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Mice with gene alterations in the GH and IGF family

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Abstract

Much of our understanding of GH's action stems from animal models and the generation and characterization of genetically altered or modified mice. Manipulation of genes in the GH/IGF1 family in animals started in 1982 when the first GH transgenic mice were produced. Since then, multiple laboratories have altered mouse DNA to globally disrupt *Gh*, *Ghr*, and other genes upstream or downstream of GH or its receptor. The ability to stay current with the various genetically manipulated mouse lines within the realm of GH/IGF1 research has been daunting. As such, this review attempts to consolidate and summarize the literature related to the initial characterization of many of the known gene-manipulated mice relating to the actions of GH, PRL and IGF1. We have organized the mouse lines by modifications made to constituents of the GH/IGF1 family either upstream or downstream of GHR or to the GHR itself. Available data on the effect of altered gene expression on growth, GH/IGF1 levels, body composition, reproduction, diabetes, metabolism, cancer, and aging are summarized. For the ease of finding this information, key words are highlighted in bold throughout the main text for each mouse line and this information is summarized in Tables 1, 2, 3 and 4. Most importantly, the collective data derived from and reported for these mice have enhanced our understanding of GH action.

Keywords Growth hormone · Prolactin · Insulin-like growth factor 1 · Transgenic mice · Knockout mice · Metabolism · Cancer · Aging

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Introduction

Growth hormone (GH) helps regulate and coordinate growth and other physiological processes, including metabolism, fluid balance, immunity, and aging. The investigation of GH's actions has an extensive history. The growth-promoting activity of GH was recognized in 1921 when chronic administration of extracts from bovine pituitary glands resulted in enhanced weight gain when injected into rats [1, 2]. Then in 1936, Houssay demonstrated both the diabetogenic activity of anterior pituitary extracts and the decreased severity of diabetes in anterior-hypophysectomized dogs [3]. The protein responsible, GH, was first purified from bovine (b) pituitary extracts in 1944 [4]. Human (h) GH was purified in 1956 from cadaver pituitary glands [5], and its efficacy was established in the treatment of pediatric GH-deficient patients [6]. Purified hGH was approved for use in the US in 1958 and became the standard treatment

Table 1 Mouse lines upstream of GHR

Mouse line	Discovery (year/ lab/1st author)	Expression control	Serum GH	Serum IGF1	Size / weight	Body composition	Insulin sensitivity	Reproductive capacity	Cancer incidence	Lifespan	Original references
Snell (*Pit1*$^{-/-}$)	1929 Snell	spontaneous mutation in *Pou1f1*	↓	↓	↓	↑ muscle at 3 mo, though lower quality	↑	↓	↓	↑	[67]
Ames (*Prop1*$^{-/-}$)	1961 Schaible and Gowen	spontaneous mutation in *Prop1*	↓	↓	↓	↑ fat	↑	↓	↓	↑	[83]
hGHRH	1994 Hyde (Moore)	*Mt1*	↑	↑	n/a	n/a	n/a	n/a	n/a	n/a	[99]
GHRH-/-	2004 Salvatori (Alba)	NeoR replacing amino acid residues 1–42	↓	↓	↓ size; – weight	↑ fat	↑	↓	n/a	↑	[108]
lit/lit (*Ghrhr*$^{-/-}$)	1976 Eicher (Beamer)	spontaneous mutation in *Ghrhr*	↓	↓	↓	↑ fat	↑	↓	↓	↑	[115, 116]
MT1-hGH	1983 Brinster (Palmiter)	*Mt1*	↑	↑	↑	↑ soleus weight	n/a	↓	↑♀	n/a	[124]
hGH	1991 Isaksson (Tornell)	*Mt1*	↑	n/a	↑ size	n/a	n/a	n/a	↑♀	n/a	[132]
171hGH/CS-TG	2009 Cattini (Jin)	*LCR*	n/a	n/a	–	n/a	n/a	n/a	n/a	n/a	[135]
hGH	1991 Brem (Gunzburg)	*Wap*	n/a	n/a	–	↑ heart weight ♂	n/a	n/a	n/a	n/a	[139]
hGH	1994 Houdebine (Devinoy)	*(Rabbit) Wap*	n/a	n/a	n/a	n/a	n/a	↓	n/a	n/a	[140]
GHv	1988 Goodman (Selden)	*Mt1*	n/a	↑	↑	n/a	n/a	↓	n/a	n/a	[128]
bGH MT	1985 Palmiter (Hammer)	*Mt1*	↑	↑	↑ weight	↑ lean; ↓ fat	dysregulated	↓	↑	↓	[145]
bGH PEPCK	1988 Handon (McGrane)	*Pck1*	↑	↑	↑ weight	↑ lean; ↓ fat	↓serum glucose	↓	n/a	↓	[155]
GH-/-	2019 Kopchick (List)	VelociGene KOMP null allele/ZENUB1 reporter	↓	↓	↓	↑ fat; ↓ lean	↑; glucose intolerant	n/a	n/a	n/a	[167]
AOiGHD	2011 Kineman (Luque)	r*Gh*/Cre with iDTR	↓	↓	–	↑ fat (HFD)	↑	n/a	n/a	n/a	[168]
GHA	1990-mGHA; 1991-hGHA Kopchick (Chen)	*Mt1*	↑	↓	↓	↑ fat; ↓ lean	↑	↓	↓	–	[26, 169, 170]

"—" indicates no change; n/a indicates not available; "mo" indicates months of age; ♂ *indicates males;* ♀ indicates females

until recombinant human GH was approved for use in 1986 [7–9]. The interesting history of GH discoveries, both basic and clinical, has been recently reviewed [2, 10].

In humans, a GH-related gene cluster is located in a 78 k base pair portion of chromosome 17 [11] and contains five tandemly linked GH-related genes, in which one (*GH1*) present at the 5' end of the cluster is expressed in the anterior pituitary. Three of the other genes are expressed in the placenta, and one is a non-expressed pseudogene. *GH1* encodes a 22 kDa protein consisting of 191 amino acids following cleavage of the 26-amino acid secretory signal peptide. It contains four antiparallel α helices and has significant structural homology with prolactin (PRL) and placental lactogen [12].

GH exerts its actions by binding to a specific cell surface receptor (R). The hGH receptor (GHR) gene is located on chromosome 5, encodes a single-chain transmembrane glycoprotein composed of 638 amino acids, and is a member of the type I cytokine receptor family. After removal of its 18-amino acid secretory signal peptide, hGHR is composed of a N-terminal, 246-amino acid extracellular domain; a 24-amino acid transmembrane domain; and a C-terminal, 350-amino acid intracellular domain [13, 14]. The extracellular domain contains three disulfide bonds; two of which are essential for ligand binding [15]. The cytoplasmic domain contains two highly conserved sequences among cytokine receptors, Box 1 and Box 2. Box 1 contains nine amino acids with proline-rich and hydrophobic residues and acts as a binding site for a signal-transducing Janus kinase 2 (JAK2). The elegant work of Waters et al. provided a mechanistic model for this initiation of GH-GHR-induced intracellular signal transduction via JAK2 activation [14, 16]. That is, GHRs exist as preformed dimers in the absence of ligands [17]. Two JAK2 molecules, each bound to a GHR, are closely located; however, trans-interaction of the kinase domain of one JAK2 molecule and the pseudokinase domain of the other JAK2 inhibit each other, and the JAK2 stays inactive. Upon GH binding, the relative position of GHRs changes, resulting in JAK2 activation [16]. Activated JAK2 further phosphorylates multiple tyrosine residues on the intracellular domain of the GHR [18–20], which serves as binding sites for proteins possessing SH2 domains. The most common and best described of the GH induced intracellular signaling pathways involves signal transducer and activator of transcription (STAT) 5a and 5b molecules. STAT5 molecules are recruited to the phosphotyrosine residues on the GHR and become activated through tyrosine-phosphorylation by JAK2. Tyrosine phosphorylation of STAT molecules results in the dissociation of the STAT molecules from the receptor followed by homo- or heterodimerization and translocation to the nucleus, where they regulate the expression of GH target genes [21].

One of the negative regulators of the JAK-STAT signaling pathway is the suppressor of the cytokine signaling (SOCS) protein family. SOCS1-3 and cytokine-inducible SH2-containing protein (CISH) are implicated in the negative regulation of GH action, of which SOCS2 appears to play a major role [22]. All SOCS proteins are able to direct the ubiquitination of SH2 and N-terminal bound substrates for degradation [23]. Additionally, SOCS3 has been shown to directly inhibit the enzyme activity of JAK2 by its kinase-inhibitory region [24].

Manipulation of GH genes in animals started in 1982 when the first GH transgenic mouse was produced by Palmiter et al. using a fusion gene consisting of the promoter/enhancer of the mouse metallothionein-1 (*Mt1)* gene and the rat (r) *Gh* gene [25]. The fusion gene was microinjected into the pronuclei of fertilized mouse eggs and gave rise to giant mice, featured on the cover of a 1982 issue of Nature [25]. Usually, mice generated in this manner (microinjection of cloned DNA in fertilized mouse eggs) are termed hemizygous, signifying random incorporation of the injected DNA into the mouse genome. Breeding of hemizygous mice can result in new mouse strains containing two or more copies/alleles of the injected DNA.

Our group has employed a structure/function experimental design using transgenic mice for the past three decades. During this time, we discovered that substitution of one amino acid, Gly119 in bGH (Gly120 in hGH), by several amino acids (except Ala) resulted in a competitive antagonist of the GHR [26–28]. Expression of this GHR antagonist in vivo resulted in dwarf mice [26]. At that time, we predicted that GH interacted with a secondary target protein to explain the mechanism of GHR antagonism [26]. Later, Cunningham et al. demonstrated that, indeed, one GH molecule interacted with two GHR molecules to initiate signal transduction [29].

We and others have also employed gene disruption, or 'knockout' (KO) technology, to globally disrupt *Gh*, *Ghr*, or other genes upstream or downstream of GH or its receptor. Our group has focused on *Ghr* gene disruptions. *Ghr-/-* (also called GHRKO or GHR-/-) mice are dwarf and obese, with low insulin-like growth factor 1 (IGF1) and high GH levels [30]. Importantly, since the mice lack functional GHRs, they are GH insensitive or resistant and, thus, more insulin sensitive than wild-type (WT) littermates [31]. They are also resistant to high-fat diet (HFD)-induced type 2 diabetes (T2D) [32] and cancer [33–36]. Surprisingly, GHR-/- mice have a longer lifespan than WT mice [37]. One GHR-/- mouse lived a week short of five years and set the standard for the Methuselah Mouse Prize as the world's longest-lived laboratory mouse (http://reason.com/archives/2004/08/18/methuselah-mouse). Since then, our group, as well as others, have developed many tissue-specific GHRKO mice, which will be described below [38–65].

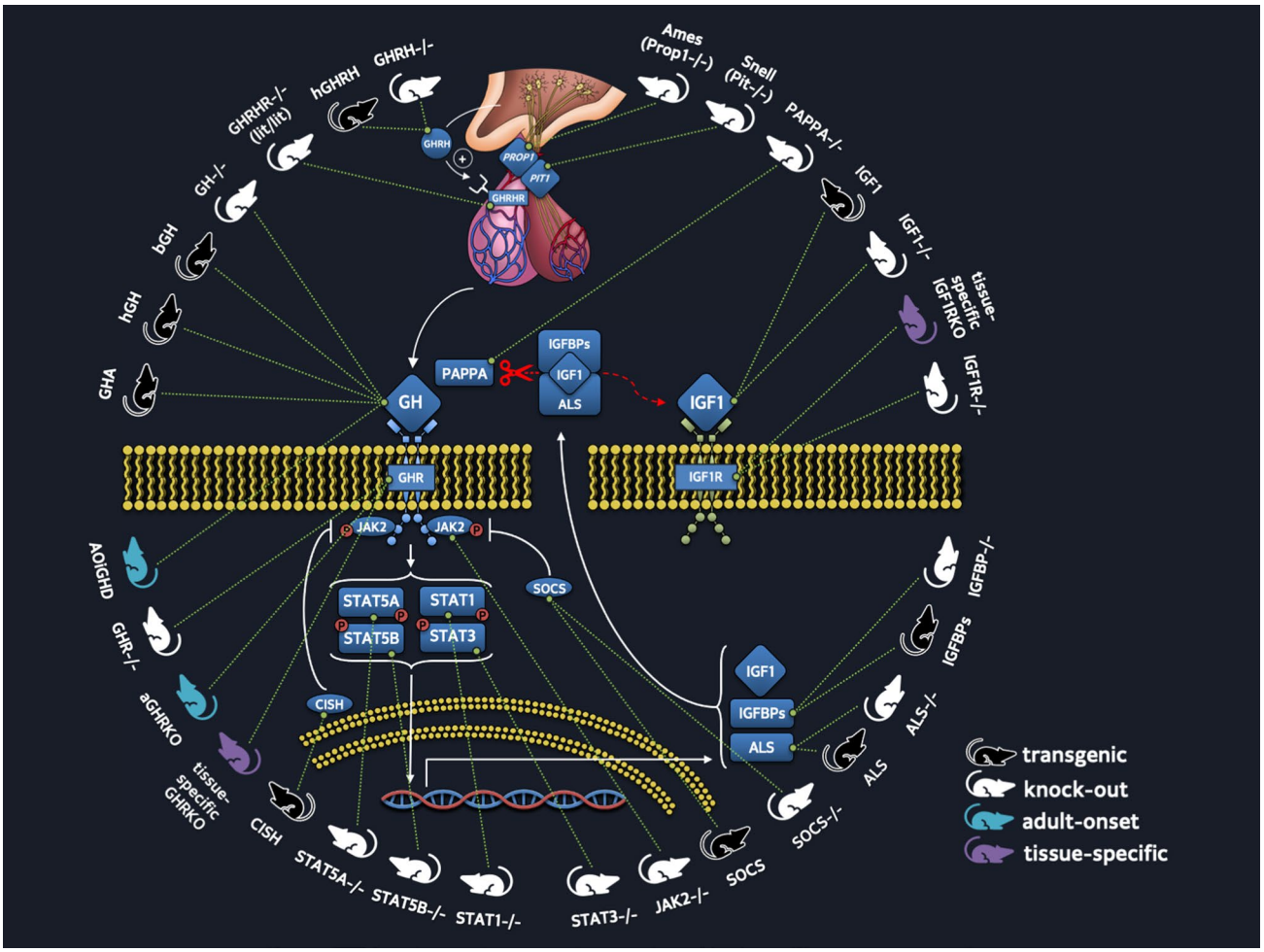

Fig. 1 Summary of transgenic and knockout mouse lines with altered GH/IGF action. The diagram shows proteins involved in the regulation of GH secretion, GH induced intracellular signaling, and the production of IGF1, ALS, IGFBP3. The different mouse colors represent mice with a transgene overexpressed (black), mice with genes that have been knocked out globally (white), adult-onset knockouts (blue) or tissue-specific knockouts (purple) (Color figurre online)

Internally, the task of 'keeping up' with the various genetically manipulated mouse lines within the GH/IGF1 family has been daunting. Thus, in this review, we have critically reviewed the literature related to the initial characterization of many natural and gene-manipulated mice related to the actions of GH, prolactin (PRL), and IGF1. We acknowledge that additional phenotypic/biochemical/endocrine data may exist for these mouse lines but consider this beyond the scope of this review.

Below, we have organized the mouse lines by modifications made to constituents of the GH/IGF family either upstream or downstream of GHR or to the GHR itself. Throughout this review, we define global homozygous null (-/-) mice as knockouts (KOs) and heterozygotes as +/−. For all mouse lines discussed, we recognize the individual(s) who generated the mice along with the date and laboratory name. Mice with 'upstream' modifications include GH transgenic, GH-/-, GH releasing hormone (GHRH) transgenic, GHRH-/-, GHRHR-/-, GHR antagonist, PRL-/-, PRLR-/-, and PRLR antagonist transgenic mouse lines. GHR modifications include global GHRKO (GHR-/-), various tissue-specific GHRKOs, and temporal GHRKOs. Modifications downstream of GHR include those made to several signal transduction molecules including JAK2 and STAT5, IGF1 and IGF1R, IGF binding proteins (BPs), ALS and PAPP-A transgenic, and KOs. Importantly, available data on the effect of altered gene expression on growth, GH/IGF1 levels, body composition, reproduction, diabetes, metabolism, cancer, and aging are summarized for each mouse line and in Tables 1, 2, 3, and 4. For ease of finding this information, key words are shown in bold throughout the main text. Also, to aid the reader, we have divided each section into "origin" and "phenotype". To provide additional context, Fig. 1 illustrates the mouse lines with alteration in

the GH/IGF family and relevant upstream and downstream constituents referred to in this review. Figure 2 compares several transgenic and null mouse lines related to GH action for adiposity, metabolism, cancer incidence, and longevity. Figure 3 provides a timeline of when the mouse lines were generated. Overall, we hope this review will provide a comprehensive reference to investigators by collating numerous results and references relating to specific mouse lines within the GH/IGF1 family. Importantly, the collective data derived and reported for these mice have enhanced our understanding of GH action.

Mouse lines upstream of GHR

Circulating GH is produced by the acidophilic somatotroph cells of the anterior pituitary gland. The transcription factors Prophet of Pit-1 (PROP1, gene product of *Prop1*), pituitary-specific transcription factor 1 (PIT1; gene product of *Pou1f1*), and GH releasing hormone receptor (*Ghrhr*) are sequentially expressed in the developing pituitary and are together responsible for the regulation of GH production. PROP1 is critical for both the development of anterior pituitary cell types (somatotrophs, gonadotrophs, lactotrophs, thyrotropes) and in inducing PIT1 expression. PIT1 regulates further differentiation of the pituitary cell lineages, as well as the expression of the *Ghrhr* gene, which in turn, promotes the clonal expansion of these cells [66]. Pituitary GH production is positively regulated by hypothalamic GHRH and gastric ghrelin, and negatively by hypothalamic somatostatin (SRIF) and endocrine IGF1. Each of these proteins binds to its cognate receptors – GHRH receptor (GHRHR), ghrelin receptor / GH secretagogue receptor (GHS-R), SRIF receptor subtypes, and IGF1R, in order to elicit their actions [66]. Mouse lines discovered or engineered to focus on each of these regulatory nodes of GH production have allowed us to understand developmental regulation and downstream physiological effects in a new light. Each will be discussed briefly below and is summarized in Table 1.

Snell (*Pit1-/-*) and Ames (*Prop1-/-*)

The earliest mouse lines discovered to have a somatotrophic deficiency in GH production resulting in distinctive phenotypes were Snell and Ames mice. These mice have been at the center of hundreds of published research reports since their discovery and are essential in the current understanding of the action of GH.

Snell dwarf mice (*dw/dw; Pit1-/-; Pou1f1-/-*)

Origin In 1921, George Snell (Nobel Prize, 1980) observed a new recessive Mendelian genotype of dwarfism in his mouse stock [67]. This Snell dwarf mouse (also termed *dw/dw* or *Pit1-/- or Pou1f1-/-*) represented the second case of hereditary dwarfism in rodents, following a previous report in guinea pigs [67].

