The level of fasting serum insulin, but not adiponectin, is associated with the prognosis of early stage hepatocellular carcinoma

SATOSHI MIUMA[1], TATSUKI ICHIKAWA[1], NAOTA TAURA[2], HIDETAKA SHIBATA[1], SHIGEYUKI TAKESHITA[1], MOTOHISA AKIYAMA[1], YASUHIDE MOTOYOSHI[1], EISUKE OZAWA[1], MASUMI FUJIMOTO[1], HIROSHI KAWASHIMO[1], HISAMITSU MIYAAKI[1], KATSUMI EGUCHI[1] and KAZUHIKO NAKAO[1]

[1]Department of Gastroenterology and Hepatology Graduate School of Biomedical Sciences, Nagasaki University, Sakamoto 1-7-1, Nagasaki 852-8501; [2]Clinical Research Center, National Nagasaki Medical Center, Kubara 2-1001-1, Omura, Nagasaki 856-8562, Japan

Received July 10, 2009; Accepted August 26, 2009

DOI: 10.3892/or_00000583

Abstract. Impaired glucose tolerance influences the prognosis of hepatocellular carcinoma (HCC), but this mechanism is still not fully understood. We investigated the impact of the fasting serum levels of insulin and adiponectin on the prognosis of HCC and its recurrence. One hundred and forty patients with newly diagnosed HCC were enrolled in the prognosis study. Their fasting serum levels of insulin and adiponectin were determined. Of 140 patients, 59 patients who underwent curative treatment were subjected to analysis of the recurrence-free survival. The 140 patients were divided into two groups by the 50th percentile value of insulin (7.73 μIU/ml) or total adiponectin (6.95 μg/ml). Kaplan-Meier analysis indicated that high insulin group (>7.73 μIU/ml) exhibited a significantly poorer prognosis than low insulin group (<7.73 μIU/ml) in early stage HCC (P=0.018). In contrast, the level of total adiponectin had no impact on the prognosis of HCC. Multivariate analysis indicated that fasting hyperinsulinemia was an independent risk factor for a poorer prognosis in early stage HCC (P=0.044). Likewise, Kaplan-Meier analysis indicated that the recurrence-free survival of high insulin group was significantly lower than that of low insulin group (P=0.017). The level of total adiponectin had no impact on the recurrence-free survival of HCC. Multivariate analysis indicated that fasting hyperinsulinemia was an independent risk factor for the lower recurrence-free survival of HCC (P=0.049). In conclusion, our study suggests that the fasting insulin level affects the clinical course of early stage HCC.

Correspondence to: Dr Satoshi Miuma, Department of Gastroenterology and Hepatology Graduate School of Biomedical Sciences, Nagasaki University, Sakamoto 1-7-1, Nagasaki 852-8501, Japan
E-mail: miuma-gi@umin.net

Key words: hepatocellular carcinoma, insulin, adiponectin, prognosis, recurrence

Introduction

Hepatocellular carcinoma (HCC) is the fifth most frequent malignant neoplasm in the world (1). Extensive evidence of the rising incidence of HCC has been reported in the United States, Japan, and several other countries (2,3). In addition to chronic infection by the hepatitis C virus (HCV), diabetes mellitus (DM) is thought to be a rising risk factor of note, because it is associated with nonalcoholic fatty liver disease (NAFLD) including its severe form, nonalcoholic steatohepatitis (NASH) (4). NASH is a chronic necroinflammatory condition that can lead to liver fibrosis, cirrhosis, and subsequently to HCC (5,6). In earlier studies it was suggested that there was no evidence linking DM to HCC, whereas more recent studies have indicated the association between DM and HCC (6-9). Moreover, several studies have reported that the coexistence of DM in chronic liver disease caused by chronic infection of HBV and HCV is closely related to the risk of not only the development of HCC, but also a poor prognosis (10,11). However, it is not clear why coexisting DM influences the development and progression of HCC in chronic liver disease.

The abnormality of the glucose metabolism found in chronic liver disease is due to the existence of a decline of insulin degradation, and peripheral insulin resistance (12). We have also reported that the developing of liver fibrosis is closely associated with insulin resistance in HCV infected patients (13). Taken together, it is likely that insulin resistance in chronic liver disease triggers hyperinsulinemia, and it may modulate the biological characteristics of HCC cells. It has been reported that insulin displays growth promoting and anti-apoptotic effects on human hepatoma cells *in vitro* and in animal models (10,14,15). Moreover, Saito *et al* have

previously reported that postprandial hyperinsulinemia is associated with the accelerated growth of HCC (16).

Recently, adiponectin, a physiologically active polypeptide secreted exclusively by adipose tissue, has been the focus of research interest as a factor involved in glucose metabolism. This hormone has a potent insulin-sensitizing effect (17-19), and a low level of circulating adiponectin is found in several types of metabolic syndrome including insulin resistance and type 2 diabetes (20). Several studies have reported the association between the values of circulating adiponectin and liver disease. Xu *et al* have reported that adiponectin administration alleviates hepatomegaly and steatosis and also significantly attenuates the inflammation and the elevated levels of serum alanine aminotransferase in alcoholic and nonalcoholic fatty liver murine models (21). In humans, the level of circulating adiponectin has been found to increase in patients with advanced cirrhosis (22-24). In addition, Hui *et al* reported that the level of serum adiponectin increases in advancing liver fibrosis and declines with a reduction in fibrosis in chronic hepatitis B (25).

The aim of the present study was to clarify whether the levels of fasting serum insulin and adiponectin are relevant to the prognosis of HCC in patients newly diagnosed to have HCC and the recurrence of HCC in those who underwent curative therapy.

Patients and methods

Patients. A total of 140 patients, who were newly diagnosed to have HCC between January 1995 and December 2004 at the First Department of Internal Medicine in Nagasaki University Hospital and fulfilled the criteria specified below, were enrolled in the current cohort. The inclusion criteria were: 1) not diagnosed as having DM on admission, in other words, no dietary intervention and no regular use of medication to affect insulin sensitivity or insulin secretion, 2) on admission a fasting serum sample was drawn and stored, and 3) no life-threatening illness other than liver disease.

