

REVIEW

The GH/IGF1 axis and signaling pathways in the muscle and bone: mechanisms underlying age-related skeletal muscle wasting and osteoporosis

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Abstract

The widespread increase in life expectancy is accompanied by an increased prevalence of features of physical frailty. Signs and symptoms may include sarcopenia and osteopenia, reduced exercise capacity, and diminished sense of well-being. The pathogenesis of age-associated sarcopenia and osteopenia is multifactorial, and hormonal decline may be a contributing factor. Aging is associated with a progressive decrease in GH secretion, and more than 30% of elderly people have circulating IGF1 levels below the normal range found in the young. GH acts directly on target tissues, including skeletal muscle and bone among many others, but many effects are mediated indirectly by circulating

(liver-derived) or locally produced IGF1. Aging is also associated with reduced insulin sensitivity which, in turn, may contribute to the impairment of IGF1 action. Recent experimental evidence suggests that besides the age-dependent decline in GH and IGF1 serum levels, the dysregulation of GH and IGF1 actions due to impairment of the post-receptor signaling machinery may contribute to the loss of muscle mass and osteopenia. This article will focus on the molecular mechanisms of impaired GH and IGF1 signaling and action in aging, and their role in the pathogenesis of sarcopenia and osteoporosis.

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Introduction

Age-related muscle wasting and sarcopenia are characterized by the loss of skeletal muscle mass with a gradual decline in muscle function, including a decrease in power-producing capacity, contraction, and relaxation, but also by metabolic abnormalities, including reduced insulin sensitivity, impaired oxidative defense, and decreased mitochondrial function (Dela & Kjaer 2006). During aging, the loss of muscle mass and strength is due to progressive atrophy, loss of muscle fibers, and reduction in muscle capacities. Although these changes are, to some extent, the consequence of the reduced level of physical activity, they can also be influenced by biological processes, including infiltration of fat and connective tissue into the muscle, changes in muscle metabolism, insulin resistance, reduced levels of specific hormones, and the oxidative stress accumulated during the lifespan (Cree *et al.* 2004, Hagen *et al.* 2004). The oxidative stress leads to mitochondrial DNA mutations resulting in dysfunctional mitochondria, and this promotes a reduced efficiency of skeletal muscle, which is particularly susceptible to this

biological phenomenon (Hagen *et al.* 2004). When muscle activity is reduced, metabolites and survival factors that are normally stored in the muscle are no longer available. In addition, aging is typically associated with bone loss, leading to bone fragility and increased risk for osteoporosis and fractures. In fact, age-related wasting may coexist with osteoporosis, establishing a vicious circle between dysfunctional muscle and bone.

GH regulates important physiological processes, including somatic growth and development (Giustina *et al.* 2008), as well as carbohydrate and lipid metabolism (Perrini *et al.* 2008a,b, Møller *et al.* 2009), directly through the activation of specific GH receptors (GHRs; Giustina *et al.* 2008), or indirectly through insulin-like growth factor 1 (IGF1; Laviola *et al.* 2007), which is produced mainly in the liver in response to GH stimulation (Ohlsson *et al.* 2009; Fig. 1). IGF1 is also produced by a variety of cell types, and acts in a paracrine fashion in most tissues (see below). Circulating GH levels decline progressively after 30 years of age at a rate of ~1% per year (Hermann & Berger 2001). In the skeletal muscle, GH promotes cell growth by favoring the fusion of myogenic

