Determinants of GH resistance in malnutrition

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Abstract

States of undernutrition are characterized by GH resistance. Decreased total energy intake, as well as isolated protein–calorie malnutrition and isolated nutrient deficiencies, result in elevated GH levels and low levels of IGF1. We review various states of malnutrition and a disease state characterized by chronic undernutrition – anorexia nervosa – and discuss possible mechanisms contributing to the state of GH resistance, including fibroblast growth factor 21 and Sirtuin 1. We conclude by examining the hypothesis that GH resistance is an adaptive response to states of undernutrition, in order to maintain euglycemia and preserve energy.

Key Words
- growth hormone
- IGF
- neuroendocrinology
- pituitary

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Introduction

Insulin-like growth factor 1 (IGF1) is a hormone produced primarily by the liver and regulated by growth hormone (GH) secretion by the somatotroph cells of the anterior pituitary gland. Many, but not all, of the actions of GH are mediated by IGF1. In addition to regulation by GH, IGF1 secretion is also responsive to nutritional cues. In obesity, a state of overnutrition, GH secretion is decreased and may result in IGF1 levels that are lower than those in normal-weight individuals (Maccario *et al.* 1997), although bioactive IGF1 levels may be comparable to those of lean controls (Frystyk *et al.* 2009). In states of undernutrition, GH levels are normal or elevated in the setting of low IGF1 levels; therefore, there is a state of GH resistance, with an inappropriate response to GH at the level of the liver. This state of acquired GH resistance is likely an adaptive response to decreased energy intake. A number of potential mechanisms of GH resistance in malnutrition have been elucidated, including possible hormonal determinants. This review will examine the current evidence of determinants of GH resistance in states of malnutrition (Fig. 1).

States of malnutrition

A number of models of malnutrition have been studied to better understand the state of GH resistance in undernutrition. These models include total energy deficiency, isolated protein deficiency (kwashiorkor) – a state in which individuals consume an appropriate number of calories but have profoundly low levels of protein intake – and anorexia nervosa, a psychiatric disease that is characterized by low body weight and self-induced starvation. There are a number of other types of malnutrition as well, such as isolated nutrient deficiencies, which may also result in GH resistance. These various states of malnutrition will be discussed in detail below.

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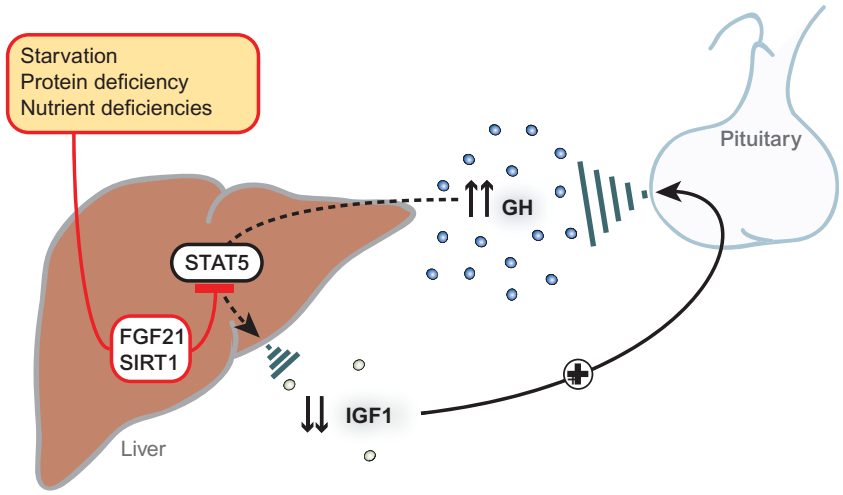

Figure 1
Potential causes and mediators of growth hormone (GH) resistance. Starvation, protein–calorie malnutrition, and isolated vitamin deficiencies can lead to a state of GH resistance, with normal or elevated levels of GH coincident with low levels of insulin-like growth factor 1 (IGF1). Recent evidence suggests that the state of GH resistance in starvation may result from a decrease in STAT5 phosphorylation, mediated by fibroblast growth factor 21 (FGF21) and/or Sirtuin 1 (SIRT1).