The diagnosis of HCC was based on the typical findings detected by ultrasonography (US), dynamic computerized tomography (CT), magnetic resonance imaging (MRI), abdominal angiography, and/or histological manifestation of liver tumor. Underlying liver diseases, such as chronic hepatitis (CH) and liver cirrhosis (LC) were diagnosed by liver histologic examination following a liver biopsy and/or by the findings of US, dynamic CT, and MRI. LC was graded according to the Child-Pugh classification (26). The body mass index (BMI) was calculated as body weight in kilograms divided by the square of the height in meters (kg/m^2). The alcohol intake was assessed by interview and recorded in grams per day. The patients were divided into two groups according to the mean alcohol consumption per day; not excessive drinkers (<80 g/day) and excessive drinkers (≥80 g/day). Fasting blood samples were obtained in the early morning for an analysis of biochemical and hematological data or fasting blood glucose, and serum samples were stored at -80˚C until further assay. Hepatitis B surface antigen (HBsAg) and hepatitis C virus antibody (HCVAb) were tested by commercial immunoassays (Fuji Rebio, Tokyo). The serum AFP level was measured by enzyme immunoassay (AxSYMAFP, Abbott Japan, Tokyo).

Table I. Clinical and laboratory characteristics of the study subjects.

Variable	Number or mean (SD)
Patients	140
Onset age, y.o.	65.1 (9.5)
Gender	
Male	110
Female	30
BMI, kg/m^2	23.1 (3.1)
Alcohol intake	
<80 g/day	108
≥80 g/day	32
Etiology	
HBsAg(+)	29
HCVAb(+)	99
non-B, non-C	12
Underling liver diseases and Child-Pugh grade	
CH	30
LC grade A	72
LC grade B	31
LC grade C	7
Total bilirubin, mg/dl	1.4 (2.2)
Ferritin, ng/ml	304.2 (345.2)
Serum iron, μg/ml	151 (75)
Fasting insulin, μIU/ml	10.0 (9.6)
Fasting blood glucose, mg/dl (n=62)	102.7 (51.5)
HOMA-R, % (n=28)	3.0 (2.6)
Total adiponectin, μg/m	8.1 (4.8)
HMW, μg/ml	3.9 (3.0)
MMW, μg/ml	1.8 (1.2)
LMW, μg/ml	2.4 (1.3

Measurement of insulin and adiponectin. Fasting serum samples stored at -80˚C were used for the assay. Fasting serum insulin was measured by enzyme immunoassay (Fuji Rebio). Fasting total adiponectin was measured by an enzyme immunoassay (Daiichi Pure Chemicals Co., Ltd., Tokyo). Serum adiponectin exists in a complex form and is classified according to its molecular weight. Therefore, high molecular weight (HMW), middle molecular weight (MMW), and low molecular weight (LMW) adiponectins were also measured separately by enzyme immunoassay (Daiichi Pure Chemicals Co.).

HCC assessment. The size and number of HCCs were confirmed by US, CT, MRI, or angiography. We used the

Table II. HCC characteristics of the study subjects.

Variable	Number or mean (SD)
Tumor size, cm	3.4 (2.8)
<2 cm	57
2 - <5 cm	62
≥5 cm	21
Number of tumor lesions	
1	75
2	29
3 - and diffuse	36
TNM stage	
I	39
II	53
III	37
IV	11
AFP, ng/ml	7792.9 (62230.8)
Therapy	
Surgical resection	7
PEIT and/or RFA	53
TACE or TAI	73
Others	7

tumor-node-metastasis (TNM) classification system of the Liver Cancer Study Group (LCSG) of Japan in 2000 (27). The T category is determined by the 3 factors of number, size, and vascular or bile duct invasion. The N category is the presence of lymph node metastasis, and the M category is the presence of distant metastasis. TNM staging has four stages according to the T, N, and M categories. The therapy for HCC was divided into four groups; the surgical resection group, percutaneous ethanol injection therapy (PEIT) and/or radiofrequency ablation (RFA) group, transcatheter arterial chemoembolization (TACE) or transarterial infusion (TAI) group, and other therapy or palliative therapy group. In this study, the curative therapy was defined as a condition characterized by the no findings of recurrence over six months after the initial therapy for HCC, including surgical resection, PEIT, RFA, and TACE or TAI.

Statistical analysis. The data were analyzed by the Mann-Whitney test for continuous ordinal data, χ^2 test with Yates' correction and Fischer exact test for the association between 2 qualitative variables, and Kaplan-Meier survival analysis. Parametric comparisons were assessed by analyses of variance. The significance of individual differences was evaluated by use of Scheffe's test. $P<0.05$ was considered to be statistically significant.

Results

Clinical features of studied patients. A total of 140 HCC patients were enrolled in this study. Patient characteristics are presented in Table I. There were 110 men (78.6%) and 30 women (21.4%). The mean age was 65.1 years. The mean BMI was 23.1 kg/m^2. Excessive drinkers comprised 22.9% (32 of 140) and not excessive was 77.1% (108 of 140). Patients who were HBsAg positive was 20.7% (29 of 140), whereas 70.7% (99 of 140) were HCVAb positive, and 8.6% (12 of 140) were negative for both (non-B, non-C). Chronic hepatitis (CH) was 21.4% (30 of 140). Liver cirrhosis (LC) was 78.6% (110 of 140). The Child-Pugh grade of LC patients was: grade A: 51.4% (72 of 140), grade B: 22.1% (31 of 140), and grade C: 5.0% (7 of 140). The mean level of total bilirubin was 1.4 mg/dl. The mean levels of ferritin and serum iron were 304.2 ng/ml and 151 μg/ml, respectively. The mean level of fasting insulin was 10.0 μIU/ml. The mean level of fasting blood sugar in 62 patients measured during the hospital stay was 102.7 mg/dl. In a similar fashion, the level of HOMA-R in the 28 patients calculated was 3.0%. The mean values of total, HMW, MMW, and LMW adiponectins were 8.1, 3.9, 1.8 and 2.4 μg/ml, respectively.

The characteristics of newly diagnosed HCC on admission are presented in Table II. The mean size of HCC was 3.4 cm and its distribution was: <2 cm: 57 (40.7%), ≥2 cm and <5 cm: 62 (44.3%), and ≥5 cm: 21 (15.0%). The number of HCCs in the subjects was: 1: 75 (53.6%), 2: 29 (20.7%), and 3 or more and diffuse: 36 (25.7%). The TNM stage of the HCC was: stage I: 39 (27.9%), stage II: 53 (37.9%), stage III: 37 (26.4%), and stage IV: 11 (7.9%). The mean level of AFP was 7792.9 ng/ml. Of the studied patients, 5.0% (7 of 140) underwent surgical resection, 37.9% (53 of 140) underwent PEIT and/or RFA, 52.1% (73 of 140) underwent TACE or TAI, and 5.0% (7 of 140) received other therapy or palliative care only.