Phenotype Snell reported the **length** of this mature dwarf mouse reaches that of a 16–17-day-old 'normal' mouse. The Snell dwarf mice also weigh only one fourth of their

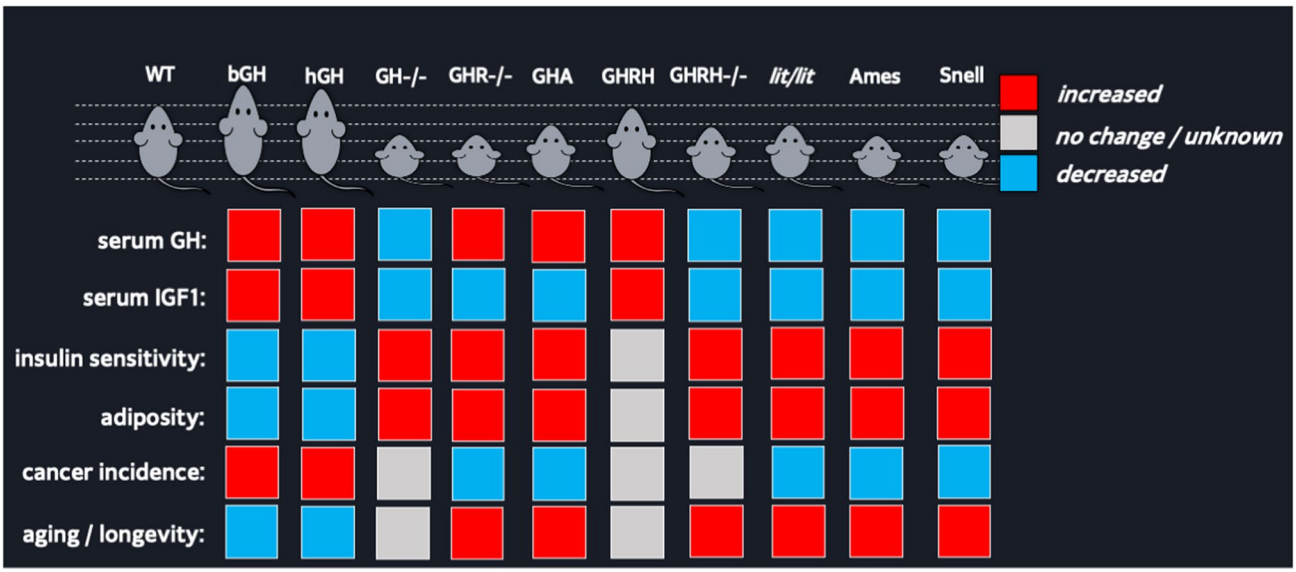

Fig. 2 Phenotypic comparison among several transgenic and knockout mouse lines with altered GH action. The mice compared are depicted at the top of the figure along with their relative size and the altered genes. The red box indicates increased growth, the blue box indicates decreased growth, and the grey box indicates no change in growth relative to WT controls (Color figure online)

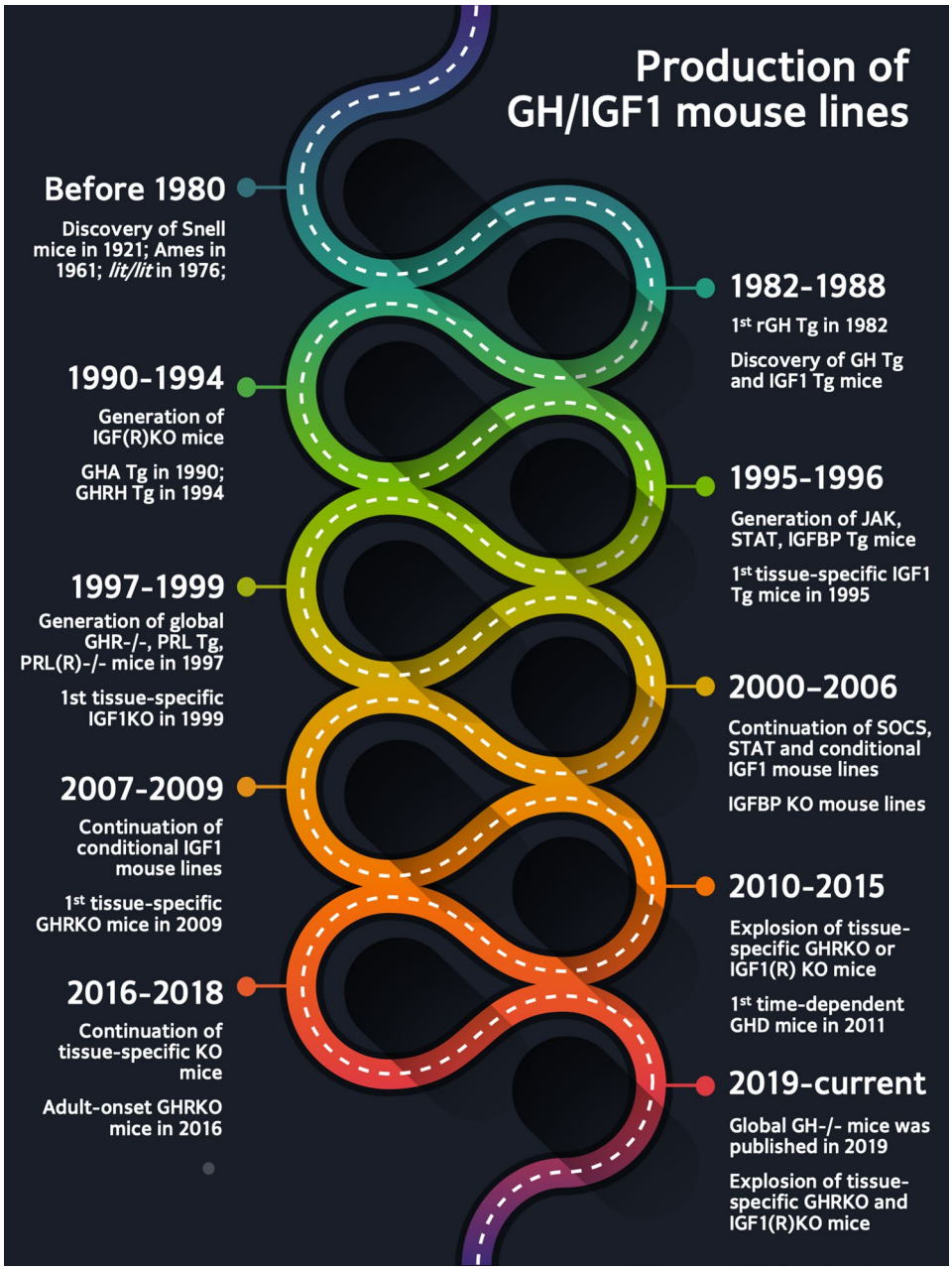

Fig. 3 Production timeline of GH/IGF1 mouse lines. Before 1980, only three mouse lines related to the GH/IGF family had been discovered (Snell, Ames and *lit/lit* mice). Through transgenic and 'knock-out' technology, generation of different mouse lines with altered GH/IGF family signaling increased. The 1990s were mainly dedicated to transgenic and global knockouts associated with altered GHR, IGF1, PRL and JAK/STAT. The first tissue-specific mouse line was generated in 1999, foretelling more than two decades of additional conditional GHR and IGF1 knockouts. To date, there are 137 mouse lines dedicated to exploring the effects of the GH/IGF family; all these mouse lines contribute to a deeper understanding of the impact of GH and IGF1 on health and disease. *Tg* transgenic mouse lines, *KO* knockout mouse lines

wild-type counterparts [67]. Snell mice have since been part of numerous studies worldwide and are characterized by pituitary hypoplasia, with a combined deficiency of **GH**, PRL, and thyroid-stimulating hormone (TSH), which later was found to be due to a spontaneous point mutation in the *Pou1f1* gene [68]. This mutation abrogates the interaction of the PIT1, a POU family transcription factor, with its target transcriptional regulatory sequence. This, in turn, leads to improper formation and dysfunction of the pituitary somatotrophs, lactotrophs, and thyrotrophs [68], as well as nearly undetectable levels of serum **IGF1** [69]. The severely suppressed growth of Snell dwarf mice [70] is partially restored following thyroxine and GH replacement therapy [71].

A 'diabetogenic' effect of GH has been known since 1930s [72]. Subsequent studies have revealed that GH induces insulin resistance primarily in peripheral tissues [73] via (i) elevated free fatty acid (FFA) from increased lipolysis leading to increase of diacylglycerol and ceramides and suppression of IRS1 activation in liver and skeletal muscle; (ii) elevated FFA induced increase of acetyl-CoA, leading to increased gluconeogenesis in liver and kidney; (iii) upregulation of PI3K regulatory p85a subunit in mouse white adipose tissues (AT); and (iv) upregulated SOCS expression [74]. Insulin resistance is an important metabolic hallmark in patients with acromegaly [75] while congenital GH insensitivity in Laron Syndrome (LS) individuals is associated

Table 2 Global, temporal and tissue-specific GHRKO mouse lines

Mouse line		Discovery (year/lab/1st author)	Expression control	Serum GH	Serum IGF1	Size / weight	Body composition	Insulin sensitivity	Lifespan	Original references
Global GHRKO	GHR-/-	1997 Kopchick (Zhou)	NeoR replacing exon4 of *Ghr*	↑	↓	↓	↑ fat; ↓ lean	↑; glucose intolerant	↑	[30, 31]
Global Adult Onset	aGHRKO	2016 Kopchick (Junnila)	ROSA26/Cre	↑	↓	↓	↑ fat; ↓ lean	↑; glucose intolerant	↑ maximal lifespan ♀	[60]
Liver-specific KO	GHRLD	2009 Sperling (Fan)	albumin/Cre	↑	↓	–	–	↓; glucose intolerant	n/a	[38]
	LiGHRKO	2014 Kopchick (List)	albumin/Cre	↑	↓	↓ after 5 mo	↑ fat at early ages; ↓ adulthood	↓ glucose homeostasis	–	[48]
	aLivGHRkd	2015 Kineman (Cordoba)	thyroxin-binding promotor/Cre	↑♂	↓	n/a	n/a	n/a	n/a	[55]
	Li-GHRKO	2016 Yakar (Liu)	albumin/Cre	n/a	↓	–	↑ fat	↓; increased blood glucose	n/a	[59]
	L-Ghr-/-	2019 Liang (Fang)	albumin/Cre	↑	↓	–	–	hypoglycemic under CR	n/a	[63]
Muscle-specific KO	ΔGHR	2010 Clemens (Mavalli)	fem-2c-73 k/Cre	–	–	↑ weight	↑ fat	↓	n/a	[40]
	mGHRKO	2012 LeRoith (Vijayakumar)	*Ckmm*/Cre	–	–	–	↓ lean	–	n/a	[42]
	MuGHRKO	2015 Kopchick (List)	*Ckmm*/Cre	–	–	–	–	↑ glucose homeostasis ♂	↑	[54]
Brain-specific KO	$Lepr^{EYFP\Delta GHR}$	2017 Sadagurski (Cady)	leptin receptor/Cre	–	–	–	–	↓ glucose homeostasis	n/a	[61]
	AgRP-IRES-Cre	2019 Donato (Furigo)	AgRP IRES/Cre	n/a	n/a	–	–	–	n/a	[64]
	LepR-IRES-Cre	2019 Donato (Furigo)	LepR IRES/Cre	n/a	n/a	↑	↓ fat	n/a	n/a	[64]
	Nestin-Cre	2019 Donato (Furigo)	Nestin/Cre	n/a	n/a	↑	↑ lean	n/a	n/a	[64]
Fat-specific KO	FaGHRKO	2013 Kopchick (List)	aP2/Cre (aka, FABP4/Cre)	–	↑♂	↑ weight	↑ fat, ↑fluid; ↑ lean mass ♀	–	↓	[44]
	AdGHRKO	2019 Kopchick (List)	adiponectin/Cre	–	–	–	↑ fat; ↑brown AT ♀	↑	n/a	[220]
	Fat-Ghr-/-	2019 Liang (Fang)	adiponectin/Cre	n/a	n/a	n/a	–	n/a	n/a	[63]
Macrophage/monocyte-specific KO	GHRMacD	2010 Menon (Lu)	*Lyzs*/Cre	n/a	n/a	–	↑ epididymal fat on HFD	–; ↓ on HFD	n/a	[39, 47]
Beta cell-specific KO	βGHRKO	2011 LeRoith (Wu)	insulin/Cre	–	–	–	–	n/a	n/a	[41]

Table 2 (continued)

Mouse line		Discovery (year/lab/1st author)	Expression control	Serum GH	Serum IGF1	Size / weight	Body composition	Insulin sensitivity	Lifespan	Original references
HSC-specific KO	$Ghr^{fl/fl}$;$Vav1^{Cre/+}$	2014 Rossi (Stewart)	*Vav1*/Cre	n/a	n/a	n/a	n/a	n/a	n/a	[50]
Bone-specific KO	DMP-GHRKO	2016 Yakar (Liu)	*Dmp1*/Cre	↑ 8 wk, – 12 wk	–	–	–	n/a	n/a	[224]
Heart-specific KO	iC-GHRKO	2016 Kopchick (Jara)	*Myh6*/Cre	n/a	–; ↓ 12.5 mo	–	↓ fat; ↑ lean	↑ 6.5 mo; ↓ 12.5 mo	n/a	[58]
Intestine-specific KO	IntGHRKO	2019 Kopchick (Young)	villin/Cre	n/a	n/a	–	–	↓♀	n/a	[65]

"—" indicates no change; n/a indicates not available. "wk" indicates weeks of age, "mo" indicates months of age; ♂ *indicates males;* ♀ indicates females

with improved insulin sensitivity [76]. In agreement, GH-deficient Snell mice display a low utilization of circulating glucose, reduced serum insulin levels, and increased **insulin sensitivity**, as well as decreased free radical-induced damage (lower protein carbonyl content) [77].

Snell mice show an increase in **lifespan** compared to WT mice, with a 50% and 29% increase in males and females, respectively [52, 70] and are protected from a number of age-related pathophysiologies, including neurological decline [78], collagen denaturation [70], cataract development, glomerular damage and **cancer** [79]. However, these dwarf mice have defects in hearing, musculature, immunity, and **reproduction**. At three months of age, Snell mice have more muscle mass as compared to WT but also a compromised muscle quality and poor fatigue recovery [80]. Defects in reproductive capacity of Snell mice include sterility and delayed testicular growth [81]. Hormone replacement (GH + thyroxine + TSH) restores fertility in male mice but not in females [71]. Congenital deafness due to a lack of TSH is partially rescued in these mice by thyroid hormone treatment [82]. Overall, results from the Snell mouse were the first to strongly implicate GH in lifespan determination.

Ames mice *(Prop1-/-)*

Origin The Ames mouse was first reported in 1961 by Schaible and Gowen [83]. These mice have a spontaneous recessive mutation in the *Prop1* gene, necessary for expression of PIT1, which results in the lack of somatotrophs, lactotrophs, and thyrotrophs similar to that seen in Snell mice.

Phenotype Ames mice have a severe lack of **GH**, PRL, and TSH, and very low circulating **IGF1** [84]. Ames mice are small with a low **body weight** [85] and are one-third the **body size** of WT mice. Although Ames mice have increased **adiposity**, they exhibit lower circulating blood glucose and enhanced **insulin sensitivity** due to the lack of GH's diabetogenic effect [86, 87]. These dwarf mice are protected from HFD-induced insulin resistance unlike age-matched WT mice [88]. Additional distinct physiological characteristics of Ames mice include significantly higher brown AT [89], lower resting core body temperature [90], and a reduced senescent cell burden in white AT [91]. Similar to Snell mice, Ames mice are also markedly resistant to standard oxidative stress inducers like paraquat and diaquat, even at older ages [92]. Moreover, Ames dwarf mice have a significantly lower incidence of fatal **neoplasms**, including lung adenocarcinoma [93]. Similar to Snell mice, Ames mice also exhibit a markedly increased **lifespan**, with males living an average of ~ 50% longer and females living > 60% longer than their WT littermates [94, 95]. Interestingly, a further extension of lifespan is observed in these mice when subjected to caloric restriction (CR), which indicates that the anti-aging effects exhibited via CR and the *Prop1* gene

Table 3 Global and tissue-specific IGF1 transgenic and IGF1(R) KO mouse lines

Mouse lines		Discovery (year/lab/first)	Expression control	Serum GH	Serum IGF1	Size / weight	Body composition	Insulin sensitivity	Reproductive capacity	Cancer incidence	Lifespan	Original references
Global	IGF1	1988 Palmiter (Mathews)	*Mt1* + human *IGF1* cDNA	↓	↑	↑ weight	n/a	n/a	–	n/a	n/a	[257]
	IGF2+/–	1990 Robertson (DeChiara)	NeoR replacing exon 2 of *Igf2*	n/a	n/a	↓	n/a	n/a	–	n/a	n/a	[259]
	IGF1-/-	1993 Efstratiadis (Liu)	deleting exon 4 of *Igf1*	n/a	↓	↓	n/a	n/a	n/a	n/a	neonatal lethality; ↓	[260]
	IGF1R-/-	1993 Efstratiadis (Liu)	deleting exon 3 of *Igf1r*	n/a	n/a	↓	n/a	n/a	n/a	n/a	complete neonatal lethality	[260]
Liver	TTR-IGF-1	2006 Xu (Liao)	transthyretin	↓	↑	↑	↑ lean	↑ glucose tolerance	–	–	n/a	[263]
	HIT	2009 LeRoith (Wu)	transthyretin	–	↑	↑;↑L,K,S	↓fat	–	–	n/a	n/a	[265]
	KO-HIT	2009 LeRoith (Wu)	transthyretin + IGF1 null	–	↑	↓at birth; – at 16 wk	↓fat	–	–♂;↓♀	n/a	No prenatal lethailty	[265]
	GHRKO-HIT	2013 Yakar (Wu)	transthyretin	n/a	–	↓; ↓K,S,H	↓ muscle; ↑brown AT	–	n/a	n/a	n/a	[267]
	LID	1999 LeRoith (Yakar)	albumin/Cre	↑	↓	–	–	↓	–	↓	↑ ♀	[268]
	LI-IGF1-/-	1999 Ohlsson (Sjögren)	Mx/Cre induced at ~ 1 mo	↑	↓	–; ↓K, ↑L at 3mo; ↓ wight at 13 mo	↓ femur length at 3 mo; ↓ fat at 13 mo	↓	–	n/a	↑ mean lifespan ♀	[270]
Adipose	IGF-1R^{aP2Cre}	2008 Blüher (Kloting)	aP2/Cre	–	↑	↑	↑ fat	–	n/a	n/a	n/a	[273]
	F-IGFRKO	2016 Kahn (Boucher)	Adipo/Cre	n/a	↑	–	↓ fat	–	n/a	n/a	n/a	[277]
Brain	bIGF1RKO+/–	2008 Holzenberger (Kappeler)	nestin/Cre	↓	↓	↓	↑ fat	↓	infertile (-/-)	↓	↑ mean lifespan (+/–); – (-/-)	[278]

Table 3 (continued)

Mouse lines		Discovery (year/lab/first)	Expression control	Serum GH	Serum IGF1	Size / weight	Body composition	Insulin sensitivity	Reproductive capacity	Cancer incidence	Lifespan	Original references
Muscle	Skeletal Muscle IGF1	1995 Schwartz (Coleman)	avian skeletal α-actin driving human *IGF1*	n/a	–	–	↑ superficial gluteus muscle mass	n/a	n/a	n/a	n/a	[279]
	MKR	2001 LeRoith (Fernandez)	MCK + dominant-negative IGF1R	n/a	n/a	↓ weight	n/a	↓	n/a	n/a	n/a	[280]
	MIGIRKO	2015 Kahn (O'Neil)	ACTA1/Cre	n/a	n/a	↓	↓ muscle	–	n/a	n/a	↓	[281]
	M-IGF1R KO	2016 Kahn (O'Neil)	ACTA1/Cre	n/a	n/a	–	–	n/a	n/a	n/a	↓	[281, 282]
Cardiac	IGF1	1996 Anversa (Reiss)	α-MHC + human *IGF1*	n/a	↑	– at birth, ↑ on day 210; ↑ organ weights	–	n/a	↓ using both Tg mice as breeders	n/a	–	[283]
	Cardiomyocyte IGF-1	2007 Rosenthal (Santini)	α-MHC + rat *mIGF1*	n/a	n/a	n/a	n/a	n/a	Hets as breeders	n/a	n/a	[284]
	CIGF1RKO	2008 Abel (Kim)	Myosin 6/Cre	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	[285]
	iCMIGF-IRKO	2012 Gödecke (Moellendorf)	4-OHTX inducible Myosin 6/Cre at 3&11 mo	n/a	↑	↓ size in newborns	n/a	–	n/a	n/a	n/a	[286]
Endothelial	hIGFREO	2012 Kearney (Imrie)	TIE2 + h*IGF1R*	n/a	n/a	–	n/a	–	n/a	n/a	n/a	[287]
	EC IGF-1RKO	2011 Kearney (Abbas)	TIE2/Cre, male 3–5 mo	n/a	n/a	n/a	n/a	–	n/a	n/a	n/a	[288]
	Endothelial IGF-1RKO	2015 Cheng (Liang)	VE-Cadherin/Cre, male 3–4 mo	n/a	n/a	–	n/a	n/a	–	n/a	n/a	[289]
Myeloid (Macrophage)	MIKO	2016 Dixit (Spadaro)	LysM/Cre	n/a	n/a	n/a	↑ fat	↓ on HFD	n/a	n/a	↑ pro-longevity effects	[290]
	MΦ-IGF1RKO	2016 Delafontaine (Higashi)	LysM/Cre x IGF1R/APOE-/-FLOX	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	[292]

Table 3 (continued)

Mouse lines		Discovery (year/lab/first)	Expression control	Serum GH	Serum IGF1	Size / weight	Body composition	Insulin sensitivity	Reproductive capacity	Cancer incidence	Lifespan	Original references
Bone	OC-IGF-1 Tg	2000 Clemens (Zhao)	osteocalcin + rat *IGF1*	n/a	–	–	n/a	n/a	n/a	n/a	n/a	[293]
	Osteoblast IGF1 Tg	2006 Kream (Jiang)	rat Col1a1 + murine *Igf1*	n/a	↑	↑ weight ♂	n/a	n/a	n/a	n/a	n/a	[294]
	Osteoblast IGF1R KO	2002 Clemens (Zhang)	osteocalcin/Cre	n/a	n/a	–	n/a	n/a	n/a	n/a	n/a	[296]
	OBIGF1R$^{-/-}$	2015 Bikle (Wang)	Col1α1/Cre	n/a	n/a	–	n/a	n/a	n/a	n/a	n/a	[298]
	Chondrocyte IGF1 KO	2007 Mohan (Govoni)	Col2α1/Cre	n/a	–	↓ length	↓ bone size, weight	n/a	n/a	n/a	n/a	[299]
	Osteocyte IGF1 KO	2013 Lau (Sheng)	*Dmp-1*/Cre	n/a	–	n/a	↓ femur length	n/a	n/a	n/a	n/a	[301]
	DMP-IGF-1R KO	2016 Yakar (Liu)	DMP-1/Cre	↑ at 8 wk; – at 16 wk	–	–	n/a	n/a	n/a	n/a	n/a	[224]
Ovarian granulosa cells	IGF1R^{gcko}	2017 Stocco (Baumgarten)	ESR2 + CYp19	n/a	n/a	n/a	n/a	–	infertile	n/a	n/a	[303]
Pancreatic beta cells	β cell IGF-1R KO	2002 Efstratiadis (Xuan); Kahn (Kulkarni)	rat insulin/Cre	n/a	n/a	n/a	n/a	glucose intolerant; ↓ insulin secretion	n/a	n/a	n/a	[304, 305]
Steroidogenic cells	Steroidogenic cells IGF-1R KO	2018 Nef (Neirijnck)	human P450SCC/Cre	n/a	n/a	–	↓ testicular weight	n/a	–	n/a	n/a	[307]
Somatotroph	SIGFRKO	2010 Radovick (Romero)	rGHpCre	↑	↑	↓ weight;— length; ↑ L, S	↓ fat	–	n/a	n/a	n/a	[308]
Thyrocyte	Thyrocyte specific IGF-1R KO	2011 Krohn (Muller)	thyroglobulin/Cre	n/a	–	↑weight in males; ↓ in females	alteration in perigonadal fat mass	↑ males	n/a	n/a	n/a	[310]

"—" indicates no change; n/a indicates not available. "wk" indicates weeks of age, "mo" indicates months of age. "Tg" indicates transgenic mice. " +/– " indicates heterozygous; "-/- " indicates homozygous. ♂ *indicates males;* ♀ indicates females; K, kidney, L, liver, S, spleen, H, heart.

