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Review

Essential roles of growth hormone (GH) and insulin-like growth factor-I (IGF-I) in the liver

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Abstract. Growth hormone (GH) and insulin-like growth factor-I (IGF-I) play essential roles in growth in childhood, and continue to have important metabolic actions in adults. Adult growth hormone deficiency (AGHD) is characterized by increased visceral adiposity, abnormal lipid profiles, premature atherosclerosis, decreased quality of life, and increased mortality. Recently, case reports and several clinical studies suggest that GHD state in adults is associated with an increased prevalence of nonalcoholic fatty liver disease (NAFLD) and progression to nonalcoholic steatohepatitis (NASH) or liver cirrhosis. As a mechanistic insight, growing evidence has revealed that GH as well as IGF-I play essential roles in the liver. Further investigation is necessary to clarify the precise mechanisms by which GH and IGF-I exert their effects in the liver; however, it should be noted that NAFLD/NASH has emerged as an important comorbidity in AGHD.

Key words: Growth hormone (GH), Insulin-like growth factor-I (IGF-I), Adult growth hormone deficiency (AGHD), Nonalcoholic fatty liver disease (NAFLD), Nonalcoholic steatohepatitis (NASH)

GROWTH HORMONE (GH) and insulin-like growth factor-I (IGF-I) play essential roles in linear growth in childhood, and continue to have important metabolic actions throughout life. Adult growth hormone deficiency (AGHD) is characterized by increased visceral adiposity, abnormal lipid profiles, premature atherosclerosis, decreased muscle mass, osteoporosis, and decreased quality of life [1, 2]. Epidemiological studies have shown that AGHD is associated with increased mortality compared with age- and gender-matched populations [3]. The main causes of premature mortality were cardiovascular and cerebrovascular disease and malignancies [4]. Recently, the significance of nonalcoholic fatty liver disease (NAFLD)/nonalcoholic steatohepatitis (NASH) as a metabolic comorbidity in AGHD has emerged. In this review, I will discuss roles of GH and IGF-I in the liver.

NAFLD and NASH

With the increasing prevalence of obesity, metabolic syndrome, and diabetes, NAFLD is now recognized as the most common cause of chronic liver disease in Asian and Western countries [5, 6]. NAFLD consists of a wide spectrum of conditions, ranging from simple steatosis to NASH. The diagnosis of NASH is based on the histologic examination of liver biopsy specimens [7]. In addition to steatosis, NASH is characterized histologically by inflammatory cell infiltration, hepatocyte ballooning, and fibrosis, which can progress to cirrhosis and hepatocellular carcinoma; thus, the prognosis of NASH is poor [8]. Obesity, metabolic syndrome, type 2 diabetes, and hyperlipidemia are frequently associated with NAFLD/NASH as risk factors. In particular, insulin resistance is the most common feature observed in NAFLD/NASH. Although the cause of NASH appears multifactorial, it has been proposed that the molecular pathogenesis of NASH consists of 2 steps: first, insulin resistance status with an accumulation of fat within hepatocyte; and, second, reactive oxygen species cause lipid peroxidation, cytokine induction, and inflammation, leading to activation of hepatic stellate cells (HSCs) and resulting in fibrogenesis [9].

Metabolic Actions of GH and IGF-I

GH functions as a major metabolic hormone in adults by optimizing body composition and regulating energy

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and substrate metabolism. GH promotes fat metabolism by enhancing lipolysis and fatty acid oxidation and activates hormone sensitive lipase indirectly through β adrenergic stimulation. GH also enhances the clearance of low-density lipoprotein (LDL) by activating the expression of hepatic LDL receptors [10]. GH regulates glucose metabolism both directly and by antagonizing insulin action. Furthermore, GH suppresses glucose oxidation and utilization while enhancing hepatic glucose production [11]. In protein metabolism, GH reduces urea synthesis, blood urea concentration, and urinary urea excretion. GH reduces protein oxidation and stimulates proteins synthesis. Although these effects are mainly mediated by IGF-I, a direct effect of GH is also speculated [12]. In contrast, IGF-I improves glucose sensitivity *via* direct action [13, 14] and indirect action by reducing GH secretion from the pituitary as a negative feedback mechanism [15]. IGF-I strongly stimulates protein synthesis and inhibits protein breakdown [16]. Generally, GH and IGF-I cooperatively exert their effects in various tissues with the exception of the insulin-like action of IGF-I.

