Role of growth hormone, insulin-like growth factor 1 and insulin-like growth factor-binding protein 3 in development of non-alcoholic fatty liver disease

Tatsuki Ichikawa · Kazuhiko Nakao · Keisuke Hamasaki ·
Ryuji Furukawa · Shotarou Tsuruta · Yasuo Ueda ·
Naota Taura · Hidetaka Shibata · Masumi Fujimoto ·
Kan Toriyama · Katsumi Eguchi

Received: 14 April 2007 / Published online: 1 June 2007
© Asian Pacific Association for the Study of the Liver 2007

Abstract

Background and aims Pituitary dysfunction including growth hormone (GH) deficiency may be associated with non-alcoholic fatty liver disease (NAFLD). Since the relationships among GH, IGF-1, IGFBP-3, and development of NAFLD without hypopituitarism are unclear, we examined the role of these hormones in the development of NAFLD based on clinical, laboratory and liver histology data.

Patients and methods A total of 55 consecutive patients (20 males and 35 females) with NAFLD.

Results Aspartate amino transferase (AST), AST/ALT, platelet count and IGF-1, levels were significantly associated with differences in fibrosis, since these variables differed between stage 0–1 and stage 2–3 NAFLD. In multivariate analysis, platelet count ($P = 0.0223$, relative risk (RR), 5.899; 95% confidence interval (CI), 1.288–27.017), and IGF-1 ($P = 0.0363$, RR, 4.568; 95% CI, 1.101–18.945) showed significant associations with stage 2–3 NAFLD. Additionally, hyaluronic acid levels had a negative relationship with IGF-1 and the IGF-1/IGFBP-3 ratio. There was no relationship of fibrosis with GH level, but decreased GH ($P = 0.0414$, RR, 0.199; 95% CI, 0.042–0.989) was significantly associated with steatosis of stage

2–3. Low GH/IGF-1 and GH/IGFBP-3 ratios were found in advanced steatosis.

Conclusion GH, IGF-1 and IGFBP-3 are associated with hepatic fibrosis and steatosis in NAFLD. Low levels of IGF-1 might be associated with fibrosis while low level of GH with hepatic steatosis.

Keywords Non-alcoholic fatty liver disease · Growth hormone · Insulin-like growth factor 1 · Insulin-like growth factor-binding protein 3 · Stage

Abbreviations

ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
γ -GTP	γ -Glutamyltranspeptidase
BMI	Body mass index
CT	Computed tomography
FMV	Flow-mediated vasodilatation
GH	Growth hormone
IGF-1	Insulin-like growth factor 1
IGFBP-3	Insulin-like growth factor-binding protein 3
IMT	Intima-media thickness
NAFLD	Non-alcoholic fatty liver disease
NASH	Non-alcoholic steatohepatitis
STAT	Signal transducers and activators of transcription
US	Ultrasonography

T. Ichikawa (✉) · K. Nakao · K. Hamasaki ·
N. Taura · H. Shibata · M. Fujimoto · K. Eguchi
The First department of Internal Medicine, Graduate school
of Biomedical science, Nagasaki University, 1-7-1 Sakamoto,
Nagasaki 852-8501, Japan
e-mail: ichikawa@net.nagasaki-u.ac.jp

R. Furukawa · S. Tsuruta · Y. Ueda
Department of Internal Medicine, The Japanese Red Cross
Nagasaki Atomic Bomb Hospital, Nagasaki, Japan

K. Toriyama
Department of Pathology, Institute of Tropical medicine,
Nagasaki University, Nagasaki, Japan

Introduction

Striking similarities exist between the metabolic syndrome and untreated GH deficiency (GHD) in adults, and

undetectable and low levels of GH may be of importance in the metabolic aberrations observed in both conditions [1]. Together with atherosclerosis, visceral obesity, hypertension, hyperlipidemia, and insulin resistance. Non-alcoholic fatty liver disease (NAFLD) is a common feature in metabolic syndrome and in hypopituitary and hypothalamic dysfunction [2, 3]. Lonardo et al. showed that low levels of GH are independent predictors of NAFLD in male patients [4], and recent reports suggest that hypothalamic and/or pituitary disease including GHD involves a rapid progressive NAFLD [3]; the reason for rapid progression was speculated to be body weight gain, and not GHD itself. We have previously found an association of adult onset GHD with NAFLD [2], and shown that hepatic steatosis is more frequently observed in patients with GHD than in those without GHD, suggesting that adult onset GHD is a possible risk factor for NAFLD. However, the role of GH in NAFLD remains unclear, despite the growing knowledge of the activity of this hormone in induction of lipolysis and lipid oxidation [5].