Table 4 IGFBP transgenic and KO mouse lines

Mouse lines		Discovery (Year/Lab/first)	Expression control	Serum GH	Serum IGF1	Size / weight	Body composition	Insulin sensitivity	Reproductive capacity	Cancer incidence	Lifespan	Original references
IGFBP1	human IGFBP1 transgene	1995 Dai (D'Ercole)	mouse *Mt1*	n/a	n/a	↓ weight	not consistent	↓	↓ ♀	n/a	n/a	[312]
	IGFBP1 KO	2003 Taub (Leu)	NeoR replacement of promotor and exons 1–2	n/a	↑ before 4 mo	–	n/a	–	n/a	↓ prostate tumor size; ↓ proliferation, but not incidence	n/a	[318]
IGFBP2	IGFBP2 transgene	1999 Wolf (Hoeflich)	CMV	–	–	–	↑ fat	↑ sensitivity; ↓ serum insulin	n/a	↓ colorectal tumor growth with induced carcinogenesis	n/a	[320]
	IGFBP2 KO	2000 Pintar (Wood)	NeoR replacement of exon 3	–	–	–weight;↑L; ↓S,H,K	n/a	–	–	n/a	n/a	[323]
IGFBP3	human IGFBP3 transgene	1995 Molnar (Murphy)	mouse *Mt1*	n/a	n/a	↑ S,L,H	↑ fat	n/a	–	n/a	n/a	[325]
	IGFBP3 KO	2006 Pintar (Ning)	NeoR replacement of exon1-3	n/a	n/a	↑ weight until 22 wk; – afterward	–	–; impaired glucose homeostasis on HFD	n/a	↑ lung cancer tumorigenesis	n/a	[328]
IGFBP4	IGFBP4 transgene	1998 Fagin (Wang)	murine cDNA driven by α-actin	n/a	n/a	↓ thymus	n/a	n/a	n/a	n/a	n/a	[331]
	IGFBP4 KO	2006 Pintar (Ning)	NeoR replacement of exon1	n/a	–	↓	↓ fat, femur length	n/a	–	n/a	n/a	[328]
IGFBP5	IGFBP5 transgene	2002 Flint (Tonner)	β-lactoglobulin	n/a	↑	↓ weight	↓ lean	n/a	↓ fertility ♀	n/a	↑ neonatal mortality	[335]
	IGFBP5 KO	2006 Pintar (Ning)	NeoR replacement of exon1	n/a	–	–	↑ fat	mild glucose intolerance	–	n/a	n/a	[328]
IGFBP6	IGFBP6 transgene	2004 Babajko (Bienvenu)	glial fibrillary acidic protein promoter/ enhancer	n/a	↓ 15 d; – 1, 3 mo	↓ weight; growth retardation up to 3 mo	n/a	mild insulin resistance w/ diet-induced obesity	↓	n/a	n/a	[340]

"—" indicates no changes; n/a indicates not available; "d" indicates days of age, "wk" indicates weeks of age, "mo" indicates months of age; ♀ indicates female. K, kidney, L, liver, S, spleen, H, heart

mutation occur through independent mechanisms [96, 97]. Despite these positive attributes, Ames mice suffer from a number of **reproductive** deficiencies. They are hypogonadal with decreased levels of gonadotropin and testosterone [98]. Although some male Ames mice remain fertile, all females are sterile. Further, Ames mice suffer from auditory deficits but, unlike in the Snell mice, are almost completely rescued by early life thyroid hormone treatment [82].

Overall, Ames and Snell mice present similar deficiencies in three pituitary hormones (GH, PRL, TSH), resulting in similar phenotypes of extended lifespan, improved oxidative stress response, improved insulin sensitivity, and reduced incidence of cancer. Numerous studies surrounding them have deepened our understanding of the endocrine control of specific aspects of health, disease, and lifespan.

Growth hormone releasing hormone (GHRH) and its receptor (GHRHR)

GHRH and its cognate receptor, GHRH receptor (GHRHR), promote GH release primarily along the hypothalamus-pituitary axis. Human patients with isolated GH deficiency (IGHD) are often found to have inactivating mutations in the *GHRHR* or *GHRHR* gene locus. Therefore, a better understanding of this ligand-receptor pair in modulating the physiological effects of GH has clinical relevance. Below we discuss three mouse lines associated with the GHRH-GHRHR pair.

Human GHRH transgenic mice

Origin In order to study the effects of GHRH in modulating the GH/IGF axis, Hyde and colleagues developed a human GHRH transgenic mouse (hGHRH) in 1994 using the *Mt1* gene promoter/enhancer to drive expression of the *hGHRH* gene [99].

Phenotype GHRH transgenic mice have increased serum concentrations of mouse **GH**, PRL and **IGF1** and are significantly larger in **body size** than WT mice [99]. As such, they are a mouse model of pituitary associated acromegaly. Also, endogenous hypothalamic GHRH levels are significantly suppressed, while levels of somatostatin (SST or SRIF) and SST receptor subtypes are elevated compared to littermate controls [100]. The upregulation of GHRH action in mice leads to massive hyperplasia of mammosomatotrophs observable at 8-months of age [101]. In adulthood (16–24-months age), pituitary adenomas immunoreactive for GH and PRL are often observed [102–104]. Transgenic GHRH mice were employed to study the effect of GH in regulating the production of neuropeptides from the anterior pituitary. In the anterior pituitary of GHRH transgenic mice, the tachykinins (substance-P and neurokinin A) are markedly increased in males and females [105]. Tachykinins, found in nearly all vertebrates, are one of the largest family of neuropeptides involved in neuronal excitation, behavioral response, vasodilation, and regulation of smooth muscle contraction. Also, the hyperpolarizing neuropeptide galanin, found in human central nervous system (CNS) and gut, are known to be produced by pituitary cells following GH stimulation in vitro [106]. In the pituitaries of male GHRH transgenic mice, galanin mRNA and peptide contents are also highly upregulated [99]. However, the levels of the neuropeptide, vasoactive intestinal polypeptide, in the anterior pituitary of male hGHRH transgenic mice are half of that of nontransgenic animals [107]. No data on the lifespan of these hGHRH transgenic mice are available.

GHRHKO mice (*Ghrh*-/- or GHRH-/-)

Origin The GHRHKO mouse (*Ghrh-/-* or GHRH-/-) was generated as a new mouse line of congenital GH deficiency in 2004 by Alba and Salvatori. Amino acid residues 1–42 of the *Ghrh* gene were replaced by a neomycin resistance (NeoR) gene [108].

Phenotype GHRH-/- (*Ghrh-/-*) mutant mice exhibit highly reduced levels of pituitary *Gh* mRNA and protein and reduced liver ***Igf1*** mRNA and serum IGF1 [108]. Growth retardation in the null animals is first detected at 3 weeks of age, and null mice are 60% the **body size** of either *Ghrh* +/+ or *Ghrh* +/− littermates by 12-weeks [108]. GHRH analogs, acting as agonists of the cognate receptor, improve body length and body weight [109]. GHRH-/- mice have increased intra-abdominal and subcutaneous fat depots, concomitant with an increase in food intake [110, 111]. Increased body temperature, intrascapular brown AT, and thermogenesis is observed in GHRH-/- mice, which could be a function of the increased metabolic rate of a smaller sized mouse to maintain body-temperature [111, 112]. Adiponectin levels are suppressed in both intra-abdominal and subcutaneous white AT depots, while it is elevated in the serum of these animals [110]. Despite an increased adiposity, **insulin sensitivity** is markedly improved in null mice and is found to be associated with decreased TOR signaling in white AT [113]. These GHRH-/- dwarf mice are long-lived with median **lifespan** increased in males and females by 50% and 43%, respectively. Maximal lifespan is increased by 18% in males and 33% in females [113]. CR also significantly increases overall survival along with both relative and maximal lifespan, indicating an additive effect especially in females [113]. Microarray analysis reveals several differentially regulated genes in the liver of GHRH-/- mice compared to WT littermates, wherein expression of multiple xenobiotic detoxification genes are dramatically increased [113]. The reported **reproductive deficiencies** in these null mice include suppressed rates of apoptosis and lipid peroxidation in testes of adult GHRH-/- mice compared to controls

[114]. As another model of congenital GH/IGF1 deficiency, GHRH-/- mice share multiple phenotypes of the Snell and Ames mice.

GHRHRKO mice (little; *lit/lit*; *Ghrhr-/-* or GHRHR-/-)

Origin In 1976, Beamer and Eicher first reported the 'little' (or *lit/lit*) mouse, a new dwarf mouse deficient in GH and PRL due to a homozygous missense mutation in the *Ghrhr* gene [115, 116].

Phenotype This dwarf had very low levels of **GH** and, consequently, IGF1 [117]. The serum GH levels in these mice are only 1% of those of WT controls [118], and serum **IGF1** and **IGFBP3** are also highly reduced [118, 119], while IGFBP1, 2, and 4 remain unaffected [119]. Low serum leptin [70] and reduced PRL levels are observed in the *lit/lit* mice [115]. The **body weight** of these mice is about 2/3rd that of WT mice [70, 118], along with reduced levels of body fluid, protein and minerals. The *lit/lit* mice exhibit abnormally larger AT, especially in males [119]. Numerous results show that the growth of several **cancers**, including sarcoma and prostate tumor implants [120, 121], is reduced in this GH deficient mouse. Also, MCF7 breast cancer xenograft growth is reduced by almost half in *lit/lit* mice compared to WT controls [122]. The femoral lengths, periosteal circumference, and bone mineral density (BMD) are reduced in the *lit/lit* mice [123], and these mice have an extension in **lifespan** by 23% in males and 25% in females[70]. Thus, both GHRH-/- and GHRHR-/- mice have significantly suppressed GH/IGF action, resulting in considerably smaller body size, increased adiposity, reduced cancer growth, and extended lifespan.

Growth Hormone (GH)

The clinical relevance of GH treatment for GH deficient children and adults, as well as the extended lifespans of both Ames and Snell mice, fueled interest in the study of GH action in genetically altered mice. The first GH transgenic mouse with the rat *Gh* gene expressed under the mouse *Mt1* promoter/enhancer developed by Palmiter et al. in 1982, grew almost twice as large as the littermate controls [25] and opened up a transformative scope of studying human conditions in laboratory mice. Beginning there, several mouse lines, transgenic for both human (h) and mouse (m) GH have been produced, which partially recapitulated several features of the human condition of GH excess found in patients with acromegaly.

Human GH transgenic mice (hGH)

MT1-hGH transgenic mice *Origin* The first hGH transgenic mouse was generated by Palmiter and Brinster in 1983 using the *Mt1* promoter/enhancer driving h*GH* expression [124].

Phenotype Zn or Cd treatment of the MT1-bGH mice further increase the *Mt1*-promoter/enhancer activity by up to tenfold [124]. The serum **hGH** levels in these mice are reported to be as high as 3000–900,000 ng/mL [125]. Expectedly, serum **IGF1** levels in hGH mice are also significantly higher than those of WT mice [124]. In addition, the serum PRL level is reduced [126] while hypothalamic somatostatin expression is twofold higher than normal [127]. These MT1-hGH transgenic mice are larger in **body size** the wild type (WT) littermates [128] with markedly increased **body weight** and greater muscle mass with more and larger type-1 and type-2 fibers [129]. However, hGH transgenic mice suffer from **reproductive defects**, including a dramatic decrease in ability of males to impregnate females possibly due to the lactogenic effects of ectopically expressed hGH. This occurs despite enlarged testes and seminal vesicles [130]. Likewise, female mice are sterile, possibly due to a dysregulated PRL axis. Daily progesterone injections as well as PRL-secreting ectopic pituitary transplants from WT female mice reverses this reproductive defect [126]. Other abnormalities include severe kidney lesions, glomerular hypertrophy with sclerosis, and hyalinosis associated with tubule-interstitial changes [125]. Transgenic female mice also have a markedly higher incidence of malignant mammary **tumors** at 27–43 weeks of age [131]. No reports on the lifespan of hGH mice are available.

MT1-hGH transgenic mice *Origin* A second transgenic mouse line expressing h*GH* under the mouse *Mt1* promoter/enhancer was produced in 1991 by Tornell and Isaksson [132].

Phenotype These transgenic mice resemble those produced by Palmiter and Brinster with larger **body size** and higher levels of circulating **hGH** than WT mice. Female transgenic mice also have markedly higher levels of spontaneous mammary **carcinomas** similar to those described above [131, 132]. This high frequency of spontaneous mammary carcinomas is probably due to hGH-mediated activation of the mouse PRLRs rather than GHRs [133, 134]. This finding is later clarified by the same group via generation of bovine (b) GH transgenic mice in the same genetic background as the hGH mice, which did not exhibit spontaneous mammary carcinomas, as only hGH binds and activates both the GHR and PRLR [133].

171hGH/CS mice *Origin* Cattini and colleagues in 2009 generated a third hGH transgenic mouse line named 171hGH/CS [135] to analyze the pituitary regulation of human GH

production. These 171hGH/CS-TG mice contain a 171-kb DNA fragment containing the intact h*GH* / chorionic somatomammotropin (*GH/CS*) gene locus, along with the locus control region (LCR) from chromosome-17, including sequences required for pituitary specific expression [135].

Phenotype Both pituitary and placental expression of hCS-A, hCS-B, and placental hGH-variant are detected in these transgenic mice during gestation, in proportions comparable to that in the human placenta, along with high **hGH** levels [135, 136]. Corticosteroid treatments increase both human and mouse GH levels as well as the *Ghrhr* mRNA in primary pituitary cells from 171hGH/CS-TG mice [136]. Studies using these mice reveal that hGH production is impacted by the circadian rhythm via direct binding of circadian transcription factors at an enhancer motif in the hGH promoter locus. GH production is suppressed in these mice by acute sleep deprivation [137] and by HFD feeding only during the light (inactive) stage of daily cycle [138].

Wap-hGH mice *Origin* In a fourth transgenic mouse line expressing human GH generated by Gunzburg et al. in 1991, the mammary specific whey acidic protein (*Wap*) promoter/enhancer was used to drive ectopic expression of hGH in mouse milk [139]. Another attempt at producing hGH in the milk of transgenic mice driven by a 6.3 kb long 5'-flanking region of the rabbit *WAP* promoter/enhancer was undertaken in 1994 by Houdebine and colleagues [140]. These models highlight the important lactogenic effect of human GH, given its unique ability to bind to and activate both GH and PRL receptors [141].

Phenotype Male transgenic mice from Gunzburg have higher plasma LDL-cholesterol and lipid peroxides and increased heart weights and lipid accumulation in liver compared to WT counterparts [142], suggesting a potential cardiac risk for male mice chronically exposed to hGH via the mammary gland. The **body size** of these mice does not differ from controls. The second Wap-hGH mouse from Houdebine produce up to 22 mg/ml of hGH in the milk but the lactogenic activity of hGH induces multiple dysfunctions including **sterility** in some of the transgenic females [140]. The same group generated another mouse line using the same transcriptional regulatory system, which express up to 16 mg/mL of bGH in the milk [143].

MT1-GHv mice *Origin* A fifth transgenic mouse line, expressing the human placental GH-variant (*GH2, or GH-V; GHv*) under the mouse *Mt1* promoter/enhancer was created by Selden and colleagues in 1988 [128].

Phenotype These mice, similar to the MT1-hGH animals, have a larger **body size** than normal with elevated **IGF1** levels and present a range of **reproductive defects** including small litter size (significantly lower than the expected at 50%), reduced fetal growth, increased pre- and post-natal mortality, as well as a 20% infertility rate in females[144]. Male MT1-hGHv mice are unable to impregnate the females in most cases and have increased testes and seminal vesicle weights like the MT1-hGH mice [130]. In both the MT1-hGH and MT1-GHv mice, spermatogenesis is unaffected [130].

Bovine GH transgenic mice (MT1-bGH and PEPCK-bGH)

MT1-bGH mice *Origin* The first mouse overexpressing bGH (b*GH*) driven by the *Mt1* promoter/enhancer was generated by Hammer et al. in 1985 [145].

Phenotype In the MT1-bGH mice, **bGH** concentrations are 40- to 400-fold those of WT mice (m) GH, and the transgene is expressed in almost all tissues [145, 146]. Serum IGF1 as well as somatostatin levels are markedly upregulated [127, 147]. The MT1-bGH mice **weigh** significantly more than controls with increased organ weights and higher lean mass and reduced fat mass [146, 148, 149]. They have larger **body size** and also model the human condition of acromegaly. These transgenic mice exhibit dysregulated **insulin sensitivity** as they are hyperinsulinemic at young ages but hypoinsulinemic and hypoglycemic at older ages [150]. Interestingly, both male and female mice also have increased adiposity in early life but switch to a leaner than normal phenotype at four (males) to six (females) months of age [146]. While GH is known to increase gluconeogenesis, MT1-bGH mice surprisingly exhibit suppressed glucose production following a pyruvate challenge, which could be confounded by higher insulin levels [151]. On HFDs, they are resistant to diet-induced obesity but develop dyslipidemia and diabetes [152]. Further, a dysregulated adipokine profile with decreased adiponectin and increased inflammatory IL-6, TNFα, and increased serum cholesterol have been reported [153, 154].

PEPCK-bGH mice *Origin* McGrane et al. developed a second bGH mouse line in 1988, employing the phosphoenolpyruvate carboxykinase (PEPCK; *Pck1*) transcriptional regulatory region ligated upstream to the b*GH* gene [155].

Phenotype PEPCK-bGH mice have serum **bGH** levels higher than that of MT1-bGH mice [127], reaching up to 2300 ng/mL, and cAMP administration causes a further twofold increase in bGH levels. As the bGH transgene in these mice is driven by the PEPCK promoter/enhancer, interventions such as a high carbohydrate diet that can suppress PEPCK mRNA, might also suppress the bGH transgene expression. Accordingly, a carbohydrate-rich diet intake by these mice does result in suppressed gluconeogenesis and hence PEPCK expression and in turn, suppresses GH expression by 90%, while increasing serum insulin levels. The PEPCK-bGH animals have a twofold higher **growth rate** despite the transgene being expressed in the liver and

kidney, indicating an endocrine effect of the bGH transgene. The pituitary weight of PEPCK-bGH mice is elevated, with smaller Golgi in pituitary somatotrophs. Serum **IGF1** concentrations of these transgenic mice range between 2–threefold higher than those of WT mice [156] along with upregulated somatostatin levels similar to the MT1-bGH transgenic animals [127]. PEPCK-bGH mice **weigh** approximately 1.5-times more than WT mice [156], with increased lean mass[146, 148] and increased weights of internal organs, including kidney, liver, and heart [149]. In addition, seven-month-old PEPCK-bGH mice display improved glucose clearance, and lower blood glucose and HbA1c levels, while **glucose and insulin sensitivities** are comparable to WT mice [156]. These mice also develop inflammatory arthritis with production of autoantibodies [157].

Similar to hGH transgenic mice, a range of **reproductive disorders** are observed in the females of both MT1- and PEPCK-bGH mice, including an increased interval between pairing with a male and conception, increased interval between litters, reduced number of litters, reduced fetal growth, increased pre- and postnatal mortality and alterations in sex ratio [144]. More than 60% of the PEPCK-bGH and 20% of the MT1-bGH female mice are infertile, concomitant with the higher level of circulating bGH in PEPCK compared to MT1 animals [144]. Male bGH transgenic mice (both MT1 and PEPCK) have significantly higher weight of the testes and seminal vesicles but spermatogenesis or fertility is unaffected [130].

There is a significant decrease in the **lifespan** of both MT1- and PEPCK- driven bGH transgenic, giant mice. MT1-bGH mice have a maximal lifespan of 24-months and a 1-year survival-rate of 44%, while PEPCK-bGH mice have a maximal lifespan of only 18-months and a 1-year survival-rate of 25% [158, 159]. The early morbidity of bGH mice recapitulates several factors underlying the shortened lifespan observed in untreated human patients with acromegaly. MT1-bGH mice suffer from renal and cardiac defects [160]. These mice exhibit **renal disorders** like hypercellular glomeruli early in life, advancing to increased glomerular size and progressive glomerulosclerosis at adulthood [160]. A significantly increased heart mass concomitant with impaired systolic function and a decreased energy reserve in the myocardium is also observed [149]. An increased mitogenic action of excess GH on its main target organ – the liver – leads to a number of **hepatic abnormalities** in both bGH transgenic mouse lines. For example, in both MT1- and PEPCK-bGH mice, hepatomegaly is observed as early as 2 weeks of age and progresses maximally into young adulthood, with an enhanced expression of proto-oncogenes and activation of multiple mitogenic signaling intermediates like c-SRC, mTOR, STAT3, GSK3, NFkB, c-fos, c-jun, and c-myc [153, 154, 161]. Additionally, pro-tumorigenic hepatocellular events, including upregulation of tumorigenic galectin-1 [162], and elevated oncogenic signaling pathways, are observed in the livers of both male and female PEPCK-bGH mice [163]. Both MT1- and PEPCK-bGH mice are known to develop spontaneous liver **tumors** [164]. In both mouse lines, a sustained hepatic hypertrophy and inflammation lead to a significantly higher rate of spontaneous hepatocellular carcinogenesis compared to WT controls [164–166].

In summary, human and bovine GH transgenic mice have a decreased fat mass, with increased body size and lean mass. However, these mice have fertility defects, exhibit kidney and cardiovascular dysfunction, and have elevated neoplasm incidence along with a decreased lifespan.

GH-/- mice (*Gh*-/- or GHKO)

Origin In order to investigate the effects of GH absence and GH replacement on phenotypic variables, GH-/- mice were generated in the Kopchick laboratory in 2019, using a VelociGene KOMP definitive null allele that replaces the *Gh* gene with a ZEN-UB1 selectable reporter [167].