The values of fasting insulin and adiponectin in subjects. We evaluated the values of fasting insulin and total adiponectin in underlying liver diseases or in the TNM stage of HCC. The mean values of insulin of CH, LC with Child-Pugh grade A, B, and C were 6.9, 10.3, 11.9, and 12.7 μIU/ml, respectively (Fig. 1A). The mean value of insulin seemed to increase in LC (Child-Pugh grade A, B, C) compared to CH, but no significant differences were observed between them. The mean values of insulin of TNM stage I, II, III, and IV were 8.1, 11.1, 11.5, and 7.1 μIU/ml, respectively. No significant differences were observed in these groups.

The mean values of total adiponectin of CH, LC with Child-Pugh grade A, B, and C were 6.4, 7.7, 9.5, and 13.4 μg/ml, respectively (Fig. 1B). In parallel with the decline of liver function, the mean value of total adiponectin increased obviously, and the mean value of total adiponectin of LC with Child-Pugh grade C showed a significantly higher level than that of CH and LC with Child-Pugh grade A. In contrast, the mean values of total adiponectin of TNM stage I, II, III, and IV were 8.5, 8.0, 8.3, and 6.6 μg/ml, and there were no significant differences.

Association of fasting insulin level with prognosis of HCC. To evaluate the association of fasting insulin level with the prognosis of HCC, the 140 patients were divided into two groups in terms of the 50th percentile of the value of insulin (7.73 μIU/ml). The mean level of insulin in the low insulin

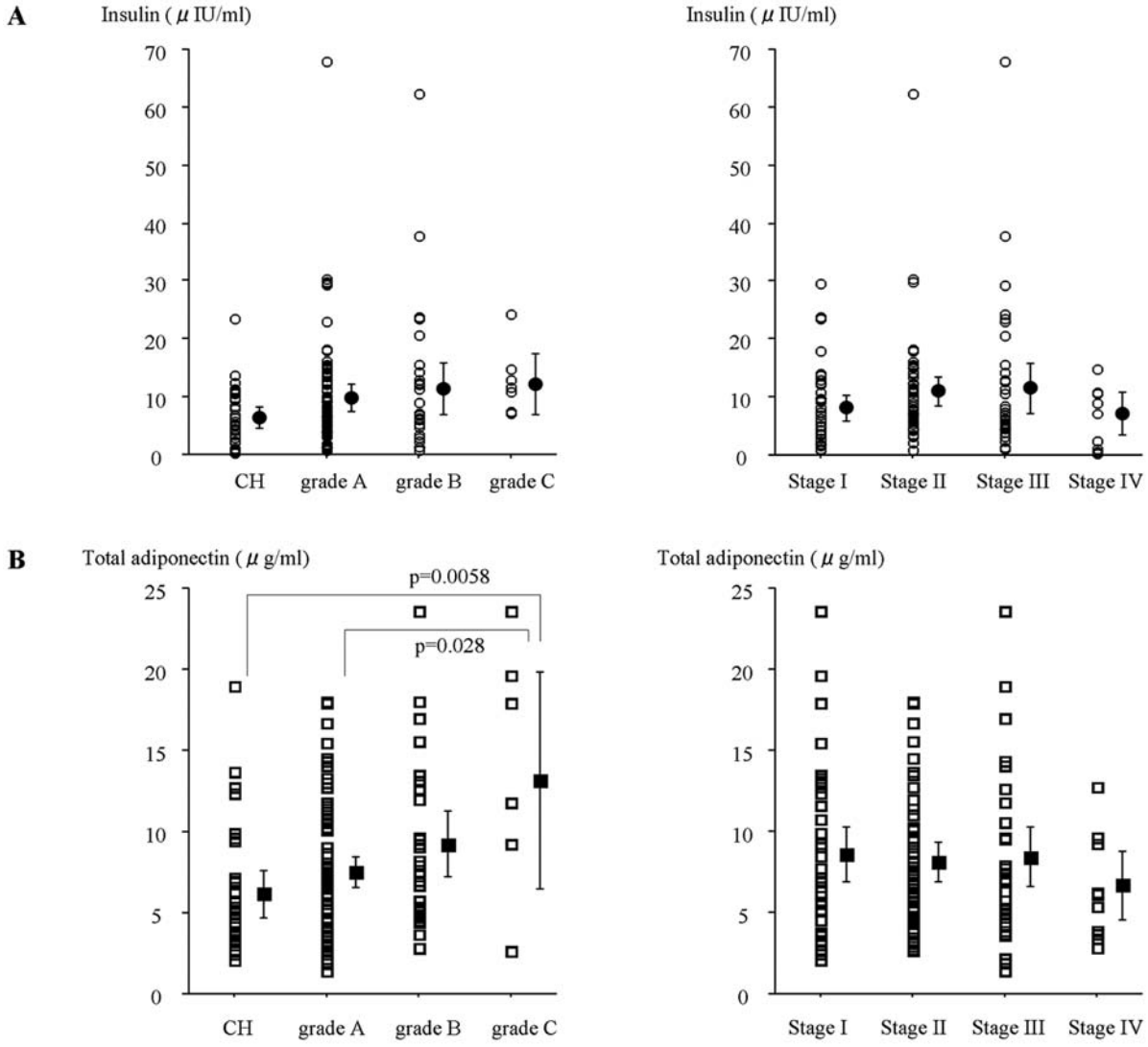

Figure 1. The mean ± SD values of fasting insulin in each underlying liver disease (CH and Child-Pugh grade) (A) and TNM stage (B), the mean ± SD values of total adiponectin in each underlying liver disease (CH and Child-Pugh grade) (C) and TNM stage (D).

