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Review

Influence of insulin on growth hormone secretion, level and growth hormone signalling

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Abstract: Growth hormone (GH), as a vital hormone, has to experience a series of processes to fulfill its function including secretion, entering the circulation to reach target tissues (pre-receptor process), binding on the GH receptor (GHR) and triggering signaling inside cells (post-GHR process). Insulin can directly or indirectly influence part of these processes. GH secretion from pituitary somatotropes is regulated by GH-releasing hormone (GHRH) and somatostatin (SS) from hypothalamus. Insulin may exert positive or negative effects on the neurons expressing GHRH and SS and somatotropes under healthy and pathological conditions including obesity and diabetes. Glucose and lipid levels in circulation and dietary habits may influence the effect of insulin on GH secretion. Insulin may also affect GHR sensitivity and the level of insulin-like growth factor 1 (IGF-1), thus influence the level of GH. The GH signaling is also important for GH to play its role. GH signaling involves GHR/JAK2/STATs, GHR/JAK2/SHC/MAPK and GH/insulin receptor substrate (IRS)/PI3K/Akt pathways. These pathways may be shared by insulin, which is the basis for the interaction between insulin and GH, and insulin may attenuate or facilitate the GH signal by influencing molecules in the pathways. Many factors are related to the effect of insulin, among them the most important ones are duration of action and amount of insulin. The tendency of insulin-reduced GH signaling becomes obvious with increased dose and acting time of insulin. The participation of suppressor of cytokine signaling (SOCS), the interaction between JAK2 and IRS, and GHR sensitivity should also be considered when discovering GH signal. The involvement of SS in response to insulin is not clear yet. The details of how GH secretion, level and signaling change in response to time and dose of insulin treatment warrant further studies.

Key words: insulin; growth hormone; somatostatin; obesity; diabetes; signal pathway

胰岛素对生长激素的分泌和细胞内信号传导的影响

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摘 要：生长激素(growth hormone, GH)在行使其功能时需要经历一系列的过程，包括从垂体分泌和进入血液循环到达靶器官或细胞(受体前过程)以及和生长激素受体(GH receptor, GHR)结合并引发细胞内信号转导(受体后过程)。胰岛素可以直接或间接地影响这些过程。GH从垂体的生长激素分泌细胞中分泌需要依赖于下丘脑释放的生长激素释放激素(GH-releasing hormone, GHRH)和生长激素抑制素(somatostatin, SS)，在生理或病理条件下，胰岛素可以对这两种激素以及GH分泌细胞施加不同影响，从而干预GH的分泌及循环水平。血糖、血脂以及饮食习惯都可以改变胰岛素对GH的影响。胰岛素还能通过影响GHR的敏感性，以及影响胰岛素样生长因子-1 (insulin-like growth factor 1, IGF-1)，进而影响GH。受体后过程也是GH行使功能的重要一环，细胞内信号转导依赖于信号通路完成。GH信号转导通路和胰岛素的信号通路有部分交叉，这使得两者的信号可以相互作用，胰岛素通过这种作用对GH的信号转导产生影响。还有很多因素可以改变胰岛素对GH的影响，包括细胞

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因子信号抑制物、GHR敏感性以及JAK2蛋白和胰岛素受体底物间的相互作用，且随着胰岛素浓度升高和作用时间延长，胰岛素对GH的影响趋向于增强。但胰岛素的浓度和时间对GH分泌和细胞内信号转导的具体影响还未完全阐明。胰岛素和SS的关系也有待进一步研究。

关键词：胰岛素；生长激素；生长激素抑制素；肥胖；糖尿病；信号转导通路
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Growth hormone (GH) plays an important role in physical growth and development by maintaining the normal structure and functionality of the body through cell regeneration and protein synthesis [1, 2]. Insulin is a vital hormone playing a number of roles in the body metabolism, especially in the regulation of blood glucose levels by signaling the liver, muscle and adipose cells to take in glucose from the blood circulation. As very important metabolic regulatory hormones, both GH and insulin work in concert to implement their effects on the cellular metabolism and biogenic activities. Their interaction and balance are critical under both pathological and physiological conditions. Understanding the interaction between GH and insulin is the prerequisite of understanding the mechanism of many endocrine processes in maintaining normal metabolic balance, as well as in metabolic disorders. The effect of GH on insulin, especially on insulin's sensitivity, has been intensively investigated in past few decades since 1930s [3]. However, given the increasing use of insulin in hormone-replacement therapies to treat diabetes, it is of great importance and interest to clarify the influence of insulin on GH, which is in the process of exploration.

About 50 years ago, Hazelwood and his colleagues found that diabetes could exert an impact on GH content inside pituitary gland [4]. In 1978, it was shown that insulin receptors (IR) were widely distributed in the central nervous system [5]. Then in 1992, Menon *et al*. found that GH-binding protein decreased in children with type 1 diabetes (T1D) [6]. In 1999, it has been demonstrated that insulin may inhibit GH signaling [7]. All these studies have indicated complicated actions of insulin on GH, even though the mechanisms are not totally understood. To better discuss insulin's action on GH, we divide the roles played by GH into two parts: the pre-receptor process including GH secretion and the maintenance of GH levels, and the post-GH receptor (GHR) process which mainly refers to GH signaling.

GH secretion is regulated in a hypothalamus-controlled pattern, which involves the stimulation or inhibition on somatotropes by GH-releasing hormone (GHRH) or somatostatin (SS) respectively. GH secretion is also influenced by insulin-like growth factor 1 (IGF-1), which involves negative feedback mechanism. GH level is greatly determined by GH secretion, but it is also influenced by insulin to a great extent in both physiological and pathological conditions. The profile of plasma GH is related to GH's functions. The GH level in circulation changes with a number of factors. The matters affected by insulin, including GHRH, SS, IGF-1, the blood glucose and lipid concentration, can directly or indirectly influence GH [8–12]. GH signaling is indispensable for GH to play its part. GH binds with the GHR, setting up the post-GHR process. The most essential signal pathways mediated by GHR include signal transducer and activator of transcription (STAT) pathway, mitogen-activated protein kinase (MAPK) pathway and phosphoinositide 3-kinase (PI3K)/Akt pathway [13]. Previous studies have implied that insulin may affect GH hormone and downstream signals in a time- and dose-dependent manner both *in vivo* and *in vitro*, even though there are conflicting results under different experimental conditions [12–15].

1 GH secretion, GH level and GH signalling: Functioning process of GH

GH, a single-chain polypeptide containing 191 amino acids, also known as somatotropin, is a hormone that motivates physical growth and organism development in human and animals. It also plays an important role in sustaining the normal structure of the whole body. Besides the well-known function to facilitate long term postnatal growth, GH has a vital metabolic regulatory effect alone or with other hormones on reproduction and aging (cell reproduction and regeneration), as well as metabolic processes [2, 16–18].

To function inside the body, GH has to be synthesized, stored, and secreted by somatotropic cells (located in anterior pituitary gland) by receiving the stimulation of GHRH from hypothalamus. Then GH goes into the blood circulation and to distant organs including liver,

muscles and adipose tissues, to act on all cells possessing GHR [18]. These processes are pre-GHR processes which involve two fundamental elements: the secretion of GH and the maintenance of GH level in blood circulation. During these processes, GH can affect and interact with other hormones, i.e., taking action on pancreatic islets to regulate insulin and glucagon. Also, it can be influenced by other hormones or matters. After GH is recognized by GHR, it can bind to the receptor, go into the cell and pass down its signal to motivate a variety of cellular activities through different signal pathways. The integrity of GH signal pathways is indispensable for the transmission of GH signal and implementation of its function in cell level. This process is the post-GHR process.

1.1 The secretion of GH

GH secretion is completed and regulated in an axial pattern. Hypothalamus perceives physiological stimulus and regulates rhythm of life and releases GHRH, which is an endogenous hormone. It is also a peptide hormone, containing 43–44 amino acids. GHRH binds to the GHRH-receptor of somatotropes in the anterior pituitary gland. Then, the stored GH inside somatotropes can be released into circulation and flow through various organs and cells to implement its function, including going back to the hypothalamus and pituitary to give feedback. GHRH is released in a pulsatile way, which leads to the pulsatile secretion of GH. There is another hormone existing in the hypothalamus called somatotropin-releasing inhibitory factor (SRIF, also known as SS or SST), which is also known as GH- inhibiting hormone (GHIH) that exists in the digestive organs too, participating in the secretion of GH[19]. SS is also a peptide hormone, and it has six different constitutions from six different genes in zebrafish, but humans only possess one: *SS*. SS can inhibit GH secretion by inhibiting the release of GH from somatotropes, thus opposing the effects of GHRH. SS also has many kinds of receptors exerting different functions besides regulating GH secretion, such as inhibiting the glucagon to control glucose in blood [20]. As regulators of GH secretion, GHRH is more systemic than SS. SS usually plays an assistant role and works together with other hormones. GHRH and SS regulate GH secretion together to keep the normal concentration of GH in circulation [9, 21, 22]. Some hormones or drugs can exert influence on GH secretion by exerting action on GHRH, SS, and somatotropes. Apart from GHRH and SS, GH secretagogue (GHS) ghrelin also facilitates the secretion of GH. The axial regulation pattern involves negative feedback mechanism: GHR exists on pituitary GH cells and hypothalamus GHRH/SS neurons to receive feedback and regulate GH release. GH/GHR/IGF-1 axis is another important axis for regulation of GH secretion which involves negative feedback mechanism. IGF-1 is considered GH secretion inhibitor. When IGF-1 gene was deleted from liver, the level of GHS receptor in pituitary went up, leading to an improved GH secretion [23]. The GH/IGF-1 system plays a role in the interaction among adipose tissue, liver, and pituitary [24].