Phenotype Circulating **GH** in GH-/- mice is reduced to an undetectable level compared with that of WT controls [167]. Serum **IGF1** levels are also significantly reduced (~90%). Disruption of the *Gh* gene significantly reduces nasal-anal **body length** (>30%), and body composition is significantly altered in both sexes, with **body weight** and lean mass significantly decreased and fat mass significantly increased relative to controls. GH-/- mice of both sexes demonstrate greatly enhanced **insulin sensitivity** probably due to the lack of GH's diabetogenic effect. However, GH-/- mice are significantly glucose intolerant (although greater in males than females), which is attributed to their decreased pancreatic islet size. Liver, kidney, heart, spleen, gastrocnemius, soleus, and quadriceps masses are also significantly decreased, whereas AT mass and relative brain weight are significantly increased. Liver triglyceride content and adipocyte size in the subcutaneous depot are elevated in both male and female GH-/- mice. White AT fibrosis is significantly decreased in the subcutaneous white AT depot of both sexes compared to controls, suggesting depot-specific effects of GH. In summary, GH-/- mice show similar phenotypes as other mouse lines that lack GH action, although their cancer incidence and lifespan have not been reported at the time of this publication.

Adult onset-isolated GH deficiency mice (AOiGHD)

Origin To better understand the metabolic effects of somatopause – the progressive decline of hormones in the hypothalamic-pituitary-somatotrophic axis with age – a mouse line of adult onset-isolated GH deficiency (AOiGHD) was created by Kineman and colleagues in 2011 by breeding the inducible monkey diphtheria toxin receptor mice (iDTR)

with mice having a rat *Gh* promoter/enhancer driven Cre recombinase [168].

Phenotype The adult $Cre^{+/-}iDTR^{+/-}$ offspring are treated with diptheria toxin (DT) to selectively ablate somatotroph cells expressing diphtheria toxin receptor at 10–12 weeks of age, resulting in a ~ 50% decrease in circulating **GH** and **IGF1** levels [168]. These mice also have lower fasting insulin levels and improved whole-body **insulin sensitivity** when fed either low-fat or HFD relative to WT littermates. Indirect calorimetry suggested that these mice utilize mainly carbohydrates for energy metabolism. Furthermore, detrimental physiological effects are seen only in HFD animals including increased fat mass, decreased hepatic lipids, and impaired glucose clearance and insulin output. The AOiGHD mice also have decreased liver weight accompanied with reduced liver triglyceride content. Overall, the mouse line shows that reduction in circulating GH and IGF1 levels with age improves insulin sensitivity and prevents metabolic dysfunction under moderated caloric intake.

GHR antagonist transgenic mice (GHA)

Origin To understand some of the effects of pharmacological perturbations to GH action, a transgenic mouse line that expresses a mutated-bovine GH gene that effectively antagonizes endogenous GH action was created. These transgenic GHR antagonist (GHA) mice were generated in a C57BL/6 J background in the Kopchick laboratory between 1990 and 1991 via the fusion of the mutated *GH* transgene downstream of the mouse *Mt1* promoter/enhancer [26, 169, 170]. The mutated bovine GH gene differs from its WT counterpart in that it encodes a single amino acid substitution at position 119. The glycine that typically occupies this position, found in the third alpha-helix of bGH (G119 in bovine GH; G120 in human GH), is critical for the successful activation of the GHR [171]. When glycine 119 is substituted with arginine, the resulting molecule competitively inhibits the association of mouse GH with the GHR [172]. Similarly, when a lysine is substituted for the glycine at position 120 of the human *GH* gene, an effective human GHR antagonist is produced. Following these discoveries, Kopchick and colleagues went on to develop the novel drug, SOMAVERT® (Pegvisomant for injection), which is a GHR antagonist that inhibits the interaction of endogenous GH with GHR and is now used world-wide for the treatment of patients with acromegaly [173].

Phenotype As a result of the overexpression of the GHR antagonist, GHA mice have smaller **body size** and show a 30% lower mean growth ratio [26, 170] with significantly reduced **body weight** [147], wherein lean mass is reduced, and body fat is increased compared to controls [174]. GHA mice have increased lipid storage in the inguinal subcutaneous white AT depot and a relative increase in extra-peritoneal to intra-peritoneal white AT [174]. Additionally, GHA mice have markedly lower serum **IGF1** [26, 147, 170, 172], lower serum **IGFBP3** [37], and higher pituitary mouse (m) **GH** levels than nontransgenic littermates [26, 170]. The pituitary weight of GHA mice is about half that of controls, with moderate to sparsely granulated somatotrophs compared to those densely granulated in WT mice [147]. Despite increased obesity [175], GHA mice are more **insulin sensitive** than controls [174]. Increased brown AT mass accompanied by higher expression of thermogenic factors has also been reported [175]. On a HFD, although GHA mice gain more weight than WT controls (males > females), they are protected from HFD-induced glucose intolerance and hyperinsulinemia [176]. Additionally, GHA mice are protected from streptozotocin-induced diabetic **kidney lesions** [175] and from **cancer**. For example, after treatment with the mammary carcinogen DMBA, ~ 66% of GHA mice remain tumor-free compared to only $1/3^{rd}$ of the controls and have less tumors and a smaller tumor burden [172]. Although no significant difference in **lifespan** between GHA and WT mice has been reported [158], female GHA mice tend to live longer than controls.

In summary, the phenotypes observed in GH transgenic mice with elevated GH action contrast significantly to those seen in the GH-/- or GHA mice. All these observations strongly suggest that GH plays a critical role in promoting growth, body size, lean mass, glucose intolerance, and reproductive deficiency, while the absence or deficiency of GH improves glucose homeostasis, adiposity, cancer resistance, and longevity.

Prolactin and prolactin receptor (PRL and PRLR)

Prolactin (PRL) is a protein secreted from the lactotrophs of the anterior pituitary gland [177] and has a structure similar to that of GH. PRL secretion is stimulated by PRL releasing factors such as thyrotropin releasing hormone, oxytocin and neurotensin [178]. On the contrary, PRL secretion is inhibited by dopamine and somatostatin [179] and induced by gamma-aminobutyric acid [180]. PRL binds to PRL receptors (PRLR), which are a member of cytokine receptors that lack intrinsic kinase domains but possess JAK2 associating regions; thus, PRL resembles the GHR and transduces similar intracellular signals. Human PRLR can bind at least three ligands including PRL, placental lactogen and hGH. Like GHR, PRLR consists of an extracellular domain for ligand binding, a helical transmembrane portion and an intracellular region. However, alternative precursor mRNA splicing leads to different isoforms of the PRLR with identical extracellular domains while the intracellular domains differ in size (referred to as 'long' or 'short' PRLR) [177]. The receptor homodimer is constitutively expressed on cell surfaces in a ligand-independent manner in several tissues and

peripheral organs including the breast, prostate, brain, pituitary gland, heart, uterus and skin [178]. Although hundreds of actions of PRL have been reported [181, 182], a main function of PRL is to promote both growth of the mammary gland and to induce and maintain lactation. In the following section, PRL transgenic, PRL-/-, PRLR-/-, PRLR variants, and PRLR antagonist mice will be discussed.

MT1-PRL transgenic mice

Origin To study prostate hyperplasia, Tornell and colleagues in 1997 generated MT1-PRL transgenic mouse lines which overexpressed PRL [183].

Phenotype These transgenic mice have ubiquitous expression of rat PRL (r*Prl*) under the control of *Mt1* promoter/enhancer. Three mouse lines generated, L1, L2 and L3, have an increase in serum rat PRL by ~ 250 ng/ml, 15 ng/ml, and 100 ng/ml respectively [183]. The endogenous mouse PRL serum levels are not reported though mouse PRL mRNA is detected in all parts of the prostate glands. The three PRL transgenic mouse lines exhibit enlarged prostates due to increased PRL secretion along with increased prostate weight and hyperplasia compared to the controls. Interestingly, these mice also have elevated **IGF1** levels close to that of bGH mice although the GH levels are not reported. L1 and L2 mice remain **fertile**, while L3 mice, with the highest PRL levels, are infertile [183].

Local prostate specific prolactin expression: Pb-PRL transgenic mice

Origin To assess the role of PRL in abnormal prostate growth in transgenic animals that overexpress PRL, Kindblom et al. in 2003, developed a Pb-PRL transgenic mouse line, which locally produce PRL in the prostate [184].

Phenotype In this mouse line, the minimal probasin (*Pb*) promoter/enhancer is used to direct r*Prl* expression in the epithelial cells of dorsolateral, ventral, and anterior of prostate lobes. Marked enlargement of prostate glands is observed in the transgenic males, which is also observed in the MT1-PRL mice. Though both MT1-PRL and Pb-PRL have marked ductal dilation and elongation, MT1-PRL mice have significantly elevated ductal branching points and tips while Pb-PRL mice have normal branching points. The data suggest that PRL action can differentially impact a variety of prostate cells. The heterozygous Pb-PRL animals remain **fertile** [184].

Mammary epithelial PRL overexpressing mice (NRL-PRL)

Origin PRL is crucial in development and differentiation of the mammary gland. Many epidemiological studies have linked PRL with increased risk of estrogen receptor positive (ERα +) breast tumors [185]. To specifically study the role of PRL in breast cancer, Schuler and colleagues developed a PRL transgenic mouse line in 2003 [186]. This mouse line called NRL-PRL has locally overexpressed r*Prl* transgene in mammary epithelia driven by a hormonally nonresponsive promoter/enhancer – neu-related lipocalin (NRL).

Phenotype The NRL-PRL females develop mammary pathology and ERα + and ERα- carcinomas [186, 187]. Overall, breast **cancer** development in NRL-PRL mice strongly implicates PRL in development of ERα + cancers.

PRL knockout mice (*Prl-/-* or PRL-/-)

Origin To determine the effects of a lack of PRL, Nelson Horseman et al. generated the PRL-/- mouse line in 1997 through a targeted insertion of a NeoR gene into the region of the PRL gene encoding the second α helix [188].

Phenotype Although no detectable effect on **growth** or adiposity at any age is observed [188], male PRL-/- mice exhibit impaired glucose tolerance at 4 weeks of age [189]. Also, higher leptin concentrations are found in PRL-/- mice on normal chow compared to WT mice [189]. Females are **sterile**, indicating that PRL is essential for female fertility, whereas males remain reproductively viable [188]. Since PRL has been found to influence the immune system, it was expected that these mice would be immunocompromised. However, no significant difference is reported in the number of B- and T-cells in PRL-/- mice compared to controls [188].

PRLR knockout mice (*Prlr-/-* or PRLR-/-)

Origin To determine the effects of a lack of PRL action, Ormandy et al. in the laboratory of Paul Kelly generated a prolactin receptor knockout mouse line (PRLR-/-) in 1997 [190].

Phenotype These mice present features like those noted in PRL-/- mice. Female PRLR-/- mice are **sterile** and show changes in estrous cyclicity when compared to WT mice [190]. Heterozygous female mice are fertile but display abnormal maternal behavior including decreased pup retrieval, leaving pups unattended or scattering them around the cage [190]. However, males are 'partially infertile' [190], with 20% of all tested males exhibiting delayed fertility [181]. Both male and female PRLR-/- mice experience a significant decrease in bone formation and a reduction in bone mineral density compared to controls [191]. In terms of glucose homeostasis, PRLR-/- mice have reduced pancreatic islet density and β-cell mass, as well as reduced pancreatic insulin mRNA levels in both sexes [192]. There is also a marked reduction in abdominal fat mass in both sexes. Importantly, PRLR-/- mice are protected from prostate **carcinogenesis** [193], suggesting that abrogated PRL action might be protective against prostate cancer.

PRLR variants

PRLR is expressed ubiquitously with various proportions of long and short isoforms in different tissues. In mice, four PRLR variants have been classified as one long (LPRLR) and three short forms (S1PRLR, S2PRLR, S3PRLR). S1PRLR and S2PRLR forms are mouse specific while LPRLR and S3PRLR are homologous in other species [194]. Only LPRLR has been shown to induce transcription of milk producing genes while both LPRLR and S1PRLR have been shown to modulate cell proliferation. Similarly, rat PRLR has a long (LPRLR), a variant (Nb2), and a short (F3-SPRLR) form. Interestingly, F3-SPRLR results in formation of inactive heterodimer resulting in absence of downstream signaling in vitro. Several mouse lines have been generated to study the individual effects of each type of isoforms.

F3-SPRLR mice *Origin* To assess the dominant negative effects of SPRLR and role of PRL in normal mammary gland development, Saunier et al. developed a transgenic mouse line in which the F3-short form of the rat PRLR (F3-SPRLR) was expressed in mouse mammary epithelium driven by mouse mammary tumor virus-long terminal repeat (MMTV-LTR) in 2003 [194].

Phenotype Mice with low levels of transgene expression exhibit phenotypes similar to WT animals while mice expressing high levels of transgene show impaired mammary gland development and lactation although **fertility** is unaffected [194]. Hence, locally blocking PRL/PRLR at the mammary gland hinders mammary gland development indicating the crucial role of PRLR signaling in mammary tumors.

PR-1 mice *Origin* To assess the signal transduction of the short PRLR isoform, Binart et al. in the Kelly laboratory developed a mouse line with overexpression of the short isoform of the mouse PRLR (originally called PR-1, also known as S1PRLR) in 2003 [195]. The *Pr1* gene is expressed in heterozygous *Prlr*+/− mice driven by the elongation factor 1α (EF1A) promoter/enhancer.

Phenotype Previous studies have shown that heterozygote *Prlr*+/− mice exhibit severe defects in lactation after the first pregnancy [190]. Interestingly, introducing the short form of the gene *(Pr1)* in *Prlr*+/− mice results in normal mammary ductal development and the ability to lactate after the first pregnancy. The results from this study strongly indicate that the short form of PRLR is specifically involved in mammary stem cell formation.

Tg-RL and CL-RL mice *Origin* PRL is involved in corpus luteum (CL) formation and progesterone production crucial in embryo implantation and maintenance of pregnancy. To delineate the role of PRLR long form in CL function, Le et al. in 2012 developed two transgenic mouse lines expressing only PRLR long form—one ubiquitously expressed and named Tg-RL driven by the EF1A promoter/enhancer, and the other in CL-specific manner and named CL-RL driven by the transcriptional regulatory region of the hydroxysteroid 17-beta dehydrogenase 7 (*hsd17b7*) CL-specific gene [196].

Phenotype Both mouse lines have normal follicular development and ovulation rates. An interesting malformation of vasculature is observed in both mouse lines, which can be attributed to lack of PRLRs (short form) function [196].

PRLR antagonist transgenic mice *Origin* The rational design for competitive PRLR antagonist where it competes with endogenous PRL and binds but does not activate the PRLR was based on the pioneering work on the GHR antagonist (Pegvisomant) by the Kopchick laboratory. Goffin and colleagues in 2003 generated the first PRLR antagonist by replacing the glycine in the 3rd PRL α-helix. This glycine, when replaced with arginine at position 129 (G129R), resulted in a strong antagonist of the PRLR [197]. Also, deleting the first nine residues (Δ1–9) at the N-terminus in the G129R-hPRL proved to enhance the effectiveness of the antagonist [197]. To study the effects of blocking the PRL action in prostate tumorigenesis, Rouet et al. in 2010 developed the Δ1–9-G129R–hPRL transgenic mouse line driven by *Mt1* promoter/enhancer for ubiquitous expression of the antagonist [198].

Phenotype These mice express about 200 ng/ml of circulating PRL antagonist. No prostate hypertrophy is observed in these mice. However, increased pituitary weight is observed in both sexes [199, 200]. Inhibition of lactotroph cell proliferation and increased apoptosis are also observed when mice are treated with dopamine agonist (D2R) and then treated with PRL [200]. In 2010, the latter team also generated a double transgenic mouse by crossing Pb-PRL (rat *Prl* expressed only in the prostates) with Δ1–9-G129R-hPRL mice. The weight of dorsal prostate in these mice is reduced at 6-month of age as compared to Pb-PRL mice. These mice also had a stark reduction in STAT5 phosphorylation in dorsal prostates and reduced **tumorigenesis**. Overall, these findings point to the role of endocrine PRLR antagonists in preventing early prostate tumorigenesis [198].

In summary, PRL is closely related to GH as both belong to the same cytokine family, have approximately the same mass, similar quaternary structures, bind to a homo-dimerized cognate receptor like GHR, and activate STAT5 in their downstream signaling. PRL-PRLR axis plays important physiological roles especially in lactation and in maintaining fertility. Also, blocking PRL can retard/inhibit prostate tumorigenesis.

Global, temporal and tissue-specific GHRKO mice

For GH to elicit a response in cells, it must bind to its cognate receptor, the GHR, which is a pre-formed single membrane spanning dimer and a member of the cytokine family receptors—all lacking a kinase domain. After GH binds to the preformed GHR homodimer, the intracellular domain associated JAK2 kinases then phosphorylate one another and begin the process of GH induced GHR signal transduction. Inactivating mutations in the GHR or down-stream signaling intermediates lead to GH insensitivity. In humans this condition is called Laron Syndrome (LS). LS is characterized by low IGF1, elevated GH, short stature, obesity, and resistance to cancer [76]. Furthermore, the Ecuadorian cohort which is the largest cohort of individuals with LS, exhibit extreme insulin sensitivity and resistance to cancer and diabetes. In mice, GHR gene disruption (GHR-/-) produces a similar phenotype to humans with LS [31]. To date, GHR-/- mice have been used in over 130 published studies that have greatly enhanced our knowledge of GH action in vivo. In addition to global GHR-/- mice, temporal and tissue-specific GHR gene disrupted mice have been generated (Table 2) as will be discussed in the subsequent section.

Global GHRKO

GHR knockout mice (*Ghr-/-*; GHR-/- or GHRKO)

Origin To determine the effects of a lack of GH action, the GHR null or GHR-/- or GHRKO mouse line was developed by Zhou et al. in the Kopchick laboratory in 1997 through a targeted mutation in which a NeoR gene was used to replace a major portion of exon 4 of the *Ghr* along with ~ 500 bp of intron 4/5 [30].

Phenotype The resulting homozygous null mice are dwarf with decreased **body length** and **weight**. These mice experience delayed **sexual maturation** and decreased **litter sizes** [30, 201]. GHR-/- mice have ~ 50–100 fold increase in serum **GH** and a ~ 90% decrease in serum **IGF1** levels [30]. In regard to **body composition**, these mice have increased fat mass and decreased lean mass [202]. Surprisingly, the largest increase in adiposity occurs in the subcutaneous white AT depot. Although obese, these mice show improved **insulin sensitivity** and decreased serum insulin [31]. However, GHR -/- mice have impaired glucose tolerance due to decreased pancreatic islet size and function [203, 204]. Additionally, these mice have normal to high levels of serum leptin [148, 205, 206] and adiponectin [148, 205, 207] with normal to low levels of cholesterol [206, 208] and T3 and T4 [209]. GHR-/- mice have increased oxygen consumption and lower respiratory quotient values, which indicate a shift towards fat oxidation [210, 211]. Additionally, these mice show 23–26% greater neuron density in the somatosensory cortex of the brain along with improved memory retention and reduced memory loss with age [212]. Remarkably, these mice display resistance to several disease states, including the development of certain types of **cancer** [33–35], nephropathy when type 1 diabetes is induced [213], resistance to T2D when placed on a HFD [32] and age-related loss of grip strength [214]. Finally, these mice have increased **longevity** [37, 215] and hold a world record for the longest-lived laboratory mouse [31].

Temporal GHRKO

Global adult onset—aGHRKO mice

Origin To investigate the physiological effects of disrupting GH action in adulthood, Junnila et al. in the Kopchick laboratory in 2016 generated a mouse line with ablated GHR at 1.5 months of age using the Cre gene transcriptionally driven by ROSA26 gene promotor/enhancer [60].

Phenotype Adult-onset GHRKO (aGHRKO) mice have a variable but significant decrease in tissue specific GHR gene expression, with liver and AT showing the greatest reduction, and skeletal muscle and heart, the least [60]. In terms of phenotype, the aGHRKO mice have reduced circulating **IGF1** and elevated circulating **GH** when compared to control mice. These mice have reduced **body weight and body size** (5–10%), with an increase in **fat mass** and a decrease in **lean mass** when compared to controls. Despite the increased adiposity, both male and female aGHRKO mice show increased **insulin sensitivity** and decreased circulating insulin levels. Similar to the germline GHR-/- mice, aGHRKO mice have decreased **glucose tolerance** in comparison to controls. The adipokine profile is altered in these mice with increased adiponectin but no difference in leptin levels. Changes in circulating IGFBPs were also seen in the aGHRKO mice when compared to WT mice. That is, similar to GHR-/- mice, aGHRKO mice exhibit a decrease in IGFBP3 and an increase in IGFBP1, 2 and 6. Therefore, while IGFBP3 is known to be positively associated with GH action, IGFBP1, 2, and 6 appear to be negatively associated with it. Finally, **longevity** studies show that aGHRKO females have an increased maximal lifespan when compared to female controls.

Tissue-specific GHRKO

Liver-specific GHR knockout mice

Liver is one of the most important organs in the GH/IGF1 axis since it is the site where the majority of circulating GH-stimulated IGF1 is produced. It is estimated that 75–90%

of circulating IGF1 is produced from the liver [48, 216]. Indicative of this importance, there are five liver-specific GHR knockout mouse lines that have been independently produced by different laboratories between 2009–2019.

GHRLD *Origin* In 2009, Fan et al. produced the first liver-specific GHR knockout (GHRLD) mouse in the laboratory of Mark Sperling [38]. To produce these mice, an albumin promoter/enhancer was used to drive Cre recombinase in liver hepatocytes.

Phenotype These mice have decreased serum **IGF1** and elevated serum **GH** levels [38]. Despite the reduction to circulating IGF1, these mice show no change in **body weight, body length**, tibia length or **body composition**. Several organs are altered in size in these mice including increased liver weight and decreased kidney weight. Glucose homeostasis in these mice is negatively affected, as GHRLD mice are **glucose intolerant and insulin resistant**. Additionally, male mice exhibit increased liver steatosis. Finally, these mice have increased hepatic fibrosis, circulating inflammatory cytokines and decreased bone density.

LiGHRKO *Origin* In 2014, List et al. in the Kopchick laboratory produced the second liver-specific GHR knockout mouse (LiGHRKO) [48]. To produce these mice, an albumin promoter/enhancer was used to drive Cre recombinase specifically in the liver hepatocytes.