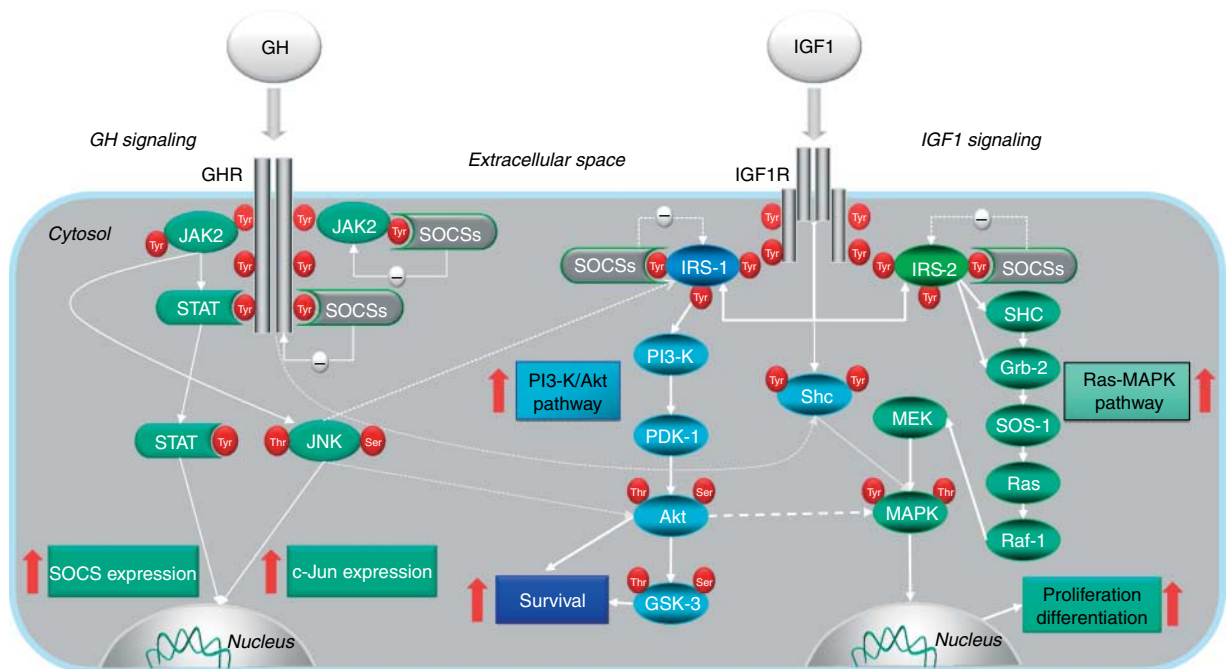


Figure 1 GH and IGF1 signaling and action in skeletal muscle and osteoblastic cells. Individual signaling molecules with the phosphorylated amino acid residues are indicated. GHR, GH receptor. IGF1R, insulin growth factor 1 receptor.

precursor cells into existing myotubes, and this requires the IGF1-independent activation of nuclear factor of activated T-cell C2 (Sotiropoulos *et al.* 2006). Acute administration of GH regulates muscle mitochondrial function by increasing the levels of several key mitochondrial proteins, and by switching fuel utilization toward fat oxidation (Short *et al.* 2008). In addition, exogenous administration of IGF1 induces an increase in muscle oxidative enzymes and fatigue resistance (Schertzer *et al.* 2006). In bone, both GH and IGF1 are essential for the development and growth of the skeleton and maintenance of bone mass. IGF1, which mediates most of the effects of GH on skeletal metabolism, promotes chondrogenesis and increases bone formation by regulating the functions of the differentiated osteoblasts (Giustina *et al.* 2008).

This review will summarize current information on the mechanisms and intracellular signaling involved in the development of age-related changes in muscle mass and bone. In addition, the specific role of the GH/IGF1 system in the pathogenesis of age-related skeletal muscle wasting and osteoporosis will be discussed.

Regulation of the GH/IGF1 axis during aging

GH

GH is a single-chain peptide of 191 amino acids produced and secreted mainly by the somatotrophs of the anterior pituitary gland (Davidson 1987). GH secretion occurs in a pulsatile manner with a major surge at the onset of slow-wave sleep

and less conspicuous secretory episodes a few hours after meals (Ho *et al.* 1988, Hartman *et al.* 1991), and is controlled by the action of two hypothalamic factors, GHRH, which stimulates GH secretion, and somatostatin, which inhibits GH secretion (Giustina *et al.* 2008). During fasting, GH secretion is amplified, whereas excess of glucose and lipids inhibits GH release (Ho *et al.* 1988). In addition, different stimuli, including gender, age, adiposity, sleep, diet, and exercise, affect the frequency and magnitude of the GH pulses. The secretion of GH is maximal at puberty accompanied by very high circulating IGF1 levels (Moran *et al.* 2002), with a gradual decline during adulthood (Corpas *et al.* 1993). Indeed, in aged men, daily GH secretion is 5- to 20-fold lower than that in young adults (Ryall *et al.* 2008). The age-dependent decline in GH secretion is secondary to a decrease in GHRH, and to an increase in somatostatin secretion (Veldhuis & Iranmanesh 1996). These changes occur at the hypothalamic level, although their cause is still unknown. As a consequence of the decline in GH synthesis and release, systemic IGF1 levels decline with advancing age (Landin-Wilhelmsen *et al.* 1994). The changes in GH and IGF1 secretion that occur with aging are paralleled by a progressive loss of muscle mass and bone mineral density (BMD; Bohannon 1997). Overload and stretch-dependent IGF1 and IGF2 production in the skeletal muscle has been found to be impaired in older humans (Hameed *et al.* 2004).