IGF-1 is secreted from hepatocytes by GH stimulation. It is a catabolic hormone with a role in protein synthesis, and can also stimulate secretion of IGFBP-3 from Kupffer cells [6]. The biological activity of IGF-1 is strongly influenced by several IGF-specific binding proteins (IGFBP-1 to 6), of which IGFBP-3 carries >80% of circulating IGF-1 [7]. The presence of IGFBP-3 lowers IGF-1 bioactivity [8], and the ratio between IGF-1 and IGFBP-3 levels decreases with increasing age in adults, resulting in decreased levels of free and biologically active IGF-1 [9]. IGF-1 has been shown to have a protective effect on ischemic heart disease, cardiovascular disease mortality and atherosclerosis [7, 10, 11], whereas high levels of IGFBP-3 and a low IGF-1/IGFBP-3 ratio are correlated with atherosclerosis [9]. Cardiovascular disease is a serious problem in NAFLD patients, and ischemic heart disease is a major cause of death among these patients [12]. NAFLD is associated with advanced carotid atherosclerosis [13] and endothelial dysfunction, and the 10-year probability of occurrence of cardiovascular events [14], and especially intima-media thickness (IMT) has a strong association with the degree of hepatic fibrosis in NAFLD patients [15].

Although GH levels have been correlated with metabolic syndrome, and IGF-1 and IGFBP-3 are related to cardiovascular disease, the relationships among GH, IGF-1, IGFBP-3, and the severity of NAFLD have not been examined. Therefore, the aim of this study was to compare GH, IGF-1, and IGFBP-3 levels with the degree of fibrosis, inflammation and steatosis in NAFLD patients, and to clarify the relationship between the development of NAFLD and the levels of these hormones.

Patients and methods

Patients

The study included 55 consecutive patients (20 males and 35 females) with NAFLD who attended the First Department of Internal Medicine at the Graduate School of Biomedical Science, Nagasaki University and the Department of Internal Medicine at the Japanese Red Cross Nagasaki Atomic Bomb Hospital, with an initial visit between April 1998 and December 2005. NAFLD was diagnosed on the basis of a persistently raised ALT level (>1.5 times the upper normal limit for 6 months or more) and on US and CT images. A percutaneous liver biopsy assisted by US was performed in all cases, and scored using the criteria proposed by Brunt et al. [16]. Liver biopsy specimens were fixed in 10% formalin, embedded in paraffin, cut to a thickness of 4 μ m, and subjected to hematoxylin-eosin, and Azan-Mallory staining. All liver tissue specimens were evaluated by a single pathologist who was blinded to the clinical condition of the patient. Patients with positive hepatitis B and C serology or with evidence of inherited, autoimmune, cholestatic, or drug-induced liver disease were excluded using standard clinical, laboratory, imaging, and histological criteria. In addition, the subjects included in the study had no history of current or past excessive alcohol intake, as defined by an average daily consumption of more than 20 g of alcohol. At the time of the study, none of the patients showed clinical, biochemical or histological evidence of cirrhosis. Of the 55 cases that were originally included in the study, three secondary cases of NAFLD were subsequently excluded: two of these cases were steatohepatitis after pancreatoduodenal resection, and the third was a case of adult onset hypopituitarism.

Clinical and laboratory measurements

Body mass index (BMI) was calculated as weight (kg) divided by the square of height (m). Subjects fasted overnight before blood samples were obtained. Venous plasma glucose was measured with an automated analyzer, and basal serum insulin was measured using a standard radioimmunoassay. The index of insulin resistance was calculated using the fasting value of plasma glucose, (we excluded the patients with greater than 130 mg/dl), and the serum insulin level, according to the homeostasis model assessment (HOMA) method. Hemoglobin A1c, ALT, AST, γ -GTP, ALP, total cholesterol, triglyceride, C-reactive protein and ferritin, and hyaluronic acid were determined by standard hematology and laboratory techniques. Measurements of GH, IGF-1, and IGFBP-3 were performed using commercially available kits. The sensitivity

of the serum GH, IGF-1, and IGFBP-3 assay was 0.02 ng/ml, 4 µg/l, and 0.20 mg/l, respectively. The GH/IGF-1, GH/IGFBP-3, and IGF-1/IGFBP-3 concentration ratios were estimated to get a better understanding of the relative changes in concentration of GH, IGF-1, and IGFBP-3.

Statistical analysis

Data were processed on a personal computer and analyzed using StatView 5.0 (SAS Institute, Inc., Cary, NC). Differences between groups were analyzed by Mann–Whitney test and Pearson χ^2 test. Correlation analysis was carried out using Spearman rank correlation. Logistic regression analysis was used to test the association of variables with different stages of NAFLD and steatosis. All data in the text and tables are given as means, unless otherwise indicated. Variables that achieved statistical significance in the univariate analysis were subsequently included in a multivariate analysis using a logistic regression model, and described as odds ratios (OR) with 95% CI. Spearman rank correlation was used to examine the relationship between variables. Values of $P < 0.05$ were considered statistically significant.

