Liver INTERNATIONAL
OFFICIAL JOURNAL OF THE INTERNATIONAL ASSOCIATION FOR THE STUDY OF THE LIVER

Liver International ISSN 1478-3223

VIRAL HEPATITIS

Independent factors associated with altered plasma active ghrelin levels in HCV-infected patients

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Keywords
albumin – ghrelin – gut hormone – hepatitis C virus – liver cirrhosis

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Received 3 July 2012
Accepted 22 May 2013

DOI:10.1111/liv.12235
Liver Int. 2013: 33: 1510–1516

Abstract

Background & Aims: Metabolic disorders are frequently seen in hepatitis C virus (HCV)-infected patients. Ghrelin, a gut hormone, regulates hepatic metabolisms, and must be activated to exert its biological effects. The aims of this study were to investigate changes in plasma active ghrelin levels and identify independent factors associated with plasma active ghrelin levels in HCV-infected patients. *Methods:* We enrolled patients with HCV infection (n = 96), hepatitis B virus (HBV) infection (n = 49), non-alcoholic fatty liver disease (NAFLD; n = 20) and healthy subjects (CON; n = 16). Plasma active ghrelin levels were measured using ELISA. Factors associated with plasma active ghrelin levels were assessed by multivariate and Spearman's correlation analyses. *Results:* Plasma active ghrelin levels were significantly lower in relation to the severity of liver disease in both the HBV and HCV groups. Furthermore, HCV infection was identified as an independent factor associated with decreased plasma active ghrelin levels in the multivariate analysis (OR −3.05; 95% CI −0.93 to −19.51; P = 0.0192). Plasma active ghrelin levels were significantly correlated with serum albumin levels in the HCV group (ρ = 0.497, $P < 0.0001$). *Conclusions:* We demonstrated that liver cirrhosis and HCV infection were independent factors associated plasma active ghrelin levels. Moreover, plasma active ghrelin levels were positively correlated with serum albumin levels among HCV-infected patients. Therefore, active ghrelin levels may be regulated by both progression of liver disease and HCV infection and could be involved in the regulation of serum albumin levels in HCV-infected patients.

Various metabolic disorders are more frequently seen in patients with chronic hepatitis C virus (HCV) infection than in those with other hepatobiliary diseases (1, 2). Metabolic disorders including insulin resistance and hypoalbuminemia are risk factors for the development of hepatocellular carcinoma and oesophageal varices, and thus, for increased mortality (3, 4). These metabolic disorders are seen even in early stage chronic liver disease (1, 5) and are partly caused by HCV-induced metabolic dysregulation in multiple organs including the liver, pancreas and intestine (6–8).

Ghrelin, a gut hormone, predominantly secreted from X/A-like stomach cells, was originally identified as a potent growth hormone secretagogue (9). Ghrelin also regulates appetite and its downregulation is involved in the development of malnutrition (10–12). Basic studies have demonstrated that ghrelin plays a significant role in glucose metabolism, fatty acid beta oxidation, hepatic fibrosis and cell proliferation (13–17). Therefore, changes in ghrelin could be associated with various metabolic disorders in HCV-infected patients.

Ghrelin is secreted as a preprohormone; therefore, it must be activated to exert its biological effects, which are tightly regulated by a unique mechanism. Preproghrelin is acylated by ghrelin O-acyltransferase in the serine-3 residue. The acylated peptide is then cleaved by a processing protease prohormone convertase 1/3 to produce active ghrelin (18, 19). Thus, ghrelin is classified into 2 isoforms: des-acyl ghrelin (the inactive form) and acylated ghrelin, so-called "active ghrelin." In healthy adults, approximately 90% of serum ghrelin exists in the inactive form and 10% exists in the active form (20). Although several previous studies have shown inconsistent results regarding serum total ghrelin levels among patients with chronic liver diseases (12, 21–24), changes in plasma active ghrelin level and their clinical association with metabolic disorders have not yet been investigated in HCV-infected patients.

The aims of this study were to investigate changes in plasma active ghrelin levels and identify independent factors associated with plasma active ghrelin levels in HCV-infected patients.

Subjects and methods

Ethics statement

The study protocol was approved by The Ethics Committee of Kurume University. All experiments were carried out in accordance with the Declaration of Helsinki. No subjects were institutionalized.