AGHD and NAFLD/NASH

A case report published in 1997 noted improvements in fatty liver associated with panhypopituitarism after GH administration, suggesting that fatty liver is at least partly attributable to GH deficiency [17]. Liver dysfunction and hyperlipidemia are frequently observed in AGHD [18]. Ichikawa *et al.* compared 5 hypopituitary patients without AGHD and 13 patients with AGHD and found that hepatic steatosis evaluated by CT was more prevalent in the AGHD group. A diagnosis of NASH was made by liver biopsy specimens in 1 of the patients with AGHD [19]. Furthermore, it was reported that patients with hypothalamic and pituitary dysfunction had excessive weight gain, impaired glucose tolerance, and dyslipidemia with subsequent development of NAFLD/NASH and a high prevalence of cirrhosis, increasing the risk of liver-related death [20]. Given the fact that GH secretion is most frequently impaired in patients with hypothalamic-pituitary organic disease, it is speculated that NAFLD/NASH is attributable to GH deficiency. Fukuda *et al.* reported that metabolic comorbidities including NAFLD increased after the cessation of GH in adult patients with childhood onset-GHD depending on its duration. In this retrospective analysis of 44 patients with AGHD, the prevalence of NAFLD increased by 29% at a mean age of 30 years [21]. Intriguingly, it has been reported that GH replacement therapy drastically reversed NASH in a case of AGHD, suggesting a beneficial effect of GH on NASH with AGHD [22]. Moreover, fatty liver severity was shown to be related to GH levels in hypopituitary patients [23]. Interestingly, GH, IGF-I, and IGF-binding protein 3 (IGFBP3) were associated with hepatic steatosis and fibrosis in patients with NAFLD even in non-GH-deficient population [24], suggesting that the GH-IGF-I axis may play a role in the liver under physiological conditions as well as in GHD. These data strongly suggest that an association exists between AGHD status and NAFLD/NASH. More convincingly, Nishizawa *et al.* [25] recently reported that the prevalence of NAFLD was significantly higher in 66 Japanese patients with AGHD compared to age-, gender-, and BMI-matched controls (77 *vs*. 12%, $p < 0.001$). At least 21% of patients with AGHD were diagnosed with NASH by liver biopsy. Moreover, GH replacement therapy significantly reduced serum liver enzyme concentrations in patients with AGHD and improved the histological changes in the liver concomitant with a reduction in the fibrotic marker concentrations in patients with NASH. These effects of GH replacement therapy suggest that NAFLD/NASH is predominantly attributable to GHD. On the other hand, Gardner *et al.* [26] reported that NAFLD is equally common in patients with GHD and in age- and BMI-matched control subjects. Several factors may explain this discrepancy. Race, age, gender, and BMI are important factors associated with the prevalence of NAFLD [27]. Generally, the prevalence of NAFLD is higher in the Caucasian population than in the Asian population. The prevalence of NAFLD and NAFLD-related fibrosis increases with age [27]. Obesity is closely associated with an increase in the prevalence of NAFLD. For example, according to the annual health check in Japan, the prevalence of NAFLD as detected by ultrasound increased with BMI: the prevalence was 10-20% in non-obese individuals, approximately 50% in those with a BMI between 25 and 30 kg/m^2, and around 80% in those with a BMI over 30 kg/m^2 [6]. Comparing these 2 studies, both the age and BMI of the subjects were higher (age: 48.2 *vs*. 52.6, BMI: 25.2 *vs*. 27.9) in the later study, which may be critical reason for the discrepancy because the prevalence of NAFLD increases with age and BMI. Likewise, the mean waist circumference of the control group was 101 cm in the

later study, suggesting the presence of visceral obesity, which is also closely associated with the development of NAFLD; this could have increased the prevalence of NAFLD in the control group. Indeed, the prevalence of NAFLD was 50% in the control group, which was obviously high in general population. Moreover, although it was not statistically significant, GH replacement therapy showed a tendency to decrease liver fat content ($p < 0.07$), suggesting that the patient sample size might have been too small to detect the effect of GH. Nishizawa *et al.* reported that increased BMI, visceral adiposity, dyslipidemia, and insulin resistance were associated with NAFLD in AGHD patients, suggesting that the susceptibility to NAFLD for general risk factors was increased in patients with AGHD [25]. Taken together, the accumulating evidence strongly suggests that a GH deficient state in adults is closely associated with the development of NAFLD/NASH.