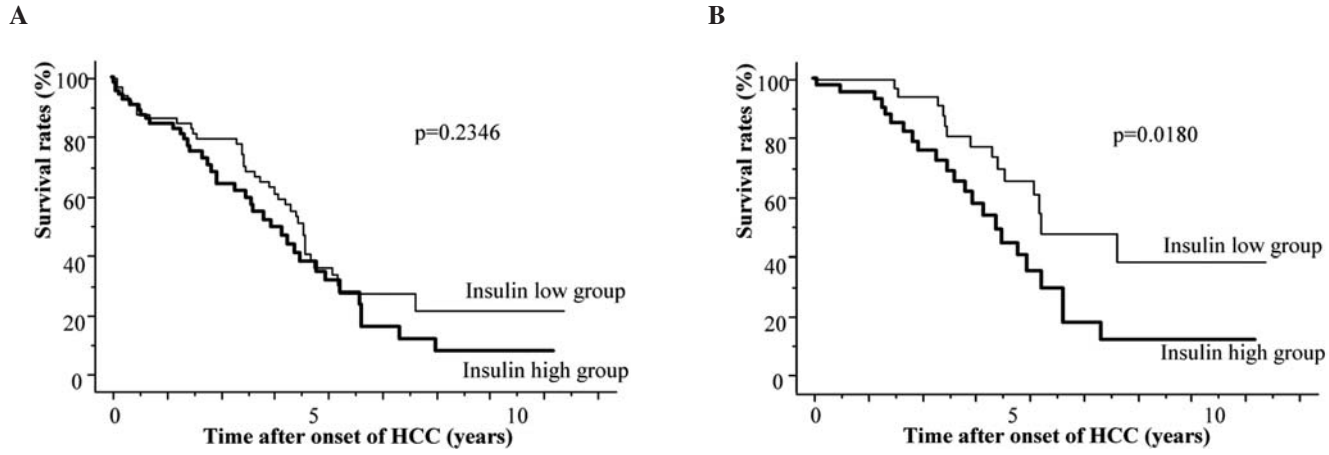

Figure 2. Kaplan-Meier curves for survival between low insulin group (thin line) and high insulin group (heavy line) in all stages of HCC patients (n=140) (A) and in TNM stage I + II HCC patients (n=92) (B).

group (<7.73 μIU/ml, n=70) was 4.3 μIU/ml. On the other hand, that in the high insulin group (>7.73 μIU/ml, n=70) was 15.8 μIU/ml. Table III shows the comparison of patient characteristics between the low and high insulin groups. The BMI in the high insulin group was significantly higher than it was in the low insulin group. The HOMA-R level was

Table III. Comparison of characteristics between low insulin group and high insulin group.

Variable	Number or mean (SD)		P
	Low insulin group (n=70)	High insulin group (n=70)	
Onset age, y.o.	64.7 (9.5)	65.5 (9.5)	0.620
Gender			0.999
Male	55	55	
Female	15	15	
BMI, kg/m^2	22.5 (3.0)	23.7 (3.0)	0.019
Alcohol intake			0.687
<80 g/day	53	55	
≥80 g/day	17	15	
Etiology			0.818
HBsAg(+)	16	13	
HCVAb(+)	48	51	
Non-B, non-C	6	6	
Underlying liver diseases and Child-Pugh grade			0.539
CH	17	13	
LC grade A	37	35	
LC grade B	14	17	
LC grade C	2	5	
Total bilirubin, ng/ml	1.5 (2.9)	1.3 (1.3)	0.634
Ferritin, ng/ml	305.2 (351.7)	303.2 (341.2)	0.974
Serum iron, μg/ml	155 (77)	148 (74)	0.571
Fasting insulin, μIU/ml	4.3 (2.2)	15.8 (10.6)	<0.001
Fasting blood glucose, mg/dl	94.0 (16.6) (n=31)	111.4 (70.5) (n=31)	0.185
HOMA-R, %	0.9 (0.6) (n=13)	4.8 (2.4) (n=15)	<0.001
Total adiponectin, μg/ml	7.9 (4.3)	8.3 (5.3)	0.611
HMW, μg/ml	3.7 (2.7)	4.1 (3.3)	0.473
MMW, μg/ml	1.9 (1.2)	1.8 (1.1)	0.570
LMW, μg/ml	2.3 (1.1)	2.5 (1.4)	0.600

calculated in 28 patients, and the level of HOMA-R in the high insulin group was significantly higher than that in the low insulin group. Table IV shows the comparison of characteristics of HCC between the two groups. Patients with more than three HCC lesions and diffuse HCC were more prevalent in the low insulin group than in the high insulin group. The other characteristics of HCC did not differ substantially between the two groups.

Fig. 2A indicates the cumulative survival rates of all stage HCC patients between the low insulin group (70 of 140) and the high insulin group (70 of 140). There was no significant difference between the two groups (P=0.235). Next, to evaluate the relationship of the fasting insulin level with the prognosis of early stage HCC patients, we analyzed the cumulative survival rates in HCC patients with TNM stage I and II disease (n=92). As shown in Fig. 2B, the high insulin group (49 of 92) exhibited a poor prognosis with a significant difference in comparison to the low insulin group (43 of 92) (P=0.018).

Association of fasting total adiponectin level with prognosis of HCC. Similarly, we evaluated the association of the total adiponectin level with the prognosis of HCC. One hundred and forty patients were divided into 2 groups in terms of the 50th percentile of the value of total adiponectin (6.95 μIU/ml). The mean level of total adiponectin in the low adiponectin group (<6.95 μIU/ml, n=70) was 4.5 μg/ml. That in the high adiponectin group (≥6.95 μIU/ml, n=70) was 11.8 μg/ml. We estimated the cumulative survival rates of all stages of HCC and early stage HCC between the low adiponectin group and the high adiponectin group. Fig. 3A and B show each result. No significant differences were found in all stages of HCC, or in the early stage of HCC (all stage HCC: low group vs. high group; P=0.886, early stage HCC: low group vs. high group; P=0.804).

Univariate and multivariate analyses of the factors associated with HCC prognosis. Univariate and multivariate analyses

Table IV. Comparison of HCC characteristics between low insulin group and high insulin group.

Variable	Number or mean (SD)		P
	Low insulin group (n=70)	High insulin group (n=70)	
Tumor size, cm	3.6 (3.2)	3.2 (2.5)	0.479
Number of tumor lesion			0.032
1	36	39	
2	10	19	
3 - and diffuse	24	12	
TNM stage			0.300
I	22	17	
II	21	32	
III	21	16	
IV	6	5	
AFP, ng/ml	5136.8 (30566.9)	10487.6 (83045.4)	0.614
Therapy			0.492
Surgical resection	5	2	
PEIT and/or RFA	27	26	
TACE or TAI	35	38	
Others	3	4	