Materials

All reagents were purchased from Wako Pure Chemical Industries (Osaka, Japan) unless otherwise indicated.

Patients

We enrolled consecutive patients with HCV-related liver disease (HCV; $n = 96$), hepatitis B virus (HBV)-related liver disease (HBV; $n = 20$), non-alcoholic fatty liver disease (NAFLD; $n = 49$) and healthy control subjects (CON; $n = 16$). All patients and controls were Asian. All diagnoses were based on clinical, serological and/or histological evidence. All data were collected on the same day as collecting blood. Body mass index (BMI) was calculated as body weight in kilograms divided by the square of height in metres (kg/m^2).

Laboratory tests

Venous blood samples were taken in the morning after a 12-h overnight fast. Plasma glucose, serum aspartate aminotransferase (AST), alanine aminotransferase, lactate dehydrogenase, alkaline phosphatase, gamma-glutamyl transpeptidase, total protein, albumin, prothrombin activity, blood glucose, immunoreactive insulin (IRI), haemoglobin A1c, total cholesterol, free fatty acids, total bilirubin, type IV collagen, hyaluronic acid, blood urea nitrogen, creatinine, alpha-foetoprotein and protein induced by vitamin K absence or antagonist-II levels were measured using standard clinical methods (Department of Clinical Laboratory, Kurume University Hospital) as previously described (25). Insulin resistance was calculated on the basis of fasting levels of plasma glucose and IRI using the homoeostasis model assessment for insulin resistance (HOMA-IR) equation: fasting glucose (mg/dl) × fasting IRI (mU/ml)/405 (26).

Determination of HCV genotype and measurement of HCV viral load

HCV genotyping was performed using Okamoto's method (27), and genotypes were classified according to Simmonds' classification system (28). An Amplicor-HCV-Monitor 1.0 (Roche Diagnostics K.K., Tokyo, Japan) was used to quantify HCV RNA levels.

Diagnosis of cirrhosis

Cirrhosis was diagnosed by liver histology or AST to platelet ratio index (APRI): serum AST level (U/L)/upper limit of normal AST (33 U/L) × 100/platelet count ($\times 10^4$/ml). APRI is a non-invasive index and can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. Patients with APRI values above 1.5 were diagnosed with cirrhosis as previously described (29). In this study, out of 96 patients in the HCV group, 18 patients were diagnosed by liver histology and the remaining 78 patients were diagnosed by APRI.

Plasma active ghrelin level assay

For measurement of plasma active ghrelin levels, 1 ml of blood was collected in a tube containing 1.25 mg of ethylenediaminetetraacetic acid and 500 KIU of aprotinin, a serine protease inhibitor. Each sample was immediately centrifuged at 1500*g* for 15 min at 4°C and treated with 10% vol/vol of 1 mM hydrochloric acid. Quantification of active ghrelin in plasma samples was accomplished with an Active Ghrelin ELISA Kit (SCETI K.K., Tokyo, Japan) that specifically quantifies active ghrelin. This ELISA kit consists of monoclonal antibodies for both the C-terminal and the acylated N-terminal of ghrelin. The absorbance (450 nm) of each well was then measured with a Bio-Rad Model 550 microplate reader (Bio-Rad, Hercules, CA, USA). Each serum sample was assayed in duplicate and the values were averaged.

Statistical analysis

All data are expressed as the mean ± standard deviation (SD). Statistical comparisons among multiple groups were performed by analysis of variance (ANOVA) followed by Scheffé's *post-hoc* test. Stepwise forward-selection multivariate linear regression analysis was used to identify any independent variables related to plasma active ghrelin levels, as previously described (30, 31). Spearman's correlation coefficient was calculated to test the relationship between different quantities in a bivariate regression model using JMP version 9.0 (SAS Institute, Cary, NC, USA). P values < 0.05 were considered significant.