In addition, in the last 10 years, several studies have demonstrated a significant inverse relationship between peak stimulated GH levels and body mass index (BMI; Bonert *et al.* 2004, Corneli *et al.* 2005, Franco *et al.* 2006). Multiple lines

of evidence indicate that apparently healthy overweight and obese subjects meet standard diagnostic criteria used to diagnose GH deficiency (GHD) in hypopituitarism (Johannsson & Bengtsson 1999). On the other hand, the role of the peripheral fat in impairing the regular pattern of GH release was recently shown in postmenopausal women with abdominal obesity. In this clinical context, the pulsatile GH secretion was negatively associated with the amount of thigh intermuscular adipose tissue, whereas basal GH secretion demonstrated an independent negative association with visceral adipose tissue (Franco *et al.* 2006). Furthermore, Møller *et al.* (2009) have recently shown that lipid infusion during GH stimulation decreases signal transducer and activator of transcription 5 (STAT5) phosphorylation (the major target of GH intracellular signaling) by about 40%, an effect that was evident already at low lipid infusion rates. This suggests that whenever free fatty acid (FFA) levels are high, e.g. during fasting/protracted exercise, dyslipidemia, excess body fat, type 2 diabetes, or inflammatory illness, the activation of intracellular GH signaling and its related growth-promoting and anabolic effects are restricted. Thus, the direct correlation between high FFA levels and reduced intracellular GH signaling and action may be applied to subjects with central obesity and/or high BMI. Since recent studies have demonstrated a significant increase in BMI and visceral fat accumulation with aging (Rössner 2001, Hajian-Tilaki & Heidari 2007), the reciprocal interplay of increasing BMI, central obesity, and FFA excess may be relevant to the age-dependent decline in GH secretion and action.

Insulin-like growth factor 1

IGF1 is perhaps the most important mediator of muscle and bone growth (Goldspink 2007). Systemic IGF1 is synthesized primarily in the liver, where its synthesis is GH dependent; IGF1 is also produced in multiple extrahepatic tissues, where it acts locally as an autocrine/paracrine growth factor under the control of multiple hormones (Laviola *et al.* 2007). The IGFs, including IGF1 and IGF2, are single-chain polypeptides, which share a similar secondary structure, with three α -helices and three disulfide bonds (Mathews *et al.* 1986). Despite a significant structural similarity, each ligand can result in unique signaling outcomes. As an example, IGF2 is unable to compensate for the loss of IGF1 activity in patients with IGF1 deficiency, leading to severe growth and mental retardation (Chen *et al.* 2001). Similarly, mice with targeted disruption of the *IGF1* or *IGF2* genes are born at 60% birth weight compared with wild-type littermates (Baker *et al.* 1993). Although in rodents IGF2 is predominantly expressed in fetal life whereas IGF1 is considered an adult growth factor, this expression pattern is not observed in humans, as both ligands are produced in multiple human tissues throughout life, which is consistent with the concept that IGF1 and IGF2 have potentially divergent roles in human physiology (Rosenfeld & Hwa 2009). It has been shown that IGF1 exists

in at least two isoforms as a result of alternative splicing of the *IGF1* gene. IGF1Ea, which is produced in both liver and muscle tissues, was the first isoform to be discovered and is often referred to as liver-type or systemic IGF1. IGF1Eb (rodent form) and IGF1Ec (human form) are produced mainly by the skeletal muscle, and are usually referred to as mechano growth factors (MGFs; Goldspink 2004). Unlike MGFs, liver-type IGF1 is glycosylated, and this modification protects it from proteolysis and confers a relatively long half-life. In addition, IGF1 half-life in plasma and the interaction of IGF1 with the IGF1 receptor (IGF1R) are regulated either positively or negatively by a family of six high-affinity IGF binding proteins (IGFBPs; IGFBP1 to IGFBP6; Laviola *et al.* 2007, Rosenfeld & Hwa 2009). In tissues, IGFBPs can both inhibit or potentiate IGF1 actions either by sequestering IGF1 from the IGF1R or by releasing free IGF1 available for receptor binding. IGF1 is released from the complex by either proteolysis of IGFBPs or binding of IGFBPs to the extracellular matrix. IGFBP phosphorylation can also alter the affinity for the IGFs (Rosenfeld & Hwa 2009). IGF-independent actions have also been recently described for most IGFBPs, and can involve intracellular localization or integrin binding (Woodhouse *et al.* 2006).

Unlike GH, serum IGF1 levels are stable without significant variability in healthy individuals. In contrast to GH, the circulating IGF1 levels are reduced during fasting, and IGF1 infusion suppresses GH release, suggesting a negative feedback loop between the two hormones. Recently, in an 8-year prospective study conducted in elderly men, Brughts *et al.* (2008) have shown that higher circulating IGF1 bioactivity was associated with better overall survival, since individuals in the lowest quartile of IGF1 bioactivity had a 1.8-fold increased mortality risk compared with individuals in the highest quartile. Besides the age-dependent decline in GH and IGF1 serum levels, the increased mortality risk and diminished sense of well-being may be also due to the impairment of the GH and IGF1 post-receptor signaling machinery. In line with these findings, Dennis *et al.* (2008) have reported that median *IGF1* and *IGFBP5* mRNA levels in resting young muscle are more than twice higher than those in elderly muscle. In general, tissue responsiveness to IGF1 is altered with aging. Aging is associated with decreases in IGF1R content and IGF1R phosphorylation in muscle (Li *et al.* 2003). Furthermore, in fibroblasts, DNA synthesis and cell proliferation in response to IGF1 decrease with older age (Sell *et al.* 1993). Bone responsiveness to IGF1 also decreases with aging (Kveiborg *et al.* 2000). The skeletal anabolic response to IGF1 administration is weakened in the elderly, and biopsy-derived osteoblasts from patients of different ages respond to IGF1 by increasing proliferation, but the dose needed to elicit a response is an order of magnitude greater for cells from aged patients (Pfeilschifter *et al.* 1993). These studies suggest that low circulating IGF1 bioactivity and abnormalities of IGF1 signaling in elderly subjects, especially in those individuals with an age-related proinflammatory state (i.e. subjects with visceral or central obesity), may play an important role in age-related sarcopenia and osteopenia (Fig. 2).

