# Serum Leptin Concentrations in Chronic Hepatitis

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*The objectives of this research were to investigate the leptin levels among Chronic Hepatitis B Virus (HBV), Chronic Hepatitis C Virus (HCV) and non-alcoholic steatosis hepatitis (NASH) diseases of Thai patients compared with controls. Twenty of each HBV, HCV and NASH patients compared with sixty people as the control group from the Outpatient Department at the Hospital for Tropical Diseases, Bangkok, Thailand were investigated. Fasting blood samples were collected for investigation of leptin concentration, liver enzyme function tests and hematological variables. The serum leptin concentration of liver patients was significantly higher than that of control subjects. It might be due to the accumulations of fat cells in liver disease patients. However, there is no relationship between leptin level and other parameters such as BMI, ALT, AST, ALP and hematological variables. Liver enzyme functions levels are much higher in patients groups. White blood cells counts, platelets and hematocrit values are slightly lower in liver disease patients. Therefore, it is concluded that physiological regulation of leptin maintains in relation to body fat, even in chronic viral liver diseases. This finding and the apparent stage suggest the possibility that in the course of chronic viral diseases, serum leptin levels may reflect the extent of liver dysfunction.*

***Keywords:*** *Leptin, BMI, Chronic Hepatitis B Virus (HBV), Chronic Hepatitis C Virus (HCV), Nonalcoholic Steatosis (NASH)*

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Leptin, the adipocyte-derived protein product of the *ob* gene, is involved in appetite regulation and obesity through central effects at the hypothalamus[1]. Leptin is related to amount of body fat[2]. Leptin is also associated with increased heart rate[2], blood pressure[3], and sympathetic neural activity[4] and may contribute to platelet aggregation[5,6]. The importance of leptin in the regulation of energy balance, body composition, and food intake has been demonstrated in both animal and human studies[7-9].

While many studies have been conducted to establish the relevance of leptin in the pathogenesis of obesity, the role of leptin in the states of negative energy balance that frequently accompany chronic diseases has not been well elucidated until now. Among these, a high prevalence disease is represented by liver cirrhosis. The identification of obese patients who may progress from steatosis to non-alcoholic steatosis hepatitis (NASH) to fibrosis/cirrhosis is an important clinical challenge. It has recently been reported that most cases of obesity in humans are associated with high leptin levels. Thus, in humans, obesity may represent a state of leptin resistance. According to Tungtrongchitr et al obesity is found in 11% of Thai elderly and moderate to severe obesity is increasingly found[10]. Also, obesity might be associated with clear health risks, including hypertension, diabetes and dyslipidemia and liver diseases. Moreover, a recent study has also indicated that obesity may play a more important role than alcohol intake in the development of steatosis in chronic HCV infection[11]. This overlap in host risk factors for the development of steatosis in chronic hepatitis C and non-alcoholic fatty liver

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disease (NAFLD) suggests a common pathogenesis, although several questions remain unanswered. There are common links between the host, viral, and other environmental factors that predispose to steatosis. One such common link may be leptin, which is increased in most cases of obesity, indicating leptin insensitivity or resistance. It is also required for hepatic fibrogenesis in toxic and metabolic forms of chronic liver injury. Several studies have shown that serum leptin levels are increased in patients with cirrhosis. Although, the decrease of leptin level in chronic hepatitis was reported[5], however, there are controversal reports of high circulating leptin levels in chronic hepatitis C related fatigue[12]. The exploration leptin study of different kind in chronic hepatitis such as hepatitis B virus, chronic hepatitis C virus and fatty liver had not yet been reported especially in Thai patients. Therefore, the aim of the present study was to investigate serum leptin levels in relation to anthropometric features and liver function in patients with viral chronic hepatitis or liver cirrhosis and non-alcoholic steatosis hepatitis. Furthermore, it still wonders whether there is a pathophysiological role of leptin in Thai chronic hepatitis patients such as chronic hepatitis B virus (HBV), chronic hepatitis C virus (HCV) and fatty liver.

**Material and Method**

The subjects used for the present study consisted of Thai participants who attended the outpatient department at the Hospital for Tropical Diseases. The objectives of the present study were explained to the volunteers. Twenty of each Chronic HBV, Chronic HCV, fatty liver patients (non-alcoholic steatosis) who participated voluntarily in the present study were investigated. Chronic liver disease patients were diagnosed on the basis of biochemical serum aminotransferase greater than or equal to 1.5 times the upper normal values for at least 6 months. Another sixty people who were matched with patients were selected as controls. The study protocol was approved by the Ethics Committee of the Faculty of Tropical Medicine, Mahidol University, Bangkok and informed consent was obtained from each participant.