Phenotype The resulting mice are significantly smaller with decreased **body weight and body length** at 6 months of age [48]. Analysis of **body composition** shows a higher percentage of body fat at early ages followed by a lower percentage in adulthood similar to the body composition profile of bGH mice that results from elevated **GH** levels. In some sense, these animals could be considered mice with 'extrahepatic acromegaly'. For example, liver IGF1 mRNA is quite low yet the levels are increased in skeletal muscle and AT. Interestingly, there is a male-specific development of fatty liver. Similar to GHRLD, LiGHRKO mice have impaired **glucose homeostasis** with an increase in several adipokines, including leptin, resistin and adiponectin, and increased inflammatory cytokines (IL-6 and MCP-1). These null mice also have increased grip strength compared to controls. Additionally, LiGHRKO mice have smaller kidneys and spleens and increased liver, heart and lung mass relative to body weight. Aging studies at two separate institutions reveal that liver-specific disruption of the GHR does not alter **lifespan** in LiGHRKO mice [52] despite severe reductions to circulating **IGF1** [48]. We suspect that the benefits of lower circulating IGF1 in LiGHRKO— which normally favors lifespan extension— were offset by impaired glucose homeostasis and elevated circulating GH, that in turn increased local IGF1 in non-hepatic tissues. To date, no other liver-specific mouse lines have been evaluated for lifespan.

aLivGHRkd *Origin* To investigate the role of GH in hepatic fat production and accumulation, Cordoba et al. produced an adult-onset (induction at 10–12 weeks of age), liver GHR knockdown mouse (aLivGHRkd) in the laboratory of Rhonda Kineman in 2015 [55]. These mice were generated utilizing a Cre system driven by the thyroxine-binding promoter/enhancer.

Phenotype Both male and female mice have reduced circulating **IGF1** and hepatic *Igf1* mRNA levels, although the reduction is less pronounced in females [55]. There is also an increase in **GH**, GHRHR and the ghrelin receptor (previously known as the GH secretagogue receptor 1a) in male mice. These mice have increased liver weight, hepatic de novo lipogenesis, triglycerides, and glycolysis-driving factors, such as glucokinase and fructose 2,6-bisphosphate.

Li-GHRKO *Origin* To investigate the role of hepatic GH on lipid and carbohydrate metabolism, Liu et al. produced the liver-specific GHR deletion mouse (Li-GHRKO) in the laboratory of Shoshana Yakar in 2016 [59]. These mice were produced using a Cre system driven by an albumin promoter/enhancer.

Phenotype There is no change in **body weight** of these mice, but there is an increase in **fat mass**, as seen before in other mouse lines [59]. Similarly, these mice have reduced serum **IGF1** levels with increased blood glucose and serum insulin, as well as impaired **insulin tolerance**. They also have increased serum triglycerides, cholesterol, FFAs and leptin levels. Furthermore, the liver weight of these mice is increased, as well as hepatic triglyceride and fatty acid content. Finally, hepatic glycogen is increased, as well as enzyme markers for gluconeogenesis (i.e., glucokinase, PCK1).

L-Ghr-/- *Origin* To investigate the role of liver-specific GH on CR, Fang et al. produced the L-Ghr-/- mouse using a Cre system driven by an albumin promoter/enhancer in the laboratory of Guosheng Liang in 2019 [63].

Phenotype There is no resulting change in **body weight** or **body composition** [63]. Additionally, these mice have **blood glucose** levels comparable to controls. However, when placed on a CR diet, the mice have decreased blood glucose resulting in a hypoglycemic state. They also have an increase in plasma **GH** and ghrelin. Differing from previous findings in the other liver-specific GHR KO mouse lines, the livers of these mice have decreased triglycerides and reduction in autophagic vacuoles.

Overall, physiological data obtained from these five liver-specific *Ghr* gene disrupted mouse lines are in agreement with only a few discrepancies. Most notably, Fan et al.

reports that deletion of GHR in liver does not affect body composition or growth as measured by total body weight and body length. In contrast, List et al. found that LiGHRKO mice have a higher percentage of adiposity at a young age, then a lower percentage in adulthood when compared to controls. Furthermore, List et al. found that body weight and body length are all significantly decreased in LiGHRKO mice compared to controls. While the precise reason for the inconsistencies is unknown, we suspect that they may be due to the age at which these measures are recorded, and/or the numbers of mice used in each study. Specifically, Fan et al. evaluated these parameters at 16 weeks of age using a *n* of 6 to 8, while List et al. measured growth factors at 6 months of age using a *n* of 15 to 16 and body composition over time up to 22 months of age using a *n* of 13 to 19. Importantly, List et al. observed no changes in weight until later in life, which may explain why Fan et al. observed no differences in growth.

Muscle-specific GHRKO mice

Since GH has significant anabolic effects on muscle, three muscle-specific GHR knockout mouse lines have been generated independently to understand the roles of the GH-axis on muscle size, fiber type, metabolism, glucose homeostasis and longevity.

ΔGHR *Origin* In 2010, Mavelli et al. created a muscle-specific GHR knockout mouse (ΔGHR) in the laboratory of Thomas Clemens [40]. These mice were produced using the *Mef-2c-73 k* promoter/enhancer to drive Cre expression in muscle. However, off target expression is reported for this Cre line (described below in comparison of the three muscle-specific knockout mouse lines).

Phenotype These mice show no change in either serum **GH** or **IGF1** levels [40]. In terms of phenotype, these mice reveal an increase in **body weight** over controls, starting at 12 weeks of age. **Body composition** analysis shows that these mice also have increased fat mass compared to controls. Additionally, ΔGHR mice have increased glucose and triglyceride levels, indicating the development of **insulin resistance**.

mGHRKO *Origin* In 2012, Vijayakumar et al. produced the muscle GHRKO mouse (mGHRKO) in the laboratory of Derek LeRoith [42].

Phenotype These mice were produced utilizing the Cre system driven by the mouse muscle creatine kinase (*Ckmm*) transcriptional regulatory region [42]. No difference is seen in **GH** and **IGF1** levels. While **body size** is comparable to WT controls, the **lean mass** of the mGHRKO mice is significantly decreased. Additionally, both **subcutaneous and gonadal AT** are significantly reduced along with an increase in serum adiponectin levels.

MuGHRKO *Origin* In 2015, to understand the effects of muscle GHR on glucose homeostasis and aging, List et al. in the Kopchick laboratory produced the MuGHRKO mouse [54]. These mice were produced utilizing the Cre system driven by the mouse muscle creatine kinase *(Ckmm)* promoter/enhancer, which is specifically expressed in skeletal and cardiac muscle.

Phenotype No changes to the **GH/IGF1** axis are found [54]. **Body length** and **weight** are comparable to controls, and no difference is observed in **fat or lean mass** as measured over time. Male MuGHRKO mice have enhanced **insulin sensitivity** and increased **lifespan** although this increase does not recapitulate that seen in global GHR-/- mice.

Comparison of the three muscle-specific *Ghr* gene disrupted mouse lines shows conflicting results. Mavalli et al. [40] report that muscle-specific disruption of the GHR in male mice produces increased adiposity with insulin resistance and glucose intolerance. In contrast, both List et al. and Vijayakumar et al. report reduced adiposity and overall improvement in glucose homeostasis [42, 54]. The difference among Mavalli's results [40] and those of the two other laboratories [42, 54] likely reflects the use of different promoter/enhancers driving Cre expression. Both List et al. and Vijayakumar et al. used muscle creatine kinase *(Ckmm)* promoter/enhancer [42, 54], which drives Cre expression in postnatal skeletal and cardiac muscle [217] while Mavalli et al. used the *mef-2c* promoter/enhancer, which directs Cre expression in postnatal skeletal muscle [44]. Unfortunately, while *mef-2c* Cre expression was thought to exclusively target skeletal muscle, more recently it has been shown that it is an important regulator of brain, bone, lymphocyte, blood vessel, endothelium, neural crest, craniofacial, and melanocyte development [218, 219]. Therefore, it is likely that unanticipated expression of Cre by the *mef-2c* promoter/enhancer in tissues other than muscle accounts for the differences between mice generated by Mavalli et al. versus other two mouse lines.

Brain-specific GHRKO mice

To understand the roles of GH axis on brain, four independent GHR brain-specific mouse lines have been generated between 2017–2019.

Lepr$^{EYFP\Delta GHR}$ *Origin* To comprehend the role of GHR signaling on the CNS, Cady et al. produced the Lepr$^{EYFP\Delta GHR}$ mouse in the laboratory of Marianna Sadagurski in 2017 [61]. A Cre/loxP system was used to ablate *Ghr* in the leptin receptor-expressing neurons.

Phenotype No changes to **body weight, length** or **composition** are observed, and there is no change in serum **IGF1** or **GH** levels [61]. Lepr$^{EYFP\Delta GHR}$ mice do show impaired **glucose homeostasis** when compared to controls but normal insulin tolerance. This impaired glucose homeostasis may be due to the observed increase in hepatic gluconeogenesis.

AgRP-IRES-Cre *Origin* To investigate the role of brain-specific GH action on energy homeostasis, Furigo et al. produced an agouti-related protein (AgRP) GHR knockout (AgRP-IRES-Cre) mouse in the laboratory of Jose Donato in 2019 [64]. These mice were produced by crossing mice carrying loxP-flanked *Ghr* alleles with AgRP-IRES-Cre mouse (Agrp$^{tm1(cre)Lowl}$/J).

Phenotype There is no change in **glucose tolerance** or insulin sensitivity, **body weight, length** or **composition** of these mice [64]. Also, no changes are observed in leptin sensitivity, ghrelin-induced food intake or ghrelin-induced c-Fos expression in the arcuate nucleus (ARH) of these mice. While there is no change in number of AgRP cells of the ARH, there is a reduction in c-Fos positive cells in a food-deprived state. These mice also show an attenuated neuroendocrine response that normally aids in energy conservation, when under food deprivation. Moreover, in this state, these mice show increased weight loss and decreased blood glucose compared to controls.

LepR-IRES-Cre *Origin* In the same publication from the laboratory of Jose Donato (mentioned above) for AgRP-IRES-Cre mice, Furigo et al. reported the generation of a leptin receptor-presenting-cell-specific GHR knockout (LepR-IRES-Cre) mouse [64]. These mice were produced by crossing mice carrying loxP-flanked *Ghr* alleles with LepR-IRES-Cre mouse (B6.129-Lepr$^{tm2(cre)Rck}$/J).

Phenotype These mice have an increase in **body weight** and **body length**, as well as a reduction in body **fat mass** [64]. There are no changes to food intake, leptin sensitivity or energy expenditure, but there is a decrease in serum leptin levels. Under food deprivation, these mice have increased weight loss with some mice becoming lethargic.

Nestin-Cre *Origin* Furigo et al. produced an entire brain GHR knockout (Nestin-Cre) mouse in their 2019 publication [64]. As the name implies, these mice were produced using a Cre system driven by the nestin promoter/enhancer.

Phenotype Nestin-Cre mice have increased **body weight** and **body length** with an increase in **lean mass** [64]. There is also an upregulation of GHRH expression in the hypothalamus. While there is no change in food intake, leptin sensitivity or energy expenditure, an increase in weight loss is observed during food deprivation in these mice compared to controls.

Disruption of GHR in the brain has helped establish that GH has a role in neurological processes. By targeting *Ghr* in various regions of the brain, researchers have established that hypothalamic GHR controls hepatic glucose production in nutrient-sensing, leptin receptor-expressing neurons [61], and GH regulates responses to weight loss in AgRP neurons [64]. Given the intricacies of the brain and the vast number of cell populations, we anticipate that many more brain-specific GHR knockout mice will be generated and evaluated.

Fat-specific GHRKO mice

GH plays an important role in AT catabolism. To understand how the GH-axis in AT affects glucose homeostasis and longevity, three fat-specific GHR knockout mice have been independently generated and characterized.

FaGHRKO *Origin* The first fat-specific GHR knockout mouse line (FaGHRKO) was produced by List et al. in the Kopchick laboratory in 2013, utilizing the Cre/LoxP system driven by *aP2*, also known as *Fabp4* promotor/enhancer [44].

Phenotype These mice show increased **body weight** with a 96% increase in total **fat mass** and an overall increase in **body fluid** when compared to controls [44]. Additionally, female mice show an 8% increase in **lean mass**. Both brown AT and all white AT depots are significantly increased in these mice. While no change is seen in **insulin sensitivity**, female mice show an increase in IGFBP5, IL-6 and leptin. Both male and female mice show a decrease in adipsin, with male mice displaying an additional decrease in adiponectin and **IGF1** levels. Finally, these mice have a shortened **lifespan** when compared to WT controls.

AdGHRKO *Origin* Later, expression of the *aP2* promoter/enhancer was found in non-ATs, interfering with the interpretation of results seen in the FaGHRKO mice. To use a more reliable and robust model to investigate the direct effects of GH on AT, List et al. produced the AdGHRKO mouse, an adipocyte-specific GHR knockout mouse driven by adipoenctin/Cre [220].

Phenotype These mice exhibit no change in **body length** or **body weight**, though they have increased **fat mass** [220]. More specifically, all white AT depots had increased mass in female mice, and all but the perigonadal depot are increased in males. There is also an increase in brown AT in female mice. Adipocyte size is increased in these mice, with the only exception, again, being the perigonadal depot in male mice. These mice have improved **glucose homeostasis** with an increase in insulin sensitivity and no change in glucose tolerance. Furthermore, there is no change in serum **GH**, **IGF1** or fasting blood glucose, but there is a decrease in total insulin in male mice. These mice also have a reduction

in liver triglycerides. Overall, the more recent AdGHRKO mouse line has an AT profile remarkably like the previously reported FaGHRKO produced in the same laboratory.

Fat-Ghr-/- *Origin* To investigate the role of adipocyte-specific GH-action on CR, Fang et al. produced the Fat-Ghr-/- mouse in the laboratory of Guosheng Liang in 2019 using the Cre/LoxP system driven by an adiponectin promoter/enhancer [63].

Phenotype Differing from AdGHRKO mice, Fat-Ghr-/- mice have no change in **body fat mass**. When placed on CR, there is no change in blood glucose, plasma ghrelin or plasma GH levels [63].

Three distinct fat-specific GHRKO mouse lines have been created. FaGHRKO and AdGHRKO lines generated in the same laboratory by List et al. have a similar AT profile, with increased adiposity resulting in an overall increase in percent body fat. In contrast, Fat-Ghr-/- mice generated by Fang et al. have no phenotypic change in any parameter including percent body fat. The difference between these mouse lines is unknown but may result from incomplete disruption of the GHR in AT. Genetic background is likely not a factor since all three lines were produced in a mixed C57BL/6 N x C57BL/6 J background, where floxed mice were generated in C57BL/6 N then crossed to Cre mice in a C57BL/6 J background. It should be noted that both FaGHRKO and AdGHRKO mouse lines were generated using the same floxed mouse – generated in the Kopchick laboratory, while Fat-Ghr-/- mice were generated using floxed mice generated in the Liang laboratory. Thus, it is possible that differences in the floxed mouse lines may exist.

Other GHRKO mouse lines

In the following section, we will discuss several individual mouse lines generated to explore the tissue-specific effects of GH and GHR on the heart, bone and intestines or cell types such as macrophages, beta-cells and hematopoietic stem cells.

Macrophage—GHRMacD

Origin While GHRs are expressed on macrophages, little is known about the role of GH in macrophage function. Accordingly, in 2010, Lu et al. produced the GHRMacD mouse in the laboratory of Ram Menon [39]. These mice were produced using the Cre/LoxP system driven by the *Lyzs* locus, expressed specifically in macrophages, monocytes and granulocytes (neutrophils, basophils, etc.).

Phenotype In vivo characterization is not described for this mouse line in this initial paper; however, in vitro studies show that cultured media collected from primary macrophages in the stromal vascular compartment (SVC) of AT from GHRMacD mice have an inhibitory effect on preadipocyte differentiation when placed on 3T3-L1 cells [39]. This finding indicates that intact GH-action in primary macrophages increases preadipocyte differentiation. However, GH does not increase IGF1 expression in macrophages. There is no difference between **IGF1** levels in GHRMacD macrophages and control macrophages when treated with GH. In a follow up study in live mice, the Menon laboratory showed that GHRMacD mice (also called MacGHR KO mice in this paper) have no observable **phenotypic changes** except when challenged with a HFD [47]. When fed a HFD, GHRMacD mice had increased macrophage abundance in AT resulting in increased AT crown like structures and increased expression of pro-inflammatory cytokines (IL-1β, TNF-α, IL-6, and osteopontin) in AT stromal vascular fraction. These results support the possibility that GH may have beneficial effects on diet induced obesity related chronic inflammation.

Beta cell—βGHRKO

Origin To determine the role of the GHR in β-cell mass and function, Wu et al. created the β-cell GHR knockout mouse (βGHRKO) in the laboratory of Derek LeRoith in 2011 [41]. βGHRKO mice were generated by crossing GHR floxed mice with a rat insulin 2 promoter (RIP)/Cre/hGH mouse line constructed by Pedro Herrera [221].

Phenotype When fed a standard chow diet, βGHRKO mice show no difference in **body weight, body composition** or **IGF1** and **insulin** levels compared to controls [221]. However, these mice show a significant decrease in islet cell size and number, suggesting that GH stimulates the growth and proliferation of islet cells. On a HFD, βGHRKO mice show a significant decrease in β-cell mass and higher glucose levels.

It should be noted that there is controversy surrounding the Cre line used in this study as it inadvertently expresses h*GH*, thus results obtained by studies using βGHRKO mice are difficult to interpret. More specifically, multiple laboratories have demonstrated that fusion genes containing the h*GH* minigene used to enhance transgene expression and thought to not be transcribed or translated do in fact produce significant amounts of hGH [222, 223]. Furthermore, GH expressed in pancreatic islets can bind to the PRLR thus augmenting phenotypic factors such as beta cell mass and insulin content [222].

Hematopoietic stem cells (HSC)

Origin To investigate the impact of GH signaling on hematopoietic stem cells (HSC), Stewart et al. produced the $Ghr^{fl/fl}$;$Vav1^{Cre/+}$mouse in the laboratory of Rossi in 2014,

using the Cre/LoxP system driven by *vav1* gene transcriptional regulatory sequences [50].

Phenotype Ex vivo analyses conducted using primary hematopoietic stem cells from these mice show no significant 'blood cell' differences apart from a decrease in number of platelets [50]. Also, there are no changes in progenitor compartments, progenitor cell action or in peripheral blood engraftment following the primary and secondary competitive transplants. These results suggest that GH signaling is dispensable for HSC function.

Bone—DMP-GHRKO

Origin To investigate the role of GHR action on bone growth, Liu et al. produced the DMP-GHRKO mouse in the laboratory of Shoshana Yakar in 2016, using the Cre/LoxP system driven by a dentin matrix protein 1 (*Dmp1*) promoter/enhancer [224].

Phenotype These mice show no change in **body weight** or **composition** [224]. Also, there is no change in osteocyte morphology or serum **IGF1** levels. Serum **GH** is increased at 8 weeks and is similar to controls by 16 weeks of age. The DMP-GHRKO mice have decreased lacunae and cross-sectional area, resulting in a slender bone phenotype. Additionally, female mice have similar cortical bone thickness to controls but decreased bone marrow area. Males, however, have decreased cortical bone thickness and increased marrow area. DMP-GHRKO mice also have reduced levels of parathyroid hormone. To further understand the role of autocrine/paracrine IGF1 in bones, a DMP-IGF1RKO mouse, as more thoroughly described below, was produced. These mice have increased cortical bone cross-sectional area and reduced bone thickness and marrow area. Thus, the authors suggest that IGF1R and GHR may have overlapping as well as distinct effects on osteocytes [224].

Heart—iC-GHRKO

Origin To study the role of GH-action on the heart, Jara et al. produced the adult-inducible cardiac-specific GHR knockout mouse (iC-GHRKO) in the Kopchick laboratory in 2016, using a Cre/LoxP system driven by myosin heavy chain 6 promoter/enhancer [58].

Phenotype These mice show no change in **body weight or length**; however, they do have changes in **body composition** [58]. That is, the knockout mice have reduced fat mass and increased lean mass when compared to controls. There is no change in circulating insulin, with a decrease in circulating **IGF1** only at 12.5 months of age. At 6.5 months, there is no change in glucose tolerance, but an increase in **insulin sensitivity** is observed. At 12.5 months, however, these mice have decreased glucose tolerance and increased insulin resistance. The iC-GHRKO mice have no changes in cardiac dimension but have decreased cardiac wall thickness. Additionally, blood pressure is unaltered in iC-GHRKO mice compared to age matched controls. Thus, taken together, removal of GHR in cardiac tissue specifically, has no observable effect on cardiac physiology but results in a decreased cardiac wall thickness and altered whole body glucose homeostasis.

Intestine—IntGHRKO

Origin In 2019, to investigate the effect of GH on the intestines, Young et al. produced the intestinal epithelial cell-specific GHR knockout mouse (IntGHRKO) in the Kopchick laboratory, utilizing the Cre/LoxP system driven by a villin promoter/enhancer [65].

Phenotype These mice have comparable **body weights** to controls, with no persistent **body composition** differences [65]. In male mice, there is a decrease in large intestine length. Also, there is a trend, albeit not significant, towards shorter villi in the small intestine, as well as decreased crypt depth in both small and large intestines. Female mice have decreased glucose tolerance and show **insulin resistance**, while males do not. In terms of intestinal permeability measurements, male mice have increased expression of occludin and females have decreased fecal albumin, indicating that there is a modest improvement to barrier function. Finally, males present with decreased fat absorption. These results demonstrate that removal of GH-action in the intestinal epithelial cells has modest and sex-specific effects on intestinal morphology and function.