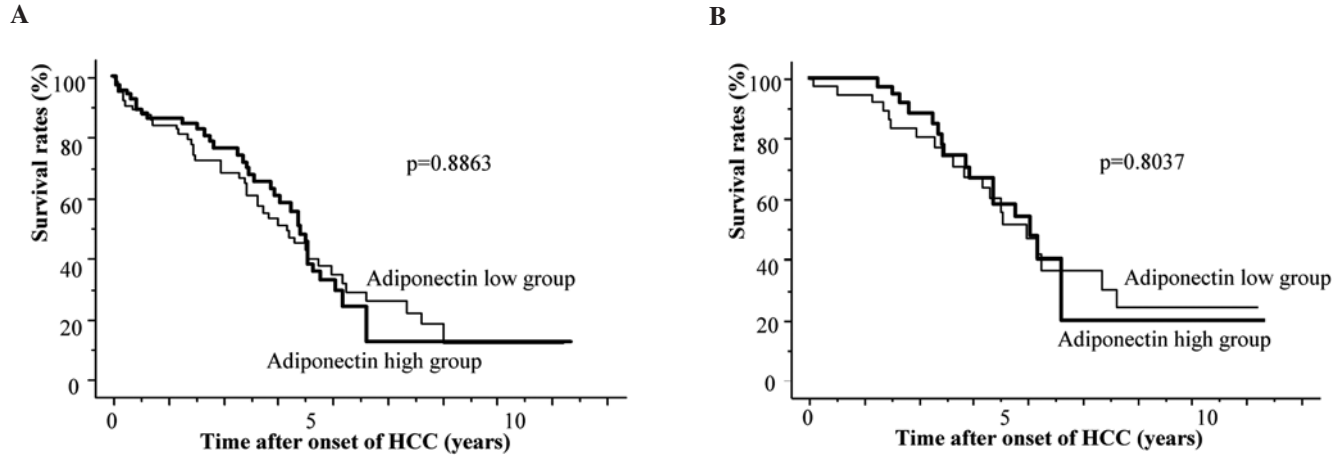

Figure 3. Kaplan-Meier curves for survival between low adiponectin group (thin line) and high adiponectin group (heavy line) in all stages of HCC patients (n=140) (A) and in TNM stage I + II HCC patients (n=92) (B).

using the Cox proportional hazards model in 140 patients diagnosed with HCC were performed to identify the relevant independent prognostic factors in all stages of HCC. In a univariate analysis, the following three factors significantly influenced the prognosis: alcohol intake (excessive drinker, RR 2.033, 95% CI 1.206-3.425, P=0.008), Child-Pugh grade (grade C, RR 9.906, 95% CI 3.547-27.666, P<0.001), and therapy for HCC (TACE or TAI, RR 1.856, 95% CI 1.143-3.015, P=0.012). However, a multivariate analysis revealed that only two factors influenced the HCC prognosis significantly: Child-Pugh grade (grade C, RR 9.807, 95% CI 2.710-30.471, P<0.001) and therapy for HCC (TACE or TAI, RR 1.803, 95% CI 1.104-2.943, P=0.018).

Next, univariate and multivariate analyses in 92 patients diagnosed with HCC, all TNM stage I or II, were performed to identify the independent prognostic factors of early stage HCC. In the univariate analysis, the following three factors significantly influenced prognosis: alcohol intake (excessive drinker, RR 2.488, 95% CI 1.160-5.319, P=0.019), Child-Pugh grade (grade B, RR 4.582, 95% CI 1.370-15.323, P=0.014, grade C, RR 41.104, 95% CI 6.403-263.831, P<0.001), and the value of insulin (>7.73 μIU/ml, RR 2.196, 95% CI 1.126-4.292, P=0.021) (Table V).

Similarly, when we performed multivariate analysis, only two factors, the Child-Pugh grade and the level of fasting insulin influenced the prognosis of early stage HCC with a

Table V. Univariate analyses of prognosis factors for HCC of TNM stage I and II.

Variable	Relative risk (95% CI)	P
Onset age, >60 y.o.	1.248 (0.536-2.907)	0.606
Gender, male	1.637 (0.715-3.745)	0.244
BMI, >25.0 kg/m^2	1.488 (0.746-2.967)	0.260
Alcohol intake, ≥80g/day	2.488 (1.160-5.319)	0.019
Background		0.647
non-B, non-C	-	-
HBsAg(+)	1.111 (0.230-5.369)	0.896
HCVAb(+)	1.566 (0.366-6.700)	0.545
Underlying liver diseases and Child-Pugh grade		<0.001
CH	-	-
LC Child-Pugh grade A	2.531 (0.866-7.395)	0.090
LC Child-Pugh grade B	4.582 (1.370-15.323)	0.014
LC Child-Pugh grade C	41.104 (6.403-263.831)	<0.001
Serum ferritin, <185 ng/ml	1.193 (0.621-2.295)	0.596
Serum iron, <141 μg/ml	1.222 (0.641-2.331)	0.542
Fasting insulin, >7.73 μIU/ml	2.196 (1.126-4.292)	0.021
Fasting blood glucose, >110 mg/dl	0.949 (0.118-7.634)	0.961
HOMA-R, >2.0%	4.762 (0.475-47.619)	0.184
Total adiponectin, >6.95 μg/ml	0.921 (0.479-1.767)	0.804
HMW, 3.0 μg/ml	0.799 (0.418-1.529)	0.498
MMW, >1.6 μg/ml	1.171 (0.613-2.232)	0.633
LMW, >2.1 μg/ml	1.038 (0.544-1.984)	0.908
Therapy, TACE or TAI	1.429 (0.743-2.748)	0.285

Table VI. Multivariate analyses of prognosis factors for HCC of TNM stage I and II.

Variable	Relative risk (95% CI)	P
Alcohol intake, ≥80 g/day	2.217 (0.933-5.263)	0.071
Underlying liver diseases and Child-Pugh grade		0.022
CH	-	-
LC Child-Pugh grade A	2.884 (0.975-8.531)	0.056
LC Child-Pugh grade B	3.771 (1.099-12.529)	0.035
LC Child-Pugh grade C	19.039 (2.782-130.298)	0.003
Fasting insulin, >7.73 μIU/ml	2.033 (1.019-4.049)	0.044

significant difference: Child-Pugh grade (grade B, RR 3.771, 95% CI 1.099-12.529, P=0.035, grade C, RR 19.039, 95% CI 2.782-130.298, P=0.003), and the level of fasting insulin (>7.73 μIU/ml, RR 2.033, 95% CI 1.019-4.049, P=0.044) (Table VI).

Association of fasting insulin and total adiponectin level with recurrence-free survival. To evaluate the association of fasting insulin level with the recurrence-free survival time, 59 patients who underwent curative therapy, defined as a condition characterized by the no findings of recurrence over six months after the initial therapy, were extracted from 140 patients and subjected to analysis. Of 59 patients, the mean level of insulin in the low insulin group (<7.73 μIU/ml, n=32) or that in the high insulin group (>7.73 μIU/ml, n=27) was 3.8 μIU/ml or 14.4 μIU/ml, respectively. Fig. 4A indicates the cumulative recurrence-free survival rates of 59 patients who underwent curative therapy. The high insulin group exhibited a lower recurrence-free survival with a significant difference in comparison to the low insulin group (P=0.017).