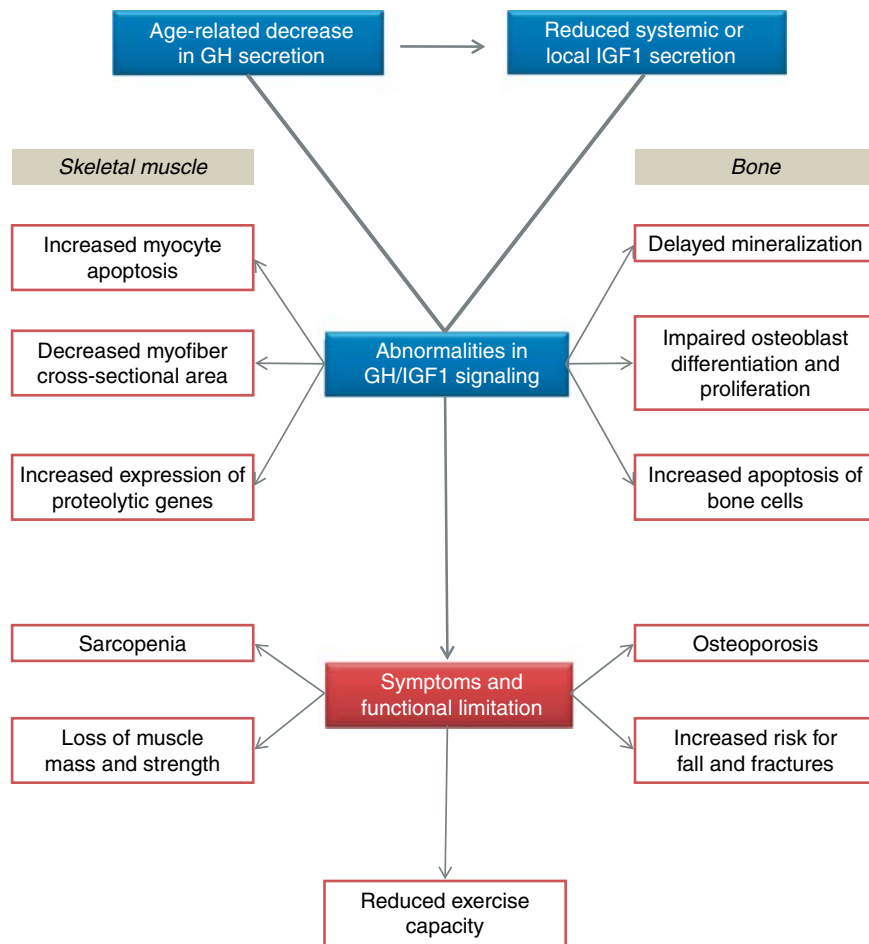


Figure 2 A schematic model of the mechanisms leading to sarcopenia, osteoporosis, physical impairment, and functional limitations and disabilities in aging subjects.

GH intracellular signaling

GH effects are mediated via the GHR, which is a member of the cytokine receptor superfamily. The critical step in initiating GH signaling is the activation of receptor-associated Janus kinase (JAK) 2, which induces cross-phosphorylation of tyrosine residues in the kinase domain of JAK2 and GHR (Fig. 1). Phosphorylated residues on JAK2 and GHR form docking sites for the members of the STAT family of transcription factors (Perrini *et al.* 2008b). Phosphorylation of the STATs by JAK2 results in their dissociation from the receptor and translocation to the nucleus, with subsequent binding to DNA and regulation of gene expression. Among the target genes, GH regulates the expression of suppressor of cytokine signaling (SOCS), a family of negative regulators that terminate the GH signaling cascade (Hansen *et al.* 1999). SOCS proteins bind to phosphotyrosine residues on the GHR or JAK2 and suppress GH signaling by inhibiting JAK2 activity, competing with STATs for binding to the GHR, or inducing degradation of the GHR complex (Fig. 1).