Results

Patient characteristics

The characteristics of enrolled patients are summarized in Table 1. Although serum levels of AST and ALT were significantly higher in the NALFD, HBV and HCV groups than those in the CON group, these levels were not significantly different among the NAFLD, HBV and HCV groups. Serum albumin levels were significantly lower in the HBV and HCV groups than in the CON and NAFLD groups. Serum total cholesterol levels revealed significant depletion in the HCV group compared with the other groups. Significantly elevated

Table 1. Patient characteristics

	Reference value	CON	NAFLD	HBV	HCV
n	18.5–22	16	20	49	96
Age (yr)	N/A	67.5 ± 5.3	62.6 ± 10.8	57.3 ± 3.1*	63.5 ± 12.5
Sex (Female/Male)	N/A	7/9	12/8	29/20	58/38
BMI (m^2/kg)	18.5–22	22.3 ± 0.9	26.8 ± 2.2*	22.3 ± 2.8	23.5 ± 3.5
AST (U/L)	13–33	10.6 ± 3.4	35.5 ± 22.6†	31.5 ± 23.8†	47.9 ± 28.5†
ALT (U/L)	8–42	12.7 ± 5.1	41.2 ± 24.4†	35.9 ± 27.8†	45.2 ± 34.6†
LDH (U/L)	119–229	192 ± 51	201 ± 35	201 ± 52	210 ± 42
ALP (U/L)	115–359	242 ± 53	262 ± 106	261 ± 125	281 ± 119
GGT (U/L)	10–47	31.5 ± 17.2	51.6 ± 53.4†	42.2 ± 28.6†	53.2 ± 68.1†
Total protein (g/dl)	6.70–8.30	8.1 ± 0.5	7.7 ± 0.3	7.3 ± 0.5†	7.1 ± 0.6†
Albumin (g/dl)	4.00–5.00	4.3 ± 0.3	4.3 ± 0.5	3.8 ± 0.7‡	3.7 ± 0.6‡
Prothrombin activity (%)	60–130	105.2 ± 13.3	108.7 ± 25.0	94.0 ± 8.8‡	91.6 ± 19.3*
Total bilirubin (mg/dl)	0.30–1.50	0.6 ± 0.2	0.9 ± 0.5	0.8 ± 0.7	1.1 ± 0.5†
C-reactive protein (mg/dl)	<0.40	0.02 ± 0.02	0.03 ± 0.02	0.02 ± 0.01	0.02 ± 0.01
Total cholesterol (mg/dl)	128–220	197 ± 41	251 ± 66*	199 ± 42	163 ± 42*
Free fatty acids (μEq/L)	100–540	286 ± 75	383 ± 186†	375 ± 131†	665 ± 362*
Fasting blood glucose (mg/dl)	80–109	96 ± 5	121 ± 35†	103 ± 12†	109 ± 31†
Haemoglobin A1c (%)	4.3–5.8	5.3 ± 0.4	6.0 ± 0.7*	5.6 ± 0.4	5.3 ± 0.9
IRI (μIU/ml)	5.0–20.0	6.1 ± 2.3	9.5 ± 6.5§	7.5 ± 4.4	14.3 ± 8.5§
HOMA-IR	<2.5	1.3 ± 0.4	2.6 ± 1.6§	2.0 ± 1.1	3.8 ± 1.5§
BUN (mg/dl)	8.0–22.0	14.5 ± 7.1	14.2 ± 4.3	17.2 ± 2.8	15.8 ± 4.3
Creatinine (mg/dl)	0.4–0.7	0.6 ± 0.3	0.6 ± 0.2	0.7 ± 0.3	0.6 ± 0.2
Hyaluronic acid (ng/ml)	< 50	N/A	31.6 ± 12.5	98.5 ± 72.5‖	182.8 ± 155.2*
Type IV collagen (ng/ml)	< 140	N/A	88 ± 68	198 ± 121‖	252 ± 146*
AFP (ng/ml)	< 8.7	N/A	3.4 ± 1.2	7.8 ± 2.1‖	14.8 ± 36.2*
PIVKA-II (mAU/ml)	<40	N/A	18.5 ± 12.2	21.5 ± 16.2	28.5 ± 18.8

Data are expressed as mean ± SD or number of patients.

*$P < 0.05$ compared to all of other groups.

†$P < 0.05$ compared to the CON group.

‡$P < 0.05$ compared to the CON and NAFLD groups.

§$P < 0.05$ compared to the CON and HBV groups.

‖$P < 0.05$ compared to the HBV group.