Mouse lines downstream of GHR

GH induced intracellular signaling molecules downstream of the GHR have been manipulated in mice and include Janus kinases (JAK), signal transducers and activators of transcription (STAT), suppressors of cytokine signaling (SOCS), acid-labile subunits (ALS), IGF1 and IGFBPs. These molecules play critical roles in growth and development, glucose homeostasis and other physiological processes; thus, mouse lines with alterations in the levels or actions of these molecules are of interest and will be discussed below. Results related to some of these mouse lines are summarized in Tables 3 and 4.

JAKs, STATs, SOCSs

The canonical GH intracellular signaling pathway, through JAK2 and STAT5b phosphorylation, has been targeted in addition to the other JAK and STAT proteins. In fact, almost every member of the JAK family and the STAT family has been knocked out in a mouse line, and a transgenic line overexpressing STAT4 has also been reported. Importantly, the

JAK/STAT pathway is shared among many different hormones and cytokines; as such, disruption of genes in this pathway generally results in impaired immune response and decreased growth and are difficult to attribute solely to GH action. Further downstream from JAK/STAT are SOCS proteins that serve as important inhibitors of this signaling pathway. Specific phenotypes of each gene disruption or transgenic mouse will be discussed below. All of the knockouts discussed in this section were generated using homologous recombination; for detailed description of the methods used for each mouse line, the reader is referred to the original publications.

JAK family knockout mice

Global JAK knockout mice *Origin* Janus kinase proteins are intracellular tyrosine kinases that transduce signals of many cytokines. There are four members of the family: JAK1, JAK2, JAK3 and Tyrosine Kinase 2 (TYK2), each of which has been disrupted in a mouse line. *Jak1-/-* mice were first reported in 1998 by Rodig et al. [225]. *Jak2-/-* mice were produced in 1998 by both the Pfeffer laboratory and Ihle laboratory [226, 227]. JAK3 expression is more limited than JAK1 or JAK2, specific to hematopoietic cells and epithelial cells, so the creation of *Jak3-/-* mice was driven in part by the desire to develop a new mouse line of immunodeficiency. *Jak3-/-* mice were produced by Park et al. in 1995 [228]. TYK2 is ubiquitously expressed, and its disruption in a mouse was first reported by Shimoda et al. in 2000 [229].

Phenotype Jak1-/- mice have decreased **size** compared to controls and an impaired immune response [225]. They also have a failure to nurse, leading to death within days of birth, indicating a broad range of cytokine signaling disruptions. In contrast, *Jak2-/-* mice die in utero, presumably due to their impaired erythropoiesis, as stem cells from *Jak2-/-* mice respond to interferon α but not to erythropoietin or interferon γ [226, 227]. *Jak3-/-* mice are born in the expected Mendelian ratio (when heterozygous mice are bred, 25% of the resultant offspring are *Jak3-/-*) and survive to adulthood but have impaired lymphocyte development [228]. Specifically, they have decreased B and T cells and lack peripheral lymph nodes, natural killer cells and γδ T cells in the skin and intestines. *Tyk2-/- mice* develop normally, but have impaired IFNα signaling and their response to interleukin (IL)-12 is completely disrupted [229]. Interestingly, these mice also develop **obesity** and **glucose intolerance** due to abnormal BAT development [230].

Mice with tissue specific disruption of JAK2 *Origin* Although systemic JAK2 gene disruption is fatal, at least two tissue-specific JAK2 gene disrupted mice with direct relevance to GH's metabolic effects have been reported. Liver-specific disruption of JAK2 (JAK2L mice) was first reported by Sos et al. in 2011, using the Cre/LoxP system with albumin promoter/enhancer to drive Cre [231]. To further explore the relationship between JAK2 and metabolism, the same laboratory developed an adipose-specific JAK2 disrupted (JAK2A) mouse line (first reported in 2013), also using the Cre/LoxP system with adiponectin promoter/enhancer driving Cre expression [232].

Phenotype JAK2L mice exhibit impaired lipid metabolism, with increased liver triglycerides and serum free fatty acids [231]. JAK2A mice have decreased lipolysis and increased **body fat**, as one would expect when GH signaling is disrupted in fat [232]. Interestingly, when the JAK2L and JAK2A mice are crossed to produce JAK2LA mice, those with JAK2 disruption in both tissues show the same increase in **body fat** and decreased lipolysis, but without interfering with liver lipid metabolism seen in JAK2L mice, indicating that the regulation of lipid metabolism through JAK2 involves coordination among multiple tissues [232].

STAT transgenic and STAT knockout mice

The STAT family are proteins that lie downstream of JAKs in various cytokine signaling pathways. The STAT family consists of 7 members—STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b, and STAT6—each of which has been disrupted in a mouse line; STAT4 transgenic mice have also been reported.

STAT1-/- mice *Origin* Because STAT1 is a central factor in interferon signaling and involved in the signaling of other cytokines, *Stat1-/-* mice were generated in 1996 by Meraz et al. and Durbin et al. to determine whether STAT1 is necessary for all interferon-induced signaling, as well as signaling of other cytokines [233, 234].

Phenotype STAT1 deficient mice have normal **reproduction** [233, 234]. Despite GH's ability to activate STAT1, this mouse line has no change in **body size** and responds normally to GH administration, gaining the same amount of weight as vehicle-treated controls. *Stat1-/-* mice also have normal responses to epidermal growth factor (EGF), IL-10, and IL-6. The most prominent phenotype of *Stat1-/-* mice is their complete lack of responsiveness to interferon (α and γ) [233, 234], leading to a strong susceptibility to infection by both bacteria and viruses, despite normal immune cell populations. The specificity of interferon signaling disruption in *Stat1-/-* mice has led to their common use as a model of interferon deficiency.

STAT2-/- and STAT3-/- mice *Origin* STAT2, in contrast to STAT1, is specific to type 1 interferon (α and β) signaling. *Stat2-/-* mice were first reported by Park et al. in 2000 [235]. STAT3 was initially identified as a downstream effector of IL-6, but later found to be activated in response to other

cytokines. In an attempt to clarify the role of STAT3 in cytokine signaling, *Stat3-/-* mice were developed in 1997 by Takeda et al. [236].

Phenotype As expected, *Stat2-/-* animals have increased susceptibility to infection but have unique deficiencies in T cells and macrophages as well as decreased STAT1 expression in some tissues [235]. STAT3 knockout mice **die early** in embryogenesis, thus limiting the utility of this mouse line [236].

STAT4 transgenic and STAT4-/- mice *Origin* STAT4 is predominantly associated with IL-12 signaling, and both STAT4 transgenic and null mice were generated to confirm this specificity. STAT4 transgenic mice were first reported in 1999 by Wirtz et al. using the cytomegalovirus (*CMV*) promoter/enhancer to drive expression of murine *Stat4* cDNA [237]. *Stat4-/-* mice were first reported in 1996 by Thierfelder et al. and Kaplan et al. [238, 239].

Phenotype Although no transgenic *Stat4* mRNA is initially detected in the colon, STAT4 expression is induced by injecting dinitrophenyl-keyhole limpet hemocyanin, and upon this treatment, colitis developed in the transgenic mice [237]. This phenotype agrees with the finding that IL-12 is associated with Crohn's disease in humans. In contrast to STAT4 transgenic mice, *Stat4-/-* mice also have normal **growth** and **reproduction** but an impaired immune system [238, 239]. STAT4 ablation in mice did result in disrupted IL-12 signaling, which causes decreased interferon-γ secretion, decreased T cell proliferation, decreased natural killer cell toxicity, and a shift from Th1 to Th2 cell differentiation.

STAT5a-/-, STAT5b-/- and STAT5a-/-5b-/- mice *Origin* STAT5 denotes two highly similar proteins, STAT5a and STAT5b, which have unique and overlapping functions, and may work together through the formation of heterodimers. As such, each has been knocked out in mice individually, as well as jointly. *Stat5a-/-* mice were first reported in 1997 by Liu et al. [240], while *Stat5b-/-* mice were reported in the same year by Udy et al. [241], and *Stat5a-/-5b-/-* were reported the following year (1998) by Teglund et al. [242].

Phenotype *Stat5a-/-* mice exhibit normal **size, weight**, and **fertility**, but they are unable to lactate, indicating a probable disruption of PRL signaling [240]. These mice also exhibit an impaired IL-2 response in T cells that can be overcome by IL-2 administration. STAT5b is part of the canonical GH signaling pathway and thus, disruption of the *Stat5b* gene, yields an expected decrease in **growth** [241]. Unexpectedly, this growth deficit is limited to males. The ablation of STAT5b also results in a sex-specific pattern of gene expression in the liver (e.g. CYP and MUP). IL-2 resistance is more pronounced in *Stat5b-/-* mice, as excess IL-2 does not ameliorate this resistance. Stat5b-/- mice also exhibit IL-15 resistance. When STAT5a and STAT5b are knocked out in combination, a stronger phenotype is observed [242]. These double null mice have decreased lymphocytes in circulation and are **infertile** due to impaired corpus luteum formation. Similar to GHR-/- animals, *Stat5a-/-5b-/-* mice are **dwarf** and have low serum **IGF1** levels and decreased epididymal **fat**. Interestingly, about 1/3 of the double knockout mice in the initial study died within 48 h of birth. The results from these three mouse lines underscore the importance of STAT5a and STAT5b in growth, lactation, and reproduction.

STAT6-/- mice *Origin* STAT6 is considered a key component in IL-4 signaling. To examine this relationship, Takeda et al. generated *Stat6-/-* mice [243].

Phenotype: *Stat6-/-* mice are similar in phenotype to many of the other STAT null mice and are also relatively "normal", with no reported change to **body length, body weight**, or **reproduction**. As expected, *Stat6-/-* mice experience disrupted IL-4 signaling, resulting in decreased MHC class II and CD23 expression. *Stat6-/-* animals also have impaired immunoglobin class switching, lymphocyte proliferation, and Th2 cell development [243]. Thus, STAT6 is important in adaptive and humoral immunity.

SOCSs transgenic and SOCS-/- mice

Further downstream of the GHR are SOCS proteins. As their name implies, SOCS proteins inhibit cytokine signaling in the JAK-STAT pathway. There are eight members of the SOCS family: SOCS1-SOCS7 and CISH, and each has been disrupted in a mouse line (except for SOCS4 and CISH). Transgenic models overexpressing CISH, SOCS1, SOCS2, SOCS3, SOCS5, and SOCS6 have also been reported. For more detail on SOCS family transgenic and null mouse lines, see a previous review on the subject [244].

SOCS1 transgenic and SOCS1-/- mice *Origin* The SOCS1 protein has been shown to inhibit GHR signaling [245], and thus *Socs1-/-* mice were generated by Starr et al. in 1998 [246]. SOCS1 transgenic mice were generated by expressing the transgene in the T cell lineage of mice via fusion of the cDNA to the *lck* tyrosine kinase proximal promoter / enhancer (first reported by Fujimoto et al. in 2000) [247].

Phenotype *Socs1-/-* mice are normal size at birth but show decreased **growth** and **die** before weaning [246]. The role of SOCS1 in immune development is further substantiated by the principal phenotypes of both SOCS1 transgenic and null animals. In the T-cell specific SOCS1 transgenic mice, impaired T cell development is observed. In addition, *Socs1-/-* mice present phenotypic differences associated with alterations to the interferon gamma (IFNγ) pathway, ranging from lymphopenia to monocyte infiltration into organs [246]. When IFNγ is knocked out alongside SOCS1, chronic

inflammation and perturbed T cell development is detected, along with polycystic kidneys [248].

SOCS2, SOCS3 transgenic and SOCS2-/-, SOCS3-/- mice *Origin* SOCS2 and SOCS3 were among the earliest SOCS proteins discovered, and both transgenic and null animals were generated for each gene/protein. *Socs2-/-* mice were reported by Metcalf et al., in 2000 [22], while SOCS2 transgenic mice were generated by Greenhalgh et al. in 2002 using the UBC promoter to drive gene expression [249]. SOCS3 transgenic and *Socs3-/-* mice were reported by Marine et al. in 1999 [250].

Phenotype Due to the role of SOCS2 in inhibiting the GH axis, *Socs2-/-* mice display **gigantism** [22]. These mice also show increased collagen deposition in their skin, another indication of increased GH action, and decreased levels of major urinary protein (MUP) in the urine. Interestingly, in SOCS2 transgenic mice, a counterintuitive result is observed; that is, the mice are **giant** [249]. These results suggest that excess or deficit of SOCS2 activate the GH/IGF axis, while moderate levels inhibit GH action. It is hypothesized that this activation of the GH/IGF axis is due to SOCS2 outcompeting SOCS3 (a more potent GHR inhibitor) for GHR-binding at high concentrations. The status of SOCS3 as a more potent GHR inhibitor is demonstrated by the more extreme phenotype seen when SOCS3 is altered. Because of embryonic **lethality**, no growth-associated phenotypes could be assessed in SOCS3 null and transgenic mice [250].

SOCS5 transgenic and SOCS5-/- mice *Origin* Another member of the SOCS family, SOCS5 is also believed to be involved in immune development, but knowledge of its association lags that surrounding other SOCS proteins. To help rectify this, SOCS5 transgenic and null mice were developed, in 2002 by Seki et al. [251] and in 2004 by Brender et al. [252], respectively. In the transgenic mice, a FLAG tagged SOCS5 protein is expressed in mice under the control of the *lck* proximal promoter/enhancer.

Phenotype Alterations to SOCS5 seem to have milder phenotypes than those seen with SOCS1 manipulation, which may explain why relatively little was known about SOCS5. In SOCS5 transgenic mice, the phenotype is limited to decreased Th2 cell differentiation [251]. *Socs5-/-* mice, on the other hand, have no alteration in phenotype [252].

SOCS6, CISH transgenic and SOCS6-/-, SOCS7-/- mice *Origin* Transgenic mice that overexpress SOCS6 were generated by Li et al. in 2004 using the elongation factor 1 (EF1) promoter/enhancer to drive Socs6 expression [253], and CISH transgenic mice were generated by Matsumoto et al. in 1999 using the β-actin promoter to drive Cis1 expression [254]. The SOCS6 and SOCS7 genes have also been disrupted in mouse lines, with *Socs6-/-* mice being reported by Krebs et al. in 2002 [255] and *Socs7-/-* mice reported in 2005 by Banks et al. [256].

Phenotype SOCS6 and SOCS7 manipulation results in phenotypes marked by alterations in **glucose metabolism**. Specifically, in SOCS6 transgenic mice, an improvement in **glucose metabolism** is observed [253]. *Socs7-/-* exhibit increased pancreatic islet size and improved **glucose metabolism** [256]. *Socs6* gene disruption (*Socs6-/-*), on the other hand, causes mild **dwarfism** with no reported change to glucose metabolism [255]. CISH transgenic mice phenotypically resemble *Stat5-/-* mice with normal development but with a defect in GH signaling [254]. Features of CISH transgenic mice include lactation deficiencies, indicating prolactin inhibition, as well as decreased **body size**, indicating the inhibition of the GH axis. However, CISH transgenic mice have normal **fertility**, differentiating them from *Stat5-/-* mice. CISH transgenic mice also have alterations to their T cells [decreased γδ T cells, natural killer (NK) cells, and NK T Cells and a shift in Th1/Th2 differentiation towards Th2 cells], further illustrating the many roles of CISH.

The strong phenotypes of some of the molecules downstream of the GHR demonstrate the complex regulation of GH signaling even before the main effector of GH action, IGF1, is taken into account.

IGF1, IGF1R, and tissue-specific KO

As one of the most important products of GH action, IGF1 and its receptor have been manipulated in numerous mouse lines to study its endocrine, autocrine and paracrine effects both globally and in specific tissues or cells. The IGFs are synthesized by almost all tissues and are important mediators of cell growth, differentiation, and transformation. IGFs have a fundamental role in both prenatal and postnatal development and exert their physiologic effects by binding to the IGF receptors or, albeit with less affinity, the insulin receptor. In addition, IGF1's effects are modulated by multiple IGF binding proteins (BP). In the following section, we will summarize the transgenic and knockout mouse lines relating to both IGF1 and its receptor. Details regarding each mouse line can also be found in Table 3.

IGF1 transgenic mice

Origin In 1988, Palmiter's laboratory generated IGF1 transgenic mice containing a fusion chimeric gene with *Mt1* promoter/enhancer, a sequence encoding the rat somatostatin secretory signal sequence to allow for secretion, the human *IGF1* cDNA, and a sequence containing the human *GH* 3'-RNA processing signals [257].

Phenotype These mice express 1.5 times higher circulating **IGF1** levels than controls and, as expected, decreased

GH levels [257]. No phenotypic differences are evident until 6–8 weeks of age. Overall, IGF1 transgenic mice display 1.3 times higher **weight** gain compared to WT mice though no increase in skeletal **growth** is observed. The spleen, pancreas, kidneys, and brain display increased growth. Also, **fertility** is not affected. Notably, changes in kidney structure have been identified in IGF1 transgenic mice by Striker's laboratory [258]. That is, IGF1 transgenic mice have enlarged glomeruli without glomerulosclerosis, in contrast to GH transgenic mice that display enlarged glomeruli with sclerosis. This implies that GH plays a direct role in the formation of kidney sclerosis while IGF1 stimulates increased glomerular size.

IGF2+/− mice (*Igf2*+/−)

Origin IGF2+/− (or *Igf2*+/−) mice were generated in 1990 by T. DeChiara in Robertson's laboratory by deleting a portion of exon 2 of the mouse *Igf2* gene [259].

Phenotype No homozygous *Igf2-/-* pups survive [259]. Heterozygous *Igf2*+/− pups display considerably smaller **body size** (60% of normal size). Genotyping of heterozygous embryos reveals that the mutant allele exerts its effect in the early embryonic stage (earlier than day 16) and maintains its effect in post-natal growth. Despite their diminutive size, the heterozygous mice appear normal and display normal **reproductive capacity**. Interestingly, this was the first study to identify the presence of imprinted genes (paternal), verifying previous hypotheses regarding this epigenetic phenomenon.

IGF1-/- and IGF1R-/- mice, and associated double mutants

IGF1-/- mice *(Igf1-/-)* *Origin* In 1993, the Efstratiadis laboratory reported the generation of the IGF1-/- (*Igf1-/-*) mouse generated via the deletion of exon 4 of the mouse *Igf1* gene [260].

Phenotype *Igf1-/-* mice experience increased neonatal lethality, although the rate of survivability is 10–68%, which is dependent on genetic background [260]. At birth, *Igf1-/-* mice display decreased **body mass** (65% of normal size). Post-natal effects include a progressively decreased **growth** rate, displaying 30% of control mouse size in adulthood [261]. The heterozygous *Igf1*+/− progeny do not display any obvious phenotypic difference from control littermates.

IGF1R-/- mice *(Igf1r-/-)* *Origin* In the same 1993 publication for the generation of IGF1-/- mice, the Efstratiadis group also reported generation of the *Igf1r-/-* mice via the deletion of exon 3 in the gene encoding *Igf1r* [260].

Phenotype These mice display severe **growth** deficiency with a **body mass** reduction of 45% compared to WT mice at birth. The mutant neonates, however, are not viable due to respiratory issues, and unlike IGF1-/- mice, lethality appears independent of the genetic background strain of the mice. In addition, mutant IGF1R-/- mice exhibit delayed ossification of bones in the extremities and trunk by 1–2 days post-birth.

IGF1-/- with IGF1R-/- mice *Origin* This same paper by Estratiadis also describes double mutants (*Igf1-/-* with *Igf1r-/-*) [260].

Phenotype The phenotype of the double knockout does not differ from the IGF1R-/- mice [260].

Overall, the role of IGF1/IGF1R in mouse embryonic development appears essential for viability, and the absence of which shows a considerable impact on bone development, muscle development and growth.

IGF1R-/- with GHR-/- mice *Origin* In 2001, the Efstratiadis laboratory also reported the crossing of mutant mice lacking either IGF1, GHR or both simultaneously to examine the impact of GH and IGF1 in controlling postnatal growth [262]. Note that GHR null mice were generated using a targeting vector that replace exons 7, 8a, and 8, distinct from that reported by Zhou et al., which is described above, but with a similar growth phenotype [30].

Phenotype With respect to **growth**, these studies estimated that 17% of **body weight** is attributed to processes unrelated to GH or IGF1 while IGF1 accounts for 35% of growth and 14% for GH [262]. Importantly, the study reveals that 34% of growth is associated with overlapping functions of GH and IGF1. This study also assesses chondrocytes and **bone ossification** and reports that GH and IGF1 have independent and overlapping functions in chondrocytes since the phenotype of double mutants is more severe than that manifested in either class of single mutant. Thus, these mutants provide conclusive evidence of the importance of both of these hormones acting independently and in concert to support body growth.

Tissue-specific IGF1 and IGF1R manipulation

To understand the role of IGF1 in specific tissues and cell types, IGF1 and IGF1R have been either knocked in or out in specific tissues and cell types. In the following section, we describe numerous tissue-specific mouse lines and provide additional details about each in Table 3.

Liver-specific IGF1 transgenic and KO mice (i) Hepatic IGF1 transgenic (TTR-IGF-I) mice

Origin In 2006, Xu's laboratory created hepatic IGF1 transgenic (TTR-IGF-I) mice using a fusion gene consisting of the promoter/enhancer of the transthyretin (TTR) gene, the mouse *Igf1* cDNA, and the SV40t polyadenylation-signal [263]. Note that the TTR promoter/enhancer targets transgene expression specifically to the liver, and the

authors estimate approximately three copies of the TTR-IGF1 transgene in these mice.

Phenotype As expected with increased circulating levels of **IGF1**, these mice show decreased levels of **GH** and increased IGFBP3 levels [263]. As Pegvisomant treatment does not alter IGFBP3 levels in WT mice, these results collectively indicate that IGFBP3 is not a direct target of the GH signaling pathway. The authors suggest that liver-expressed IGF1 can stimulate IGFBP3 expression and stabilize IGF1 under GH-deficient conditions. These mice display a larger **body size** and organ weight, presumably due to the higher circulating IGF1 levels. When TTR-IGF-I mice are bred with MMTV-ErbB2 mice to investigate the effect of elevated IGF1 on ErbB2 driven mammary carcinogenesis, the high levels of systemic IGF1 appear to have no effect on promoting ErbB2 driven **mammary carcinogenesis** [264].