The RAS/mitogen-activated protein kinase (MAPK) pathway has also been shown to be activated by GH. The GHR–JAK2 complex has been shown to recruit the adapter protein SHC, resulting in SHC tyrosine phosphorylation and binding to GRB2, and activation of RAS, RAF, MAPK/extracellular-regulated protein kinase (MEK), and ERK-1/2 (Vanderkuur *et al.* 1997; Fig. 1). Alternatively, it has been suggested that GH might also regulate the activation of ERK-1/2 by a SRC-dependent, JAK2-independent mechanism which involves phospholipase D (Zhu *et al.* 2002). Tyrosine phosphorylation of a GRB2-binding site in the epidermal growth factor receptor could also be involved in GH-mediated MAPK activation (Yamauchi *et al.* 1997).

GH has also been shown to stimulate the phosphatidylinositol 3-kinase (PI3-K) pathway through JAK2-mediated tyrosine phosphorylation of the insulin receptor substrates (IRS-1 to IRS-3), leading to their association with PI3-K regulatory subunits (Zhu *et al.* 2001). In addition, direct binding of the p85 α and p85 β subunits to phosphotyrosine residues in the carboxyl terminus domain of the GHR

has also been demonstrated (Moutoussamy *et al.* 1998). GH stimulation of PI3-K is linked to the stimulation of the antiapoptotic serine protein kinase B or AKT (Costoya *et al.* 1999). Akt activation has been shown to be dependent on the presence of the JAK2-binding region of GHR, and to promote cell survival through the inhibition of the proapoptotic protein caspase 3 (Sanders *et al.* 2006).

IGF1 intracellular signaling

Most biological actions of IGF1 are mediated through the type I IGF receptor (IGF1R), which is a transmembrane tyrosine kinase that is structurally and functionally related to the IR. Both receptors show a heterotetrameric structure (two α -subunits and two β -subunits), with an extracellular hormone-binding domain, a transmembrane region, and an intracellular portion, which contains the kinase domain and multiple regulatory residues. Upon ligand binding, the intrinsic tyrosine kinase of the IGF1R is activated, and this results in autophosphorylation of tyrosine residues in the intracellular portion of the β -subunit, including tyrosine residues in the juxtamembrane and C-terminal domains. Once phosphorylated, tyrosine 950 in the juxtamembrane domain can serve as a docking site for several receptor substrates, including the IRSs and Shc (Laviola *et al.* 2008). These substrates initiate phosphorylation cascades that serve to transmit the IGF1R signal (Fig. 1). Phosphorylated IRS-1 can activate the p85 regulatory subunit of PI3-K, leading to the activation of several downstream substrates, including Akt (Giorgetti *et al.* 1993). Akt phosphorylation, in turn, enhances protein synthesis through mechanistic target of rapamycin (mTOR) and p70S6 kinase activation, and mediates the antiapoptotic effects of the IGF1R through phosphorylation and inactivation of BAD (Petley *et al.* 1999). In parallel to PI3-K-driven signaling, recruitment of GRB2/SOS by phosphorylated IRS-1 or SHC leads to recruitment of Ras and activation of the RAF-1/MEK/ERK pathway and downstream nuclear factors, resulting in the induction of cell proliferation (Fig. 1; Grey *et al.* 2003). Shc may actually compete with IRS-1 for a limited cellular pool of GRB2, and the extent of SHC/GRB2 binding appears to correlate with the amount of insulin-activated ERK and *c-fos* transcription (Yamauchi & Pessin 1994). Therefore, the SHC/GRB2 pathway represents the predominant mechanism activating the RAS/ERK signaling pathway in response to IGF1. Recent experimental evidence has shown that distinct SHC isoforms exert opposite effects on the ERK signaling cascade. The SHC proteins originate by alternative use of three distinct translation starting points on a longer transcript (p46^{Shc}, p52^{Shc}, and p66^{Shc}) and two translation starting points on a shorter transcript (p46^{Shc} and p52^{Shc}); the two mRNA transcripts are generated by alternative splicing from a single gene (Pelicci *et al.* 1992, Natalicchio *et al.* 2004). Although all three SHC isoforms can be tyrosine phosphorylated upon growth factor stimulation, p46/p52^{Shc} transduce growth and survival signals, whereas

p66^{Shc} is also modified by serine phosphorylation and plays an important role in mediating oxidative stress-dependent cell damage and apoptosis (Migliaccio *et al.* 1999). Indeed, the p66^{Shc} protein has been shown to regulate intracellular oxidant levels and hydrogen peroxide-mediated forkhead inactivation (Nemoto & Finkel 2002), effects that are probably relevant to the reported ability of p66^{Shc} to control lifespan in mammals (Migliaccio *et al.* 1999). Furthermore, while p46/p52^{Shc} trigger the activation of the ERK pathway via GRB2/SOS/RAS, p66^{Shc} exerts an inhibitory effect on ERK, because reduced p66^{Shc} expression levels are associated with persistent ERK activation (Natalicchio *et al.* 2004). The opposite regulation of ERK effects by p46/p52^{Shc} and p66^{Shc} respectively may be physiologically relevant, since human breast cancer tissues with high p46/p52^{Shc} to p66^{Shc} expression ratios are characterized by increased cell proliferation and poor prognosis (Davalò *et al.* 2003).