Similarly, we evaluated the association of the total adiponectin level with the recurrence-free survival time. The mean level of total adiponectin in the low adiponectin group (<6.95 μIU/ml, n=28) or that in the high adiponectin group (≥6.95 μIU/ml, n=31) was 4.5 μIU/ml or 11.7 μIU/ml, respectively. We compared the cumulative recurrence-free survival rates of HCC between the low adiponectin group and the high adiponectin group, but no significant difference was found (Fig. 4B).

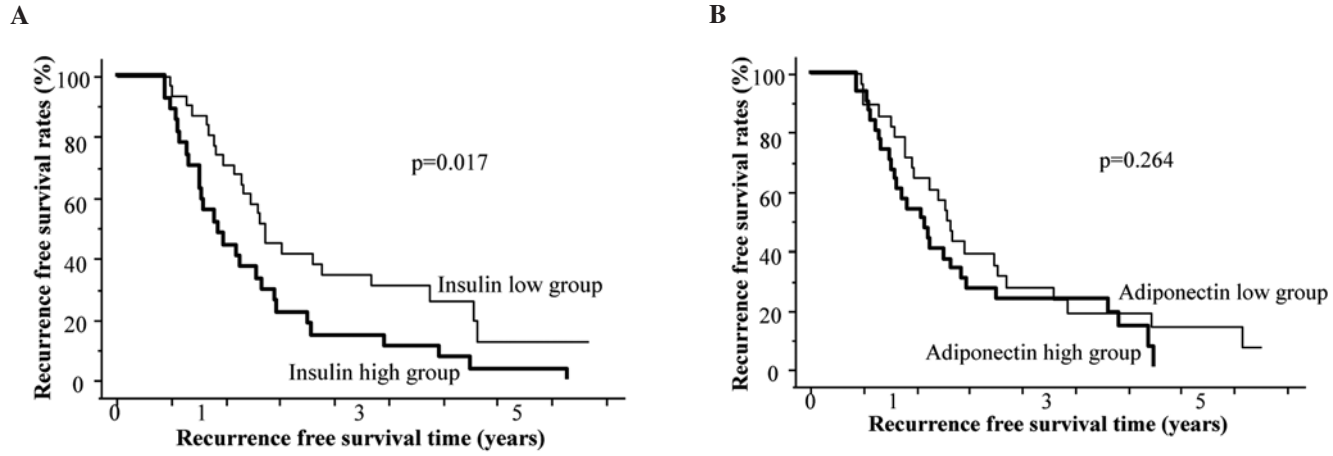

Figure 4. Kaplan-Meier curves for recurrence-free survival in HCC patients who underwent curative therapy (n=59) between low insulin group (thin line) and high insulin group (heavy line) (A) and between low adiponectin group (thin line) and high adiponectin group (heavy line) (B).

Table VII. Univariate analyses of the factor that contribute to recurrence-free survival.

Variable	Relative risk (95% CI)	P
Onset age, >60 y.o.	1.905 (0.951-3.871)	0.069
Gender, male	1.984 (0.355-1.243)	0.201
BMI, >25.0 kg/m^2	2.268 (0.805-4.367)	0.014
Alcohol intake, ≥80 g/day	1.289 (0.653-2.538)	0.465
Background		0.671
Non-B, non-C	-	-
HBsAg(+)	1.302 (0.289-5.855)	0.731
HCVAb(+)	1.566 (0.390-6.773)	0.505
Underlying liver diseases and Child-Pugh grade		0.093
CH	-	-
LC Child-Pugh grade A	2.300 (1.095-4.831)	0.028
LC Child-Pugh grade B	2.883 (1.086-7.650)	0.034
LC Child-Pugh grade C	3.655 (0.774-17.263)	0.102
Serum ferritin, <185 ng/ml	1.157 (0.663-2.019)	0.607
Serum iron, <141 μg/ml	1.379 (0.772-2.464)	0.278
Fasting insulin, >7.73 μIU/ml	1.946 (1.117-3.378)	0.019
Fasting blood glucose, >110 mg/dl	4.975 (0.903-27.778)	0.065
HOMA-R, >2.0%	4.255 (0.816-22.222)	0.086
Total adiponectin, >6.95 μg/ml	1.376 (0.784-2.410)	0.266
HMW, >3.0 μg/ml	1.076 (0.611-1.893)	0.799
MMW, >1.6 μg/ml	1.258 (0.711-2.222)	0.430
LMW, >2.1 μg/ml	1.012 (0.572-1.792)	0.967
Therapy, TACE or TAI	1.165 (0.646-2.101)	0.610

Univariate and multivariate analyses of the factors associated with recurrence-free survival. To clarify the factors that contribute to recurrence-free survival except for tumoral factors, univariate and multivariate analyses were performed using the Cox proportional hazards model in 59 patients who underwent curative therapy. In a univariate analysis, only two

Table VIII. Multivariate analyses of the factors that contribute to recurrence-free survival.

Variable	Relative risk (95% CI)	P
BMI, >25.0 kg/m^2	1.992 (1.026-3.861)	0.042
Fasting insulin, >7.73 μIU/ml	1.767 (1.004-3.117)	0.049

factors significantly influenced the recurrence-free survival: BMI (>25.0 kg/m^2, RR 2.268, 95% CI 0.805-4.367, P=0.014) and the value of insulin (>7.73 μIU/ml, RR 1.946, 95% CI 1.117-3.378, P=0.019) (Table VII). Multivariate analysis showed that both factors influenced the recurrence-free survival with a significant difference: BMI (>25.0 kg/m^2, RR 1.992, 95% CI 1.026-3.861, P=0.042) and the value of insulin (>7.73 μIU/ml, RR 1.767, 95% CI 1.004-3.117, P=0.049) (Table VIII).

Discussion

Several prior studies have reported that the coexistence of DM influences the prognosis of HCC patients (10,11,28,29). However, the mechanism responsible for this finding remains unclear. Since the glucose tolerance of an individual is defined by the potential insulin secretion from ß-cells and by the insulin sensitivity of target tissues including the liver, serum levels of fasting and postprandial insulin could differ in each HCC patient. In addition, advanced liver fibrosis is directly linked to an increase in the insulin resistance in HCV-infected patients (13,30).