CON, healthy control subjects; NAFLD, non-alcoholic fatty liver disease; HBV, hepatitis B virus; HCV, hepatitis C virus; N/A, not applicable; AST, aspartate transaminase; ALT, alanine aminotransferase; LDH, lactate dehydrogenase; ALP, alkaline phosphatase; GGT, γ-glutamyl transpeptidase; IRI, Immunoreactive insulin; HOMA-IR, homoeostasis model assessment for insulin resistance; BUN, blood urea nitrogen; AFP, α-foetoprotein; PIVKA-II, protein induced by vitamin K absence.

serum insulin and HOMA-IR levels were observed in the HCV group compared with those in the CON and HBV groups. Serum levels of hyaluronic acid and type IV collagen were significantly higher in the HCV group than in the other groups.

Changes in plasma active ghrelin levels in chronic liver disease

There were no significant differences in plasma active ghrelin levels among the CON, NAFLD and HBV groups. However, plasma active ghrelin levels were significantly lower in the HCV group than in the other groups (Fig. 1).

Changes in plasma active ghrelin levels in relation to the progression of liver disease

In the HBV groups, plasma active ghrelin levels were significantly decreased in relation to the severity of liver disease (compare with dotted lines in Fig. 2). Similarly, significant decreases in plasma active ghrelin levels in relation to severity of liver disease were seen in the HCV group (compare with dotted lines in Fig. 2).

The association of plasma active ghrelin levels with virological factors and disease severity in HCV-infected patients

No significant differences in plasma active ghrelin levels were seen among HCV genotypes 1b, 2a and 2b. There was no significant correlation between plasma active ghrelin levels and HCV viral load ($\rho = 0.589$, $P < 0.396$).

Stage-matched comparisons of plasma active ghrelin levels

In patients with chronic hepatitis, plasma active ghrelin levels were significantly lower in the HCV group than in the HBV or NAFLD group (compare with solid lines in

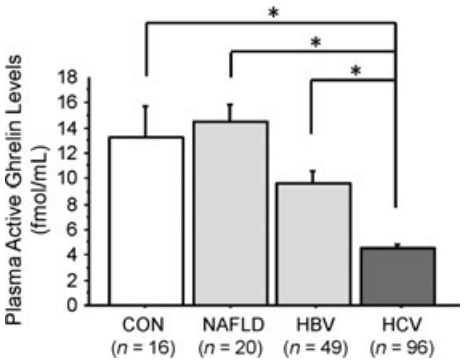

Fig. 1. Changes in plasma active ghrelin levels in the CON, NAFLD, HBV and HCV groups. Values are expressed as mean ± SD. Comparisons among groups were made using analysis of variance with Scheffé's *post-hoc* test. *, $P < 0.01$.

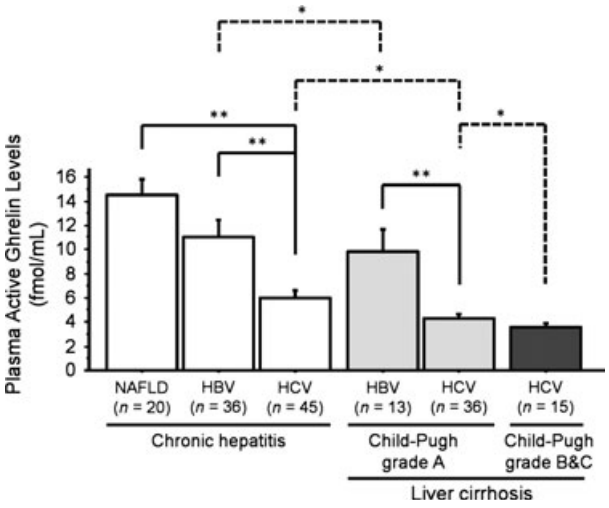

Fig. 2. Changes in plasma active ghrelin levels stratified by aetiology and progression of liver disease. Values are expressed as mean ± SD. Comparisons among the groups were made using analysis of variance with Scheffé's *post-hoc* test. *, $P < 0.05$; **, $P < 0.01$.

Fig. 2). In cirrhotic patients with Child-Pugh grade A, a significant decrease in plasma active ghrelin levels was also seen in the HCV group compared with the HBV group (compare with solid lines in Fig. 2).

Multivariate analysis of plasma active ghrelin levels

In the multivariate analysis, the presence of cirrhosis and low serum albumin levels were independent factors associated with decreased plasma active ghrelin levels (Table 2). In addition, the presence of HCV infection was identified as the most significant independent factor associated with lowering of plasma active ghrelin levels (Table 2).