Abnormalities in GH/IGF1 signaling and age-related muscle mass wasting

Age-related skeletal muscle wasting affects all elderly subjects by impairing their functional independence and increasing their risk for falls and fractures (Szulc *et al.* 2005). It has been suggested that during aging, the loss of muscle mass and strength is due to the progressive atrophy, decrease in myofiber cross-sectional area (CSA), loss of muscle fibers (Ryall *et al.* 2008), and reduction in muscle capacities (Szulc *et al.* 2005). An age-related reduced efficiency of the multistep GH/IGF signaling machinery may also play a role in the reduced skeletal muscle structure and function. Indeed, reduced mRNA expression levels of the GHR in skeletal muscle have been observed in older versus younger healthy men, exhibiting a significant negative relationship with myostatin levels (Marcell *et al.* 2001). Myostatin functionally impairs satellite cell (myoblast) activity by inhibiting protein and DNA synthesis and cell proliferation (Taylor *et al.* 2001), and counteracting GH-dependent promotion of muscle protein synthesis and activation of satellite cells (Marcell *et al.* 2001). In addition, myostatin null mutations (Zhu *et al.* 2000) and gene knockout experiments (McPherron *et al.* 1997) result in a significant increase in muscle mass. Thus, the observed increased expression of muscle myostatin with aging may be due, at least in part, to the age-related decrease in endogenous GH, contributing to age-related sarcopenia. It has also been shown that mice lacking GHR (GHR^{-/-}) exhibit proportional growth retardation and delayed and/or diminished responses in IRS-1 phosphorylation following *in vivo* insulin stimulation in muscle (Robertson *et al.* 2006). Dysregulation of the IRS/PI3-K/AKT pathway appears to be particularly relevant to the development of age-related muscle wasting, because *in vitro* studies have implicated this signaling pathway in myocyte growth and muscle mass regulation (Rommel *et al.* 2001). Skeletal muscle biopsies from older male subjects show a reduction in the CSA of type II muscle

fibers by 40–45%, in parallel with a 45% decreased GHR protein and *IGF1* mRNA levels, as well as increased *TNF- α* and *SOCS-3* mRNA levels, when compared with younger donors (Léger *et al.* 2008). Furthermore, total AKT, but not phosphorylated AKT, proteins levels are increased by 2–5-fold, resulting in a 30% reduction in the efficiency of AKT phosphorylation in older subjects. Indeed, multiple studies have shown that *SOCS-3* has been causally linked to insulin resistance either by associating with the IR and suppressing its tyrosine kinase activity, or by decreasing IRS phosphorylation and downstream signaling reactions. Deletion of PI3-K in the muscle *per se* results in decreased muscle size and inefficient muscle metabolism (Luo *et al.* 2006), and the inhibition of PI3-K or AKT prevents the anabolic effects of IGF1 in cultured cells (Giorgino *et al.* 1997). In fact, an age-related deficit in IGF1R activation has been demonstrated in the muscle of old rats, and this was associated with a reduced activity of the IRS-1/PI3-K pathway (Haddad & Adams 2006). Conversely, AKT hyperactivation leads to muscle hypertrophy *in vivo* (Lai *et al.* 2004). At the molecular level, myocyte growth is thought to be promoted by AKT signaling through MTOR and GSK-3 (Bodine *et al.* 2001, Rommel *et al.* 2001). In addition, AKT-mediated phosphorylation of forkhead transcription factors results in reduced expression of atrogin-1 (also known as MAFbx or FBXO) and MuRF1, two E3 ubiquitin ligases that promote muscle protein degradation (Sandri *et al.* 2004). More recently, an impaired AKT kinase activity in myocytes has been found to be associated with higher levels of apoptosis, lower abundance of the contractile proteins myosin and actin, and a smaller muscle fiber CSA (Wu *et al.* 2009). Interestingly, the restoration of AKT kinase activity was associated with increased amount of contractile proteins and myocyte size, and decreased apoptotic rates. More limited studies are available in humans. Recently, a reduced effect of insulin on leg protein breakdown has been observed in older versus younger subjects, and this appears to be associated with blunted AKT activation (Wilkes *et al.* 2009).