(ii) Hepatic IGF1 transgenic (HIT) mice and KO-HIT mice

Origin In 2009, LeRoith and colleagues, developed the hepatic IGF1 transgenic (HIT) mice, which overexpresses the rat *Igf1* transgene in the liver of mice, as well as KO-HIT mice, in which only the liver produces IGF1 (i.e. mice that have a null *Igf1* gene in all tissues but overexpress a rat *Igf1* transgene specifically in the liver) [265].

Phenotype HIT mice have increased **IGF1**, unaffected **GH** levels, increased **body mass**, organ sizes and skeletal sizes, but decreased **adiposity** [265]. In contrast, KO-HIT mice have total absence of tissue IGF1, but elevated levels of serum IGF1, which can support normal **body size** and **weight** at puberty and postpubertal ages. Early deficits in skeletal structure of KO-HIT mice are restored by adulthood [266]. **Insulin sensitivity** is not altered by elevated levels of serum IGF1. Female KO-HIT mice have insufficient tissue IGF1 to fully support the female reproductive system, while male mice reproductive function is not affected. Overall, KO-HIT mice show that most autocrine/paracrine actions of IGF1 related to tissue growth and function can be offset by elevated levels of endocrine IGF1 although autocrine/paracrine IGF1 appears critical for neonatal development.

(iii) GHRKO-HIT mice

Origin In 2013 Yakar et al. combined the GHRKO mouse with the HIT mouse to generate the GHRKO-HIT mouse [267].

Phenotype The results with GHRKO-HIT suggest that, with the absence of GH-GHR mediated action, **serum IGF1** is not sufficient to restore body and **skeletal size**, but sufficient to restore impaired glucose tolerance in GHRKO mice [267].

(iv) LID mice

Origin The first liver specific IGF1 KO (LID) mouse line was produced by Yakar and LeRoith in 1999 via crossing albumin Cre mice with *Igf1* floxed mice.

Phenotype LID mice have increased **GH** and decreased **IGF1** levels due to IGF1 ablation in the liver [268]. Their **body weight**, selected organ weights (kidney, fat, muscle, spleen, and heart), **body length** and femur length are not different from WT controls. LID mice exhibit decreased **insulin sensitivity** and display normal **reproductive** capacity. Interestingly, when treated with GH, female LID mice exhibit an accelerated **growth** rate compared to males [216]. LID mice also show decreased **cancer** incidence and an increased **lifespan** in females compared to controls [21, 269] presumably attributing to lowered levels of circulating IGF1. These results challenged the idea that circulating IGF1 is critical for normal growth and development and suggest that growth is preserved even when IGF1 is absent from the liver and/or the importance of the autocrine/paracrine role of IGF1.

(v) Conditional liver IGF1KO mice (LI-IGF-I-/-)

Origin In 1999, Sjögren et al. produced conditional liver IGF1KO mice (referred to in the paper as LI-IGF-I-/- mice) by crossing mice with a Mx Cre (Mx dynamin-like GTPase 1) promoter/enhancer, which is activated in an interferon-dependent manner, to *Igf1* floxed mice [270].

Phenotype Similar to LID mice, these mice have increased **GH** levels, decreased **IGF1** levels in serum (~75%) and exhibit no changes in **postnatal growth** with induction of interferon at ~1 month and measurements at ~2 months after induction. Interestingly, kidneys are slightly smaller and the livers larger in LI-IGF-I-/- mice than in controls [270]. At 13 months of age, these mice have decreased **fat mass** and become **insulin resistant** [271]. The female mice also have an increased mean **lifespan** [272].

In summary, these results suggest that decreased endocrine IGF1 has a critical role in decreasing cancer incidence and extending lifespan, but it does not affect growth and development significantly. These findings are in contrast to what is observed in LiGHRKO mice in which lifespan is not altered and body size is decreased [48]. In these cases, GH and local IGF1 may be able to sustain growth of the whole organism and organs. On the other hand, increased IGF1 levels could further increase body size, organ weight and glucose tolerance.

Adipose-specific IGF1R KO mice (i) *aP2* adipose-specific IGF1R KO mice

Origin Different transcriptional regulators have been used to determine the physiological role of the IGF1R signaling in AT. Initially, an *aP2* promoter/enhancer-driven Cre was utilized by Kloting et al. in 2008 [273].

Phenotype These ap2 adipose-specific IGF1R KO mice have a marked increase in somatic growth with increases in both **body weight** and **body length** [273]. They also have elevated circulating **IGF1** and **IGFBP3** levels with no change in GH. The authors suggest that the ~20% increase in circulating **IGF1** is responsible for the increased growth.

Other notable metabolic features in these mice include elevated **glucose** levels and suppressed adiponectin levels, despite normal glucose and insulin tolerance. Regarding their AT phenotype, these mice have increased **fat mass**, more prominent in the gonadal region versus the subcutaneous region, and significant increases in adipocyte size. The increase in lipid accumulation is attributed to an increase in IRs and **insulin-stimulated glucose uptake** into adipocytes with the deletion of the IGF1R. Importantly, these authors reveal a decrease in IGF1R protein not only in AT but also in the brain. More recently, other groups have confirmed the promiscuity of the *ap2* promoter in several tissues including regions of the brain [274–276]. The "leaky" nature of this promoter sheds doubts on whether the phenotype observed in these mice is due to a deletion of IGF1R AT or other tissues. Regardless, the authors conclude that IGF1R signaling in adipocytes is not crucial for the development and differentiation of AT/adipocytes but does seem to participate in regulating circulating IGF1 levels.

(ii) *Adiponectin* adipose-specific IGF1R KO mice

Origin To uncover the specific role of IGF1R in adipocytes, a second adipocyte-specific IGF1R KO mouse was created using the adiponectin promoter/enhancer by Ron Kahn's group in 2016 [277].

Phenotype These mice have a distinct phenotype as compared to the first mouse line made with the ap2 Cre. The adipo-Cre IGF1R KO mice have modest reductions in both **white AT** and **brown AT** mass (~ 25%), despite a 73% increase in circulating **IGF1** levels [277]. They also have reduced expression of lipogenic genes in intra-abdominal fat depots, reduced levels of circulating leptin and adiponectin [277] with no change in ectopic fat deposition. However, these mice have no appreciable changes in response to a **glucose** or **insulin** challenge or basal insulin or glucose levels. In comparison, insulin receptor adipocyte-specific KO results in a severe lipodystrophic state, severely impaired glucose metabolism (higher basal glucose and insulin, impaired GTT and ITT), and increased ectopic fat deposition than IGF1R KO mice. Thus, the authors conclude that insulin and IGF1 signaling play essential but distinct roles in the development and function of white and brown fat.

Brain-specific IGF1R KO mice (bIGF1RKO +/− and bIGF1RKO −/−) *Origin* In 2008, Holzenberger's laboratory generated brain-specific IGF1R KO mice by crossing *Igf1* floxed females with Nestin-Cre transgenic males (flox/+; NesCre +/0) [278]. Nestin driven Cre recombinase is specific to neural and glial precursors early in neural development.

Phenotype Homozygous double mutants express no IGF1R on CNS neurons or glia [278]. The homozygous animals have microcephaly with severe **growth retardation**; they are also **infertile** and exhibit abnormal behavior (e.g. male KO mice have impaired exploratory behavior and are less anxious) but have normal **lifespans**. On the other hand, heterozygotes, whose IGF1R levels are depleted by half in the neurons and glia, exhibit healthier aging (delayed **mortality** and longer mean **lifespan**) and behave normally. By 90 days, heterozygote adults weigh 90% of WT controls and are 5% shorter in length. They have normal **IGF1** levels in peripheral tissues but lower plasma **GH** and IGF1 levels. Adult pituitaries are 30–40% smaller with markedly fewer somatotrophs, and most other organs are smaller in adult bIGF1RKO +/− mice with the exception of **AT**, which is significantly increased in both adult males and females. Adult heterozygous males also have significantly higher circulating lipid levels (triglyceride, HDL, total cholesterol and free fatty acid) compared to WT animals. Both sexes of heterozygous mice have impaired **glucose tolerance**. Like homozygous mice, there is no change in maximum lifespan of heterozygous mice; however, heterozygous mice do have an increase in mean **lifespan**, which is attributed to fewer degenerative diseases as well as tumors compared to WT. Overall, the authors conclude that partially lowered GH/IGF1 signaling in the brain favors lifespan extension and that the ability to alter somatotropic function in stressful environments allows the organism to decelerate growth and preserve resources, and thereby improve health span.

Muscle-specific IGF1 transgenic and IGF1R KO mice (i) Skeletal muscle IGF1 transgenic Mice

Origin Striated muscle-specific IGF1 transgenic mice were created in 1995 by the Schwartz laboratory using the avian skeletal α-actin gene proximal promoter/enhancer appended to the human *IGF1* gene [279].

Phenotype Striated muscle-specific IGF1 transgenic mice have no changes in serum **IGF1** levels or **body weight** [279]. However, concentrations of **IGF1** in muscle are 47-fold greater in transgenic mice compared to WT controls, causing myofiber hypertrophy with a change in overall fiber types and increased superficial gluteus muscle.

(ii) MKR Mice

Origin In 2001, Le Roith's laboratory generated skeletal muscle-specific transgenic mice by overexpressing a dominant-negative IGF1R (MKR mice) via fusion of mutant *IGF1R* (KR-hIGF1R) cDNA downstream of the muscle-creatine kinase (MCK) promoter/enhancer [280]. In these mice, the mutated gene encodes a protein that has lysine at position 1003 changed to arginine (KR mutant), which abolishes the ATP-binding within the β-subunit of the human *IGF1R* cDNA.

Phenotype In these mice, expression at the protein level results in the formation of hybrid receptors between mutant and endogenous IGF1R and IRs, abrogating their normal function and resulting in a marked decrease in glucose uptake upon stimulation with either IGF1 or insulin [280].

Although normal glucose tolerance is maintained, peripheral **insulin resistance** and pancreatic beta cell dysfunction develop by seven to twelve weeks of age in MKR mice, contributing to a chronic hyperglycemic state. Overall, body glucose disposal, glycolysis and glycogen synthesis are significantly reduced in MKR mice. In the skeletal muscle and brown AT of these mice, glucose transport activity is reduced by 50%. There is also a marked increase in the number of glycogen deposits, FFAs, and triglycerides in the livers consistent with an aggravation of the insulin-resistant state. MKR mice also exhibit a 10–20% reduction in **body weight** relative to WT controls.

(iii) MIGIRKO Mice

Origin A double knockout mouse (MIGIRKO), which has a loss of both IR and IGF1R signaling reported by O'Neill et al., in 2015, was generated via the use of a Cre/LoxP system using a skeletal muscle actin promoter/enhancer [281].

Phenotype MIGIRKO mice exhibit a 60% decrease in muscle mass, accompanied by loss of both muscle strength and endurance, and a shortened **lifespan** (6 months) due to atrophy of the diaphragm [281]. These mice have normal glucose and **insulin tolerance** but lower fasting glucose levels and increased basal glucose uptake. The alteration in glucose metabolism is due to increased membrane localization of glucose transporters (Glut 4 and Glut 1) as a result of decreased TBC1D1, a protein critical to the regulation of glucose transport in muscle cells.

(iv) M-IGF1R KO Mice

Origin In 2016, O'Neil et al. also reported the characterization of muscle-specific IGF1R KO mice (M-IGF1R KO) [282].

Phenotype M-IGF1R KO has no significant reduction in muscle mass in contrast to MIGIRKO, most likely due to compensation on behalf of functional IR signaling [282]. Overall, these mice do not display a dramatic phenotype resulting from disruption of solely IGF1 action in muscle, again, likely due to compensation via functional IR signaling.

Cardiac-specific IGF1 transgenic and IGF1R KO mice (i) Cardiac-specific IGF1 transgenic mice

Origin In 1996, IGF1 transgenic mice were generated by the Anversa laboratory using human *IGF1* cDNA placed under transcriptional control of rat α-myosin heavy chain promoter/enhancer [283].

Phenotype These transgenic mice have increased serum **IGF1** despite cardiomyocytes being the only source of transgenic IGF1 [283]. This finding is similar to what is reported above for cardiac-specific GHR disruption by Jara et al. [58] and emphasizes the significant contribution of cardiomyocytes to endocrine IGF1. These mice have significantly greater total heart mass, liver, brain, spleen and kidney due to the increase in IGF1. The enlarged hearts are attributed to overexpression of IGF1-induced myocyte proliferation, suggesting that local and endocrine IGF1 increase **organ sizes** and promote myocyte proliferation.

(ii) Cardiomyocyte IGF1 transgenic mice

Origin Another cardiomyocyte IGF1 transgenic mouse line was created using mouse α-myosin heavy chain promoter/enhancer by the Rosenthal group in 2007 [284].

Phenotype In these mice, local IGF1 expression results in accelerated postnatal cardiac growth and greater heart size [284]. These mice have the capacity to repair their hearts more efficiently both morphologically and functionally in response to injuries induced by cardiotoxin or ligation.

(iii) CIGF1RKO mice

Origin In 2008, the laboratory of Abel developed a constitutive cardiac-specific IGF1R knockout mouse (CIGF1RKO) [285].

Phenotype These mice are resistant to exercise-induce cardiac hypertrophy, implicating IGF1 in this process [285].

(iv) iCMIGF-IRKO mice

Origin Adult heart, tamoxifen-inducible, cardiomyocyte-specific IGF1R KO mice (iCMIGF-IRKO) were reported in 2012 by Gödecke et al. [286]. Mice with tamoxifen induction at 3 months and 11 months of age, with measurements taken 6 weeks after gene deletion, are described.

Phenotype Younger induction (3 months) results in no functional or structural consequences; however, induction at the older age (11 months) results in cardiac dysfunction without structural abnormality [286].

In summary, these studies show that autocrine/paracrine IGF1 promotes heart repair in response to injury and conservation of cardiac function. However, the absence of IGF1 signaling in cardiomyocytes does not affect the morphology or function of hearts significantly, unless induction occurs at a later age (11-month-old). Similarly, the removal of GH action in heart at adult age (4-month-old) affects neither the local IGF1 levels nor the function of hearts [58] even though endocrine IGF1 levels are altered.

Endothelial IGF1R transgenic and KO mice (i) Endothelial IGF1R transgenic mice (hIGFREO)

Origin Generated by Kearney et al. team in 2012, the endothelial IGF1R transgenic mice (hIGFREO) were produced by overexpressing human *IGF1R* following the *Tie2* (mouse endothelial-specific receptor tyrosine kinase) promoter/enhancer [287].

Phenotype These transgenic mice exhibit no change in **size/weight** or **glucose homeostasis** [287]. Reduced basal and insulin-stimulated eNOS activity is reported in these mice. As for cardiac function, no difference in endothelial cell eNOS is observed with only enhanced aortic constriction in response to phenylephrine. These mice have normal blood pressure and aortic response to acetylcholine (ACH)

and nitroprusside but increased endothelial cell migration and regeneration.

(ii) EC IGF-1R KO mice

Origin Also reported by Kearney et al. in 2011, opposite results are observed for the endothelium-specific IGF1R KO mouse (EC IGF-1R KO) produced by *Tie2* Cre [288]. A second endothelial cell (EC)-specific IGF1-R KO mouse line was generated by Cheng and colleague in 2015 within the context of chronic kidney disease (CDK)-induced pathology via vascular epithelial (VE)-cadherin-Cre [289].

Phenotype Kearney et al. report that male EC IGF-1R KO mice show normal **glucose homeostasis** with enhanced basal and insulin-stimulated eNOS phosphorylation[288]. As for cardiac function, there is blunted aortic constriction in response to phenylephrine and enhanced aortic constriction in response to l-*N*G-nitro-l-arginine methyl ester (L-NMMA). No difference in endothelial cell eNOS is observed. The EC specific IGF1-R KO mice produced by Cheng et al. have no changes in overall **body size, weight**, or **reproductive** capacity [289]. However, these KO mice display significantly more severe tubular injury and interstitial collagen deposition in obstructed kidneys compared to WT. The phosphorylation state of VE-cadherin, correlating with the disassembly of EC junctions, is significantly higher, along with markedly increased platelet accumulation and vascular permeability in null animals.

Collectively, these results support an important role for IGF1R within a physiological range in regulating nitric oxide bioavailability and vascular repair, which are hallmarks of several human diseases involving tissue growth and vascularization.

Myeloid and macrophage-specific IGF1R KO mice (i) MIKO mice

Origin In 2016, Dixit and colleagues at Yale University created myeloid-specific IGF1R KO mice (MIKO) with Cre driven by *LysM* promotor/enhancer [290, 291].

Phenotype MIKO mice have decreased NLRP3 (NOD-, LRR- and pyrin domain-containing protein 3) inflammasome activation in aging macrophages [290]. They also exhibit increased **adiposity**, with fewer macrophages in the stromal vascular fraction of visceral AT and a decrease in M2 macrophage activation, unlike the increase in visceral AT M2 macrophage polarization reported in GHRKO mice [291]. Interestingly, these mice show delayed resolution from helminth infection (which induces an adaptive immune response characterized by a distinct T helper cell driven cellular and cytokine profile) and have increased **insulin resistance** when placed on a HFD.

(ii) MΦ-IGF1RKO mice

Origin As autocrine/paracrine action of IGF1 plays an important role in increasing macrophage activities [290], in 2016, Delafontaine and colleagues created a monocyte/macrophage-specific IGF1R KO mouse (MΦ-IGF1RKO), bred on an apolipoprotein E-deficient genetic background [292].

Phenotype These mice show increased atherosclerotic lesion formation with less stable plaques and marked by increased macrophage content [292]. Plaque-associated macrophages exhibit increased inflammatory responses to stimulation, as well as increased expression of antioxidant genes. Production of cytokines or chemokines such as IL-1α, IL-6, TNFα, MPC1 and fractalkine (an unusual chemokine encoded by the gene CX3CL that can act as either a soluble or membrane-bound mediator), are associated with increases in NFκB activity. These macrophages also demonstrate decreased expression of ATP-binding cassette transporters ABCA1 and ABCG1 and, therefore, a significant reduction in HDL-dependent cholesterol efflux, which leads to atherogenesis.

In conclusion, these studies suggest that macrophage IGF1 signaling exerts anti-atherogenic effects through suppressing macrophage activities, atherosclerotic lesion formation, and reducing plaque vulnerability.

Bone-specific IGF1 transgenic and IGF1(R) KO mice (i) Osteoblast-specific IGF1 transgenic mice

Origin In 2000, Clemens' Laboratory created the osteoblast-specific IGF1 transgenic by fusing the r*Igf1* cDNA transgene to the human osteocalcin (*OC*) promoter/enhancer [293]. Another osteoblast-lineage IGF1 transgenic mouse reported by Kream's group in 2006 utilized the upstream regulatory sequence of rat *Col1a1* gene followed by murine *Igf1* [294].

Phenotype The mice from Clemens' group have increased bone formation rate and cortical and trabecular bone mass density [295]. The mice generated by Kream's group show increased bone formation and resorption; male transgenic mice have increased serum **IGF1** levels and **body weight** [294].

(ii) Osteoblast IGF1R KO mice and OBIGF1R-/- mice

Origin Osteoblast IGF1R KO mice were generated by Clemens's group in 2002 (human osteocalcin promoter/enhancer) [296–298] and OBIGF1R-/- mice (*Col1α*1 promotor/enhancer) by Bikle's laboratory in 2015 [296–298].

Phenotype Both mice have decreased bone formation rate and cancellous bone volume/connectivity with normal **body size** [296–298]. These mice also have increased trabecular bone separation with a decrease in trabecular number.

(iii) Chondrocyte IGF1 KO mice

Origin Chondrocyte IGF1 KO mice originated using the procollagen *Col2α1* gene promoter/enhancer driven Cre by Mohan's laboratory in 2007 [299, 300].

Phenotype These mice exhibit decreased bone mineral content, bone mineral density, **bone size**, weight and **body length** when compared to WT [299, 300].

(iv) Osteocyte IGF1 KO mice

Origin In 2013, Lau and colleagues created the osteocyte IGF1 KO mice using dentin matrix protein 1 *(Dmp1)* driven Cre [301].

Phenotype These mice have significantly smaller periosteal diameter of femurs, shorter **femur lengths**, reduction in bone mineral contents, bone formation and bone turnover [301].

(v) DMP-IGF-1R KO mice

Origin Osteocyte IGF1R KO mice (DMP-IGF-1R KO) were generated by Yakar et al. in 2016 [224].

Phenotype DMP-IGF-1R KO mice show an increase in total cross-sectional areas of femora with reductions in bone area but significant increases to marrow area. DMP-IGF-1R KO mice also exhibit cortical **bone thickness** with enlarged marrow area, which indicates increased endosteal resorption [224]. When DMP-GHR KO mice are compared with DMP-IGF-1R KO, there is a decrease in bone accrual for both [224]. These results imply that GH and IGF1 share some overlapping yet distinct effects on osteocytes.

In summary, the GH/IGF1 axis controls skeletal growth through an endocrine and autocrine/paracrine fashion. Studies above clearly show that IGF1 signaling regulates bone length, radial bone growth, cortical and trabecular bone properties through chondrocyte, osteoblast and osteocyte function. Detailed information can be found in the review from Yakar et al., 2018 [302].

Ovarian granulosa cells IGF1R KO mice *Origin* In 2017, Stocco's laboratory generated ovarian granulosa cell specific IGF1R KO mice ($IGF1R^{gcko}$) using Cre driven by the estrogen receptor β (*Esr2*) and the aromatase (*Cyp19)* promoter/enhancers [303].

Phenotype These mice do not possess antral follicles, even with gonadotropin stimulation. They are **sterile** and have smaller ovaries [303]. Serum estradiol levels are decreased by 90% compared to controls, while follicle stimulation hormone (FSH) receptor expression is not altered. Their **insulin sensitivity** is unchanged in comparison to control mice. Activation of AKT is significantly dampened, and apoptosis levels in follicles from primary to secondary stages are increased. Overall, these data suggest that IGF1R has an essential role in granulosa cell function and, as a result, in female **fertility**.