Starvation

Malnutrition may be due to isolated energy deficiencies – such as protein–calorie deficiency – or total energy deficiency, the extreme case of which is starvation. States of total energy deficiency result in a GH resistance state. IGF1 levels decrease by ~50% after only 4 days of fasting (Grinspoon *et al.* 1995). Fasting individuals have elevated GH levels coupled with the low IGF1 levels (Fichter *et al.* 1986, Ho *et al.* 1988) and may also have hyperresponsive GH secretion after thyrotropin-releasing hormone stimulation (Shimizu *et al.* 1991). The fact that endogenous hypersecretion of GH does not result in elevated IGF1 levels suggests that fasting either decreases GH receptors in the liver or there is a post-receptor defect resulting in an inability of GH to stimulate IGF1 production. In animal models of starvation, low IGF1 levels are coupled with decreased GH receptor mRNA (Straus & Takemoto 1990) and decreased GH binding (Baxter *et al.* 1981), suggesting that GH receptor downregulation in the liver contributes to the state of GH resistance.

Exogenous GH is also unable to stimulate IGF1 production during fasting. The effects of exogenous GH in the fed vs the fasted state were investigated in subjects with isolated GH deficiency (Merimee *et al.* 1982). Subjects were treated with 5 mg GH twice/day for 5 days, either while consuming an *ad libitum* diet or during a 5-day fast (Merimee *et al.* 1982). In response to GH, IGF1 levels increased tenfold in the fed state but only doubled in the fasted state, demonstrating resistance to exogenous GH during fasting (Merimee *et al.* 1982). Interestingly, in a study of acromegalics undergoing a 36-h fast, serum IGF1 levels did not decrease in the acromegalics whereas levels decreased in the fasted control patients (Grottoli *et al.* 2008). Therefore, it is likely that adaptations to fasting are abnormal in individuals with baseline GH hypersecretion, although the effects of a longer term fast in individuals with acromegaly are unknown.

Protein deficiency

Isolated protein deficiency, with otherwise normal energy intake, also results in a state of GH resistance. Studies in children with protein–calorie malnutrition demonstrate elevated GH levels and low IGF1 levels with normalization after nutritional recovery (Olusi *et al.* 1977, Robinson & Picou 1977, Hintz *et al.* 1978, Soliman *et al.* 1986). In adults who were fasted and then re-fed a protein-deficient diet, IGF1 levels increased significantly less than in adults fed an iso-caloric, protein-sufficient diet (Isley *et al.* 1983).

Importantly, in animal models with low IGF1 levels due to protein deficiency, pharmacological doses of GH do not increase IGF1 levels to the normal range or normalize growth (Thissen *et al.* 1990, 1991*a*), but increasing dietary protein does increase IGF1 levels. In animal models, varying the protein content of food did not affect plasma GH levels, whereas IGF1 levels increased significantly with increasing protein content (Reeves *et al.* 1979). Similarly, in a

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population of elderly individuals, 6 months of protein supplementation increased IGF1 levels by 85% (Schurch *et al.* 1998) and in individuals with type 2 diabetes mellitus, IGF1 levels increased by 30% in those fed a diet consisting of 30% protein for 5 weeks compared with those fed a diet consisting of 15% protein (Gannon & Nuttall 2011).

Unlike in starvation, in which a decrease in GH receptors is likely contributing to the GH-resistant state, in protein deficiency, the GH resistance state is likely due to a post-receptor defect. In animal models of protein deficiency, GH binding is not reduced (Maes *et al.* 1988, Thissen *et al.* 1990) and *Igf1* mRNA production in response to exogenous GH is similar in hypophysectomized rats on a protein-deficient vs protein-sufficient diet (Thissen *et al.* 1991*a*). Protein deficiency not only results in GH resistance but also likely results in a state of end-organ resistance to IGF1. Rats with low IGF1 levels due to protein restriction and a control population of rats with low IGF1 levels due to hypophysectomy were treated with recombinant human IGF1 at a dose of 300 μg/day (Thissen *et al.* 1991*b*). Serum IGF1 levels normalized in both groups but increases in body weight, tibial epiphyseal widening, and tail growth only occurred in the hypophysectomized rats, not in the protein-restricted rats (Thissen *et al.* 1991*b*). Therefore, in addition to a state of GH resistance, there may also be a state of IGF1 resistance in protein restriction.