Table 2. Multivariate analysis of plasma active ghrelin levels

Variable	Odds ratio	95% confidence interval	*P* value
Liver cirrhosis	−1.82	−0.37 to −4.33	0.0359
Serum albumin levels	2.39	1.32 to 7.95	0.0251
HCV infection	−3.05	−0.93 to −19.51	0.0192

HCV, hepatitis C virus.

Correlations between plasma active ghrelin levels and metabolic parameters in HCV-infected patients

A significant negative correlation was observed between plasma active ghrelin levels and BMI ($\rho = -0.137$, $P = 0.0171$) (Table 3). Although plasma active ghrelin levels were not significantly correlated with the parameters indicated in Table 3, plasma active ghrelin levels tend to be correlated with total cholesterol, total bilirubin, type IV collagen and creatinine levels. A significant positive correlation was seen only between plasma active ghrelin levels and serum albumin levels ($\rho = 0.497$, $P < 0.0001$) (Table 3 and Fig. 3).

Discussion

In this study, we demonstrated that plasma active ghrelin levels were significantly decreased in relation to the progression of liver disease in both HBV- and HCV-infected patients. In addition, HCV infection was identified as the most significant factor associated with decreased plasma active ghrelin levels, independent of the presence of cirrhosis and serum albumin levels. In HCV-infected patients, plasma active ghrelin levels were positively correlated with serum albumin levels. Taken together, these findings suggest that HCV itself, along with cirrhosis, may be involved in decreasing plasma active ghrelin levels, which could be linked to serum albumin levels.

In this study, plasma active ghrelin levels were significantly lower in the HCV group than in the other groups. To assess the effect of advanced liver disease on the plasma ghrelin levels, we performed stratified analyses based on the severity of liver disease. The results indicated that plasma active ghrelin levels significantly decreased in relation to the severity of liver disease in the HBV and HCV groups. The presence of cirrhosis was also identified as an independent factor associated with plasma active ghrelin levels in multivariate analysis. Although changes in total ghrelin levels in cirrhosis remain a controversial subject (12, 21, 24), we have demonstrated that active ghrelin was decreased in patients with cirrhosis. The causal relationship between advanced cirrhosis and changes in active ghrelin levels remains unclear. Plasma active ghrelin levels are regulated by production of preproghrelin or activation of ghrelin (11, 32, 33). Therefore, it is likely that chronic liver injury may change X/A-like stomach cells and

Table 3. Correlations between plasma active ghrelin levels and variables

Variables	ρ	*P*
Age	0.048	0.6972
BMI	−0.137	0.0171
AST	−0.068	0.5083
ALT	0.021	0.8371
LDH	0.093	0.3660
ALP	−0.139	0.1788
GGT	0.142	0.1674
Total protein	−0.049	0.6323
Albumin	0.497	<0.0001
Prothrombin activity	0.163	0.1621
Total bilirubin	−0.210	0.0533
Total cholesterol	0.194	0.0595
Free fatty acids	−0.190	0.4469
Fasting blood glucose	−0.142	0.1682
Haemoglobin A1c	0.162	0.1201
IRI	0.160	0.2591
HOMA-IR	0.120	0.3968
BUN	0.077	0.4508
Creatinine	0.182	0.0766
Type IV collagen	0.378	0.0639
Hyaluronic acid	0.273	0.2910
AFP	0.106	0.3022
PIVKA-II	0.044	0.6835

BMI, body mass index; AST, aspartate transaminase; ALT, alanine aminotransferase; LDH, lactate dehydrogenase; ALP, alkaline phosphatase; GGT, γ-glutamyl transpeptidase; IRI, Immunoreactive insulin; HOMA-IR, homoeostasis model assessment for insulin resistance; BUN, blood urea nitrogen; AFP, α-foetoprotein; PIVKA-II, protein induced by vitamin K absence.

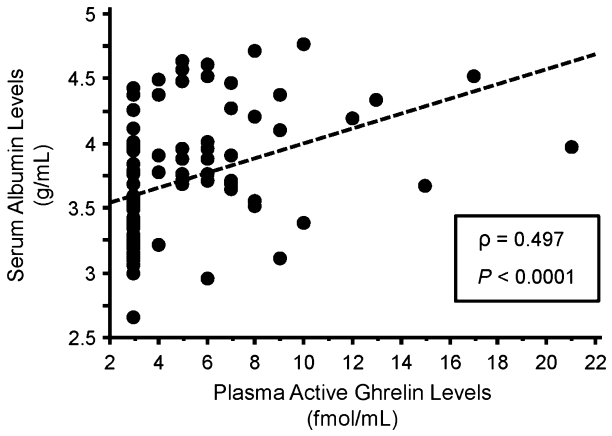

Fig. 3. The association between plasma active ghrelin levels and serum albumin levels. This association was analysed using the Spearman's correlation coefficient in a bivariate regression model.

ghrelin-O-acyl transferase and/or prohormone convertase 1/3.