Results

Subject characteristics

Clinical and laboratory data for the 52 NAFLD subjects are shown in Table 1. BMI (kg/m^2) was distributed as follows: BMI < 25 in eight cases; BMI 25–30 in 27 cases; BMI 30–35 in 13 cases, BMI 35–40 in three cases, and BMI > 40 in one case. The HOMA-IR score reflects insulin resistance, with a score of > 2.5 indicating insulin resistance in Japanese subjects [17]. The mean HOMA-IR index in our subjects was 4.05, and 26 cases (70%) had values in excess of 2.5. The normal range of serum GH concentration is < 0.17 ng/ml in males and 0.28–1.64 ng/ml in females. Of the 35 females with NAFLD in the study, 24 (73%) had GH levels less than the normal range, while in the others this was within the normal limits. In contrast, 11 of the 20 male NAFLD patients (58%) had GH levels above the normal range for males, and the remaining eight cases (42%) were within the normal limits. For IGF-1, the normal ranges in males aged 17–20, 21–30, 31–40, 41–50, 51–60, 61–70 and > 70-years-old are 219–509, 85–369, 67–318, 41–272, 59–215, 42–250, 75–218 µg/l, respectively, whereas in females these data are 264–542, 119–389, 73–311, 46–282, 37–266, 37–150, 38–207 µg/l, respectively. In this study, three patients (one male and two female) had IGF-1 levels below the normal range for their age, and

two patients (both females) had IGF-1 levels higher than the normal range. Regarding IGFBP-3, the normal ranges in people aged 17–35 and 36–70 years old were 2.29–4.17 and 2.17–4.05 mg/l, respectively, and 12 female patients (35%) and seven male patients (37%) had IGFBP-3 levels less than the normal limit for their age. Hence, aberrant values of serum IGF-1 concentration were found in a few cases, IGFBP-3 levels lower than the normal range were found in more than 30% of NAFLD patients.

Clinical and laboratory data in NAFLD patients at stages 0–1 and 2–3

The NAFLD patients who were classified into stages and clinical and laboratory data were compared between stage 0–1 and stage 2–3 patients (Table 1). There were no differences in sex, age, BMI, ALT, γ -GTP, ALP, total cholesterol, triglyceride, C-reactive protein, white blood cell count, red blood cell count, GH, IGFBP-3, insulin, HOMA-IR score, and HbA1c between the two groups. However, AST ($P = 0.0005$) and AST/ALT ($P = 0.0206$) were lower in stage 0–1 patients, and platelet count ($P = 0.002$) and IGF-1 ($P = 0.0272$) were higher in stage 0–1 patients, compared to stage 2–3 patients.

Univariate and multivariate analysis of risk factors for stage 2–3 NAFLD

The results of univariate and multivariate analyses of risk factors for stage 2–3 NAFLD are shown in Table 2. In the univariate analysis, the following three factors were significantly associated with stage 2–3 NAFLD: reduced AST (relative risk (RR), 0.238; 95% confidence interval (CI), 0.069–0.824 [$P = 0.0235$]), reduced platelet count (RR, 4.667; 95% CI, 1.301–16.739 [$P = 0.0181$]), and reduced IGF-1 level (RR, 4.643; 95% CI, 1.331–16.196 [$P = 0.016$]). In a multivariable analysis, reduced platelet count (RR, 5.899; 95% CI, 1.288–27.017 [$P = 0.0223$]) and reduced IGF-1 (RR, 4.568; 95% CI, 1.101–18.945 [$P = 0.0363$]) were significantly associated with stage 2–3 NAFLD.

Correlation between serum hyaluronic acid and the GH/IGF-1/IGFBP-3 axis

Hyaluronic acid is a hepatic fibrosis marker [18], and its correlation with GH, IGF-1 and IGFBP-3 was explored in 41 NAFLD patients in whom the level was within the detection range of the assay (Table 3). The GH level showed no correlation with hyaluronic acid, but IGF-1 and IGFBP-3 levels showed a negative correlation with the hyaluronic acid level, with the IGF-1 level showing a

Table 1 Clinical and laboratory data for NAFLD patients at stages 0–1 and 2–3

Item	Total (<i>n</i> = 52)	Stage 0–1 (<i>n</i> = 34)	Stage 2–3 (<i>n</i> = 18)	<i>P</i> -value
Sex (M/F)	19/33	13/21	6/12	NS
Age (year)	49.7 (17.1)	47.5 (16.7)	55.4 (17.5)	NS
BMI (kg/m ²)	29.0 (4.12)	29.2 (4.4)	28.7 (3.6)	NS
ALT (IU/l)	93.5 (79.1)	76.3 (53.0)	126.0 (107.8)	NS
AST (IU/l)	62.0 (49.8)	45.3 (25.2)	93.7 (67.8)	0.0005
AST/ALT	0.714 (0.226)	0.67 (0.20)	0.80 (0.26)	0.0206
γ-GTP (IU/l)	85.7 (87.2)	67.4 (46.2)	120.3 (129)	NS
ALP (IU/l)	239.0 (71.7)	234.8 (69.3)	246.8 (77.6)	NS
Total cholesterol (mg/dl)	203.1 (35.5)	209.4 (37.6)	191.5 (28.7)	NS
Triglyceride (mg/dl)	161.2 (65.8)	161.1 (64.1)	161.5 (70.7)	NS
C-reactive protein (mg/dl)	0.26 (0.25)	0.251 (0.292)	0.271 (0.187)	NS
White blood cells (μl)	6482 (1664)	6641 (1628)	6683 (1737)	NS
Red blood cells (10 ⁴ /μl)	465 (54.6)	473 (45.7)	452 (67.6)	NS
Ferritin (mg/dl)	322 (412.2)	247 (162)	415 (619)	NS
Platelets (μl)	23300 (6300)	24800 (6378)	20400 (5119)	0.002
GH (ng/ml)	0.31 (0.36)	0.298 (0.374)	0.331 (0.357)	NS
IGF-1 (μg/l)	171 (111.8)	191.8 (128)	129.2 (45.4)	0.0272
IGFBP-3 (mg/l)	2.45 (0.66)	2.56 (0.73)	2.24 (0.42)	NS
Insulin (IU/l)	14.8 (6.88)	13.9 (5.56)	16.4 (9.03)	NS
Fasting plasma glucose (mg/l)	113.0 (33.3)	109.4 (30.4)	119.8 (38.4)	NS
HOMA-IR	4.03 (2.4)	3.86 (2.18)	4.40 (2.88)	NS
HbA1c (%)	6.70 (1.9)	6.77 (2.09)	6.48 (1.63)	NS