1.2 GH level in circulation

GH level in blood circulation is essential for normal metabolism. When the frequency and quantity of GH pulsatile secretion are influenced, GH concentration fluctuates. GHR sensitivity also has an effect on GH level because normal GHR sensitivity is important to the successful GH utilization. When GHR sensitivity is impaired, body enhances GH level to make up. GH profile gives feedback to hypothalamus, the upper head of axis, thus maintaining a normal concentration. GH/IGF-1 axis also plays a part in regulating GH level. An experiment showed that liver-specific deletion of IGF-I in mice (*LI-IGF-I*$^{-/-}$) gave rise to declined circulating IGF-I and increased plasma GH levels [23].

The profile of plasma GH is correlated to physiological and pathological conditions. GH not only accelerates lipolysis, but also regulates glucose metabolism, and has the ability to enhance blood glucose concentration. Therefore, diet habits and metabolic diseases can influence GH level by changing blood glucose and lipid content. Non-esterified fatty acid (NEFA) has been considered a factor to influence GH level, for excess NEFA restrains GH level [25]. But NEFA and GH level are not always in inverse proportion to each other. The regulation of GH level may involve the participation of insulin. It was found that the GH offsets the effect of insulin on glucose homoeostasis, so glucose metabolism also accounts for regulating GH level [26, 27]. T1D patients were found to have a lower insulin level and higher GH level [28]. This implies that the maintenance of GH level can be disturbed by other hormones, especially insulin, which possesses reverse metabolic functions to GH. For the regulation pattern of GH secretion and GH level, see Fig. 1.

1.3 The signaling of GH

Signalling inside the cell is a post-GHR process, which

is the cornerstone for the cellular activities. It is the last step for GH to function. GH exerts its actions through a series of signalling pathways and stimulates the phosphorylation of groups of molecules. The beginning of GH signal transmission is the combination of GH to two GHR molecules. GHR is a member of superfamily of cytokine/hematopoietic receptors. However, GHR lacks inherent activity of tyrosine kinase. Fundamentally, it is connected with a tyrosine-kinase called Janus kinase (JAK) 2 [29, 30]. After GHR is bound, the dimerization action results in the auto-phosphorylation of one or more certain tyrosine(s) of JAK2, which brings a conformational alternation in JAK2 that changes (decreases or stimulates) JAK2 activity. For instance, phosphorylation of tyrosine 1 007 has been considered to expose the ATP or downstream binding sites, which can also be combined with the negative regulators of cytokine -- suppressor of cytokine signaling (SOCS), and then stimulates the GHR on tyrosine residues inside cells [31–33]. Phosphorylated residues on GHR and JAK2 are docked with a variety of intracellular transmission intermediators including the STAT family STAT-1, 3, 5 (including 5a and 5b), and other intracellular substrates including insulin receptor substrate 1 (IRS-1), IRS-2 and src homology and collagen (SHC) protein [34]. Replenishment of these proteins to the GHR-JAK2 complexes permits GH to execute a variety of physiological function [32]. The removal of tyrosine phosphatises in GHR-JAK2 complex draws the signal of GH to the end [30, 35]. Among various kinds of cellular activities, transcription of genes is the most important one. Three major signal pathways are of great significance in this post-GHR process: GHR/JAK2/STATs, GHR/JAK2/SHC/MAPK and GH/IRS/PI3K/Akt pathways [29].

STAT family contains many subtypes, and the close relationship between GHR and STAT5b has been

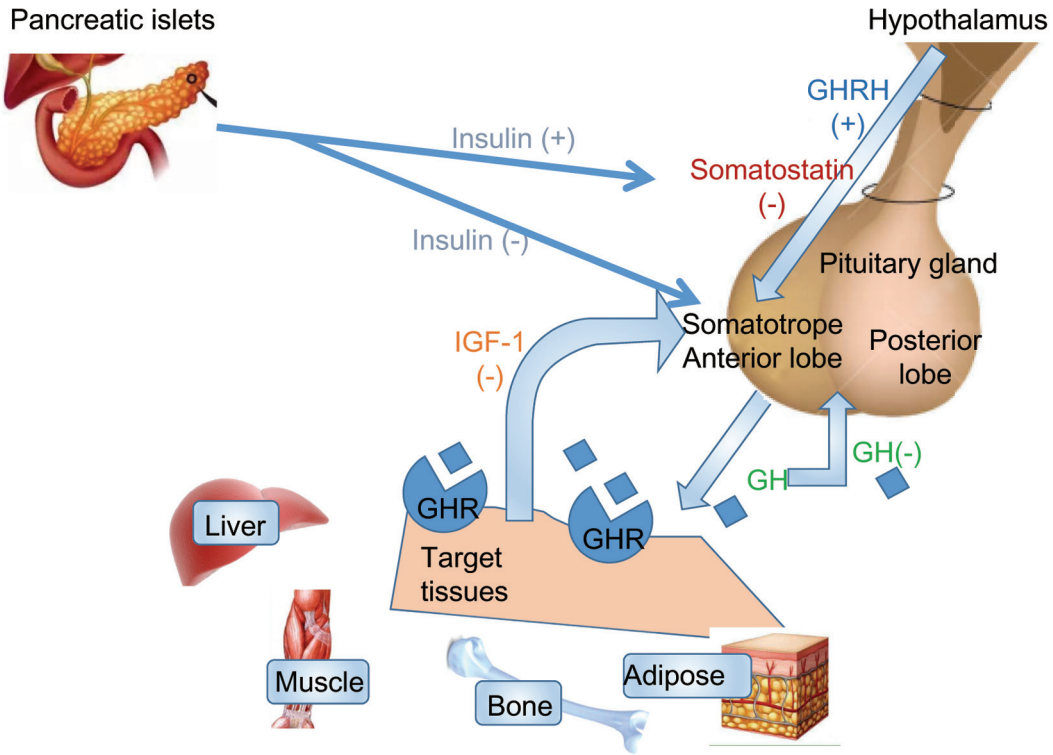

Fig. 1. The physical regulation pattern of GH profiles. GH secretion is completed and regulated in an axial pattern. GH is synthesized, stored, and secreted by somatotropic cells (located in anterior pituitary gland) by receiving the stimulation of GHRH from hypothalamus. Meanwhile, GH in pituitary is regulated by somatostatin existing in the hypothalamus, which can be stimulated by insulin from pancreatic islets and inhibit GH secretion. Insulin can also bind to the insulin receptor (IR) on somatotropes in pituitary and inhibit GH secretion. An excessive high level of GH in circulation gives negative feedback to hypothalamus and pituitary gland, preventing GH secretion. GH/GHR/IGF-1 axis is another important axis for regulation of GH secretion, which participates in controlling GH level in circulation. GH goes to distance organs including liver, muscles, bones and adipose tissues and begins to take action on all cells possessing GHR. Abbreviations: −, inhibition; +, stimulation; GH, growth hormone; GHR, growth hormone receptor; GHRH, growth hormone releasing hormone; IGF-1, insulin-like growth factor-1.

discovered, although STAT5a also plays a role in GH signal [36–38]. STAT5b is sensitive to pulsatile secretion of GH [39]. GH-induced tyrosine phosphorylation of STAT5B is followed by the relocation of cytoplasmic STAT5 proteins complex to the nuclear area and mediated by an SH2 domain-phosphorylated tyrosine action. Then target genes' transcription is motivated, mainly including *c-fos*, serine protease inhibitor (*spi 2.1*), and *SOCS*, which are related to somatic growth and development and metabolic functions of GH [30, 40–42]. The growth effect of GH on muscle relies on STAT5b: muscle IGF-1 transcript and muscle mass weaken due to the muscle-specific deletion of STAT5B [43]. In humans, clinical research showed that growth failure and disorders were related to STAT5b mutation, implying an irreplaceable post of STAT5b in GH signalling [44–46]. SOCSs are in the downstream pathway of STAT5, and GH induces four molecules in them, including SOCS-1, 2, 3 and CIS. The SOCSs family is involved in attenuating the GH-induced stimulation of JAK2. Each of them can block the auto-phosphorylation of JAK2 by competing for the binding sites in GHR with STATs [47–49].

The Ras-MAPK pathway is also crucial in GH signalling. In most cases, GH activates GHR/JAK2/SHC/MAPK pathway by JAK2 phosphorylation of the protein SHC [50, 51]. Noticeably, IRS-1 is shown to be involved in this pathway and plays a positive role by facilitating GH-induced cell regeneration mediated by MAPK [52]. GH can also induce the phosphorylation of IRS [53]. GH appears to facilitate tyrosine phosphorylation of IRSs and build up their association with PI3K in a variety of GH-responsive cells to fulfil its function in lipid and glucose metabolism [54–58]. Activation of the GH/IRS/PI3K/Akt pathway is mediated by JAK2, and there is no overt direct connection between the IRS proteins and with the GHR [59].