Anorexia nervosa

Anorexia nervosa is a psychiatric disorder, predominantly affecting women, with a lifetime prevalence of 2.2% (Keski-Rahkonen *et al.* 2007). The disease is characterized by self-imposed starvation and an inability to maintain a normal weight for height (American Psychiatric Association 1994). Individuals with anorexia nervosa are in a state of chronic starvation. Like individuals with total energy deficiency and isolated protein deficiency, individuals with anorexia nervosa are GH resistant. In girls and women with anorexia nervosa, basal and pulsatile GH secretion is increased and IGF1 levels are low (Garfinkel *et al.* 1975, Scacchi *et al.* 1997, Stoving *et al.* 1999, Misra *et al.* 2003). Systemic IGF1 levels in anorexia nervosa are ~50% of those of normal weight women and levels of IGF-binding protein 3, the predominant binding protein for IGF1, are low (Counts *et al.* 1992). Yet, bioactive IGF1 levels are also low (Stoving *et al.* 2007), suggesting that the low IGF1 levels in anorexia nervosa are not simply due to decreased levels of binding proteins but due to decreased bioactive IGF1. The low IGF1 levels in women with anorexia nervosa are exquisitely sensitive to nutritional repletion – after only 3 days of hyperalimentation therapy, levels have been shown to increase by ~50% (Hotta *et al.* 2000).

Individuals with anorexia nervosa also have an exaggerated response to GH-releasing hormone (GHRH) (Rolla *et al.* 1990) and abnormal GH suppressibility after a glucose load (Tamai *et al.* 1991). In healthy individuals, pretreatment with a cholinergic muscarinic antagonist blocks GH secretion in response to GH-releasing factor (Massara *et al.* 1984). The mechanism by which this occurs may be an increase in the release of somatostatin – a potent inhibitor of GH secretion – by the hypothalamus. By contrast, in girls with anorexia nervosa, pretreatment with a cholinergic muscarinic antagonist does not block the exaggerated GH response to GHRH (Rolla *et al.* 1990, Tamai *et al.* 1990), suggesting that there may be an abnormality in the release of or response to somatostatin.

We investigated whether supraphysiological doses of GH can overcome the state of GH resistance in women with anorexia nervosa (Fazeli *et al.* 2010*a*). Subjects ($n=21$) were randomized to either placebo or recombinant human GH treatment (mean maximum daily dose: 1.4 mg/day) for 12 weeks (Fazeli *et al.* 2010*a*). At the conclusion of the study, IGF1 levels did not differ between the groups, demonstrating that even very high levels of exogenous GH cannot overcome the insensitivity to GH, at the level of the liver, in anorexia nervosa (Fazeli *et al.* 2010*a*). One purported mechanism for the GH resistance state in anorexia nervosa is the downregulation of GH receptors in the liver (Counts *et al.* 1992). The failure of supraphysiological doses of GH to increase IGF1 levels supports this hypothesis.

Isolated nutrient deficiencies

Isolated vitamin deficiencies may also cause a state of GH resistance. Vitamin A-deficient rats have lower IGF1 levels compared with pair-fed controls despite comparable GH levels (Mohan & Jaya Rao 1980). Compared with similar weight controls, children with vitamin A deficiency have normal GH levels coincident with lower IGF1 levels (Mohan & Jaya Rao 1979). Similarly, rats fed a diet deficient in vitamin B6 for 4 weeks were found to have low IGF1 levels and normal GH levels (Rao & Mohan 1982).

In a rodent model, both zinc deficiency and magnesium deficiency are associated with decreased IGF1 levels in the setting of normal GH levels (Dorup *et al.* 1991). Young rats fed a magnesium-deficient diet were found to have significantly lower serum IGF1 levels compared with pair-fed controls, whereas basal GH levels and GH levels after stimulation with GH-releasing factor were similar in the magnesium-deficient group compared with an *ad libitum*-fed

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group (Dorup *et al.* 1991). Similarly, young rats fed a zinc-deficient diet were also found to have significantly lower serum IGF1 levels compared with pair-fed controls but levels of GH were similar in the zinc-deficient rats and the *ad libitum*-fed zinc-sufficient rats after treatment with GH-releasing factor, suggesting a state of GH resistance (Dorup *et al.* 1991). In postmenopausal women, zinc intake is positively associated with IGF1 levels, even after adjustment for possible confounders, but because GH levels were not measured in this study, it is not known whether GH resistance contributes to the lower IGF1 levels (Devine *et al.* 1998).