We also examined the effect of HCV infection on plasma ghrelin levels. HCV genotype and HCV viral load were not associated with plasma active ghrelin levels, however; plasma active ghrelin levels were significantly lower in the HCV group than in the NAFLD or HBV group in stage-matched analyses. Moreover, HCV infection was identified as the most significant factor associated with lowering of plasma active ghrelin levels, independent of the presence of cirrhosis. These findings suggest that HCV itself may be involved in decreasing plasma active ghrelin levels. Although the mechanisms of HCV-related decreases in plasma ghrelin levels remain unclear, serum leptin levels are known to be high in HCV-infected patients (34, 35). Given that leptin is a potent inhibitor of ghrelin secretion (36, 37), HCV may suppress ghrelin secretion through up-regulation of leptin.

Various metabolic disorders are frequently seen in cirrhotic patients with HCV infection (38, 39). Therefore, we investigated the correlation between plasma active ghrelin levels and various metabolic parameters in the HCV group. Consistent with the previous reports (40, 41), in this study, plasma active ghrelin levels were found to be negatively correlated with BMI. These changes in plasma active ghrelin levels may represent a physiological adaptation to the positive energy balance associated with obesity. Furthermore, albumin was the only serum parameter, which significantly positively correlated with plasma active ghrelin levels in this study. Serum albumin is a representative marker for both protein metabolism and the severity of liver disease (42); however, other protein metabolism parameters such as total protein level and prothrombin activity were not correlated with plasma active ghrelin levels in this study. Ghrelin is a potent stimulator for secretion of growth hormone (33) and also elicits marked up-regulation of the mammalian target of rapamycin (mTOR) signalling pathway (43). Both growth hormone and activation of mTOR signalling up-regulate albumin synthesis (39, 44). Taken together, these findings suggest that active ghrelin has the novel biological effect on specifically regulating albumin synthesis in hepatocytes.

One limitation of this study is that we did not investigate changes in HCV or HBV carriers with persistently normal ALT levels. As these data may further elucidate the mechanisms underlying the decrease in plasma active ghrelin in patients with liver disease, further study should be conducted while bearing these points in mind.

In conclusion, this study showed that plasma active ghrelin levels were significantly decreased with the progression of liver disease in both HBV- and HCV-infected patients. Moreover, HCV infection was identified as the most significant factor associated with decreased plasma active ghrelin levels, independent of cirrhosis. Furthermore, plasma active ghrelin levels were positively correlated with serum albumin levels in HCV-infected patients. These findings indicated that active ghrelin may be regulated by both the progression of liver disease and HCV infection, and could be related to serum albumin levels.

Acknowledgements

Financial support: This study was supported by Health and Labour Sciences Research Grants for Research on Hepatitis from the Ministry of Health, Labour and Welfare of Japan, and by a Grant from the Ishibashi Foundation for the Promotion of Science, Japan. The funders had no role in study design, data collection and analysis, decision to publish or preparation of the manuscript.