Additionally, signal molecules in GH signalling process like STAT, PI3K and MAPK do not serve for GH merely; instead they are shared by many other hormones. This is the basis for the interaction in the cellular level between GH and other hormones especially insulin.

2 The effect of insulin on the pre-receptor process: the effect on GH secretion and GH level by insulin

In early stage of research, it is noticed that there is a mutual effect of GH and insulin on each other secretion inside mammalian bodies, including human beings. Considering the inverse physiological function of these two hormones, which mainly refers to the function of regulating the lipogenesis and lipolysis, and the effect on glucose metabolism, one may easily speculate the inverse relationship between insulin and GH. With the increasing incidence of diabetes, scholars have paid more attention to the effect of GH on insulin in the past ten years because GH influences insulin sensitivity. Recent research has shown that the hyperinsulinemia leads to the change in both GH secretion and GH level in both diabetes condition and non-diabetes condition. This finding has a significant meaning for endocrine diseases or hormone disorders, and the effect of insulin on GH secretion and GH level should be emphasized [9].

GH is stimulated by endogenous GHRH, which is controlled by hypothalamus and stimulates the GH stored in pituitary to be released to the blood circulation. And it is inhibited by SRIF or SS. Importantly, SS could reduce the release of GH from GH-releasing cells (somatotropes) in pituitary, preventing excessive GH. So, GHRH and SS regulate GH secretion together to keep the normal concentration of GH in circulation [9, 21, 22]. In summary, GH secretion depends on three decisive factors: GHRH released by hypothalamus, SS in the body especially in the hypothalamus, and the GH cells which store GH in pituitary.

2.1 The effect of insulin on GH secretion and GH level under diabetes or non-diabetes condition

In a healthy body, insulin and glucagon are main factors for regulating glucose concentration. GH's effect on blood glucose is similar to glucagon, which accelerates gluconeogenesis and restrains the absorption of glucose from circulation to cells, so there is a balance between GH and insulin on regulating glycol-metabolism. However, in patients with diabetes, insulin is relatively or absolutely in deficiency. The balance between GH and insulin is disturbed, and it not only imposes influences on blood glucose, but can cause a series of impacts on GH secretion and level.

As it is mentioned, to analyse the effect of insulin on GH secretion, three elements should be considered: GHRH, SS, and somatotropes. In 1985, Shibasaki and colleagues found that pretreatment of insulin could weaken the GH response to the administration of GHRH-44, which suggests that insulin may cause the desensitization of GHRH receptors in GH secretion

cells and enhance the level of SS [60]. Then in 1990, scholars found the average GHRH levels were higher in the control group than that of T1D subjects at all stages after meals, while SS level had no difference [61]. Also, in T1D patients, the level of GH is higher, the GHR level is lower than normal, and a raise in GH secretion was observed following the reduction in insulin level [10]. All these data suggest that a higher level of insulin brings down the sensitivity of GHRH receptors in somatotropes, which restrains the hypothalamus-stimulated GH secretion. Meanwhile, insulin in normal physiological concentration maintains the level and sensitivity of GHR and GHRH action on somatotropes, promoting the normal release of GH from pituitary.

When the blood glucose concentration is influenced by some pathological conditions, SS participates in the effect of insulin on GH. Insulin-induced hypoglycaemia can stimulate GH secretion mainly through inhibiting hypothalamic SS release [62]. By contrast, when the glucose concentration of blood is overloaded in diabetic conditions, SS may be secreted in a greater level, and reduce somatotrope-released GH. Insulin maintains the balance with GH through SS. Even though one function of SS is to restrict the glucagon, which is similar to insulin, the overall effect of SS on glucose metabolism does not completely accord with the level of insulin. The further research on the interactive relationship between the SS and insulin is needed.

In conclusion, insulin could maintain a balance with GH in physiological conditions, which is essential for glucose metabolism; but in diabetes, insulin may reduce the sensitivity of GHRH receptor on somatotropes and restrain GH secretion by increasing the level of SS. GH could stay steady without changing with insulin's fluctuation because of self-regulation of GH. Insulin regulates the secretion of SS and GH to better control the blood glucose level. By realizing the relationship among GH, insulin and their mediator SS, we may treat diabetes from a new perspective, and better understand how insulin-replacement therapy influences GH level and secretion when treating diabetes. The mechanism of insulin on GH may be different in T1D and T2D. The matter about how the effect of insulin on GH changes with different conditions of body and with hormones' concentrations need to be further studied. A recent research *in vitro* has showed that somatotropes in hypophysis possess many kinds of ion channels on their membranes, especially Ca^{2+} channel, which is modified by insulin, affects the signal transmission and then exerts impact on the release of GH [19]. This is a possible regulation mechanism of insulin in a sub-cellular level.

2.2 The effect of insulin on GH secretion and GH level under obese or non-obese condition

Obesity is a complex medical condition caused by the combination of many factors, one of which is disorder of lipid metabolism [63]. Factors that can exert impacts on lipogenesis or lipolysis lead to obesity. In mammal, similar to glycometabolism, it is the balance between GH and insulin that controls lipid metabolism. The relationship between GH profiles and obesity is proved as enhanced obesity in patients lacking GH and in animals, e.g. *lit*/*lit* mice, which cannot express GH [64]. Obesity is an important factor of decreased insulin sensitivity and contributes to T2D. Therefore, obese condition can influence both GH and insulin, and influence the balance between them. The effect of insulin on GH secretion and level under obese condition also involves the effect of insulin on GHRH, SS, and GH secreting cells.

De Schepper *et al*. found no overt relationship between insulin and pulsatile GH secretion in both obese and non-obese rats in an experiment of small sample size, but the GH secretion was less and insulin level was higher in obese group than that in non-obese group [65]. This suggested that there were other factors participating in the balance between GH and insulin. In 2001, Wang *et al*. found that insulin level was higher in obese children compared with that in non-obese children, and GH levels were remarkably lower in obese boys [66]. When obesity occurs, the sensitivity of insulin is lower, which brings hyperinsulinemia. Increasing insulin stimulates SS secretion and inhibits GH secretion. A study about children with obesity has confirmed the idea that SS plays an essential role in the connection between insulin and GH in obesity condition. This study showed that, when chronic increase appeared in SS secretion, pituitary-secreted GH level would be reduced [67]. Sauter *et al.* considered that insulin firstly stimulated the release of catecholamines, and then catecholamines took a second step action to promote the increase in SS [68]. In 2006, a study showed that hypothalamus did not play an indispensable role in regulating the secretion of GH. Unlike the regulation pattern in diabetes mentioned above, the suppression of GH in obesity condition is relatively independent of GHRH and SS [69]. Insulin resistance may exist in the

somatotropes, just like it exists in many other cells [8]. Insulin signal in somatotropes is a result of systemic regulation, and suppresses GH release directly. Insulin can complete this effect even in systemic insulin resistance individuals such as T2D patients, suggesting a relatively sensitive status of somatotropes to insulin in obesity [70, 71]. It also leads to a new question: obesity impairs the insulin signaling, so logically the effect of insulin on somatotropes in pituitary should also be attenuated [72]. Why GH secretion and GH levels still be suppressed by insulin-induced SS secretion? This question is still open for further study. Gender is also a crucial factor for the effect of insulin on GH. Androgen may play an essential role in the difference [73].

Additionally, GH level in circulation is correlative with the pulsatile secretion of GHRH, but it is not absolutely depending on GHRH only. Cells expressing GHR play a role in managing the GH levels. Insulin treatment is found to enhance GH-induced JAK2 phosphorylation in accordance with the expression of surface GHRs, but to decrease circulating GH, suggesting that insulin may influence GH level by influencing GHR sensitivity [74]. Interaction of GH with other hormones on a cellular of molecular level also influences GH level, including a variety of signal proteins and many pathways, which will be discussed below. Most significantly, GH level tends to be closely related to the insulin level in circulation. Chen *et al*. have verified a reverse effect of dietary-induced weight gain on insulin and GH levels in circulation by discussing which comes first, obesity or the decline of GH [25]. Previous studies tended to support the former. However, they found the inhibition of pulsatile GH secretion coming before dietary-induced weight gain (not obese yet), and the level of GH, which showed the reverse relationship with insulin, did not change parallelly with the circulating levels of NEFAs or glucose. Their result agreed with Cornford's study, which discovered that the suppression of GH secretion and the improved insulin sensitivity might be the reason for obesity, not the result [11]. The clinical application is that we may prevent or cure obesity by monitoring and regulating the balance between GH and insulin.

In summary, the regulation of GH secretion by insulin in people with obesity is different with the way in people suffering diabetes. IR plays an important role in inhibiting GH secretion in obese condition, while SS and other hormones may mainly account for insulin-induced decline of GH secretion in diabetes. During the diet-induced weight gain, the regulatory effect of insulin on GH may be stronger than the effect of GH on insulin. For maintaining the safe lower levels of NEFA and blood glucose, insulin level will increase and cause suppression of GH secretion and decline of GH level after calorie intake [25].