The drastic effect of GH on visceral adiposity and lipid metabolism in patients with AGHD led to several pilot clinical trials that assessed the effect of GH administration on patients with obesity [28] and liver cirrhosis [29], in whom GH secretion was not impaired. Franco *et al.* analyzed the effect of GH on 40 postmenopausal women with abdominal obesity [28]. GH treatment for 1 year reduced visceral fat mass, increased thigh muscle area, and reduced LDL cholesterol. Insulin sensitivity measured by a glucose clamp was increased in the GH-treatment group and a positive correlation was found between the changes in the glucose disposal rate and liver attenuation as a measure of hepatic fat content. Donaghy *et al.* performed a randomized, double-blind, placebo-controlled pilot study of GH therapy in 20 cirrhotic patients to assess the reversibility of GH resistance and the subsequent impact on protein economy [29]. Administration of a relatively high dose of GH (0.25IU/kg body weight) for 7 days significantly increased serum IGF-I levels and improved nitrogen balance in cirrhotic patients. These results imply the GH can be applied clinically for these metabolic or chronic liver diseases, although the long-term outcome and precise underlying mechanisms remain to be clarified.

GH Action in Liver

Although GH generates IGF-I at local tissue sites for autocrine and paracrine actions [30], circulating IGF-I is derived predominantly from heptocyte [31, 32]. Indeed, liver-specific deletion of the GH receptor in mice (GHRLD) resulted in a greater than 90% reduction in serum IGF-I levels [33]. The liver-specific knockout mice exhibited normal linear growth, but decreased total bone density, which was comparable with the results of liver-specific IGF-I deficient mice [31]. Intriguingly, GHRLD mice showed insulin resistance, glucose intolerance, increased free fatty acids, decreased triglyceride efflux, and severe steatosis, indicating the physiological importance of GH signaling in the liver. Liver regeneration was impaired in this model and several GH-deficient animal models, indicating that GH also regulates the proliferation capacity of hepatocyte [34-36]. Regarding the downstream signaling of the GH receptor, liver-specific JAK2-deficient mice (JAK2L) develop hepatic steatosis [37]. Although these mice are lean, they display markedly elevated GH levels, liver triglyceride content, and plasma FFA levels. A cross between GH-deficient *lit/lit* mice and JAK2L mice resulted in reduced plasma FFA levels and hepatic steatosis, suggesting that elevated GH and GH-induced lipolysis in adipose tissue play a role in the development of hepatic steatosis. The elevated expression of peroxisome proliferator-activated receptor gamma (PPARγ) and its target gene CD36 in the liver, which leads to an increased uptake of FFA, has been proposed as an additional mechanism. Furthermore, mice with a liver-specific signal transducer and transcriptional activator 5 (STAT5) ablation developed steatosis, glucose intolerance, insulin resistance, late-onset obesity, and impaired liver regeneration. Recent studies with liver-specific STAT5-deficient mice suggest that elevated CD36, PPARγ, and PPARγ coactivator 1 alpha/beta (PGC1α/β), along with increased fatty acid synthesis, lipoprotein lipase, and VLDL receptor, are associated with hepatic steatosis in these mice [38]. GH receptor loss of function mutations in humans (Laron syndrome) also manifests NAFLD in adults and chronic replacement of IGF-I does not influence the NAFLD status, suggesting that GH has a direct action in the liver, particularly in the prevention of steatosis in hepatocytes [39] (Fig. 1).