Potassium-deficient diets have also been studied in an animal model (Flyvbjerg *et al.* 1991). Rats consuming a potassium-deficient fodder had significantly lower serum IGF1 levels compared with pair-fed controls, but in this case, the response to GH-releasing factor stimulation was significantly less in the potassium-deficient group compared with the controls (Flyvbjerg *et al.* 1991). Therefore, the low IGF1 levels in potassium-deficient rodents are not due to GH resistance but likely due to downregulation of an appropriately functioning GH–IGF1 axis.

Effects of dietary composition

In *in vitro* studies, saturated fatty acids, but not unsaturated fatty acids, inhibit promoter activity at the GH receptor gene and thereby decrease GH receptor mRNA and protein levels (Thimmarayappa *et al.* 2006), suggesting that saturated fatty acids may contribute to GH resistance. In a large cohort of healthy women ($n=1000$), a cross-sectional examination of plasma IGF1 levels and various dietary components demonstrated a positive association between IGF1 levels and total energy intake (Holmes *et al.* 2002). There was also a positive association between IGF1 levels and increasing levels of protein intake (Holmes *et al.* 2002). Smaller human studies ($n=115$) have also investigated the cross-sectional relationship between IGF1 and dietary composition (Kaklamani *et al.* 1999). Positive associations between IGF1 and fats, red meat, and oil consumption have been reported as well as an inverse association between IGF1 levels and carbohydrate intake (Kaklamani *et al.* 1999). Importantly, GH levels were not measured in these studies and therefore we do not know whether these differences in IGF1 levels are GH mediated or due to GH insensitivity.

Mechanisms of GH resistance

GH is secreted by somatotroph cells in the anterior pituitary gland and binds to GH receptors in many tissues, including hepatocytes. The binding of GH to its receptor activates JAK2, which leads to phosphorylation of STAT5. STAT5 is subsequently translocated into the nucleus where it is able to bind to regulatory elements of target genes including IGF1. Recently, two proteins have been shown to be important regulators of GH resistance in states of nutritional deprivation: fibroblast growth factor 21 (FGF21) and Sirtuin 1 (SIRT1). Both FGF21 and SIRT1 induce GH resistance via STAT5 inhibition.

Fibroblast growth factor 21

FGF21, a member of the FGF family of proteins, is a hormone produced in the liver (Nishimura *et al.* 2000) and adipocytes (Zhang *et al.* 2008). Serum FGF21 levels correlate with BMI and also with *FGF21* mRNA expression in subcutaneous fat (Zhang *et al.* 2008), suggesting that serum FGF21 levels primarily originate in adipose tissue. FGF21 also stimulates insulin-independent glucose uptake in adipocytes via the glucose transporter-1 (Kharitonenkov *et al.* 2005) and is currently being investigated as a therapeutic agent in individuals with type 2 diabetes mellitus (Gaich *et al.* 2013). In animal models, starvation induces FGF21 production in the liver via a mechanism requiring peroxisome proliferator-activated receptor α (Inagaki *et al.* 2007, Lundasen *et al.* 2007). FGF21 subsequently induces peroxisome proliferator-activated receptor γ coactivator protein 1α (PGC1α) expression, leading to fatty acid oxidation and increased gluconeogenesis (Potthoff *et al.* 2009). In humans, a very low-calorie diet and prolonged fasting result in increased levels of FGF21 (Galman *et al.* 2008, Mraz *et al.* 2009). Therefore, FGF21 may act differently in states of nutritional sufficiency compared with states of nutritional deprivation.

In a transgenic animal model, *Fgf21* transgenic mice have high levels of GH and significantly lower levels of IGF1 compared with WT mice (Inagaki *et al.* 2008), consistent with GH resistance. The mechanism of GH resistance in *Fgf21* transgenic mice is a decrease in STAT5 phosphorylation (Inagaki *et al.* 2008). Similarly, we have shown that FGF21 levels are significantly higher in adolescent girls with anorexia nervosa compared with normal-weight girls after controlling for percent body fat and insulin resistance, suggesting that the increased levels of FGF21 in anorexia nervosa are due to increased levels of liver-derived FGF21 (Fazeli *et al.* 2010*b*). We have also demonstrated a significant positive relationship between GH area under the curve and FGF21 and an inverse relationship between IGF1 and elevated levels of FGF21,

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after controlling for percent body fat and insulin resistance (Fazeli *et al*. 2010*b*). These data suggest that FGF21 may be a mediator of GH resistance in the human model of chronic starvation – anorexia nervosa.