In conclusion, the age-associated reduction in GH and IGF1 plasma levels, and their reduced intracellular signaling efficiency are associated with decreased muscle size and strength, diminished protein synthesis, and increased cell apoptosis. Therefore, age-related human sarcopenia may be linked to impaired activation of anabolic signaling pathways emanating from the GHR and IGF1R and involving the IRSs, PI3-K, and AKT (Fig. 2).

Abnormalities in GH/IGF1 signaling and age-related osteoporosis

The GH/IGF1 axis provides the main stimulus for bone growth regulation by activating the osteoblast differentiation program, stimulating chondrocyte proliferation at the growth plate, and modulating tubular re-absorption of phosphate and 25-hydroxyvitamin D3 1 α -hydroxylase activity in the kidney (Giustina *et al.* 2008). Consistent with these findings, a decline

in GH and IGF1 secretion has been correlated with BMD loss in postmenopausal women; in addition, *IGF1* promoter polymorphisms have been linked to bone mass (Zhao *et al.* 2008). GH stimulates the proliferation of cells of the osteoblastic lineage, although IGF1 is required for the selected anabolic lineage (Slootweg *et al.* 1988), suggesting either a direct effect of GH on chondrocytes or an effect mediated by the locally secreted IGF1. Indeed, GHR null mice exhibit decreased bone remodeling, which is rescued by IGF1, suggesting a role of GH/IGF1 in bone remodeling that is independent of STAT5. The fundamental role of IGF1 in regulating bone formation is demonstrated by the analysis of IGF1-deficient mice, which exhibit skeletal malformations, delayed mineralization, reduced chondrocyte proliferation, and increased chondrocyte apoptosis (Laviola *et al.* 2008). The expression of functional IGF1R and GHRs mediating effects on cell proliferation and differentiation has been reported in cultured human osteoblast-like cells (Nilsson *et al.* 1995) and in clonal rat and mouse osteoblast-like cell lines (Slootweg *et al.* 1996). In addition, it has been shown that IGF1 treatment is effective in inducing gene expression of osteoblastic markers independent of age, identifying exogenous IGF1 as a potential beneficial treatment in age-related bone loss (Tanaka *et al.* 1994). IGF1R expression has also been demonstrated in mature rabbit osteoclasts, as well as in human pre-osteoclasts (Hou *et al.* 1997), and IGF1 enhances the formation of osteoclast-like cells in long-term bone marrow cultures (Jonsson *et al.* 1996). In contrast, IGF1 has an inhibitory effect on stimulated bone resorption in bone organ cultures (Jonsson *et al.* 1996). Osteoblasts also produce IGFBPs, dependent upon the stage of maturation and stimulation with GH or IGFs. These IGFBPs regulate GH and IGF responses by modulating receptor expression and bioavailability of IGF1 and IGF2. IGFBP2 is important as a circulating carrier of IGFs, and IGFBP2 serum levels correlate with BMD and turnover in humans (Amin *et al.* 2004). The effects of IGFBP2 are complex: female *igfbp2* null mice have increased cortical bone, whereas male *igfbp2* null mice display decreased cortical and trabecular bone secondary to decreased bone formation (DeMambro *et al.* 2008). These observations suggest that IGFBP2 is required for normal bone formation in male mice, and are in agreement with clinical observations indicating a correlation between serum IGFBP2 levels following the administration of IGF2/IGFBP2 and bone remodeling and anabolic effects in disuse osteoporosis (Amin *et al.* 2007). It is also of interest that mechanical loading upregulates the expression of *IGF1* and *IGFBP2* mRNA transcripts in osteocytes (Reijnders *et al.* 2007). The IGFs increase collagen production, and are incorporated into bone matrix bound to IGFBP5. During osteoclastic resorption, IGF1 and IGF2 are released and may again regulate osteoblastic functions, thereby coupling bone resorption and formation. Finally, GH, IGFs, and IGFBPs may all regulate osteoclastic bone resorption through direct and indirect effects on osteoclast differentiation and activation.