2.3 Mechanisms behind the effect of insulin on GH

According to the discussion above, hypothalamus is an important site for insulin to exert influence on GH, especially in diabetes, with the participation of GHRH, SS and other hormones in the body, all of which lead to inhibition of GH secretion and a lower GH level. Hypothalamus is not the only target for GH level to be influenced by insulin in obesity. The mediation of SS secretion centrally and peripherally, the direct action of insulin binding to IR, is very important for the regulation of GH secretion and level. In addition, IGF plays an important role. Recently IGF-1 has been considered an inhibitor of GH secretion, and the GH/IGF-1 system accounts for the visceral adiposity [9, 24]. This may be on account of that IGF-1 and insulin have hybrid receptors [29]. Insulin can bind with the IGF receptor. If the level of insulin is excessive, the profile of plasma IGF-1 will go up, due to the lack of available receptor. IGF-1 gives negative feedback to the GHRH/GH/IGF-1 axis, resulting in the decline of GH level. Meanwhile, the expression of GHRH and SS gene has no significant change in dietary-induced obesity [75]. Somatotropes and IR may be the targets for insulin to affect GH secretion and level directly and indirectly. The body may sacrifice normal weight in order to prevent normal metabolism from hyperlipidemia. In other words, people overeating and with hyperglycemia have to pay the price -- the tendency to suffer obesity by restraining the GH secretion and level in order to sustain the normal lipid and glucose concentration. Also, according to some *in vitro* studies, this mechanism should have protected body from too much lipolysis, but it may easily lose control due to the decline of the number of GHR expressed in adipocytes in the obese [76, 77]. For the regulation pattern of the whole process, see Fig. 2.

3 The post-GHR interaction between GH and insulin: Influence of insulin on GH signalling and mechanism

The successful process of GH secretion and the mainte-

nance of GH level do not guarantee the successful performance of GH. Insulin can exert effect on the post-GHR process too. The insulin-induced impairment of GH overall function may involve insulin's influence on GH signalling inside cells. It is found that a physiological dose of insulin is required for maintaining normal liver GH signalling responsiveness both *in vivo* and *in vitro*, and the lower fasting insulin was accompanied by lower levels of GHR [6, 7, 78–80]. Crucial signal pathways of GH have been already introduced above. Insulin has different effect on these pathways, leading to the attenuation or enhancement of GH signalling. The interaction between insulin and GH signalling is the basis for understanding insulin's effect on GH.

3.1 Shared signalling: the basis for the post-GHR interaction between GH and insulin

Signalling of GH and insulin are not same in the receptor level, but they partly begin to converge in the post-GHR level. Decades ago, GH has shown the acute insulin-like effect and chronic anti-insulin effect, suggesting the shared signalling between the two hormones [26, 81]. There are three main signal pathways as mentioned above. GHR/JAK2/STATs and GHR/JAK2/SHC/MAPK pathways mainly serve the function of regulating the genes transcription for vital proteins inside the nucleus, and GH/IRS/PI3K/Akt pathway accounts for the regulation of lipometabolism and glycometabolism. These pathways are shared between GH and insulin for different purposes (see Fig. 3).

IR/IRS/PI3K/Akt is the vital pathway for insulin. It has been found that this pathway participates most of the cellular activities by insulin action such as glucose transportation, glycogen synthesis, and suppression of gluconeogenesis [82, 83]. Akt, a serine/threoine kinase, is the most essential downstream molecule of PI3K. The proteins in the downstream of Akt includes glycogen synthase kinase-3 (GSK-3), BAD, forkhead box O1 (FoxO1) transcription factor. These effectors of Akt have different destination, directly or indirectly affecting the function of insulin signalling. Noticeably, GH regulates metabolism of lipid and glucose through PI3K/Akt pathway [82, 83], so insulin can interact or cause crosstalk with GH in this pathway, through Akt [84–86]. Moreover, PI3K is indispensable for the acti-

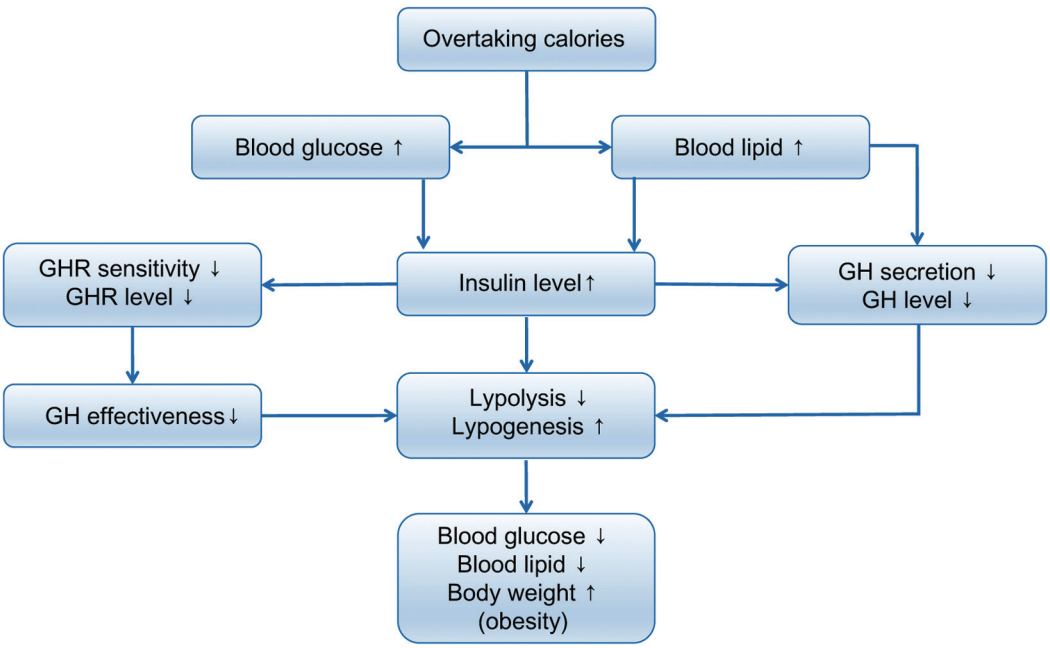

Fig. 2. The regulation pattern of GH profiles in obesity. GH and insulin levels maintain a balance in body. They jointly control fat metabolism and glucose metabolism. Overtaking calories leads to a high level of glucose and lipid in circulation, which will stimulate the secretion of insulin and somatostatin. Also, a high level of blood lipid will tend to inhibit GH secretion. As insulin and somatostatin increases, GH secretion decreases, so does its level in circulation. Moreover, GHR level decreases with its sensitivity being attenuated, leading to the dysfunction of GHR and noneffective GH signaling. As a result of these actions, lipolysis is accelerated and lipogenesis is restrained. In this condition, the concentrations of lipid and glucose can be controlled, although the weight gain increases. Abbreviations: GH, growth hormone; GHR, growth hormone receptor.