References

1. Kawaguchi T, Yoshida T, Harada M, *et al.* Hepatitis C virus down-regulates insulin receptor substrates 1 and 2 through up-regulation of suppressor of cytokine signaling 3. *Am J Pathol* 2004; **165**: 1499–508.
2. Sheikh MY, Choi J, Qadri I, Friedman JE, Sanyal AJ. Hepatitis C virus infection: molecular pathways to metabolic syndrome. *Hepatology* 2008; **47**: 2127–33.
3. Eslam M, Kawaguchi T, Del Campo JA, *et al.* Use of HOMA-IR in hepatitis C. *J Viral Hepat* 2011; **18**: 675–84.
4. Nagao Y, Sata M. Serum albumin and mortality risk in a hyperendemic area of HCV infection in Japan. *Virol J* 2010; **7**: 375.
5. Nishimura M, Yamamoto H, Yoshida T, *et al.* Decreases in the serum VLDL-TG/non-VLDL-TG ratio from early stages of chronic hepatitis C: alterations in TG-rich lipoprotein levels. *PLoS ONE* 2011; **6**: e17309.
6. Narita R, Abe S, Kihara Y, *et al.* Insulin resistance and insulin secretion in chronic hepatitis C virus infection. *J Hepatol* 2004; **41**: 132–8.
7. Itou M, Kawaguchi T, Taniguchi E, *et al.* Altered expression of glucagon-like peptide-1 and dipeptidyl peptidase IV in patients with HCV-related glucose intolerance. *J Gastroenterol Hepatol* 2008; **23**: 244–51.
8. Sakata M, Kawahara A, Kawaguchi T, *et al.* Decreased expression of insulin and increased expression of pancreatic transcription factor PDX-1 in islets in patients with liver cirrhosis: a comparative investigation using human autopsy specimens. *J Gastroenterol* 2013; **48**: 277–85.
9. Kojima M, Hosoda H, Date Y, *et al.* Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature* 1999; **402**: 656–60.
10. Kojima M, Hosoda H, Kangawa K. Clinical endocrinology and metabolism Ghrelin, a novel growth-hormone-releasing and appetite-stimulating peptide from stomach. *Best Pract Res Clin Endocrinol Metab* 2004; **18**: 517–30.
11. Kojima M, Kangawa K. Drug insight: The functions of ghrelin and its potential as a multitherapeutic hormone. *Nat Clin Pract Endocrinol Metab* 2006; **2**: 80–8.
12. Takahashi H, Kato A, Onodera K, Suzuki K. Fasting plasma ghrelin levels reflect malnutrition state in patients with liver cirrhosis. *Hepatol Res* 2006; **34**: 117–23.
13. Cao C, Chen Y, Wang W, Liu Y, Liu G. Ghrelin inhibits insulin resistance induced by glucotoxicity and lipotoxicity in cardiomyocyte. *Peptides* 2011; **32**: 209–15.
14. Moreno M, Chaves JF, Sancho-Bru P, *et al.* Ghrelin attenuates hepatocellular injury and liver fibrogenesis in rodents and influences fibrosis progression in humans. *Hepatology* 2010; **51**: 974–85.
15. Rak-Mardyla A, Gregoraszczuk EL. ERK 1/2 and PI-3 kinase pathways as a potential mechanism of ghrelin action on cell proliferation and apoptosis in the porcine ovarian follicular cells. *J Physiol Pharmacol* 2010; **61**: 451–8.
16. Rigault C, Le Borgne F, Georges B, Demarquoy J. Ghrelin reduces hepatic mitochondrial fatty acid beta oxidation. *J Endocrinol Invest* 2007; **30**: RC4–8.
17. Gauna C, Delhanty PJ, Hofland LJ, *et al.* Ghrelin stimulates, whereas des-octanoyl ghrelin inhibits, glucose output by primary hepatocytes. *J Clin Endocrinol Metab* 2005; **90**: 1055–60.
18. Takahashi T, Ida T, Sato T, *et al.* Production of n-octanoyl-modified ghrelin in cultured cells requires prohormone processing protease and ghrelin O-acyltransferase, as well as n-octanoic acid. *J Biochem* 2009; **146**: 675–82.
19. Sato T, Nakamura Y, Shiimura Y, *et al.* Structure, regulation and function of ghrelin. *J Biochem* 2012; **151**: 119–28.
20. Goodyear SJ, Mottershead M, Sung EZ, *et al.* Dysregulation of plasma ghrelin in alcoholic cirrhosis. *Clin Endocrinol (Oxf)* 2010; **73**: 323–9.
21. Tacke F, Brabant G, Kruck E, *et al.* Ghrelin in chronic liver disease. *J Hepatol* 2003; **38**: 447–54.
22. Diz-Lois MT, Garcia-Buela J, Suarez F, *et al.* Fasting and postprandial plasma ghrelin levels are decreased in patients with liver failure previous to liver transplantation. *Endocrine* 2009; **35**: 467–76.
23. Marchesini G, Bianchi G, Lucidi P, *et al.* Plasma ghrelin concentrations, food intake, and anorexia in liver failure. *J Clin Endocrinol Metab* 2004; **89**: 2136–41.
24. Kalaitzakis E, Bosaeus I, Ohman L, Bjornsson E. Altered postprandial glucose, insulin, leptin, and ghrelin in liver cirrhosis: correlations with energy intake and resting energy expenditure. *Am J Clin Nutr* 2007; **85**: 808–15.
25. Kawaguchi T, Sakisaka S, Sata M, Mori M, Tanikawa K. Different lobular distributions of altered hepatocyte tight junctions in rat models of intrahepatic and extrahepatic cholestasis. *Hepatology* 1999; **29**: 205–16.
26. Matthews DR, Hosker JP, Rudenski AS, *et al.* Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985; **28**: 412–9.
27. Okamoto H, Sugiyama Y, Okada S, *et al.* Typing hepatitis C virus by polymerase chain reaction with type-specific primers: application to clinical surveys and tracing infectious sources. *J Gen Virol* 1992; **3**: 673–9.
28. Simmonds P, Holmes EC, Cha TA, *et al.* Classification of hepatitis C virus into six major genotypes and a series of subtypes by phylogenetic analysis of the NS-5 region. *J Gen Virol* 1993; **11**: 2391–9.
29. Wai CT, Greenson JK, Fontana RJ, *et al.* A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. *Hepatology* 2003; **38**: 518–26.
30. Otsuka M, Uchida Y, Kawaguchi T, *et al.* Fish to meat intake ratio and cooking oils are associated with hepatitis C virus carriers with persistently normal alanine aminotransferase levels. *Hepatology Res* 2012; **42**: 982–9.
31. Taniguchi E, Kawaguchi T, Sakata M, *et al.* Lipid profile is associated with the incidence of cognitive dysfunction in viral cirrhotic patients: a data-mining analysis. *Hepatol Res* 2013; **43**: 418–24.
32. Kojima M, Kangawa K. Ghrelin: more than endogenous growth hormone secretagogue. *Ann NY Acad Sci* 2010; **1200**: 140–8.