Data are shown as means (standard deviation) and numbers, with statistical analysis using a Mann–Whitney test for means and a Pearson χ^2 test for numbers

Normal values in laboratory tests: ALT (IU/l), 5–40; AST (IU/l), 10–40; γ-GTP (IU/l), <70 in males, <30 in females; ALP (IU/l), 115–359; total cholesterol (mg/dl), 150–219; triglyceride (mg/dl), 50–149; C-reactive protein (mg/dl), <0.30; white blood cell count (μl), 3900–9800 in males, 3500–9100 in females; red blood cell count (10⁴/μl), 427–570 in males, 376–500 in females; ferritin (mg/dl), 27–320 in males, 3.4–89 in females; platelet (μl), 13.1–36.2 in males, 13–36.9 in females; insulin (IU/l), 3.06–16.9; fasting plasma glucose (mg/l), 70–109; HbA1c (%), 4.3–5.8. BMI, GH, IGF-1, IGFBP-3 and HOMA-IR are described in the text

Table 2 Variables independently associated with NAFLD of stages 2–3

Variable	Univariate analysis		Multivariate analysis	
	<i>P</i> -value	RR (95% CI)	<i>P</i> -value	RR (95% CI)
AST	0.0235	0.238 (0.069–0.824)	0.0834	0.282 (0.067–1.182)
Platelet count	0.0181	4.667 (1.301–16.739)	0.0223	5.899 (1.288–27.017)
IGF-1	0.016	4.643 (1.331–16.196)	0.0363	4.568 (1.101–18.945)

The reference groups are AST ≤ 50 IU/l, platelet count < 200,000/l, and IGF-1 < 140 μg/l

Table 3 Correlation between serum hyaluronic acid and the GH/IGF-1/IGFBP-3 axis (*n* = 41)

Variable	Correlation coefficient	<i>P</i> -value
GH	0.135	NS
IGF-1	−0.427	0.0043
IGFBP-3	−0.352	0.0216
GH/IGF-1	0.338	0.0282
GH/IGFBP-3	0.175	NS
IGF-1/IGFBP-3	−0.436	0.0035

particularly strong negative correlation. As a reflection of these results, the GH/IGF-1 ratio, but not the GH/IGFBP-3 ratio, showed a positive correlation with the hyaluronic acid level, and similarly the IGF-1/IGFBP-3 ratio was positively correlated with hyaluronic acid.

Histopathology and the IGF-1 axis

Due to the negative correlation between IGF-1 and fibrosis, the relationships of IGF-1 with some aspects of

Brunt's fibrosis classification were evaluated. Patients were categorized based on the presence and absence of pericellular fibrosis, portal fibrosis, bridging fibrosis, and ballooning. No differences in IGF-1 were found for groups with and without pericellular fibrosis and bridging fibrosis, but IGF-1 was significantly lower in patients with portal fibrosis (stages 1–3) than in those without portal fibrosis ($P = 0.0229$), and the IGF-1/IGFBP-3 ratio showed a tendency to decrease in patients with portal fibrosis ($P = 0.0837$). In addition, the IGF-1 level was relatively low in patients showing ballooning ($P = 0.0816$) (Fig. 1). The relationship between histological grade of stages 0–1 or 2–3 was also examined, but no correlation was found.

Clinical and laboratory data in patients with steatosis of grade 1 and grades 2–3

The role of the GH/IGF-1/IGFBP-3 axis in steatosis was investigated by grouping patients into grades 1 and grades 2–3 using Brunt's steatosis classification (Table 4). There were no differences in sex, age, BMI, AST, ALT, γ -GTP, ALP, triglyceride, C-reactive protein, white blood cell count, red blood cell count, IGFBP-3, insulin, HOMA-IR score, and HbA1c between the two groups. However, total cholesterol ($P = 0.034$) and IGF-1 level ($P = 0.0180$) were higher in steatosis 2–3 patients and the GH level ($P = 0.0236$) was higher in steatosis 1 patients. In the multivariate analysis, a reduced GH level showed a significant association with steatosis of grades 2–3: GH (RR, 0.43; 95% CI, 0.002–0.772 [$P = 0.0328$]).