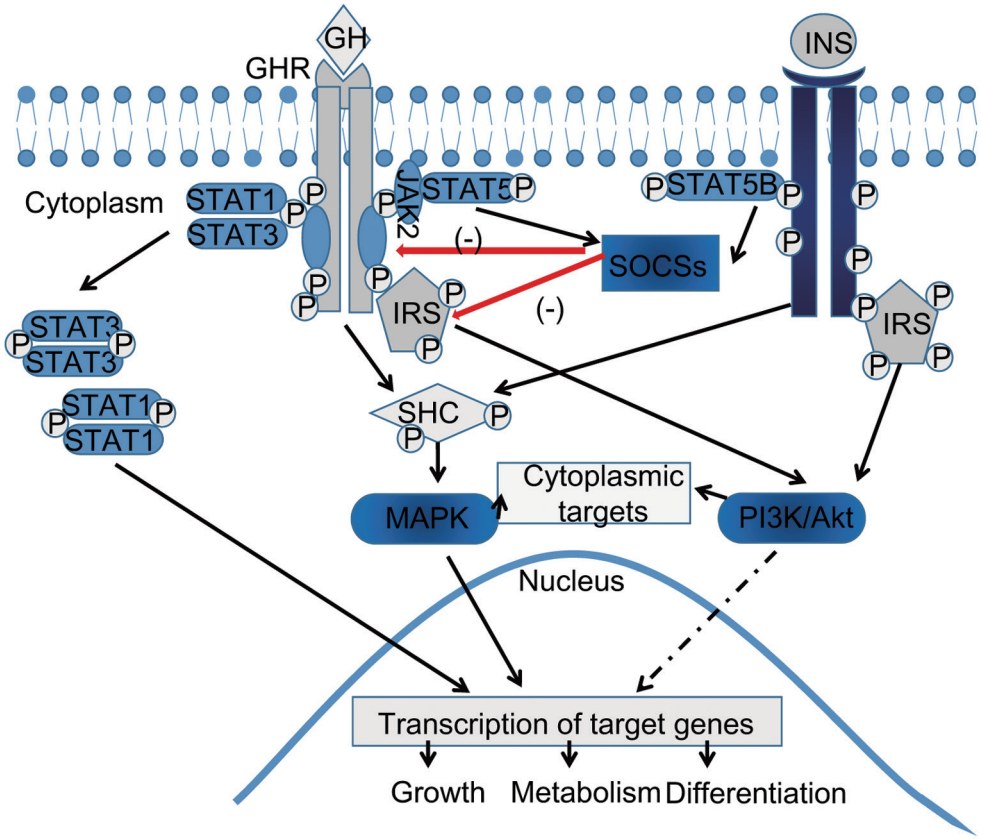

Fig. 3. Shared signal pathways between insulin and GH. GH signaling involves three signal pathways. The first one mediated by STATs proteins, which can activate SOCSs proteins, is shared by insulin with the particular participation of STAT5B. Insulin activates SOCSs, which will block the combination of JAK2 and GHR. Also, SOCSs can attenuate the interaction between GHR and IRS. The second way shared by insulin is mediated by SHC, a cytosolic protein. The engagement of SHC is responsible for the activation of MAPK cascades. Insulin can enhance the tyrosine phosphorylation of IRS-1, which can facilitate MAPK activation. Insulin shows an additive effect on GH-stimulated MAPK activation, by facilitating the activation of IRS. Another signal pathway of GH shared by insulin is PI3K/Akt pathway. Tyrosine phosphorylation of IRS-1 and -2 leads to the activation of PI3K, a SH2 domain containing protein, and its downstream Akt cascade. The activation of PI3K/Akt pathway is essential for the activation of other pathways, and insulin coordinates GH's insulin-like metabolic regulation in this pathway. Pathways mediated by STATs and MAPK are two major pathways for the regulation of gene transcription, including genes of growth, metabolism and differentiation; PI3K/Akt pathway is involved in activation of cytoplasmic targets and is important for metabolic regulation of GH and insulin, especially the regulation on glucose and lipid in circulation. The shared signaling is the basis of the effect of insulin on GH signaling. Abbreviations: −, inhibition; P, phosphotyrosine; GH, growth hormone; GHR, growth hormone receptor; INS, insulin; STAT, signal transducers and activators of transcription; IRS, insulin receptor substrate; SOCSs, suppressors of cytokine signaling; SHC, src homology and collagen proteins; MAPK, mitogen-activated protein kinase; PI3K, phosphatidylinositol 3-kinase; Akt, protein kinase B.

vation of MAPK [57, 87]. The significance of PI3K/Akt to both GH and insulin signalling is obvious.

MAPK pathway is also shared by GH and insulin. As mitogen-activated protein kinase, MAPK plays a role in promoting the growth, and has a minor role in regulating the metabolism. PI3K and Ras-Raf-Mek-Erk pathway are indispensable in stimulating the MAPK [83, 88]. Interestingly, MAPK has been shown to weaken the signal of regulating metabolism emanated by insulin [89]. Since GH and insulin have reverse effect on the regulation of glucose and lipid metabolism, MAPK may get involved in the crosstalk between GH and insulin.

STAT pathway is a necessary pathway for GH to regulate gene transcription for vital proteins, and it has been elucidated that STAT5 is sensitive to pulsatile secretion of GH [39]. According to Chen's study *in vitro*, STAT5b-Ct is phosphorylated by IR kinase domain which was depurated. In the cells which overexpress

IR, STAT5b-Ct phosphorylation and overexpressed endogenetic STAT5 were found [90]. Also, evidence showed that SOCSs mRNA expression controlled by insulin is also mediated by STAT5 [91]. Insulin interacts with many other hormones including GH, luteinizing hormone (LH), leptin and prolactin (PRL) in a STAT5-dependent way [29, 92–94]. JAK2 may play a part in the phosphorylation of STAT5 [94]. However, Zovnic *et al*. found that insulin could not stimulate the phosphorylation of STAT5 *in vitro*, instead, GH could. This study was verified *in vivo* [95]. Whether STAT5 can be activated by the insulin depends on the types of cells and tissues and the physiological condition. Nowadays, most scholars support that STAT5 is a physiological substrate of IR [90]. Therefore, interaction can be mediated by STAT5, which is important downstream protein for both insulin and GH signalling.

3.2 The effect of insulin on MAPK pathway of GH signalling

An *in vitro* study found that GH and insulin both can stimulate the maximum activation of MAPK pathway in a time- and dose- dependent way [59]. Insulin co-treatment with different concentrations of GH showed an additive effect on GH-stimulated MAPK activation when GH concentration was low (5 and 25 ng/mL). Insulin pretreatment (200 nmol/L insulin was added to GH 20 min in advance) had no effect on GH-induced MAPK activation [12]. However, *in vivo* study showed that insulin did not enhance GH-induced MAPK, suggesting that insulin might play a positive role in GH-induced MAPK signalling when the concentration ratio of GH to insulin was suitable. It is also considered that IRS-1 can facilitate GH-induced MAPK activation. In the same study, the co-treatment of insulin and GH enhanced the tyrosine phosphorylation of IRS-1, and the GH-induced MAPK activation also rose. We consider that pretreatment of insulin-induced IRS may have already been recruited before the treatment of GH, so no additive effect was observed.

Mek/Erk can be phosphorylated without the involvement of JAK2, Ras, and Raf-1, instead, with the enhanced cell membrane translocation of Mek1/2. These findings suggest that insulin is indispensable for GH-induced Erk1/2 activation and provide us a possible explanation for the mechanism of insulin's additive effect on GH-induced MAPK signalling [13]. For the effect of insulin on the GH/MAPK pathway, the role of insulin may be not only additive, but also irreplaceable for sensitivity of membrane kinase.

3.3 The effect of insulin on JAK2/STAT5 pathway of GH signalling

In 1999, Ji [7] found insulin impairs GH signalling by affecting JAK2/STAT5 pathway *in vitro*. Continuous high concentration insulin treatment inhibited GH binding and the GHR level, as well as the phosphorylation of JAK2 and STAT5B in a time-dependent way [7]. However, some studies showed that high levels of insulin and insulin treatment can enhance GH-induced JAK2 phosphorylation in accordance with expression of surface GHRs [74]. Zhang *et al*. found that insulin *per se* cannot induce STAT5 activation, conflicting with previous studies [12, 36, 90, 96]; however, insulin did improve GH-induced STAT5 tyrosine phosphorylation in 3T3-F442A adipocytes, both time- and dose-dependently. This result is consistent with the study from Xu *et al*. [13]. This additive-effect was more overt when given pre-treatment of insulin, and conclusions were confirmed *in vivo* [12]. These experiments suggest that insulin's influence on GH/GHR/JAK2/STAT5 is not monodirectional or monotypical, but depends on conditions. Normal concentration of insulin is a necessity of the responsive of GH signalling by maintaining the level of GHR and phosphorylation of proteins in JAK2/STAT5 pathway. However, insulin above suitable range causes inhibition of GH signal transmission. In the future, we need to find the relationships between effect, time and dose of insulin treatment, pre-treatment, or the co-treatment with GH. Also, the applicability in the counterpart of human still need to confirm [13, 74, 97]. A curve graph which shows how GH signalling intensity changes with time and dose of insulin treatment, as well as other factors should be made. Related endocrine diseases can be better understood, and more evidence can be provided for clinical therapeutics.

3.4 The effect of insulin on PI3K/Akt pathway of GH signalling

PI3K is the most important downstream binding protein for IRS. The IRS/PI3K/Akt pathway is essential for insulin to implement metabolic regulation function and for activation of MAPK [57, 87]. There has been substantial evidence suggesting that PI3K/Akt mediates crosstalk between GH and insulin signalling [84, 86]. A research showed that under sepsis, there was a GH-resistant condition, but when the sepsis objects were treated with insulin, GHR and IGF-1 were back to normal levels, and GH-resistance was alleviated. It was also found

that when PI3K/Akt pathway was blocked, the alleviation effect of insulin could not work anymore. This research suggested that in the pathology condition, a suitable range of insulin can help GH maintain normal function in the cellular level by regulating the IRS/PI3K/Akt pathway [98]. For GH, PI3K/Akt pathway is used for metabolic regulation in an insulin-like way and it plays a coordinate role with insulin. Therefore, another effect of insulin on PI3K/Akt pathway mainly refers to coordinating GH's insulin-like metabolic regulation, including accelerating the transportation of blood glucose and amino acid into cells, down-regulating the blood glucose level, and inhibiting the lipolysis. As to the long-term effect of GH, namely, anti-insulin effect, it may involve insulin's effect on other pathways of GH's signalling.

3.5 Mechanisms of the effect on GH signalling by insulin

In combination with previous experiment data, if insulin is pretreated on GH system, inslin is more likely to exert suppressive impact on the GH signalling when the period of insulin pretreatment is relatively long. This mechanism may involve SOCSs. Short period (less than 1 h) of pretreatment can help GHR maintain higher abundance, but with the time span increasing, the insulin will activate the STAT/SOCS pathway. SOCSs are able to restrain JAK2 activity, so long period of insulin pretreatment can interfere with the GH-induced phosphorylation of downstream protein [12].

For the co-treatment, time and dose are important factors. According to Zhang's study [12], it is hypothesized that the dose of GH is more decisive than the dose of insulin. When insulin's dose did not change, the change of GH dose could sharply affect the intensity of signal molecules' phosphorylation; however, when the dose of GH was fixed, even the dose of insulin changed in ten-fold level, it only had a little effect on the result. Both GH and insulin has the ability to activate downstream proteins, but the ability of GH may prevail within a certain range. Therefore, once GH takes control the path, insulin cannot compete. This hypothesis also explained why the pretreatment of insulin is more effective than co-treatment of GH and insulin: pretreatment of insulin can help itself to take prior control of the pathway, which can offset its weaker ability.