Sirtuin 1

SIRT1, a class III histone deacetylase, is regulated by nutrient availability and promotes gluconeogenesis and fatty acid oxidation during periods of fasting (Gillum *et al*. 2010). Recently, SIRT1 has been shown to play a role in GH resistance in states of starvation (Yamamoto *et al*. 2013). SIRT1 knockdown mice that underwent a 48-h fast had higher serum levels of IGF1 and higher *Igf1* mRNA levels in the liver when compared with WT fasted mice, suggesting that SIRT1 mediates GH resistance in states of undernutrition (Yamamoto *et al*. 2013). Like FGF21, the mechanism by which SIRT1 acts is a reduction in STAT5 phosphorylation; this decrease in STAT5 phosphorylation is a result of SIRT1 deacetylating lysine residues on STAT5 (Yamamoto *et al*. 2013).

Insulin

Type 1 diabetes mellitus is also a state of GH resistance (Yde 1969, Lundbaek *et al*. 1970, Horner *et al*. 1981). Therefore, insulin has also been investigated as a possible determinant of GH resistance. GH receptors in the liver are reduced in diabetic female rats and this decrease correlates with the decreased serum insulin levels (Baxter & Turtle 1978). GH receptors also increase in response to treatment with insulin (Baxter & Turtle 1978) and *in vitro* models demonstrate upregulation of GH receptors by insulin (Leung *et al*. 2000). Therefore, insulin levels, which are low in states of nutritional deprivation such as fasting, may in part mediate GH resistance by downregulation of GH receptors in the liver. Although, this is unlikely to be the only mechanism by which GH resistance is induced in states of undernutrition, as studies in diabetic rats have demonstrated independent effects of insulin and a low-protein diet (Maiter *et al*. 1989).

Insulin-like growth factor 1

The low IGF1 levels in the GH resistance state – and the resultant decrease in negative feedback at the level of the pituitary – contribute to the elevated GH levels characteristic of GH resistance. In a study of healthy, fasting men, a recombinant human IGF1 infusion resulted in a significant decrease in serum GH levels (Hartman *et al*. 1993). Similarly, in a study of women with anorexia nervosa, administration of recombinant human IGF1 (20 μg/kg) significantly reduced mean basal GH concentrations, although levels remained higher than those in normal weight controls (Gianotti *et al*. 2000).

Other hormonal factors

Triiodothyronine Triiodothyronine (T_3) may play a role in the GH resistance state induced by fasting (Ikeda *et al*. 1990). Both plasma IGF1 and T_3 levels are low in fasted rats (Ikeda *et al*. 1990). S.c. injections of T_3 (5 μg/kg) normalized T_3 levels in fasted rats and plasma IGF1 levels also increased significantly, suggesting that T_3 may play a role in mediating GH resistance in states of undernutrition (Ikeda *et al*. 1990).

Leptin Levels of leptin, an anorexigenic hormone secreted by adipocytes, are low in states of undernutrition (Frederich *et al*. 1995), and these low levels may mediate the GH resistance state. In sheep, an animal model that also demonstrates GH resistance in response to fasting, ovariectomized ewes that were fasted for 72 h and infused with leptin centrally had levels of GH, which were lower than those of the vehicle-treated, fasted, and ovariectomized ewes (Henry *et al*. 2004). GH levels in the leptin-treated ewes were comparable to levels in *ad libitum*-fed ewes (Henry *et al*. 2004). Therefore, central leptin may reduce GH secretion and the low levels of leptin during starvation may result in an increase in GH levels, contributing to the GH resistance state.

In humans, children with protein–energy malnutrition have significantly lower levels of leptin and IGF1 compared with healthy controls and significantly higher levels of GH (Soliman *et al*. 2000, Kilic *et al*. 2004). Leptin levels are also positively associated with IGF1 and inversely associated with GH (Soliman *et al*. 2000, Kilic *et al*. 2004). When women with hypothalamic amenorrhea, a state of negative energy balance, were treated with recombinant human methionyl leptin for 3 months – resulting in a normalization of leptin levels – IGF1 levels increased to levels comparable to euleptinemic controls, even after controlling for estradiol (E_2) levels and changes in weight (Chan *et al*. 2008). Therefore, low leptin levels may be a mediator of GH resistance in states of nutritional deprivation.