IGF-I Action in Liver

Decreased levels of free IGF-I are observed in patients with chronic liver disease and malnutrition despite normal or elevated GH secretion [40-42] because the liver is the main site of serum IGF-I production, as shown by

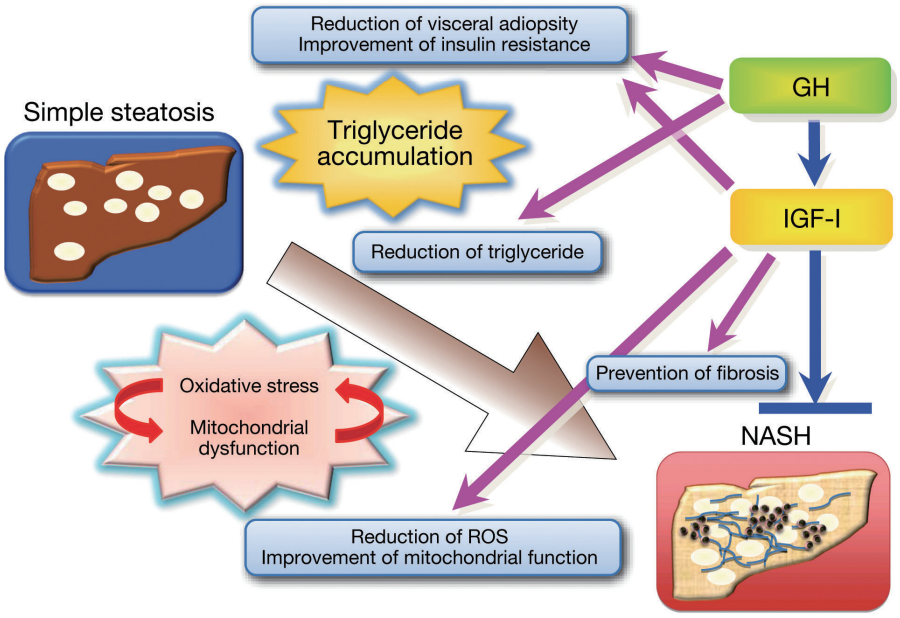

Fig. 1 GH and IGF-I action in the liver
GH improves triglyceride accumulation in hepatocytes. On the other hand, IGF-I reduces oxidative stress and improves mitochondrial function and may prevent the progression of fibrosis.

GHRLD mice [32]. On the other hand, it is believed that IGF-I does not affect hepatocyte function directly because the hepatocytes express few IGF-I receptors in normal condition [43]. However, IGF-IR overexpression in hepatocytes has been described in chronic hepatitis C [44], chronic hepatitis B, and liver cirrhosis [45, 46] when compared to that of the normal liver, suggesting that IGF-IR plays a role in these pathological conditions [43, 47]. Indeed, accumulating evidence suggests that IGF-IR has a pivotal role in the liver. Recently, Nishizawa *et al.* demonstrated that a spontaneous dwarf rat, in which GH is deficient, exhibits NASH and IGF-I as well as GH administration reversed these changes in the liver [48]. Although it remains uncertain whether these effects of IGF-I on the liver results from direct action on hepatocytes, these results clearly demonstrate that IGF-I prevents the development of NASH *via* a GH-independent mechanism. It is speculated that IGF-I exerts its effect on hepatocytes by direct action *via* aberrant expression of IGF-IR under pathological conditions, *via* insulin receptor (IR), *via* hybrid receptors that consist of IR and IGF-IR [49], or *via* indirect actions through Kupffer cells, hepatic stellate cells (HSCs), and systemic changes in metabolism and inflammation.