Considering all the work done, there are other factors that affect insulin's influence on GH signalling. Besides time and dose of insulin's and GH treatment (including the ratio of dose and time), possible factors include the schedule and order when adding reagents, the whole circumstance and condition of experiment, types of cell and tissue and creatures used, all of which should be taken into consideration. Just as it is mentioned, there are many hormones interacting with GH or insulin via their shared signalling. The reason why *in vivo* study is different from *in vitro* study is that *in vivo* study tends to be affected by a mixed regulation from other hormones, such as leptin and ghrelin [92, 99, 100]. GHR should also be taken into consideration. In 2004, Rhoads *et al*. found that insulin enhanced the richness of the GHR both in liver and adipose tissue of peri-parturient dairy cows without affecting JAK2 and STAT5 proteins. It implied that insulin can exert impact on GH signalling through GHR [97]. Excess insulin can impair the sensitivity of GHR, while suitable range of insulin maintains normal GHR level. GHR, IR and IGF-IR are concomitant in the lipid raft, which offers a site for these molecules to interact with each other, and this microcosmic reciprocity may be the cornerstone of crosstalk between signal pathways [101–103].

4 Conclusions

Increasing evidence implies that insulin can exert actions on every part when GH is performing its function. A physiological dose of insulin is necessary for the maintenance of the level and sensitivity of GHR and the action of GHRH on somatotropes, while excessive insulin restrains the hypothalamus-stimulated secretion of GH by attenuating the sensitivity of GHRH receptors on GH secretion cells. Insulin also exerts effect on GH secretion by influencing the level of blood glucose concentration and SS, especially in diabetes. Insulin's action on GH level is related to GH secretion and GHR sensitivity, as well as the condition of the body especially the obesity. Bodies with excess calories consumption have a reverse relationship between GH and insulin. Insulin implements this effect by regulating lipolysis and lipogenesis. IGF also get involved in the insulin's effect on pre-receptor process of GH. In future, we may explore other possible regulation pattern or related factors besides hypothalamus and IR, and find out how insulin works on them.

GH signalling is also affected by insulin because they share signal pathways in the post-GHR signalling. Insulin affects GH signalling in a time- and dose-dependent way, and multiple factors are involved in

this process, including IRSs, SOCSs and the sensitivity of GHR. Insulin can activate IRS, which facilitates GH-induced MAPK pathway. Insulin also stimulates the phosphorylation of STAT5, and then SOCSs, which can restrain the function of JAK2 and GHR/STAT5 pathway, but the reason of a few conflicting results from *in vivo* and *in vitro* studies needs further research. Insulin helps GH maintain normal function by regulating the IRS/PI3K/Akt pathway in the cellular level in the pathological condition. This process may involve other hormones.

In future, we should focus on the details of factors and genes responsible for the interaction between insulin and GH in different conditions. More substantial evidence is in need for diagnosis and therapies of endocrine disorders in clinical medicine field.

REFERENCES

1 Frank SJ. Growth hormone, insulin-like growth factor I, and growth: local knowledge. Endocrinology 2007; 148(4): 1486–1488.

2 Lichanska AM, Waters MJ. How growth hormone controls growth, obesity and sexual dimorphism. Trends Genet 2008; 24(1): 41–47.

3 Betteridge A, Wallis M. Biosynthesis of growth hormone in the rat anterior pituitary gland. Stimulation of biosynthesis *in vitro* by insulin. Biochem J 1973; 134(4): 1103–1113.

4 Hazelwood RL, Hazelwood BS. Influence of alloxan diabetes on growth hormone content of the rat hypophysis. Am J Physiol 1964; 206: 1137–1144.

5 Havrankova J, Roth J, Brownstein M. Insulin receptors are widely distributed in the central nervous system of the rat. Nature 1978; 272(5656): 827–829.

6 Menon RK, Arslanian S, May B, Cutfield WS, Sperling MA. Diminished growth hormone-binding protein in children with insulin-dependent diabetes mellitus. J Clin Endocrinol Metab 1992; 74(4): 934–938.

7 Ji S. Insulin inhibits growth hormone signaling via the growth hormone receptor/JAK2/STAT5B Pathway. J Biol Chem 1999; 274: 13434–13442.

8 Melmed S, Neilson L, Slanina S. Insulin suppresses rat growth hormone messenger ribonucleic acid levels in rat pituitary tumor cells. Diabetes 1985; 34(4): 409–412.

9 Steyn FJ, Tolle V, Chen C, Epelbaum J. Neuroendocrine regulation of growth hormone secretion. Compr Physiol 2016; 6(2): 687–735.

10 Manglik S, Cobanov B, Flores G, Nadjafi R, Tayek JA. Serum insulin but not leptin is associated with spontaneous and growth hormone (GH)-releasing hormone-stimulated GH secretion in normal volunteers with and without weight loss. Metabolism 1998; 47(9): 1127–1133.

11 Cornford AS, Barkan AL, Hinko A, Horowitz JF. Suppression in growth hormone during overeating ameliorates the increase in insulin resistance and cardiovascular disease risk. Am J Physiol Endocrinol Metab 2012; 303(10): E1264–E1272.

12 Zhang Y, Liu Y, Li X, Gao W, Zhang W, Guan Q, Jiang J, Frank SJ, Wang X. Effects of insulin and IGF-I on growth hormone-induced STAT5 activation in 3T3-F442A adipocytes. Lipids Health Dis 2013; 12: 56.

13 Xu J, Keeton AB, Franklin JL, Li X, Venable DY, Frank SJ, Messina JL. Insulin enhances growth hormone induction of the MEK/ERK signaling pathway. J Biol Chem 2006; 281(2): 982–992.

14 Xu J, Messina JL. Crosstalk between growth hormone and insulin signaling. Vitam Horm 2009; 80: 125–153.

15 Gao Y, Su P, Wang C, Zhu K, Chen X, Liu S, He J. The role of PTEN in chronic growth hormone-induced hepatic insulin resistance. PLoS One 2014; 8(6): e68105.

16 Ranabir S, Reetu K. Stress and hormones. Indian J Endocrinol Metab 2011; 15(1): 18–22.

17 Greenwood FC, Landon J. Growth hormone secretion in response to stress in man. Nature 1966; 210(5035): 540–541.

18 Mark PB, Watkins S, Dargie HJ. Cardiomyopathy induced by performance enhancing drugs in a competitive bodybuilder. Heart 2005; 91(7): 888.

19 Yang SK, Steyn F, Chen C. Influence of membrane ion channel in pituitary somatotrophs by hypothalamic regulators. Cell Calcium 2012; 51(3–4): 231–239.

20 Ben-Shlomo A, Melmed S. Pituitary somatostatin receptor signaling. Trends Endocrinol Metab 2010; 21(3): 123–133.

21 Cataldi M, Magnan E, Guillaume V, Dutour A, Conte-Devolx B, Lombardi G, Oliver C. Relationship between hypophyseal portal GHRH and somatostatin and peripheral GH levels in the conscious sheep. J Endocrinol Invest 1994; 17(9): 717–722.

22 Rubinow DR, Post RM, Davis CL, Doran AR. Somatostatin and GHRH: mood and behavioral regulation. Adv Biochem Psychopharmacol 1987; 43: 137–152.

23 Wallenius K, Sjogren K, Peng XD, Park S, Wallenius V, Liu JL, Umaerus M, Wennbo H, Isaksson O, Frohman L, Kineman R, Ohlsson C, Jansson JO. Liver-derived IGF-I regulates GH secretion at the pituitary level in mice. Endocrinology 2001; 142(11): 4762–4770.

24 Lewitt MS. The role of the growth hormone/insulin-like growth factor system in visceral adiposity. Biochem Insights 2017; 10: 1178626417703995.