In the present study, we therefore focused on the serum level of insulin rather than on the glucose tolerance in the HCC patients. Our study indicates that a high value of fasting insulin heralds not only a poor prognosis in the early stage of HCC but also a high recurrence rate in the curative HCC. There are a few studies on the prognostic value of hyperinsulinemia on patients with HCC. Saito *et al* have demonstrated that the area under the plasma insulin curve for the oral glucose tolerance test can serve as a significant prognostic tool, and can assist in forecasting the doubling time of HCC (16), and that continuous infusion of octreotide in five patients inhibited insulin secretion resulting in a decrease in the HCC growth rate.

Komura *et al* reported that insulin therapy for coexisting DM is an independent risk factor for HCC recurrence after a curative resection (10). Taken together, it is possible that hyperinsulinemia promotes the progression and development of HCC. This is consistent with the results from the following *in vitro* studies, that insulin has the potential to accelerate the growth of hepatoma cells and inhibits apoptosis through the upregulation of Bcl-xl (14), and that insulin stimulates the motility and invasiveness of hepatoma cells (31). In addition, there have been several clinical studies supporting the association between hyperinsulinemia and the advancement of cancers. A high level of fasting insulin is associated with distant recurrence and death in early stage breast cancer (32). High insulin levels are associated with a poorer prognosis in prostate cancer and endometrial cancer, and malignant degeneration of adenomatous polyps (33-36). These findings suggest that, in addition to an effect on glucose metabolism, insulin functions to promote the proliferation and metastasis of various types of cancer cells.

Hyperinsulinemia is inextricably linked to insulin resistance of the peripheral tissues including the liver. In our study, HOMA-R, a good indicator of insulin resistance, was not associated with a poor prognosis in early stage HCC (univariate analysis, P=0.184) and a recurrence-free survival in curative HCC (univariate analysis, P=0.086) although HOMA-R was significantly higher in the high insulin group than in the low insulin group (Table III). It is probably due to the small number of cases used to determine the HOMA-R (28 of 140 subjects or 11 of 59 subjects).

Since adiponectin has a potent insulin-sensitizing effect, we determined its value in HCC patients. In contrast to fasting serum insulin, the mean value of total adiponectin apparently increased with the decline of liver function. The HCC stage did not affect the values of total adiponectin. A similar observation has been reported by Tacke *et al* (23), in which they suggest that the elevation of adiponectin in chronic liver disease is due to the decrease of clearance from the serum, and possibly decreased biliary excretion of adiponectin, and that portal hypertension and the development of HCC do not affect the values of adiponectin. In addition to total adiponectin, we measured the levels of HMW, MMW, and LMW adiponectins. These adiponectins increased in direct relation to the decline in the liver function (data not shown), thus suggesting that higher molecular weight adiponectin is also metabolized by the liver. It is surprising that the values of total, HMW, MMW, and LMW adiponectins showed no significant differences between the high insulin group and the low group (Table III). However, Tacke *et al* have already reported a similar observation that the elevated adiponectin in LC patients is not directly involved in insulin sensitivity. Recently, adiponectin is known to possess antitumoral activity. The circulating adiponectin level is inversely associated with an increased risk of breast cancer, endometrial, prostate, gastric, and colorectal cancer (37-41). Furthermore, Miyazaki *et al* reported that adiponectin shows an antitumor effect against HepG2 hepatoma cells through JNK activation and suppression of STAT3 function (42). However, our study showed that total adiponectin has no impact on the prognosis of any stage of HCC. It is unclear why there is a discrepancy between these literature findings and our own. We are now speculating that certain cirrhotic environments such as advanced liver fibrosis, decreased liver function and portal systemic shunting may diminish the anti-tumoral activity of adiponectin against HCC. Further studies are thus needed to clarify this.

Although the present study is retrospective and involves a limited number of participants, this is a first study indicating that fasting hyperinsulinemia is a risk factor associated with a poor prognosis in the early stage of HCC and a high recurrence rate in the curative HCC. We have to validate our findings with a prospective study and also clarify the mechanism by which insulin impacts the clinical course of HCC. However, our study suggests that treatment modalities which lower the level of fasting insulin could improve the prognosis of the early stage of HCC and reduce the recurrence of HCC.