Several potential underlying mechanisms may be responsible for the effects of IGF-I in the liver. Insulin resistance, oxidative stress, mitochondrial dysfunction, and the inflammatory cascade are believed to play integral roles in the development of NASH [50]. It is well known that IGF-I improves insulin sensitivity *in vivo*. The specific deletion of IGF-I in the liver results in insulin resistance [51], indicating that hepatic IGF-I regulates systemic insulin sensitivity. The increase in circulating IGF-I may ameliorate NASH at least in part by improving insulin sensitivity. In addition, IGF-I exerts a strong anabolic action, particularly in protein metabolism in muscle, which is commonly disturbed in chronic liver disease. Furthermore, improvement of the nutritional condition may also contribute to these effects because a pilot study showed that IGF-I administration in cirrhotic patients improved serum albumin and energy metabolism after 120 days of administration [52]. Intriguingly, the mitochondrial morphology was severely impaired in the hepatocytes of GH-deficient rats and IGF-I reversed these changes [48]. IGF-I improved conditions of enhanced oxidative stress in the liver, suggesting that IGF-I regulates mitochondrial function and oxidative stress (Fig. 1). In fact, it has been reported that IGF-I improves mitochondrial function *in vitro* [53] and *in vivo* [54]. IGF-IR activation prevented oxidative stress, mitochondrial dysfunction, and apoptosis in human umbilical vein endothelial cells (HUVEC) [53]. IGF-I administration reduced oxida-

tive mitochondrial damage, corrected impaired mitochondrial function such as complex V ATPase activity, and decreased caspase activities [54]. In line with this, Perez *et al.* showed that IGF-I administration improved liver dysfunction and fibrosis in a rat cirrhosis model and mitochondrial function in aging rats [55]. Recently, it has been reported that impaired insulin signaling, which shares a common pathway with IGF-I signaling, leads to mitochondrial dysfunction in the liver *via* hyperactivation of Foxo1 [56]. The double deletion of *irs1-1* and *-2* in mice results in Foxo1 activation and an increase in its target genes including heme oxegenase-1 (Hmox1), which disrupts complex Ⅲ and Ⅳ of the respiratory chain, and lowers the NAD^+/NADH ratio and ATP production in the mitochondria. IGF-I may also regulate mitochondrial function *via* these pathways as well as insulin. Regarding the progression of fibrosis, it has been reported that targeted overexpression of IGF-I by activated HSCs restricts their activation, attenuates fibrosis, and accelerates liver regeneration in a carbon tetrachloride (CCl_4)-treated cirrhotic model [57]. These effects appear to be mediated in part by upregulation of hepatocyte growth factor (HGF) and downregulation of transforming growth factor beta 1(TGFβ1). HSC is a key player in hepatic regeneration and progression of fibrosis [58]. Activation of HSCs into the myofibroblast phenotype can be provoked by a range of chronic injuries to the liver including oxidative stress, inflammatory cytokines, and lipopolysaccharide (LPS) [59]. In cultured HSCs, IGF-I increases proliferation [60] and collagen synthesis [61]. On the other hand, quiescent HSCs do not respond to IGF-I irrespective of high IGF-I receptor expression, suggesting that IGF-I action on HSCs is dependent on the stage of differentiation [60, 62]. In addition, IGF-I stimulates production of HGF, but does not stimulate TGFβ1 production, in HSCs [63]. HGF is a mitogen for hepatocytes and appears to limit fibrosis *in vivo* [64].

In humans, NAFLD is associated with low circulating levels of IGF-I [65, 66]. IL-6 and IGF-I are independent prognostic factors of liver steatosis and NASH in morbidly obese patients [67]. In addition, hyaluronic acid levels, which are a fibrotic marker, showed a negative correlation with IGF-I and the IGF-I/IGFBP-3 ratio in patients with NAFLD [24]. Although GH obviously has an essential and direct role on heptocytes in the aspects of anti-steatotic action [33, 38] and gene expression profiles [68], these results strongly suggest that IGF-I has GH-independent actions in the liver *via* various mechanisms (Fig. 1).

Conclusions

It has been known that the liver is one of the important target tissues of GH. In somatomedin hypothesis, the liver was a simple organ that secretes IGF-I [69]. Indeed, circulating IGF-I is mainly produced by the liver. Furthermore, until recently, the physiological significance of the GH-IGF-I axis in the liver has not been fully recognized. However, growing evidence has revealed that GH as well as IGF-I play essential roles in the liver, especially in adults. Further investigation is necessary to clarify the precise mechanisms by which GH and IGF-I exert their effects in the liver. Finally, it should be noted that NAFLD/NASH has emerged as an important comorbidity in AGHD.

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