKO pituitary was not altered; however, pituitary GH and GHRH receptor (GHRHR) mRNA levels were increased, and this was associated with an increase in circulating GH and total IGF1 levels. In contrast, somatotrope-specific knockout of the INSR (SIRKO) did not influence GH and GHRHR gene expression but did enhance GH secretory vesicle release, indicating IGF1 and insulin act through unique mechanisms at the level of the pituitary to control GH output. Consistent with the parallel actions of IGF1 and insulin in suppressing GH output, mice with somatotrope-specific knockout of both the IGF1R and INSR have elevated circulating GH levels greater than those observed in SIGFRKO and SIRKO mice (Gahete et al. 2011, 2013). Of note, although the hypothalamus is commonly thought to be a master regulator of GH output, the fact that circulating GH and IGF1 are elevated in SIGFRKO mice indicates that negative feedback at the level of the hypothalamus cannot fully compensate for the loss of IGF1-negative feedback at the level of the pituitary. Therefore, both central and pituitary regulatory loops are critical in maintaining appropriate GH output.

In the first study that examined the metabolic phenotype of SIGFRKO mice (Romero et al. 2010), when knockouts were compared to pooled controls of different genotypes, body weight was reduced, and this was attributed to a decrease in fat mass, without overtly impacting systemic insulin sensitivity. In contrast, in the second report (Gahete et al. 2013), when SIGFRKO mice (Igf1rfl/fl/rGHp-Cre) were compared only to their Igf1rfl/fl littermate controls, body weight and body composition did not differ up to 20 weeks of age, when mice were maintained on either a low-fat or high-fat diet. However, in the later study, sex- and diet-dependent changes in metabolic function were observed in SIGFRKO mice that were indicative of mild insulin resistance (Gahete et al. 2013). Taken together, these studies demonstrate IGF1 directly inhibits pituitary production of GH, where IGF1-mediated changes in GH secretion impact systemic insulin sensitivity, which could ultimately remodel body composition. However, it should be noted that the rather mild insulin-resistant phenotype observed in SIGFRKO mice, compared to congenital, liver-specific loss of GHR signaling or IGF1 production, is likely due to the anti-insulin effects of elevated GH being off-set by the insulin-sensitizing effects of elevated IGF1.

IGF1 works through the IGF1R to enhance pancreatic β-cell function

Insulin promotes WAT glucose uptake, lipid synthesis and storage (anti-lipolytic) and acts directly on hepatocytes to suppress glucose production, stimulate glycogen and lipid production and suppress VLDL triglyceride release (Titchenell et al. 2017). Insulin is also required for maximum hepatocyte expression of IGF1, by augmenting IGF1 gene expression and maintaining hepatocyte GHR expression (Phillips et al. 1998, Dong et al. 2008). These actions may in part be responsible for reduced hepatic IGF1 production in states of fasting and diabetes type I.

Relevant to this review, intact IGF1 signaling is required to maintain normal β-cell function, based on reports showing rat insulin promoter 2-Cre mice crossbred to two different models of Igf1rfl/fl mice, exhibit impaired glucose tolerance, without influencing β-cell mass (Kulkarni et al. 2002, Xuan et al. 2002). The lack of an impact of IGF1R loss on β-cell mass in vivo is somewhat at odds with studies that used cell lines or overexpression models showing IGF1 augments β-cell replication (Chowdhury et al. 2014, Huang & Chang 2014). These differential effects could be related to the fact that cell lines are not fully representative of β-cells in vivo and overexpression of IGF1 may lead to activation of the INSR, which has been shown to be important in establishing β-cell mass (Otani et al. 2004).