25 Steyn FJ, Xie TY, Huang L, Ngo ST, Veldhuis JD, Waters

MJ, Chen C. Increased adiposity and insulin correlates with the progressive suppression of pulsatile GH secretion during weight gain. J Endocrinol 2013; 218(2): 233–244.
26 Davidson MB. Effect of growth hormone on carbohydrate and lipid metabolism. Endocr Rev 1987; 8(2): 115–131.
27 Altszuler N, Rathgeb I, Winkler B, De Bodo RC, Steele R. The effects of growth hormone on carbohydrate and lipid metabolism in the dog. Ann N Y Acad Sci 1968; 148(2): 441–458.
28 Raisingani M, Preneet B, Kohn B, Yakar S. Skeletal growth and bone mineral acquisition in type 1 diabetic children; abnormalities of the GH/IGF-1 axis. Growth Horm IGF Res 2017; 34: 13–21.
29 Dominici FP, Argentino DP, Munoz MC, Miquet JG, Sotelo AI, Turyn D. Influence of the crosstalk between growth hormone and insulin signalling on the modulation of insulin sensitivity. Growth Horm IGF Res 2005; 15(5): 324–336.
30 Herrington J, Carter-Su C. Signaling pathways activated by the growth hormone receptor. Trends Endocrinol Metab 2001; 12(6): 252–257.
31 Cunningham BC, Ultsch M, De Vos AM, Mulkerrin MG, Clauser KR, Wells JA. Dimerization of the extracellular domain of the human growth hormone receptor by a single hormone molecule. Science 1991; 254(5033): 821–825.
32 Lanning NJ, Carter-Su C. Recent advances in growth hormone signaling. Rev Endocr Metab Disord 2006; 7(4): 225–235.
33 Yasukawa H, Misawa H, Sakamoto H, Masuhara M, Sasaki A, Wakioka T, Ohtsuka S, Imaizumi T, Matsuda T, Ihle JN, Yoshimura A. The JAK-binding protein JAB inhibits Janus tyrosine kinase activity through binding in the activation loop. EMBO J 1999; 18(5): 1309–1320.
34 Herrington J. The role of STAT proteins in growth hormone signaling. Oncogene 2000; 19: 2585 - 2597.
35 Greenhalgh CJ, Alexander WS. Suppressors of cytokine signalling and regulation of growth hormone action. Growth Horm IGF Res 2004; 14(3): 200–206.
36 Storz P, Döppler H, Pfizenmaier K, Müller G. Insulin selectively activates STAT5b, but not STAT5a, via a JAK2-independent signalling pathway in Kym-1 rhabdomyosarcoma cells. FEBS Lett 1999; 464(3): 159–163.
37 Ji S, Frank SJ, Messina JL. Growth hormone-induced differential desensitization of STAT5, ERK, and Akt phosphorylation. J Biol Chem 2002; 277(32): 28384–28393.
38 Teglund S, McKay C, Schuetz E, van Deursen JM, Stravopodis D, Wang D, Brown M, Bodner S, Grosveld G, Ihle JN. Stat5a and Stat5b proteins have essential and nonessential, or redundant, roles in cytokine responses. Cell 1998; 93(5): 841–850.
39 Ram PA, Park SH, Choi HK, Waxman DJ. Growth hormone activation of Stat 1, Stat 3, and Stat 5 in rat liver. Differential kinetics of hormone desensitization and growth hormone stimulation of both tyrosine phosphorylation and serine/threonine phosphorylation. J Biol Chem 1996; 271(10): 5929–5940.
40 Ihle JN. STATs: Signal transducers and activators of transcription. Cell 1996; 84(3): 331–334.
41 Woelfle J, Rotwein P. *In vivo* regulation of growth hormone-stimulated gene transcription by STAT5b. Am J Physiol Endocrinol Metab 2004; 286(3): E393–E401.
42 Chia DJ. Minireview: mechanisms of growth hormone-mediated gene regulation. Mol Endocrinol 2014; 28(7): 1012–1025.
43 Klover P, Hennighausen L. Postnatal body growth is dependent on the transcription factors signal transducers and activators of transcription 5a/b in muscle: A role for autocrine/paracrine insulin-like growth factor I. Endocrinology 2007; 148(4): 1489–1497.
44 Hwa V, Little B, Adiyaman P, Kofoed EM, Pratt KL, Ocal G, Berberoglu M, Rosenfeld RG. Severe growth hormone insensitivity resulting from total absence of signal transducer and activator of transcription 5b. J Clin Endocrinol Metab 2005; 90(7): 4260–4266.
45 Rosenfeld RG, Kofoed E, Buckway C, Little B, Woods KA, Tsubaki J, Pratt KA, Bezrodnik L, Jasper H, Tepper A, Heinrich JJ, Hwa V. Identification of the first patient with a confirmed mutation of the JAK-STAT system. Pediatr Nephrol 2005; 20(3): 303–305.
46 Kofoed EM, Hwa V, Little B, Woods KA, Buckway CK, Tsubaki J, Pratt KL, Bezrodnik L, Jasper H, Tepper A, Heinrich JJ, Rosenfeld RG. Growth hormone insensitivity associated with a STAT5b mutation. N Engl J Med 2003; 349(12): 1139–1147.
47 Fasshauer M, Kralisch S, Klier M, Lossner U, Bluher M, Klein J, Paschke R. Insulin resistance-inducing cytokines differentially regulate SOCS mRNA expression via growth factor- and Jak/Stat-signaling pathways in 3T3-L1 adipocytes. J Endocrinol 2004; 181(1): 129–138.
48 Mooney RA, Senn J, Cameron S, Inamdar N, Boivin LM, Shang Y, Furlanetto RW. Suppressors of cytokine signaling-1 and -6 associate with and inhibit the insulin receptor. A potential mechanism for cytokine-mediated insulin resistance. J Biol Chem 2001; 276(28): 25889–25893.
49 Ueki K, Kondo T, Kahn CR. Suppressor of cytokine signaling 1 (SOCS-1) and SOCS-3 cause insulin resistance through inhibition of tyrosine phosphorylation of insulin receptor substrate proteins by discrete mechanisms. Mol Cell Biol 2004; 24(12): 5434–5446.
50 VanderKuur J, Allevato G, Billestrup N, Norstedt G, Carter-Su C. Growth hormone-promoted tyrosyl phosphorylation

of SHC proteins and SHC association with Grb2. J Biol Chem 1995; 270(13): 7587–7593.

51 Vanderkuur JA, Butch ER, Waters SB, Pessin JE, Guan KL, Carter-Su C. Signaling molecules involved in coupling growth hormone receptor to mitogen-activated protein kinase activation. Endocrinology 1997; 138(10): 4301–4307.

52 Liang L, Zhou T, Jiang J, Pierce JH, Gustafson TA, Frank SJ. Insulin receptor substrate-1 enhances growth hormone-induced proliferation. Endocrinology 1999; 140(5): 1972–1983.

53 Thirone AC, Carvalho CR, Saad MJ. Growth hormone stimulates the tyrosine kinase activity of JAK2 and induces tyrosine phosphorylation of insulin receptor substrates and Shc in rat tissues. Endocrinology 1999; 140(1): 55–62.

54 Argetsinger LS, Hsu GW, Myers MG, Jr., Billestrup N, White MF, Carter-Su C. Growth hormone, interferon-gamma, and leukemia inhibitory factor promoted tyrosyl phosphorylation of insulin receptor substrate-1. J Biol Chem 1995; 270(24): 14685–14692.

55 Ridderstrale M, Degerman E, Tornqvist H. Growth hormone stimulates the tyrosine phosphorylation of the insulin receptor substrate-1 and its association with phosphatidylinositol 3-kinase in primary adipocytes. J Biol Chem 1995; 270(8): 3471–3474.

56 Argetsinger LS, Norstedt G, Billestrup N, White MF, Carter-Su C. Growth hormone, interferon-gamma, and leukemia inhibitory factor utilize insulin receptor substrate-2 in intracellular signaling. J Biol Chem 1996; 271(46): 29415–29421.

57 Kilgour E, Gout I, Anderson NG. Requirement for phosphoinositide 3-OH kinase in growth hormone signalling to the mitogen-activated protein kinase and p70s6k pathways. Biochem J 1996; 315 (Pt 2): 517–522.

58 Yamauchi T, Kaburagi Y, Ueki K, Tsuji Y, Stark GR, Kerr IM, Tsushima T, Akanuma Y, Komuro I, Tobe K, Yazaki Y, Kadowaki T. Growth hormone and prolactin stimulate tyrosine phosphorylation of insulin receptor substrate-1, -2, and -3, their association with p85 phosphatidylinositol 3-kinase (PI3-kinase), and concomitantly PI3-kinase activation via JAK2 kinase. J Biol Chem 1998; 273(25): 15719–15726.

59 Liang L, Jiang J, Frank SJ. Insulin receptor substrate-1-mediated enhancement of growth hormone-induced mitogen-activated protein kinase activation. Endocrinology 2000; 141(9): 3328–3336.

60 Shibasaki T, Hotta M, Masuda A, Imaki T, Obara N, Demura H, Ling N, Shizume K. Plasma GH responses to GHRH and insulin-induced hypoglycemia in man. J Clin Endocrinol Metab 1985; 60(6): 1265–1267.

61 Foot AB, Davidson K, Edge JA, Wass JA, Dunger DB. The growth hormone releasing hormone (GHRH) response to a mixed meal is blunted in young adults with insulin-dependent diabetes mellitus whereas the somatostatin response is normal. Clin Endocrinol (Oxf) 1990; 32(2): 177–183.

62 Hanew K, Utsumi A. The role of endogenous GHRH in arginine-, insulin-, clonidine- and l-dopa-induced GH release in normal subjects. Eur J Endocrinol 2002; 146(2): 197–202.

63 Yazdi FT, Clee SM, Meyre D. Obesity genetics in mouse and human: back and forth, and back again. PeerJ 2015; 3: e856.

64 Donahue LR, Beamer WG. Growth hormone deficiency in 'little' mice results in aberrant body composition, reduced insulin-like growth factor-I and insulin-like growth factor-binding protein-3 (IGFBP-3), but does not affect IGFBP-2, -1 or -4. J Endocrinol 1993; 136(1): 91–104.

65 De Schepper JA, Smitz JP, Zhou XL, Louis O, Velkeniers BE, Vanhaelst L. Cafeteria diet-induced obesity is associated with a low spontaneous growth hormone secretion and normal plasma insulin-like growth factor-I concentrations. Growth Horm IGF Res 1998; 8(5): 397–401.

66 Wang S (王舒然), Yu C, Sun C, Liu Z. Changes and relations of leptin, growth hormone and insulin during puberty in obese and non-obese children. J Hygiene Res (卫生研究) 2001; 30(4): 219–220, back cover (in Chinese with English abstract).