The results of univariate and multivariate analyses of risk factors for steatosis of grade 2–3 are shown in Table 5. In the univariate analysis, the following three factors were significantly associated with steatosis of grade 2–3: GH (RR, 0.196; 95% CI 0.046–0.830 [$P = 0.0269$]) and IGF (RR, 4.00; 95% CI, 1.151–13.901 [$P = 0.0291$]). In a multivariable analysis, GH (RR, 0.199; 95% CI, 0.042–0.989 [$P = 0.0414$]) was significantly associated with steatosis of grade 2–3.

Hepatic steatosis score and the GH axis

Correlations of hepatic steatosis score with GH, IGF-1, and IGFBP-3 levels were explored by examining differences in the GH/IGF-1, GH/IGFBP-3, and IGF-1/IGFBP-3 ratios between patients with steatosis of grade 1 and grades 2–3. The following factors showed a significant difference between these groups of patients: GH/IGF-1 ratio, $P = 0.0009$; GH/IGFBP-3 ratio, $P = 0.0019$ (Fig. 2), but IGF-1/IGFBP-3 ratio did not have any statistical difference.

Fig. 1 Histopathologic factors and IGF-1: (A) relationship of portal fibrosis score and IGF-1 concentration ($\mu\text{g/l}$); (B) relationship of portal fibrosis score and IGF-1 concentration ($\mu\text{g/l}$)/IGFBP-3 concentration (mg/l) ratio; (C) relationship of ballooning score and IGF-1 concentration ($\mu\text{g/l}$). Statistical analysis by Mann–Whitney U test

Discussion

The data in this study shows that GH, IGF-1, and IGFBP-3 are predictors for the development of fibrosis and steatosis in NAFLD patients. Low levels of IGF-1 and IGF-1/IGFBP-3 may be associated with advanced fibrosis in NAFLD, and GH may be involved in the mechanism of

Table 4 Clinical and laboratory data for patients with steatosis of grade 1 and grades 2–3

Item	Steatosis 1 (<i>n</i> = 26)	Steatosis 2–3 (<i>n</i> = 26)	<i>P</i> -value
BMI	28.5 (4.48)	29.6 (3.75)	NS
Total cholesterol	193.7 (36.2)	211.4 (33.3)	0.034
Triglyceride	154.2 (65.6)	167.7 (66.6)	NS
GH	0.450 (0.458)	0.173 (0.162)	0.0236
IGF-1	157.1 (142.6)	184.3 (71.4)	0.0118
IGFBP-3	2.25 (0.58)	2.63 (0.68)	NS
Insulin	13.9 (5.89)	15.7 (7.83)	NS
Fasting plasma glucose	111.4 (28.2)	114.6 (38.2)	NS
HOMA-IR	4.02 (2.53)	4.04 (2.30)	NS
HbA1c	6.82 (1.75)	6.53 (2.08)	NS

Data are shown as means (standard deviation), with statistical analysis using a Mann–Whitney test

Table 5 Variables independently associated with steatosis of grade 2–3

Variable	Univariate analysis		Multivariate analysis	
	<i>P</i> -value	RR (95% CI)	<i>P</i> -value	RR (95% CI)
Total cholesterol	0.1239	2.424 (0.785–7.490)	0.3656	1.795 (0.506–6.372)
GH	0.0269	0.196 (0.046–0.830)	0.0414	0.199 (0.042–0.989)
IGF-1	0.0291	4.000 (1.151–13.901)	0.0676	3.628 (0.911–14.453)

The reference groups are total cholesterol > 200 mg/dl, GH > 0.3 ng/ml, and IGF-1 > 170 µg/l

triglyceride secretion from hepatocytes. It is of interest that our data are consistent with results of hormonal studies associated with atherosclerosis and metabolic syndrome [7, 9–11], and we speculate that the same hormonal profile results in development of hepatic steatosis and fibrosis in NAFLD, and that the mechanism of advanced fibrosis is common in development of atherosclerosis and visceral obesity.