In vitro, islets from β-cell-specific IGF1 KO mice had reduced expression of Glut2 and glucokinase and a reduction in glucose- and arginine-stimulated insulin release. Although the impact of β-cell loss of IGF1R is relatively mild, similar to that reported for β-cell loss of INSR, knockout of both IGF1R and INSR causes early-onset overt diabetes (Ueki et al. 2006). Taken together, these observations suggest IGF1 works in conjunction with insulin to establish optimal β-cell function to allow appropriate insulin release in response to nutrient stimulation. Also, early loss of one receptor may in part compensate for the loss of the other. Appropriate models
IGF1 working through the IGF1R supports thyroid hormone production

Thyroid hormone, under fasting conditions, reduces insulin sensitivity, promotes lipolysis and directly enhances hepatic glucose output (Martinez & Ortiz 2017). A direct role in thyroid hormone-mediated hepatic IGF1 production appears to be species specific and concentration dependent. However, IGF1 can directly support thyroid hormone production based on the observation that Igf1r−/− mice, crossbred to thyroglobulin promoter/enhancer-driven Cre mice showed relatively normal thyroid hormone levels associated with elevated TSH, indicative of subclinical hypothyroidism. Curiously, this was only observed in male mice, which showed an increase in weight gain, with age-associated increase in fat mass (Muller et al. 2011). This direct action of IGF1 on thyroid function may explain in part the subclinical hypothyroidism observed in patients with isolated GH deficiency (Aguiar-Oliveira et al. 2017). However, it should be noted that the GH-independent actions of IGF1 on thyroid function are somewhat controversial, as recently reviewed (Giavoli et al. 2017).

IGF1 working through the IGF1R is essential for granulosa cell (estrogen) development and function

Estrogens have profound effects on overall metabolic function, as exemplified by the enhanced prevalence of obesity, insulin resistance, diabetes and fatty liver in post-menopausal compared to normal cycling women (Clegg 2012). The positive metabolic actions of estrogen, at least on controlling excess hepatic fat accumulation, appear to be mediated by extra-hepatic actions, since hepatic lipid accumulation does not differ between liver-specific Erk-knockout and control mice, under chow or high-fat-fed conditions (Hart-Unger et al. 2017). Estrogen can enhance hepatic IGF1 production (Venken et al. 2005), but at high levels, estrogens antagonize the actions of GH (Birzniece & Ho 2017).

Recent evidence indicates IGF1 acts directly to modulate granulosa cell development and function. Inhibition of IGF1R signaling in vivo and in vitro using siRNA or pharmacologic strategies, block the ability of IGF1 to augment FSH-mediated steroidogenic enzyme production in granulosa cells (Zhou et al. 2013). In addition, granulosa cell knockout of the IGF1R by crossbreeding Igf1r−/− mice with mice heterozygous for both the Esr2-Cre and Cyp19-Cre transgenes (Baumgarten et al. 2017), required for maximal recombination, led to the impairment of follicular growth and estrogen production, as well as infertility. Of note, using the same strategy to knockout the INSR in the granulosa cells had no appreciable effect. We might speculate that reduced IGF1 input to granulosa cells during catabolic states may contribute to infertility.

IGF1R and INSR appear to fully compensate for each other to support normal adrenal and Leydig cell development and function (glucocorticoids/testosterone)

Glucocorticoids, derived from the adrenal cortex, stimulate glucose production in the liver, antagonize the actions of insulin and decrease insulin secretion from the pancreatic β-cells (Whirledge & DeFranco 2018). Glucocorticoid receptors (GRs) physically interact with STAT5B in the liver to promote IGF1 and ALS (Tronche et al. 2004), but at high levels, can decrease the production of IGF1, in part by acting on the hypothalamus to suppress GH secretion (Ferrau & Korbonits 2015). Androgens have gender-dependent effects on metabolism, many of which are mediated by tissue-specific conversion to estrogen by aromatase (Bianchi & Locatelli 2018, Birzniece 2018). Androgens work in combination with GH/IGF1 to stimulate skeletal and muscle growth (Giannoulis et al. 2012).

In order to investigate the direct role IGF1R plays in adrenal cortical and Leydig cell development/function, Igf1r−/− mice were crossbred to Cyp11a1 promoter-driven Cre mouse (SCC-Cre), which drives Cre expression in the adrenogonadal primordium (Neirijnick et al. 2018). Knockout of the IGF1R had no appreciable impact on adrenal function and resulted in a modest reduction in testicular size without altering androgen production or fertility. A similar phenotype was observed when the INSR was knockout. However, combined IGF1R/INSR knockout resulted in impaired adrenal cortical and testicular development associated with low glucocorticoid and testosterone production. Due to lack of tissue specificity, it remains to be determined how the impact of receptor loss in each tissue may indirectly impact the other tissue. However, these results clearly indicate that with respect to adrenal cortical and Leydig cell development, the IGF1R and INSR can fully compensate for each other’s loss.
Nonetheless, appropriate models remain to be developed to directly test how IGF1 directly and independently mediates adrenal cortical and Leydig cell function in adults, and how these actions would ultimately influence metabolic function. It should be noted that IGF1 may also support male fertility via regulation of Sertoli cell development (Cannarella et al. 2018); however, Sertoli-specific manipulation of IGF1/IGF1R remains to be explored.