33. Sato T, Nakamura Y, Shiimura Y, *et al.* Structure, regulation and function of ghrelin. *J Biochem* 2012; **151**: 119–28.
34. Giannini E, Botta F, Cataldi A, *et al.* Leptin levels in non-alcoholic steatohepatitis and chronic hepatitis C. *Hepato-gastroenterology* 1999; **46**: 2422–5.
35. Eguchi Y, Mizuta T, Yasutake T, *et al.* High serum leptin is an independent risk factor for non-response patients with low viremia to antiviral treatment in chronic hepatitis C. *World journal of gastroenterol* 2006; **12**: 556–60.
36. Meier U, Gressner AM. Endocrine regulation of energy metabolism: review of pathobiochemical and clinical chemical aspects of leptin, ghrelin, adiponectin, and resistin. *Clin Chem* 2004; **50**: 1511–25.
37. Kalra SP, Ueno N, Kalra PS. Stimulation of appetite by ghrelin is regulated by leptin restraint: peripheral and central sites of action. *J Nutr* 2005; **135**: 1331–5.
38. Angeli P, Wong F, Watson H, Gines P. Hyponatremia in cirrhosis: results of a patient population survey. *Hepatology* 2006; **44**: 1535–42.
39. Kawaguchi T, Izumi N, Charlton MR, Sata M. Branched-chain amino acids as pharmacological nutrients in chronic liver disease. *Hepatology* 2011; **54**: 1063–70.
40. Tschop M, Weyer C, Tataranni PA, *et al.* Circulating ghrelin levels are decreased in human obesity. *Diabetes* 2001; **50**: 707–9.
41. Shiiya T, Nakazato M, Mizuta M, *et al.* Plasma ghrelin levels in lean and obese humans and the effect of glucose on ghrelin secretion. *J Clin Endocrinol Metab* 2002; **87**: 240–4.
42. Quinlan GJ, Martin GS, Evans TW. Albumin: biochemical properties and therapeutic potential. *Hepatology* 2005; **41**: 1211–9.
43. Martins L, Fernandez-Mallo D, Novelle MG, *et al.* Hypothalamic mTOR Signaling Mediates the Orexigenic Action of Ghrelin. *PLoS ONE* 2012; **7**: e46923.
44. Zachwieja JJ, Bier DM, Yarasheski KE. Growth hormone administration in older adults: effects on albumin synthesis. *Am J Physiol* 1994; **266**: E840–4.