Hepatic fibrosis is caused by activated hepatic stellate cells (HSC), which are activated by inflammatory cytokines in NAFLD [19]. Over expression of IGF-1 in HSC attenuates fibrogenesis and accelerates liver regeneration in a mouse model of CCl₄-induced liver damage [20]. These effects appear to be mediated in part by up-regulation of HGF and down-regulation of TGF-β1. Although GH is the major stimulant of IGF-1 synthesis in hepatocytes, inflammatory cytokines such as IL-1β [21], TNF-α [22] and IL-6 [23] inhibit IGF-1 secretion from hepatocytes. These cytokines are reported to have a pivotal role in development of NAFLD [24, 25], therefore, this may account for the decreased IGF-1 level and reduced IGF-1/IGFBP-3 ratio in advanced NASH and the negative correlation of these variables with the hyaluronic acid level. Based on the success of IGF-1 replacement therapy in animal models [26, 27], this therapy has recently been used for patients with liver cirrhosis [28]. IGF-1 supplementation protects the liver from damage, increases the albumin concentration and resting energy expenditure. The protective effects of IGF-1 on vascular cells, and endothelial

function, atherosclerotic plaque development and ischemic myocardial damage all depend on nitric oxide production by activated PI3-k and Akt signaling in target cells [8]. IGF-1 induces glucose uptake in muscle [29] and low levels of IGF-1 produce different degrees of glucose tolerance in association with insulin sensitivity [30]; therefore, IGF-1 has been used successfully as an adjuvant to insulin therapy in patients with type 1 and 2 diabetes [29]. Insulin resistance is the most important factor in metabolic syndrome, including NAFLD and vascular disease, and low levels of IGF-1 may contribute to insulin resistance [29, 30]. IGF-1 has direct anti-fibrotic, cell protective and insulin-like actions and, decreases in IGF-1 and the IGF-1/IGFBP-3 ratio caused by inflammatory cytokines were associated with the development of advanced NAFLD, as well as serving as a marker for advanced NAFLD. In particular, our data show a tendency for low levels of IGF-1 and a low IGF-1/IGFBP-3 ratio in patients with portal fibrosis and ballooning. We speculate that this hormonal profile is required for progression of NAFLD, because of the emergence of portal fibrosis and ballooning in progressive NAFLD [31].

IGFBP-3 binds IGF-1 and an acid-labile subunit and forms a stable ternary complex, in which the half life of IGF-1 is prolonged and the bioactivity of IGF-1 is reduced [8]. New biological functions of IGFBP-3 have also been reported in recent years; for example, in mesangial cells, IGFBP-3 mediates TNF-α and glucose-induced apoptosis by blocking Akt phosphorylation at threonine-308 [32].

Fig. 2 Hepatic steatosis score and the GH axis: **(A)** relationship of steatosis score and GH (ng/ml)/IGF-1 concentration ($\mu\text{g/l}$) ratio; **(B)** relationship of steatosis score and GH (ng/ml)/IGFBP-1 concentration (mg/l) ratio. Statistical analysis by Mann–Whitney U test

Additionally, IGF-1 stimulates vascular endothelial growth factor (VEGF) and IGFBP-3 in retinal pigment epithelial cells, whereas, IGFBP-3 attenuates IGF-1-induced VEGF secretion [33]. IGF-1 also, inhibits $\text{TNF-}\alpha$ (interferon γ and Fas-induced apoptosis in vascular smooth muscle cells, and $\text{TNF-}\alpha$ decreases IGF-1 and increases IGFBP-3 [22]. As stated above, IGFBP-3 binding reduces IGF-1 activity and the IGF-1/IGFBP-3 ratio therefore reflects the activity of IGF-1. In our study, hyaluronic acid, a hepatic fibrosis marker, showed negative correlations with IGF-1 ($r = -0.427$), IGFBP-3 ($r = -0.352$) and the IGF-1/IGFBP-3 ratio ($r = -0.436$). Although IGF-1 causes secretion of IGFBP-3 [6], we speculate that inflammatory cytokines decrease the levels of IGF-1 rather than those of IGFBP-3 in NAFLD patients, thereby decreasing the IGF-1/IGFBP-3 ratio. Therefore, a low IGF-1/IGFBP-3 ratio is a marker of advanced NAFLD, as it is for vascular disease.

The GH level did not show an association with fibrosis, but had a close affinity with steatosis in our cases. A low level of GH is the most reliable marker for advanced steatosis (grades 2–3), but the GH/IGF-1 and GH/IGFBP-

3 ratios suggest that IGF-1 and IGFBP-3 are unrelated to steatosis. In previous reports, GH supplementation has been shown to reduce abdominal visceral fat in obese woman [34] and GHD patients [35], improve endothelial function in GHD patients [36], and decrease hepatic fat content in obese woman [34]. Such conditions are thought to promote GH-induced lipolysis in adipose tissue [37] and secretion of very low density lipoprotein from liver [38], and we suggest that low levels of GH may contribute to severe triglyceride accumulation in hepatocytes; though our results must be evaluated in a large cohort of patients.

In conclusion, GH, IGF-1 and IGFBP-3 are associated with fibrosis and steatosis of NAFLD. We speculate that a low level of IGF-1 and a low IGF-1/IGFBP-3 ratio may lead to development of fibrosis in NAFLD patients, whereas a low GH level contributes to development of hepatic steatosis. Since NAFLD is associated with metabolic syndrome, risk factors for development of these diseases show a common pattern of GH, IGF-1 and IGFBP-3 levels.