IGF1 action on bone cells requires the integrity of the IGF1R signaling pathway (Fig. 1). As an example, both IRS-1 and IRS-2 are expressed in osteocytes (Yamauchi *et al.* 1997). It has been proposed recently that IRS-2 is needed to maintain the predominance of bone formation over bone resorption, whereas IRS-1 maintains bone turnover, and that the integration of these two signals mediates a potent anabolic response to IGF1 in the bone (Ogata *et al.* 2000, Akune *et al.* 2002). The PI3-K/AKT pathway is utilized by IGF1 to decrease osteoblast apoptosis. Sustained activation of ERK-1 and ERK-2 by IGF1 is also important for the regulation of osteoblast proliferation (Zhang *et al.* 1999, Grey *et al.* 2003), whereas other MAP kinase family members, such as JNK and p38, seem to play a minor role (Grey *et al.* 2003). Abnormalities in IGF1 action and signaling occur in human osteoblasts under conditions of net bone loss. Individuals with insulin deficiency, as exemplified by type 1 diabetic patients, are susceptible to develop osteoporosis (Krakauer *et al.* 1997). Patients with Laron's syndrome caused by IGF1 deficiency are also prone to osteoporosis (Laron 1999). A reduction in IGF1 levels is implicated as an important factor in the etiology of evolutionary osteoporosis, especially of age-related bone loss (Nicolas *et al.* 1994, Rosen 2004). Interestingly, while it has been shown that recombinant human IGF1 (rhIGF1) administration increases osteoblast function in healthy females in whom IGF1 levels and bone turnover were decreased by short-term caloric deprivation (Grinspoon *et al.* 1996), rhIGF1 was ineffective in increasing BMD in women with postmenopausal osteoporosis (Ghiron *et al.* 1995). Thus, abnormalities in IGF1 signaling may be responsible for altered IGF1 action on osteoblasts in human osteoporosis. Recently, a comparative analysis of IGF1 signaling was carried out in primary cultures of human osteoblasts isolated from osteoporotic and control bone specimens (Perrini *et al.* 2008a,b). In the osteoblasts from osteoporotic bone, tyrosine phosphorylation of the IGF1R was found to be increased in the basal state, but it was poorly responsive to IGF1 stimulation. Augmentation of IGF1R phosphorylation in the basal state was associated with increases in tyrosine phosphorylation of IRS-2 and activation of ERK, which were also poorly responsive to IGF1 stimulation. By contrast, phosphorylation levels of IRS-1, AKT, and GSK-3 were similar in the basal state in control and osteoporotic osteoblasts, and showed an increase after IGF1 stimulation in both cell populations, albeit the response in the osteoporotic osteoblasts was lower. Specifically, phosphorylation of AKT on Ser473 and Thr308 following IGF1 stimulation was significantly reduced in osteoporotic cells compared with control cells, leading to decreased GSK-3 phosphorylation. The IGF1 signaling abnormalities in osteoporotic osteoblasts were associated with reduced DNA synthesis both under basal conditions and after stimulation with IGF1 (Perrini *et al.* 2008a,b). Therefore, abnormalities in IGF1 signaling may explain the impaired cell proliferation and decreased bone formation that occur in human osteoporosis (Fig. 2).

Further complexity is added to this scenario while considering the role of the insulin/IGF system throughout evolution. Indeed, strong similarities exist in the insulin/IGF1 signaling machinery in worms, flies, mammals, and humans. However, worms and flies have only one receptor for both hormones, whereas the vertebrates have at least three closely related receptors (Katic & Kahn 2005). In addition, studies in invertebrates suggest that insulin/GH/IGFs control lifespan. In fact, the impairment of the GH/IGF1 signaling has been correlated with extended longevity in worms, flies, and transgenic rodent models (Berryman *et al.* 2008). These observations are apparently in contrast with the above-described effects of reduced GH/IGF1 action on muscle strength and trabecular bone mass; however, the complete removal of GH action by disrupting or knocking out the GHR gene (*GHR*^{-/-}) in rodents (which resulted in GH resistance, elevated GH levels, and markedly reduced IGF1 levels) determined both a significant increase in lifespan and reduced muscle mass and BMD compared with littermate controls (Berryman *et al.* 2008). Thus, the insulin/GH/IGF signaling systems are involved in an integrated interplay between anabolic and life-cycle-controlling mechanisms.

Conclusions

The GH and IGF1 signaling pathways are important regulators of muscle and bone cell survival and function. The commonly observed decline in circulating GH and IGF1 levels that accompanies aging may contribute to reduced muscle strength and trabecular bone mass. Indeed, multiple long-term GH replacement trials, with a duration of at least 5 years, have shown that GH protects against the normal age-related decline in muscle strength, neuromuscular function, and bone mass (Gibney *et al.* 1999, Svensson *et al.* 2003, Götherström *et al.* 2005). However, in elderly GHD adults above 60 years of age, the main effect of GH replacement seems to be a prevention of the age-related decline in muscle efficiency, rather than a significant increase in absolute values of muscle strength. In line with these observations, a recent 10-year GH replacement trial in GHD adults has shown that the increased muscle strength and improved neuromuscular function are more evident in younger subjects than in older subjects (Götherström *et al.* 2009). In addition, subjects receiving GH demonstrated no significant changes in BMD (Fernholm *et al.* 2000, Hoffman *et al.* 2004). The reduced effect on BMD and muscle strength noted in elderly subjects during prolonged GH replacement therapy suggests that other mechanisms, including abnormalities of the GH/IGF1 signaling in muscle and bone cells, may contribute to age-related muscle wasting and osteoporosis. The identification of distinct abnormalities in GH/IGF1 signaling contributing to impaired cell proliferation and decreased functional efficiency in the muscle–bone system with aging may help to identify novel therapeutic targets for improving health and quality of life in aged humans.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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