**IGF1 works through the IGF1R to support M2-like macrophage activation**

It is now becoming appreciated that the macrophage can transition from a pro-inflammatory (M1) to an anti-inflammatory (M2) state, where differentially expressed cytokines influence adipose and liver metabolism. In general, the M1 phenotype is associated with insulin resistance (Vergadi et al. 2017). Of note, M2 macrophages show high expression of IGF1, relative to M0/M1 macrophages (Spadaro et al. 2017). Knockout of the IGF1R in the myeloid lineage, which includes macrophages, by crossbreeding Igf1r<sup>fl/fl</sup> mice with Lys-M-Cre mice (Spadaro et al. 2017) reduced expression of M2 markers. When these mice were placed on a high-fat diet, they became more obese and insulin resistant, compared to their IGF1R intact counterparts. These results indicate IGF1 supports an anti-inflammatory macrophage phenotype that may protect against the development of obesity-associated insulin resistance.

It should be noted here that some reports show an increase in hepatic expression of IGF1R in animal models and patients with chronic liver disease, including non-alcoholic steatohepatitis. At least in the early stage of the disease, this increase in IGF1R expression is likely due to non-hepatocyte cells types within the liver, which could include resident macrophages (Kupffer cells), infiltrating macrophages, activated hepatic stellate cells, cholangiocytes and vascular endothelial cells. However, very low levels of IGF1R expression in the mature hepatocyte might play a functional role, which could be tested in future investigations using adult-onset, hepatocyte-specific IGF1R knockdown. Nonetheless, treatment with IGF1 has been shown to reduce liver injury and fibrosis, at least in part acting on these non-hepatocyte cells (Takahashi 2017). As liver disease progresses to hepatocellular carcinoma, the hepatocytes dedifferentiate to again express the IGF1R, which is thought to contribute to cancer progression (Scharf & Braulke 2003, Liu et al. 2011, Yee 2018).

**IGF1 can indirectly regulate (hepatocyte) metabolism by altering the development and function of key metabolic tissues**

**IGF1 works through the IGF1R to directly enhance adipose tissue function**

Adipose tissue is essential to store nutrients and control glucose/lipid homeostasis, where lack of adipose tissue (lipodystrophy) is associated with extreme systemic insulin resistance and fatty liver. Diet-induced obesity is also associated with adipose tissue insulin resistance and development of fatty liver (Polyzos et al. 2017).

The direct impact of IGF1 on adipocyte function, *in vivo*, was first investigated by crossbreeding Igf1r<sup>fl/fl</sup> mice with aP2 promoter-driven Cre mice (Kloting et al. 2008). These mice had increased fat mass and adipocyte size. However, after this publication appeared, it was shown that the aP2 promoter-Cre mice were not adipocyte specific, where recombination of the floxed allele could also occur in peripheral and central nervous tissue and the adrenal medulla (Martens et al. 2010). Based on these observations, subsequent studies using the Cre/loxP system to manipulate floxed alleles in the adipocytes have turned to the adipocyte-specific adiponectin Cre mice. To this end, crossbreeding Igf1r<sup>fl/fl</sup> mice with adiponectin Cre mice resulted in a reduction in subcutaneous and brown fat mass, reduced expression of lipogenic genes in visceral fat depots and reduced levels of circulating leptin and adiponectin (Boucher et al. 2016). However, these changes did not result in appreciable changes in response to glucose and insulin challenge. Of note, knockout of the INSR in adipocytes had a more severe metabolic phenotype, while combined loss of both IGF1R and INSR resulted in lipodystrophy and ectopic accumulation of fat in the liver and muscle (Boucher et al. 2016, Softic et al. 2016). Although additional follow-up studies are required to understand the full metabolic impact of IGF1 signaling on adipocytes, it is clear the actions of IGF1 are distinct from insulin, where both are required for adipocyte maturation and adult function.