Testosterone Testosterone has also been shown to be a significant predictor of IGF1 levels in women with anorexia nervosa (Brick *et al*. 2010). In a group of women

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with anorexia nervosa and normal weight controls, free testosterone was found to be a significant and independent predictor of IGF1 levels and was able to account for 36% of the variability in IGF1 levels after controlling for other possible hormonal predictors including E_2 (Brick *et al*. 2010). Therefore, the low testosterone levels in anorexia nervosa (Miller *et al*. 2007) and other states of starvation (Klibanski *et al*. 1981) may contribute to the state of GH resistance.

GH resistance is an adaptive response to undernutrition

In states of undernutrition, GH resistance may be an adaptive response. Elevated GH levels may be necessary to maintain euglycemia, whereas decreased levels of IGF1 help conserve energy during periods of nutritional deprivation.

Mobilization of fat stores

Elevated GH levels may be important for mobilization of fat stores in states of malnutrition. Mice with adult-onset, isolated GH deficiency and their GH-replete littermates were calorie restricted for 11 days (Gahete *et al*. 2013); GH levels increased in the GH-replete mice but not in the GH-deficient mice in response to the calorie deprivation (Gahete *et al*. 2013). Importantly, the loss of fat mass and subsequent increase in free fatty acid levels were significantly greater in the GH-replete mice compared with the GH-deficient mice (Gahete *et al*. 2013), suggesting that mobilization of fat stores during starvation is a significant, and likely adaptive, consequence of the elevated GH levels. In humans, GH has also been shown to be a critical factor in increasing the rate of lipolysis during fasting (Sakharova *et al*. 2008).

Maintenance of euglycemia

Elevated GH levels may also be important for maintaining euglycemia in states of starvation. Ghrelin is an orexigenic hormone produced primarily in the fundus of the stomach. Importantly, there are ghrelin receptors – called GH secretagogue receptors – in the anterior pituitary gland and octanoylated ghrelin acts as a potent stimulator of GH secretion (Kojima & Kangawa 2005). In an animal model, elimination of ghrelin *O*-acyltransferase (GOAT) – which attaches octanoate to proghrelin – results in mice that lack octanoylated ghrelin or acylated ghrelin (Zhao *et al*. 2010). *Goat* knockout mice on normal or high-fat diets grow and gain weight normally but when calorie restricted, the *Goat* knockout mice become profoundly hypoglycemic in contrast to the WT, calorie-restricted mice (Zhao *et al*. 2010). GH levels were also found to be twofold higher in the WT mice compared with the *Goat* knockout mice and infusion of GH was able to rescue the *Goat* knockout mice from hypoglycemia and normalize blood sugars, suggesting that an important role of elevated GH levels in states of malnutrition is to maintain euglycemia (Zhao *et al*. 2010). Although there is recent controversy regarding the role of ghrelin and GH in protecting against hypoglycemia during periods of calorie restriction (Yi *et al*. 2012, Gahete *et al*. 2013), it is clear that the increasing levels of GH serve to mobilize energy during periods of malnutrition and are an adaptive response to undernutrition.

Decreased energy expenditure

Elevated GH levels are potentially an important means of mobilizing fat stores and maintaining euglycemia in states of undernutrition. Yet, if the GH–IGF1 axis remained intact during states of nutritional deprivation, the elevated levels of GH would result in elevated IGF1 levels, leading to increased energy expenditure on growth and therefore incur a survival disadvantage in states of malnutrition. Therefore, GH resistance, with an inability of GH to appropriately stimulate IGF1 production, is likely an adaptive mechanism to preserve energy during periods of undernutrition.

Conclusions

Our evolutionary past was marked by periods of undernutrition. These periods of undernutrition were characterized by isolated protein–calorie deficiency, isolated vitamin deficiencies, and even starvation and survival depended on the body's ability to mobilize energy stores and prevent hypoglycemia. GH plays a key role in mobilizing energy and therefore elevated GH levels confer a survival advantage during periods of undernutrition. Yet, increasing energy expenditure for growth during these periods – which would result if the GH–IGF1 axis remained intact and IGF1 levels increased – would be disadvantageous in states of undernutrition. Therefore, the elevated GH levels and low IGF1 levels characteristic of GH resistance are an important adaptive response to calorie and nutrient deprivation and an important mechanism for survival.

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