References

1. Bosch FX, Ribes J, Diaz M and Cleries R: Primary liver cancer: worldwide incidence and trends. Gastroenterology 127: S5-S16, 2004.
2. El-Serag HB and Mason AC: Risk factors for the rising rates of primary liver cancer in the United States. Arch Intern Med 160: 3227-3230, 2000.
3. Yu MC and Yuan JM: Environmental factors and risk for hepatocellular carcinoma. Gastroenterology 127: S72-S78, 2004.
4. Marchesini G, Brizi M, Morselli-Labate AM, *et al*: Association of nonalcoholic fatty liver disease with insulin resistance. Am J Med 107: 450-455, 1999.
5. Cotrim HP, Parana R, Braga E and Lyra L: Nonalcoholic steatohepatitis and hepatocellular carcinoma: natural history? Am J Gastroenterol 95: 3018-3019, 2000.
6. Shimada M, Hashimoto E, Taniai M, *et al*: Hepatocellular carcinoma in patients with non-alcoholic steatohepatitis. J Hepatol 37: 154-160, 2002.
7. Lagiou P, Kuper H, Stuver SO, Tzonou A, Trichopoulos D and Adami HO: Role of diabetes mellitus in the etiology of hepatocellular carcinoma. J Natl Cancer Inst 92: 1096-1099, 2000.
8. El-Serag HB, Richardson PA and Everhart JE: The role of diabetes in hepatocellular carcinoma: a case-control study among United States Veterans. Am J Gastroenterol 96: 2462-2467, 2001.
9. Davila JA, Morgan RO, Shaib Y, McGlynn KA and El-Serag HB: Diabetes increases the risk of hepatocellular carcinoma in the United States: a population based case control study. Gut 54: 533-539, 2005.
10. Komura T, Mizukoshi E, Kita Y, *et al*: Impact of diabetes on recurrence of hepatocellular carcinoma after surgical treatment in patients with viral hepatitis. Am J Gastroenterol 102: 1939-1946, 2007.
11. Huo TI, Wu JC, Lui WY, *et al*: Differential mechanism and prognostic impact of diabetes mellitus on patients with hepatocellular carcinoma undergoing surgical and nonsurgical treatment. Am J Gastroenterol 99: 1479-1487, 2004.
12. Perseghin G, Mazzaferro V, Sereni LP, *et al*: Contribution of reduced insulin sensitivity and secretion to the pathogenesis of hepatogenous diabetes: effect of liver transplantation. Hepatology 31: 694-703, 2000.
13. Taura N, Ichikawa T, Hamasaki K, *et al*: Association between liver fibrosis and insulin sensitivity in chronic hepatitis C patients. Am J Gastroenterol 101: 2752-2759, 2006.
14. Wanke I, Schwarz M and Buchmann A: Insulin and dexamethasone inhibit TGF-beta-induced apoptosis of hepatoma cells upstream of the caspase activation cascade. Toxicology 204: 141-154, 2004.
15. Tran TT, Medline A and Bruce WR: Insulin promotion of colon tumors in rats. Cancer Epidemiol Biomarkers Prev 5: 1013-1015, 1996.
16. Saito K, Inoue S, Saito T, *et al*: Augmentation effect of postprandial hyperinsulinaemia on growth of human hepatocellular carcinoma. Gut 51: 100-104, 2002.
17. Tschritter O, Fritsche A, Thamer C, *et al*: Plasma adiponectin concentrations predict insulin sensitivity of both glucose and lipid metabolism. Diabetes 52: 239-243, 2003.
18. Matsuzawa Y, Funahashi T, Kihara S and Shimomura I: Adiponectin and metabolic syndrome. Arterioscler Thromb Vasc Biol 24: 29-33, 2004.
19. Diez JJ and Iglesias P: The role of the novel adipocyte-derived hormone adiponectin in human disease. Eur J Endocrinol 148: 293-300, 2003.
20. Havel PJ: Update on adipocyte hormones: regulation of energy balance and carbohydrate/lipid metabolism. Diabetes 53 (Suppl 1): S143-S151, 2004.
21. Xu A, Wang Y, Keshaw H, Xu LY, Lam KS and Cooper GJ: The fat-derived hormone adiponectin alleviates alcoholic and nonalcoholic fatty liver diseases in mice. J Clin Invest 112: 91-100, 2003.
22. Tietge UJ, Boker KH, Manns MP and Bahr MJ: Elevated circulating adiponectin levels in liver cirrhosis are associated with reduced liver function and altered hepatic hemodynamics. Am J Physiol Endocrinol Metab 287: E82-E89, 2004.
23. Tacke F, Wustefeld T, Horn R, *et al*: High adiponectin in chronic liver disease and cholestasis suggests biliary route of adiponectin excretion in vivo. J Hepatol 42: 666-673, 2005.
24. Kaser S, Moschen A, Kaser A, *et al*: Circulating adiponectin reflects severity of liver disease but not insulin sensitivity in liver cirrhosis. J Intern Med 258: 274-280, 2005.
25. Hui CK, Zhang HY, Lee NP, *et al*: Serum adiponectin is increased in advancing liver fibrosis and declines with reduction in fibrosis in chronic hepatitis B. J Hepatol 47: 191-202, 2007.
26. Pugh RN, Murray-Lyon IM, Dawson JL, Pietroni MC and Williams R: Transection of the oesophagus for bleeding oesophageal varices. Br J Surg 60: 646-649, 1973.
27. Cancer Study Group of Japan: Clinical findings. In: The General Rules for the Clinical and Pathological Study of Primary Liver Cancer. Makuuchi M (ed). 2nd English edition, Kanehara Co., Tokyo, pp6-28, 2003.
28. Ikeda Y, Shimada M, Hasegawa H, *et al*: Prognosis of hepatocellular carcinoma with diabetes mellitus after hepatic resection. Hepatology 27: 1567-1571, 1998.
29. Poon RT, Fan ST and Wong J: Does diabetes mellitus influence the perioperative outcome or long term prognosis after resection of hepatocellular carcinoma? Am J Gastroenterol 97: 1480-1488, 2002.
30. Hui JM, Sud A, Farrell GC, *et al*: Insulin resistance is associated with chronic hepatitis C virus infection and fibrosis progression [corrected]. Gastroenterology 125: 1695-1704, 2003.
31. Qi HL, Zhang Y, Ma J, Guo P, Zhang XY and Chen HL: Insulin/protein kinase B signalling pathway upregulates metastasis-related phenotypes and molecules in H7721 human hepatocarcinoma cell line. Eur J Biochem 270: 3795-3805, 2003.
32. Goodwin PJ, Ennis M, Pritchard KI, *et al*: Fasting insulin and outcome in early-stage breast cancer: results of a prospective cohort study. J Clin Oncol 20: 42-51, 2002.
33. Hammarsten J and Hogstedt B: Hyperinsulinaemia: a prospective risk factor for lethal clinical prostate cancer. Eur J Cancer 41: 2887-2895, 2005.
34. Giovannucci E and Michaud D: The role of obesity and related metabolic disturbances in cancers of the colon, prostate, and pancreas. Gastroenterology 132: 2208-2225, 2007.
35. Lukanova A, Zeleniuch-Jacquotte A, Lundin E, *et al*: Prediagnostic levels of C-peptide, IGF-I, IGFBP -1, -2 and -3 and risk of endometrial cancer. Int J Cancer 108: 262-268, 2004.
36. Schoen RE, Weissfeld JL, Kuller LH, Thaete FL, Evans RW, Hayes RB and Rosen CJ: Insulin-like growth factor-I and insulin are associated with the presence and advancement of adenomatous polyps. Gastroenterology 129: 464-475, 2005.
37. Miyoshi Y, Funahashi T, Kihara S, Taguchi T, Tamaki Y, Matsuzawa Y and Noguchi S: Association of serum adiponectin levels with breast cancer risk. Clin Cancer Res 9: 5699-5704, 2003.
38. Cust AE, Kaaks R, Friedenreich C, *et al*: Plasma adiponectin levels and endometrial cancer risk in pre- and postmenopausal women. J Clin Endocrinol Metab 92: 255-263, 2007.
39. Baillargeon J, Platz EA, Rose DP, *et al*: Obesity, adipokines, and prostate cancer in a prospective population-based study. Cancer Epidemiol Biomarkers Prev 15: 1331-1335, 2006.
40. Ishikawa M, Kitayama J, Kazama S, Hiramatsu T, Hatano K and Nagawa H: Plasma adiponectin and gastric cancer. Clin Cancer Res 11: 466-472, 2005.
41. Wei EK, Giovannucci E, Fuchs CS, Willett WC and Mantzoros CS: Low plasma adiponectin levels and risk of colorectal cancer in men: a prospective study. J Natl Cancer Inst 97: 1688-1694, 2005.
42. Miyazaki T, Bub JD, Uzuki M and Iwamoto Y: Adiponectin activates c-Jun NH2-terminal kinase and inhibits signal transducer and activator of transcription 3. Biochem Biophys Res Commun 333: 79-87, 2005.