**IGF1/IGF1R plays an age-dependent role in muscle development and function**

Active muscles preferentially oxidize lipids for energy and promote the overall metabolic health (Lundsgaard et al. 2018). During myocyte development, IGF1R expression is predominant, while in the differentiated muscle, INSR expression predominates (O’Neill et al. 2016). Crossbreeding Igf1r<sup>fl/fl</sup> mice with mef-2c-73k...
promoter-Cre expressed early in development (Mavalli et al. 2010) impaired muscle fiber formation and reduced muscle weight, but did not influence glucose homeostasis. However, knockout of muscle IGF1R using the actin promoter (Acta)-driven Cre, expressed in differentiated muscle cells (O’Neill et al. 2015), did not influence normal body weight or muscle mass, but did modestly impair muscle function (wheel running). Muscle-specific INSR had a more pronounced phenotype, while combined IGFR/INSR deficiency (MIGIRKO) showed severe impairment in muscle mass leading to premature death due to respiratory complications. Surprisingly, MIGIRKO did not influence glucose or insulin tolerance.

In contrast to the muscle-specific knockout models, muscle IGFR1-lysine-arginine (MKR) mice expressing a dominant-negative mutant IGF1R in skeletal muscle and heart are diabetic with insulin resistance in muscle, liver and adipose tissue (Fernandez et al. 2001). Of note, crossbreeding the MKR mice with mice lacking both IGFR1 and INSR in the muscle (MIGIRKO) led to exacerbation of metabolic dysfunction, which could not be attributed to changes in heart function, suggesting the mutant IGFR1 can interact with receptors other than IGFR1, INSR or downstream targets to alter skeletal muscle metabolism (O’Neill et al. 2015).

Similar to skeletal muscle loss of IGFR1, loss of IGFR1 in heart using muscle creatine kinase promoter-driven Cre mice, did not promote changes in heart function or whole body glucose metabolism. However, by 6 months of age, the loss of the INSR led to a deficit in heart function, while combined loss of IGFR and INSR led to alterations in heart glucose metabolism and function, leading to death by 1 month of age (Laustsen et al. 2007). Therefore, insulin signaling, not IGF1 signaling, appears to dominate with respect to controlling aspect of mature skeletal and cardiac muscle function.

**Future lessons to be learned from age-dependent, tissue-specific inactivation/activation of IGF1/IGFR signaling**

Although tremendous progress has been made in understanding the tissue-specific role IGF1 plays in regulating metabolic function, in the majority of mouse models discussed earlier, the Igf1 or Igf1r gene is deleted early in development, where compensation by insulin/INSR or other unknown factors may contribute to the metabolic phenotype observed in the adult. Given that metabolic disease typically develops after sexual maturation, due to excess caloric intake and reduced physical activity, it is important to understand how changes in IGF1 production and tissue-specific sensitivity influence metabolic disease progression. This information is critical to identifying key targets for treatment and prevent off-target effects of pharmacologic reduction or enhancement of IGF1/IGFR signaling. To this end, animal models are required that allow for tissue-specific, inducible, Cre recombinase-mediated inactivation of floxed alleles at specific ages. As discussed previously, this strategy was first used to examine the impact of hepatocyte loss of IGF1 using the interferon- inducible Mx-Cre model (Sjogren et al. 1999, Wallenius et al. 2001). More recently, this technology has been applied to generate an adult-onset, whole-body knockdown of IGFR1 (Francois et al. 2017). Specifically, IGF1R<sup>Igf1r</sup> mice were crossed to tamoxifen-inducible ubiquitin promoter-driven Cre mice (Ubc-CRE<sup>T2</sup>), and offspring were treated with tamoxifen starting at postnatal day 3.5, 31 or 90 (adults). Complete recombination of the IGF1R<sup>Igf1r</sup> allele was confirmed in all tissues tested. Specifically focusing on adult-onset loss of IGF1R signaling, circulating GH levels were elevated leading to an increase in IGF1, consistent with the importance of negative feedback of IGF1 to suppress GH production (Romero et al. 2010, Gahete et al. 2013). This was associated with mild hyperglycemia, glucose intolerance and insulin resistance, with reduced fat mass, which may be the consequence of loss of the ‘insulin-like’ effects of IGF1, combined with the antagonist actions of GH on insulin signaling. Interestingly females, but not males, showed reduced fertility, which may be related to loss of IGF1 support on granulosa cell function (Zhou et al. 2013, Baumgarten et al. 2017). Further characterization of this unique model with respect to thyroid, adrenal, immune, adipocyte and muscle, under different physiologic and nutritional conditions, will enhance our understanding as to how IGF1/IGFR1 integrates the functions of metabolically relevant tissue to maintain adult metabolic function.