References

- Johannsson G, Bengtsson BA. Growth hormone and the metabolic syndrome. *J Endocrinol Invest* 1999;22:41–6.
- Ichikawa T, Hamasaki K, Ishikawa H, Ejima E, Eguchi K, Nakao K. Non-alcoholic steatohepatitis and hepatic steatosis in patients with adult onset growth hormone deficiency. *Gut* 2003;52:914.
- Adams LA, Feldstein A, Lindor KD, Angulo P. Nonalcoholic fatty liver disease among patients with hypothalamic and pituitary dysfunction. *Hepatology* 2004;39:909–14.
- Lonardo A, Loria P, Leonardi F, Ganazzi D, Carulli N. Growth hormone plasma levels in nonalcoholic fatty liver disease. *Am J Gastroenterol* 2002;97:1071–2.
- Mauras N, Haymond MW. Are the metabolic effects of GH and IGF-I separable? *Growth Horm Int* 2005;15:19–27.
- Scharf J, Ramadori G, Braulke T, Hartmann H. Synthesis of insulin like growth factor binding proteins and of the acid-labile subunit in primary cultures of rat hepatocytes, of Kupffer cells, and in co-cultures: regulation by insulin, insulin like growth factor, and growth hormone. *Hepatology* 1996;23:818–27.
- Juul A, Scheike T, Davidsen M, Gyllenborg J, Jorgensen T. Low serum insulin-like growth factor I is associated with increased risk of ischemic heart disease: a population-based case-control study. *Circulation* 2002;106:939–44.
- Conti E, Carozza C, Capoluongo E, Volpe M, Crea F, Zuppi C, Andreotti F. Insulin-like growth factor-1 as a vascular protective factor. *Circulation* 2004;110:2260–5.
- Colao A, Spiezia S, Di Somma C, Pivonello R, Marzullo P, Rota F, Musella T, Auremma RS, De Martino MC, Lombardi G. Circulating insulin-like growth factor-I levels are correlated with the atherosclerotic profile in healthy subjects independently of age. *J Endocrinol Invest* 2005;28:440–8.
- Laughlin GA, Barrett-Connor E, Criqui MH, Kritz-Silverstein D. The prospective association of serum insulin-like growth factor I (IGF-I) and IGF-binding protein-1 levels with all cause and cardiovascular disease mortality in older adults: the Rancho Bernardo Study. *J Clin Endocrinol Metab* 2004;89:114–20.