Pancreatic β Cell IGF1R KO mice (i) β cell IGF-1R KO mice

Origin In 2002, Efstratiadis' laboratory generated β Cell IGF-1R KO mice using Cre driven by the rat insulin promoter/enhancer (InsPr-Cre) [304]. Another β Cell IGF1R KO mouse was generated in Kahn's laboratory in 2002, also using InsPr-Cre [305].

Phenotype The lack of IGF1R in the mice produced by Efstratiadis's laboratory does not affect β cell mass but does lead to age-dependent **glucose intolerance** and decreased **insulin secretion** in response to arginine and glucose [304]. With these results, the authors suggest that IGF1R signaling is a requirement in regulating insulin secretion. The finding from Kahn's laboratory corroborates those of the Efstratiadis laboratory, except that they report normal insulin secretion in vivo in response to arginine [305]. Kahn's mice also have a decrease in insulin secretion in response to glucose.

(ii) βDKO mice

Origin βDKO mice with disruptions in both IR and IGF1R in β cells were generated using an Ins/Pr-Cre [306].

Phenotype These double KO mice have low insulin levels with high levels of glucagon and are highly **glucose-intolerant** [306]. Both the mass and insulin content of pancreatic β cells are decreased. While β cell-specific IGF-1R KO and IR KO are both glucose intolerant, a more severe intolerance is observed in βDKO mice, probably due to the overlapping functions of IGF1 and insulin.

Steroidogenic cell IGF1R KO mice (i) Steroidogenic cell IGF1R KO mice

Origin In 2018, Nef's laboratory developed IGF1R KO mice with IGF1R deleted in steroidogenic cells using Cre driven by the human $P450^{SCC}$ promoter/enhancer [307].

Phenotype IGF1R KO in steroidogenic cells result in mice that **grow** normally, have normal adrenal gland development, and no change in corticoid synthesis [307]. Male KO mice have significantly decreased **testicular weight** in comparison to control mice, but seminal vesicle size and anogenital index are unchanged. Leydig cells, which are steroidogenic cells that produce androgens, are found to have decreased responsiveness to human chorionic gonadotropin (hCG). The authors indicate that IGF1R signaling is necessary for the development of Leydig cells and their steroidogenic activity as adults. They also noted that disruption of IGF1R signaling did not have a significant effect on adrenal gland development or function.

(ii) Steroidogenic cell IGF1R;IR KO mice

Origin Nef's laboratory also developed IGF1R;IR KO mice with both IR and IGF1R deleted in steroidogenic cells using Cre driven by the human $P450^{SCC}$ promoter/enhancer [307].

Phenotype The phenotype of IGF1R;IR double KO mouse is dramatic [307]. That is, Leydig cells fail to mature, resulting in impaired steroidogenic function, decreased steroidogenic cells and serum testosterone levels. These mice have a substantial reduction in the size of the adrenal cortex and testis and are **infertile**. After weaning, survival rate of these mice is significantly reduced due to disparity in salt and water metabolism.

Somatotroph IGF1R KO mice (i) SIGFRKO mice

Origin In 2010, Radovick's laboratory generated somatotroph-specific IGF1R KO mice (SIGFRKO) using Cre driven by the GH promoter/enhancer (rGHpCre) [308].

Phenotype SIGFRKO mice do not respond to feedback by IGF1 [308]. By 14 weeks of age, these mice **grow** normally but **weigh** significantly less than controls; and **body length** is unchanged in comparison to control mice at all life stages. These mice have increases in the levels of **fasting serum GH and IGF1** and have altered **body composition** with decreased fat mass but no change in lean mass. Average weights of many tissues (brain, heart, lungs, and kidney) are unchanged although liver and spleen mass are increased. Additionally, IGFBP3 levels are unchanged and ALS levels increased. There are also decreased mRNA levels of GHRH and increased mRNA levels of somatostatin in pituitary tissue, likely contributing to the growth deficiency observed by 14 weeks of age. **Glucose and insulin tolerance** are both unchanged in SIGFRKO mice [308].

(ii) HiGH mice

Origin In 2011, Kineman's laboratory generated somatotroph IGF1R and IR KO mice (HiGH) with somatotroph specific inactivation of both the IR and IGF1R using the rat *Gh* promoter/enhancer driving Cre [309].

Phenotype HiGH mice are characterized by increased **levels of GH and IGF1** [309]. From birth to 3 weeks of age, these mice are the same **size** as control mice, but their **weight** is modestly increased in adult life. The increase in GH promotes a **lean phenotype** but has minimal effects on adiposity in males, even in response to HFD. These mice have decreased **insulin sensitivity** and elevated insulin levels. HiGH mice also have a mild elevation in the GH/IGF1 axis and provide a means to understand the role of the GH/IGF1 axis within more physiological levels than transgenic GH mice, which have extraordinarily high levels of GH.

Thyrocyte-specific IGF1R KO mice *Origin* In 2011, Müller et al. generated thyrocyte-specific IGF-1R KO mice using Cre driven by the thyroid-specific thyroglobulin promoter/enhancer [310]. Mice lacking one or two alleles of the *Igf1r* (*Igf1r*+/− *or Igf1r*-/-) were characterized.

Phenotype These mice have no difference in thyroid weights; however, both $Igf1r^{flox/wt}$ and $Igf1r^{flox/flox}$ mice exhibit a more abnormally large thyroid follicles than controls [310]. They also have a greater number of papillary structures resembling papillary thyroid hyperplasia, increased thyroid-stimulating hormone (TSH) levels, and normal thyroid hormone synthesis. $Igf1r^{flox/flox}$ males exhibit increases in **body weight** consistent with latent hypothyroidism. Conversely, the weights of female $Igf1r^{flox/flox}$ remain lower compared to WT. There is also a sex- and age-dependent alteration in **perigonadal fat mass**. Both $Igf1r^{flox/wt}$ and $Igf1r^{flox/flox}$ mice of both sexes retain normal **glucose tolerance**, though male $Igf1r^{flox/flox}$ experience lower insulin resistance. Overall, specific ablation of IGF1R in thyrocytes does not affect thyroid hormones synthesis, but it does affect thyroid homeostasis and systemic alterations in metabolism.

IGF Binding Proteins (IGFBPs) and Acid Labile Subunit (ALS)

IGFBPs transgenic and knockout mice

In circulation and in tissues, most IGF molecules are bound by one of the six distinct members of the IGF-binding protein family (IGFBP) designated as IGFBP1 through IGFBP6 [311]. IGFBPs bind to IGF molecules with high affinity, regulating their bioavailability and functions. In addition, several IGFBPs have been reported to have cellular actions that are independent of their IGF binding. To determine the specific function of each IGFBP in vivo, different mouse lines have been generated. Mice with IGFBP 1, 2, 3, 4, and 5 expressed as transgenes or knockouts have been reported. For IGFBP6, data are only published for transgenic mice. Due to the overlapping functions of IGFBPs, some phenotypes of transgenic or null mice are mild. More details for the mice described in this section are provided in Table 4.

Human IGFBP1 transgenic and IGFBP1-/- mice (i) IGFBP1 transgenic mice

Origin The hIGFBP1 transgenic mice were created by D'Ercole et al. in 1995 in which gene expression was controlled by the mouse *Mt1* promoter/enhancer [312]. The inserted transgene was a full-length human *IGFBP1* (hIGFBP1) cDNA, which was truncated at the 3' untranslated (3'UT) region.

Phenotype IGFBP1 is expressed ubiquitously in these animals [312] whereas liver is the major site of expression in nontransgenic mice [313]. Transgenic hIGFBP1 mice have lower **body weight**, smaller brains, as well as smaller and sometimes dysmorphic bone structure [312, 314]. Mice display **insulin resistance** in skeletal muscle, hyperglycemia and impaired glucose tolerance with advancing age [315], reduced **fertility** in the female mice due to changes in follicular growth [316], and increased extracellular matrix deposition and glomerulosclerosis in kidneys [317].

(ii) IGFBP1-/- mice

Origin Igfbp1-/- mice, reported by the Taub laboratory in 2003, used a *Spe*I restriction enzyme to insert a NeoR gene that disrupted the *Igfbp*1 gene [318].

Phenotype These null mice present with increased serum **IGF1** levels that normalizes by 4-months of age [318]. These mice show no major alterations in their metabolic phenotype or **insulin sensitivity**. When crossed with c-Myc transgenic mice to induce prostate **cancer**, there is no significant difference in the incidence of cancer though the prostate tumor size tends to be smaller, and proliferation is

decreased [319]. Overall, the impact of lost IGFBP1 action appears to be minimal.

IGFBP2 transgenic and IGFBP2-/- mice (i) IGFBP2 transgenic mice

Origin IGFBP2 transgenic mice were generated via the CMV promoter/enhancer fused to the *Igfbp2* cDNA by Hoeflich et al. in 1999 [320].

Phenotype The CMV transcriptional regulatory region is known to direct expression in multiple cell types with transgene expression being highest in the pancreas and stomach, whereas IGFBP2 is normally produced primarily in the liver and kidneys in adult nontransgenic mice [313]. IGFBP2 transgenic mice display no changes in circulating levels of **GH** or **IGF1**, total **body weight**, or bone size; however, a reduction in body length and a significant increase in fat mass in males is observed [321]. These mice also have reduced serum insulin levels and increased **insulin sensitivity** as well as lower systolic blood pressure [321, 322]. When subjected to HFD, IGFBP2 mice are more resistant to obesity when compared to WT controls and have decreased leptin levels, increased glucose sensitivity, and lower blood pressure. Surprisingly, IGFBP2 transgene expression has a protective effect against colon **cancer** due to decreased cell proliferation and is protective against metabolic diseases [321, 322].

(ii) IGFBP2-/- mice

Origin Igfbp2-/- mice were generated by the Pintar group in 2000 via deletion of exon 3 in the *Igfbp2* gene [323].

Phenotype Igfbp2-/- mice have no noticeable changes in circulating **GH, IGF1** or **body weight** when compared with WT controls [323]. However, organ specific differences are observed, with a notable increase in liver size and a decrease in the size of the spleen, heart, and kidneys. There are no differences **in insulin sensitivity**, other metabolic parameters or **fertility**. Blocking IGFBP2 action can improve **cancer** outcomes, at least in mice susceptible to glioblastoma due to reduced immunosuppression caused by IGFBP2 [324]. This indicates that IGFBP2 has tissue dependent effects on susceptibility to cancers although a comprehensive analysis of cancer incidence for *Igfbp2-/-* mice are not reported.

Human IGFBP3 transgenic and IGFBP3-/- mice (i) IGFBP3 transgenic mice

Origin IGFBP3 transgenic mice were generated by Murphy et al. in 1995 using a *Mt1* promoter/enhancer and the cDNA of the human *IGFBP3* transgene [325]. IGFBP3 transgenic mice created using the CMV promotor/enhancer or the phosphoglycerate kinase (PGK) promoter/enhancer have also been reported (called CMVBP-3 and PGKBP-3 mice) [326].

Phenotype IGFBP3 is normally expressed predominantly in the kidneys of adult nontransgenic mice and is also highest in the kidneys of transgenic mice as well [313, 325]. Transgenic mouse using *Mt1* promotor/enhancer exhibits greater spleen, liver, heart, and fat **weight**. Other detectable changes include reduced alveoli size and a significant age-related decrease in pancreatic beta cell mass [313, 325, 327]. Although not assessed in IGFBP3 mice made with the *Mt1* promoter/enhancer, other IGFBP3 transgenic mice (CMVBP-3 or PGKBP-3) show no change in **fertility** for males or females [326].

(ii) IGFBP3-/- mice

Origin Igfbp3-/- mice were generated by the laboratory of Pintar in 2006 [328] using a NeoR cassette inserted between exon 1 and 3 of the *Igfbp3* gene.

Phenotype These mice have no change in **body weight** or size [328, 329]. The initial analysis of these animals reveals a decreased metabolic rate and reduced plasma triglyceride and adiponectin levels [329]. When challenged with a HFD, *Igfbp3-/-* animals also maintain hepatic **insulin sensitivity** despite impaired fasting glucose. Null mice show an increase in lung **tumorigenesis** due to IGFBP3's influence on IGF1 signaling [330]. Changes in **reproductive** capabilities are not reported. In summary, gene disruption of IGFBP3 creates a mouse that has some positive effects on metabolism, but a negative effect as it relates to at least one type of cancer.

IGFBP4 transgenic and IGFBP4-/- mice (i) IGFBP4 transgenic mice

Origin In 1998 and as first reported by Fagin's group, IGFBP4 transgenic mice were generated via microinjection of murine *Igfbp4* cDNA cloned downstream of α-actin 5′-flanking region [331].

Phenotype IGFBP4 is expressed mainly in the adult kidney, liver, and spleen of nontransgenic mice although transgene expression is highest in the bladder and the aorta of transgenic animals [313, 331]. The expression of the transgene negatively affects cellular proliferation in lymphoid tissues although total lymphocyte development is not inhibited. Further, growth of the thymus is limited via the increased stimulation of apoptosis in the thymocytes. No data about the **fertility** of IGFBP4 transgenic mice are reported.

(ii) IGFBP4-/- mice

Origin Igfbp4-/- mice were also generated by Pintar's group in 2006 [328].

Phenotype These mice have no significant changes in metabolic parameters or serum levels of any other IGFBPs or **IGF1**. Although these mice exhibit a standard **growth** rate in later life, they never catch up to achieve full WT size [332] and have reductions in **fat mass**, total **body length** and femur length [333, 334]. *Igfbp4-/-* mice are **reproductively** viable, though pups show decreased growth in utero and are 10–15% smaller than WT controls through 14 weeks of age.

IGFBP5 transgenic and IGFBP5-/- mice (i) IGFBP5 transgenic mice

Origin Expression of IGFBP5 in transgenic mice was directed to the mammary gland via the β-lactoglobulin promoter/enhancer. This mouse line was first reported by Tonner et al. in 2002 [335]. WT mice normally have highest IGFBP5 expression in the kidney, muscle, ovaries among other tissues [313].

Phenotype After birth, IGFBP5 transgenic mice exhibit a decrease in total **body weight** as compared to controls. A reduction in total **muscle mass** and a transient decrease in bone volume and mineral density through 8 weeks of age is reported [336, 337]. Females show reduced **fertility** with an increase in the mortality of neonates.

(ii) IGFBP5-/- mice

Origin In 2006, Pintar's group reported on *Igfbp5-/-* mice, which were achieved by insertion of a NeoR cassette into exon 1 of the *Igfbp5* gene [328].

Phenotype Null mice have a similar total **body size**, with a modest increase in lung weight [338], an increase in **adiposity**, a mild **glucose intolerance** and increased susceptibility to diet-induced obesity as compared to controls [339]. No impact on **fertility** of null mice is reported.

IGFBP6 transgenic mice *Origin* IGFBP6 transgenic mice were developed by Bienvenu et al. in 2004 using human *IGFBP6* cDNA with a glial fibrillary acidic protein (GFAP) promoter/enhancer [340]. *Igfbp6-/-* mice have not been reported.

Phenotype Transgene expression in these mice is high in the CNS [340], while nontransgenic mice normally express the highest levels of IGFBP6 in the lungs and heart with variable amounts in other tissues [313]. Transgenic mice exhibit increased levels of IGFBP6 between 3 and 15 days of age, along with decreased detected plasma **IGF1** levels at 15 days [340]. This change is transient with IGF1 levels being the same as the WT group at both 1 and 3 months of age. Mice have reduced litter size, with **sterility** in 5–20% of females. They also exhibit **growth retardation** as neonates through three months of age. These mice have a reduction in the size of the cerebellum [340] along with cerebellar abnormalities although changes in cognition or behavior are not reported. With diet-induced obesity, these mice develop mild **insulin resistance** and obesity. They also show a decrease in brown AT UCP-1 expression, along with an increase in plasma levels of glucose, insulin, and leptin [341].

ALS transgenic and ALS-/- mice *(or Igfals-/-)*

Origin The acid-labile subunit (ALS) is component of the IGF1 ternary complex along with IGF1 and IGFBP3, mediating the stability and bioavailability of IGF1. ALS transgenic mice were generated in 2001 by a group led by Murphy using the *ALS* cDNA driven by the CMV promoter/enhancer [342]. *Igfals-/-* mice were reported in 2000 by the Boisclair group and were made by replacing the *Igfals* gene with a neomycin phosphotransferase gene [343].

Phenotype ALS transgenic mice have decreased **body size** but no change in circulating **IGF1** or IGFBP3 levels [342]. Therefore, it is hypothesized that the decreased **body size** is due to altered tissue availability of IGF1. The authors also report decreased litter size in ALS transgenic mothers, suggested to be due to IGF1's role in ovarian follicular development. *Igfals-/-* mice have decreased **serum IGF1** and IGFBP3 levels, with a corresponding decrease in **body size** [343]. Interestingly, there is no significant change in *Igf1* or *Igfbp3* mRNA expression, indicating that the decrease in protein level is due to decreased stability in the serum, as is often seen when IGFs are not sequestered in ternary complexes. A later study published by Yakar's group in 2010 reported the same decrease in body size and serum IGF1 levels but with an additional skeletal phenotype [344], i.e. *Igfals-/-* mice had a sex- and age-dependent decrease in the periosteum formation around the femur leading to decreased bone formation. This decrease in bone thickness is compensated with an increase in the endosteal surface inside the bone that covers the bone marrow. Thus, the outer layer of the bones becomes thinner but the inner layer increases in thickness. Overall, it appears that disruption of ALS in mice decreases size in mice and affects skeletal shape.

PAPP-A-/- (Pregnancy-associated plasma protein-A)

Pregnancy-associated plasma protein A (PAPP-A) modulates the activity and bioavailability of IGF1 by cleaving IGFBP2, IGFBP4, and IGFBP5. Importantly, while IGFBP2 and IGFPB5 may be cleaved by other proteases, the proteolysis of IGFBP4 seems to be limited to PAPP-A [345].

Origin Germline *Pappa-/-* (PAPP-A-/- or PAPP-A-null) mice were reported in 2004 by Conover et al. [346]. To evaluate the phenotype of PAPP-A disruption at an adult age, a separate mouse line was reported by Bale et al., in 2017 [346]. This mouse line was generated using the tamoxifen-inducible Cre/LoxP system in which the *Pappa* gene was disrupted at five months of age (fPAPP-A/pos).

Phenotype Pappa-/- mice are dwarf with a 40% **body size** reduction and have compromised **fertility**, with an 80% reduction in litter size [346, 347]. *Pappa-/-* mice have normal circulating **GH** and **IGF1** levels compared to controls. They also have significantly increased **longevity**, with males showing a 33% and females a 41% lifespan extension [348]. Although these mice do not show changes in **glucose metabolism**, food intake, and total energy expenditure and resting energy [349], they do show a decrease in the prevalence and severity of age-related diseases, such as cardiomyopathy, nephropathy, and **cancer** [346, 350, 351]. Treadmill

experiments provide evidence of improved skeletal muscle function with a decrease in fatigue and an increase in endurance in PAPP-A-null mice [352]. Postnatal ablation of the *Pappa* gene in fPAPP-A/pos mice results in a significant extension of **lifespan** with an increase in median lifespan of 21% compared to control mice [351, 353]. As tamoxifen can induce scrotal enlargement and subsequent complications in male mice, only female mice are used in the longevity study. Thus, although germline disruption of *Pappa* shows positive results in terms of aging and some age-related diseases in both male and female mice, it is unknown if adult disruption of the *Pappa* gene will also lead to lifespan extension in male mice. It is interesting to note that disruption of GHR at an adult age also leads to lifespan extension in females [60]. As both strategies – PAPP-A and GHR ablation – lead to reduced IGF1 action, it is of interest to test if the PAPP-A system is also regulated by GH. To that end, AT of GHR-/- and bGH mice show no change in gene expression of *Igfbp4* and *Pappa* when compared to WT mice, although protein levels of IGFBP4 are increased in bGH mice compared to GHR-/- mice. Furthermore, the C terminal-IGFBP4 fragment, which is generated after PAPP-A-cleavage, does not differ among bGH, GHR-/-, and WT mice [354]. Despite no change in AT, PAPP-A is expressed in different tissues [345]; thus, it is possible that PAPP-A expression and activity is modulated by GH in other tissues.

Concluding remarks

The above review characterizes 137 mouse strains in which genes in the GH/IGF1 family have been altered. A few of these alterations are via 'natural gene mutations;' however, the majority are via genetic manipulations, namely (1) generation of transgenic mice that express a gene or cDNA encoding a component of the family or (2) disruption of specific genes within the family. A summary of the published data is presented, including authors, date of publication, corresponding references as well as salient physiological consequences of the gene alterations. Also, we had a 'personal laboratory reason' for generating this review; namely, we often need a consolidated document that can be easily used and referenced when preparing new manuscripts that refer to subsets of these mice. The reader is encouraged to use this review in a similar manner and, when doing so, a few new tidbits of information may be acquired. Although 137 different mouse lines are described, we are sure we missed some, and for that we apologize. Also, we tried to extract the salient physiological points from the published data when describing these mice. Again, if we missed some of these points or mistakenly described them, we are very sorry. Finally, I (JJK) am indebted to the authors of this review, which includes Ohio University faculty, staff of the Edison Biotechnology Institute, post-doctoral fellows, graduate students, undergraduate students, medical students, and technicians. Without their effort, this review would not have been accomplished.

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Declarations

Conflict of interest The authors have no conflicts of interest to declare that are relevant to the content of this article.

Ethical approval The authors of this review have abided by and are compliant with all typical ethical standards.

Research involving human and animal rights This review only documents mice with alterations in the GH/IGF family. Thus, no research was discussed or cited related to humans. Also, we believe that each research group involved in generation of the various mouse strains used animal use and care procedures that were approved by their individual university/hospital/institute.

Data availability Not applicable.

Code availability Not applicable.

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