Further application of the inducible Cre recombinase system is required to ultimately understand how raising or lowering IGF1 input regulates the function of adult tissues to achieve metabolic homeostasis. However, a limitation of the inducible Cre recombinase models, as in congenital Cre-mediated-knockout models, remains tissue specificity of the promoter. In addition, in some inducible models, low levels of Cre expression may occur, ‘leak’, independent of the inducer. Also, the necessity of repeated treatment of the inducer (tamoxifen, doxycycline or interferon) can have effects independent of inducing Cre recombinase expression.
Another strategy to induce efficient, age- and tissue-specific knockdown or expression of a transgene is the use of adeno-associated viral vectors (AAVs) using tissue-specific promoters, where the serotype of the AAV also preferentially targets some tissues over others. This strategy has advantages over the use of adenoviral vectors because it is more efficient, long lasting and does not evoke an acute inflammatory response. This strategy has been most applied to study the role of factors in hepatocyte metabolism using AAV8-throxine binding globulin promoter (TBGp)-driven transgenotypes. Although this strategy has not yet been directly applied to knockdown IGF1 in adult hepatocytes, it has been used to knockdown the GH receptor. Specifically, adult \( \text{Ghr}^{-/-} \) mice were treated with a single injection of AAV8-TBGp-Cre, leading to loss of the GHR exclusively in hepatocytes. As in developmental loss of the hepatocyte GHR (\( \text{Ghr}^{-/-} \) mice crossbred to albumin promoter-driven Cre), circulating IGF1 levels are dramatically reduced and GH levels are elevated. However, in contrast to the congenital model which is thought to develop fatty liver indirectly due to GH-mediated development of systemic insulin resistance and WAT lipolysis, shifting nutrient flux to the liver (Cui et al. 2007, Fan et al. 2009, Mueller et al. 2011, Sos et al. 2011, List et al. 2014), mice with adult-onset loss of the hepatocyte GHR developed fatty liver due to enhanced de novo lipogenesis, independent of systemic insulin resistance (Cordoba-Chacon et al. 2015, Wolf Greenstein et al. 2017). Taken together, these studies demonstrate the phenotype of tissue specific, adult-onset loss-of-gene function, may not replicate that observed in tissue-specific, congenital knockout models. Therefore, the AAV strategy can be used in future studies to better understand the hepatocyte-specific role of GH/IGF1 in regulating adult metabolism. Such studies may include, but are not limited to (1) determining if adult-onset loss of hepatocyte IGF1 replicates the phenotype observed in LID mice, (2) differentiating between the direct hepatocyte actions of GH from those mediated by elevation in circulating GH due to loss of IGF1 feedback by knockout of the GHR in the adult hepatocyte, combined with reconstitution of hepatocyte IGF1 expression and (3) directly testing if hepatocyte IGF1R plays any role in regulating adult hepatocyte function.

Although the AAV delivery of transgenes represents a powerful tool, once again, the tissue specificity is dependent on the promoter used to drive transgene expression. Also, AAV does not incorporate into the genome, so if applied to rapidly dividing cells or the transgene induction enhances cell division, the vector will become diluted in subsequent cell generations. In addition, antibodies do develop to AAVs, not allowing for the sequential application of AAV vectors.

**Summary**

Although all the Cre/loxP models have limitations, use of these models has allowed us to refine our knowledge regarding how IGF1/IGF1R regulates metabolism, independent of the actions of insulin. It is clear from these studies that IGF1/IGF1R acts on many tissues to fine tune metabolism, where future applications of these strategies will continue to focus our understanding of the age-dependent, tissue-specific role IGF1/IGF1R plays in health and disease.

**Declaration of interest**

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