11. Janssen JA, Stolk RP, Pols HA, Grobbee DE, Lamberts SW. Serum total IGF-I, free IGF-I, and IGFB-1 levels in an elderly population: relation to cardiovascular risk factors and disease. *Arterioscler Thromb Vasc Biol* 1998;18:277–82.
12. Adams LA, Lymp JF, St Sauver J, Sanderson SO, Lindor KD, Feldstein A, Angulo P. The natural history of nonalcoholic fatty liver disease: a population-based cohort study. *Gastroenterology* 2005;129:113–21.
13. Brea A, Mosquera D, Martin E, Arizti A, Cordero JL, Ros E. Nonalcoholic fatty liver disease is associated with carotid atherosclerosis: a case-control study. *Arterioscler Thromb Vasc Biol* 2005;25:1045–50.
14. Villanova N, Moscatiello S, Ramilli S, Bugianesi E, Magalotti D, Vanni E, Zoli M, Marchesini G. Endothelial dysfunction and cardiovascular risk profile in nonalcoholic fatty liver disease. *Hepatology* 2005;42:473–80.
15. Targher G. Associations between liver histology and early carotid atherosclerosis in subjects with nonalcoholic fatty liver disease. *Hepatology* 2005;42:974–5.
16. Brunt EM, Janney CG, Di Bisceglie AM, Neuschwander-Tetri BA, Bacon BR. Nonalcoholic steatohepatitis: a proposal for grading and staging the histological lesions. *Am J Gastroenterol* 1999;94:2467–74.
17. Taniguchi A, Fukushima M, Sakai M, Miwa K, Makita T, Nagata I, Nagasaka S, Doi K, Okumura T, Fukuda A, Kishimoto H, Fukuda T, Nakaishi S, Tokuyama K, Nakai Y. Remnant-like particle cholesterol, triglycerides, and insulin resistance in non-obese Japanese type 2 diabetic patients. *Diabetes Care* 2000;23:1766–9.
18. Zeng MD, Lu LG, Mao YM, Qiu DK, Li JQ, Wan MB, Chen CW, Wang JY, Cai X, Gao CF, Zhou XQ. Prediction of significant fibrosis in HBeAg-positive patients with chronic hepatitis B by a noninvasive model. *Hepatology* 2005;42:1437–45.
19. Elsharkawy AM, Oakley F, Mann DA. The role and regulation of hepatic stellate cell apoptosis in reversal of liver fibrosis. *Apoptosis* 2005;10:927–39.
20. Sanz S, Pucilowska JB, Liu S, Rodriguez-Ortigosa CM, Lund PK, Brenner DA, Fuller CR, Simmons JG, Pardo A, Martinez-Chantar ML, Fagin JA, Prieto J. Expression of insulin-like growth factor I by activated hepatic stellate cells reduces fibrogenesis and enhances regeneration after liver injury. *Gut* 2005;54:134–41.
21. Thissen JP, Verniers J. Inhibition by interleukin-1 beta and tumor necrosis factor-alpha of the insulin-like growth factor I messenger ribonucleic acid response to growth hormone in rat hepatocyte primary culture. *Endocrinology* 1997;138:1078–84.
22. Anwar A, Zahid AA, Scheidegger KJ, Brink M, Delafontaine P. Tumor necrosis factor-alpha regulates insulin-like growth factor-1 and insulin-like growth factor binding protein-3 expression in vascular smooth muscle. *Circulation* 2002;105:1220–5.
23. Leibelach A, Scharf JG, Ramadori G. Regulation of insulin-like growth factor-I and of insulin-like growth factor binding protein-1, -3 and -4 in cocultures of rat hepatocytes and Kupffer cells by interleukin-6. *J Hepatol* 2001;35:558–67.
24. Nozaki Y, Saibara T, Nemoto Y, Ono M, Akisawa N, Iwasaki S, Hayashi Y, Hiroi M, Enzan H, Onishi S. Polymorphisms of interleukin-1 beta and beta 3-adrenergic receptor in Japanese patients with nonalcoholic steatohepatitis. *Alcohol Clin Exp Res* 2004;28:106S–10S.
25. Larter CZ, Farrell GC. Insulin resistance, adiponectin, cytokines in NASH: which is the best target to treat? *J Hepatol* 2006;44:253–61.
26. Castilla-Cortazar I, Garcia M, Muguerza B, Quiroga J, Perez R, Santidrian S, Prieto J. Hepatoprotective effects of insulin-like growth factor I in rats with carbon tetrachloride-induced cirrhosis. *Gastroenterology* 1997;113:1682–91.
27. Garcia-Fernandez M, Castilla-Cortazar I, Diaz-Sanchez M, Navarro I, Puche JE, Castilla A, Casares AD, Clavijo E, Gonzalez-Baron S. Antioxidant effects of insulin-like growth factor-I (IGF-I) in rats with advanced liver cirrhosis. *BMC Gastroenterol* 2005;5:7.
28. Conchillo M, de Knecht RJ, Payeras M, Quiroga J, Sangro B, Herrero JJ, Castilla-Cortazar I, Frystyk J, Flyvbjerg A, Yoshizawa C, Jansen PL, Scharschmidt B, Prieto J. Insulin-like growth factor I (IGF-I) replacement therapy increases albumin concentration in liver cirrhosis: results of a pilot randomized controlled clinical trial. *J Hepatol* 2005;43:630–6.
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;15:324–36.
30. Sesti G, Sciacqua A, Cardellini M, Marini MA, Maio R, Vatrano M, Succurro E, Lauro R, Federici M, Perticone F. Plasma concentration of IGF-I is independently associated with insulin sensitivity in subjects with different degrees of glucose tolerance. *Diabetes Care* 2005;28:120–5.
31. Morita Y, Ueno T, Sasaki N, Kuhara K, Yoshioka S, Tateishi Y, Nagata E, Kage M, Sata M. Comparison of liver histology between patients with non-alcoholic steatohepatitis and patients with alcoholic steatohepatitis in Japan. *Alcohol Clin Exp Res* 2005;29:277S–81S.
32. Vasylyeva TL, Chen X, Ferry RJ Jr. Insulin-like growth factor binding protein-3 mediates cytokine-induced mesangial cell apoptosis. *Growth Horm IGF Res* 2005;15:207–14.
33. Slomiany MG, Rosenzweig SA. Autocrine effects of IGF-I-induced VEGF and IGFBP-3 secretion in retinal pigment epithelial cell line ARPE-19. *Am J Physiol Cell Physiol* 2004;287:C746–53.
34. Franco C, Brandberg J, Lonn L, Andersson B, Bengtsson BA, Johannsson G. Growth hormone treatment reduces abdominal visceral fat in postmenopausal women with abdominal obesity: a 12-month placebo-controlled trial. *J Clin Endocrinol Metab* 2005;90:1466–74.
35. Eden Engstrom B, Burman P, Holdstock C, Karlsson FA. Effects of growth hormone (GH) on ghrelin, leptin, and adiponectin in GH-deficient patients. *J Clin Endocrinol Metab* 2003;88:5193–8.
36. Abdu TA, Elhadd TA, Buch H, Barton D, Neary R, Clayton RN. Recombinant GH replacement in hypopituitary adults improves endothelial cell function and reduces calculated absolute and relative coronary risk. *Clin Endocrinol (Oxf)* 2004;61:387–93.
37. Kersten S. Mechanisms of nutritional and hormonal regulation of lipogenesis. *EMBO Rep* 2001;2:282–6.
38. Frick F, Linden D, Ameen C, Eden S, Mode A, Oscarsson J. Interaction between growth hormone and insulin in the regulation of lipoprotein metabolism in the rat. *Am J Physiol Endocrinol Metab* 2002;283:E1023–31.