67 Volta C, Bernasconi S, Iughetti L, Ghizzoni L, Rossi M, Costa M, Cozzini A. Growth hormone response to growth hormone-releasing hormone (GHRH), insulin, clonidine and arginine after GHRH pretreatment in obese children: evidence of somatostatin increase? Eur J Endocrinol 1995; 132(6): 716–721.

68 Sauter A, Goldstein M, Engel J, Ueta K. Effect of insulin on central catecholamines. Brain Res 1983; 260(2): 330–333.

69 Luque RM, Kineman RD. Impact of obesity on the growth hormone axis: evidence for a direct inhibitory effect of hyperinsulinemia on pituitary function. Endocrinology 2006; 147(6): 2754–2763.

70 Melmed S. Insulin suppresses growth hormone secretion by rat pituitary cells. J Clin Invest 1984; 73(5): 1425–1433.

71 Melmed S, Slanina SM. Insulin suppresses triiodothyronine-induced growth hormone secretion by GH3 rat pituitary cells. Endocrinology 1985; 117(2): 532–537.

72 Brons C, Jensen CB, Storgaard H, Hiscock NJ, White A, Appel JS, Jacobsen S, Nilsson E, Larsen CM, Astrup A, Quistorff B, Vaag A. Impact of short-term high-fat feeding on glucose and insulin metabolism in young healthy men. J Physiol 2009; 587(Pt 10): 2387–2397.

73 Takeuchi T, Tsutsumi O, Taketani Y. Impaired growth hormone secretion after glucose loading in non-obese women with polycystic ovary syndrome, possibly related to androgen but not insulin and free fatty acids. Gynecol Endocrinol 2007; 23(8): 468–473.

74 Leung KC, Doyle N, Ballesteros M, Waters MJ, Ho KK. Insulin regulation of human hepatic growth hormone receptors: divergent effects on biosynthesis and surface translocation. J Clin Endocrinol Metab 2000; 85(12): 4712–4720.

75 Cattaneo L, De Gennaro Colonna V, Zoli M, Muller E, Cocchi D. Characterization of the hypothalamo-pituitary-IGF-I axis in rats made obese by overfeeding. J Endocrinol 1996; 148(2): 347–353.

76 Erman A, Wabitsch M, Goodyer CG. Human growth hormone receptor (GHR) expression in obesity: II. Regulation of the human GHR gene by obesity-related factors. Int J Obes (Lond) 2011; 35(12): 1520–1529.

77 Erman A, Veilleux A, Tchernof A, Goodyer CG. Human growth hormone receptor (GHR) expression in obesity: I. GHR mRNA expression in omental and subcutaneous adipose tissues of obese women. Int J Obes (Lond) 2011; 35(12): 1511–1519.

78 Menon RK, Stephan DA, Rao RH, Shen-Orr Z, Downs LS Jr, Roberts CT Jr, LeRoith D, Sperling MA. Tissue-specific regulation of the growth hormone receptor gene in streptozocin-induced diabetes in the rat. J Endocrinol 1994; 142(3): 453–462.

79 Leung KC, Waters MJ, Markus I, Baumbach WR, Ho KK. Insulin and insulin-like growth factor-I acutely inhibit surface translocation of growth hormone receptors in osteoblasts: a novel mechanism of growth hormone receptor regulation. Proc Natl Acad Sci U S A 1997; 94(21): 11381–11386.

80 Bielohuby M, Sawitzky M, Stoehr BJ, Stock P, Menhofer D, Ebensing S, Bjerre M, Frystyk J, Binder G, Strasburger C, Wu Z, Christ B, Hoeflich A, Bidlingmaier M. Lack of dietary carbohydrates induces hepatic growth hormone (GH) resistance in rats. Endocrinology 2011; 152(5): 1948–1960.

81 Moller N, Jorgensen JO. Effects of growth hormone on glucose, lipid, and protein metabolism in human subjects. Endocr Rev 2009; 30(2): 152–177.

82 White MF. IRS proteins and the common path to diabetes. Am J Physiol Endocrinol Metab 2002; 283(3): E413–E422.

83 Saltiel AR, Kahn CR. Insulin signalling and the regulation of glucose and lipid metabolism. Nature 2001; 414(6865): 799–806.

84 Costoya JA, Finidori J, Moutoussamy S, Searis R, Devesa J, Arce VM. Activation of growth hormone receptor delivers an antiapoptotic signal: evidence for a role of Akt in this pathway. Endocrinology 1999; 140(12): 5937–5943.

85 Piwien-Pilipuk G, Van Mater D, Ross SE, MacDougald OA, Schwartz J. Growth hormone regulates phosphorylation and function of CCAAT/enhancer-binding protein beta by modulating Akt and glycogen synthase kinase-3. J Biol Chem 2001; 276(22): 19664–19671.

86 Ji SN, Frank SJ, Messina JL. Growth hormone-induced differential desensitization of STAT5, ERK, and Akt phosphorylation. J Biol Chem 2002; 277(32): 28384–28393.

87 Ridderstrale M, Tornqvist H. Effects of tyrosine kinase inhibitors on tyrosine phosphorylations and the insulin-like effects in response to human growth hormone in isolated rat adipocytes. Endocrinology 1996; 137(11): 4650–4656.

88 Lavin DP, White MF, Brazil DP. IRS proteins and diabetic complications. Diabetologia 2016; 59(11): 2280–2291.

89 Bard-Chapeau EA, Hevener AL, Long SN, Zhang EE, Olefsky JM, Feng GS. Deletion of Gab1 in the liver leads to enhanced glucose tolerance and improved hepatic insulin action. Nature Medicine 2005; 11(5): 567–571.

90 Chen J, Sadowski HB, Kohanski RA, Wang LH. Stat5 is a physiological substrate of the insulin receptor. Proc Natl Acad Sci U S A 1997; 94(6): 2295–2300.

91 Sadowski CL, Choi TS, Le M, Wheeler TT, Wang LH, Sadowski HB. Insulin induction of SOCS-2 and SOCS-3 mRNA expression in C2C12 skeletal muscle cells is mediated by Stat5*. J Biol Chem 2001; 276(23): 20703–20710.

92 Carvalho CR, Carvalheira JB, Lima MH, Zimmerman SF, Caperuto LC, Amanso A, Gasparetti AL, Meneghetti V, Zimmerman LF, Velloso LA, Saad MJ. Novel signal transduction pathway for luteinizing hormone and its interaction with insulin: activation of Janus kinase/signal transducer and activator of transcription and phosphoinositol 3-kinase/Akt pathways. Endocrinology 2003; 144(2): 638–647.

93 Yu J, Xiao F, Zhang Q, Liu B, Guo Y, Lv Z, Xia T, Chen S, Li K, Du Y, Guo F. PRLR regulates hepatic insulin sensitivity in mice via STAT5. Diabetes 2013; 62(9): 3103–3113.

94 Carvalheira JB, Ribeiro EB, Folli F, Velloso LA, Saad MJ. Interaction between leptin and insulin signaling pathways differentially affects JAK-STAT and PI3-kinase-mediated signaling in rat liver. Biol Chem 2003; 384(1): 151–159.

95 Zvonic S, Story DJ, Stephens JM, Mynatt RL. Growth hormone, but not insulin, activates STAT5 proteins in adipocytes *in vitro* and *in vivo*. Biochem Biophys Res Commun 2003; 302(2): 359–362.

96 Sawka-Verhelle D, Tartare-Deckert S, Decaux JF, Girard J, Van Obberghen E. Stat 5B, activated by insulin in a Jak-independent fashion, plays a role in glucokinase gene transcription. Endocrinology 2000; 141(6): 1977–1988.

97 Rhoads RP, Kim JW, Leury BJ, Baumgard LH, Segoale N, Frank SJ, Bauman DE, Boisclair YR. Insulin increases the

abundance of the growth hormone receptor in liver and adipose tissue of periparturient dairy cows. J Nutr 2004; 134: 1020–1027.

98 Hong Y, Wen Y, Qi C, Ning. L, Wei Z, Wei L, Tao Juan Z, Feng X. The combination of insulin and growth hormone upregulates growth hormone receptor in septic rats. Parenter Enteral Nutr 2013; 20(2): 99–102.

99 Mosa RM, Zhang Z, Shao R, Deng C, Chen J, Chen C. Implications of ghrelin and hexarelin in diabetes and diabetes-associated heart diseases. Endocrine 2015; 49(2): 307–323.

100 Choi K, Roh SG, Hong YH, Shrestha YB, Hishikawa D, Chen C, Kojima M, Kangawa K, Sasaki S. The role of ghrelin and growth hormone secretagogues receptor on rat adipogenesis. Endocrinology 2003; 144(3): 754–759.

101 Huang Y, Kim SO, Yang N, Jiang J, Frank SJ. Physical and functional interaction of growth hormone and insulin-like growth factor-I signaling elements. Mol Endocrinol 2004; 18(6): 1471–1485.

102 Vainio S, Heino S, Mansson JE, Fredman P, Kuismanen E, Vaarala O, Ikonen E. Dynamic association of human insulin receptor with lipid rafts in cells lacking caveolae. EMBO Rep 2002; 3(1): 95–100.

103 Yang N, Huang Y, Jiang J, Frank SJ. Caveolar and lipid raft localization of the growth hormone receptor and its signaling elements: impact on growth hormone signaling. J Biol Chem 2004; 279(20): 20898–20905.