In the morning, about 10 ml of venous blood was taken from each subject after an overnight fast to determine serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and leptin. Heparinised blood was also used to determine hematological variables. Blood samples were centrifuged immediately and stored at -70 C. Platelet counts, white blood cells counts and hematocrit values were determined. Routine biochemical tests were carried out using commercially available kits.

Systematic data collection was carried out by reviewing all of the medical records. Age, gender, height, and weight were documented at baseline. BMI (body mass index) was calculated by dividing weight (kg) by height squared ($m^2$). The classifications of BMI employed were those used by the WHO Expert Committee 2000: normal BMI is 18.5-24.99[13].

**Laboratory techniques**

Serum leptin was assessed using a commercially available radioimmunoassay from Linco Research Inc. utilized $^{125}$I- labeled human leptin and a human leptin antiserum.

Hepatitis B Virus and Hepatitis C Virus were tested using enzyme immunoassay (EIA). Laboratory data at baseline and during follow-up included the following values; serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP).

A liver biopsy specimen should be obtained to detect the presence of non-alcoholic steatosis hepatitis (NASH) in subjects with two or more components of the metabolism syndrome. Taking a liver biopsy is indicated in order to diagnose fatty liver or NASH and to determine its severity by evaluating the grade and the stage of the disease[14]. Biopsy specimens of fatty liver patients had been fixed in neutral buffered formation, zinc chloride Zenker's, or Hllande's fixative and embedded in paraffin. Specimens had been stained with H&E for morphological review and Masson's trichrome for assessment of fibrosis. Perl's Prussian blue was performed to evaluate iron load. Biopsy specimens were reviewed by one hepatopathologist (TG) using a systematic approach.

The hematocrit values were measured by a micro-method using calibrated heparinized capillary tubes. After filling the capillaries with blood, they were centrifuged for 5 minutes at 14,000 g in a micro-hematocrit centrifuge (IEC MB centrifuge model 3412, Massachusetts, USA). Then the hematocrit values were read using a micro-hematocrit reader (Hawksley & Son Ltd, Marlborough, UK).

Platelets in peripheral blood smears were counted using the method of Nosanchuk *et al*[15]. The morphology of white blood cells was determined using the Wedge method, which involved making blood films, staining with Wright's stain and examining under a microscope using an oil-immersion lens (1000x).

***Statistical analysis***

All data were recorded both in paper form and in an electronic database. All continuous data were examined for their distribution, skewness and kurtosis. As these were dispersed from a normal distribution, the results were expressed as median and range 95% C.I. and non-parametric statistical analysis was used. For data processing, the Minitab computer program was utilized. The Mann-Whitney U-test and the Wilcoxon rank sum W-test were used to compare medians. Correlations between results were evaluated by Pearson bivariate correlation test and by multiple stepwise regression analysis.

**Results**

Median, range and 95% confidence interval (CI) of age, anthropometric variables serum leptin concentration, liver function tests, hematological parameters of the 60 liver disease patients and 60 controls subjects are shown in Table 1. Liver disease patients are chronic hepatitis B virus (HBV), chronic hepatitis C virus (HCVg) and fatty liver (NASH) ones. BMI ranged from 16.40 to 38.86 with the median BMI considered overweight at 25.00 kg/m$^2$ (healthy BMI ranged 18.50 -24.99 kg/m$^{2)}$. The age of patients ranged from 26 to 69 years, with a median age of 47 years.

In Table 1, liver disease patients were significantly older than control subjects (p = 0.0001). The number of white blood cells (WBC) and hematocrit (Hct) were within the normal range (5900 x $10^9$ $l^{-1}$ and 43 $l^{-1}$ respectively). Moreover, anthropometric measurements such as body weight, height, BMI (body mass index) were similar in those two groups. As expected, all liver enzymes functions such as aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphate (ALP) are almost double that of the control subjects. Furthermore, leptin concentration in patients was significantly higher than that of control subjects (p = 0.0001).

Table 2 shows the median, range and 95% of confidence interval (CI) of anthropometric, biochemical characteristics in details, comparing each group of HBV, HCV, fatty liver and control subjects. Fatty liver patients had higher body weight than that of the other three groups. The body weight of fatty liver patients was significantly different from HBV, HCV and control subjects although the median heights of all subjects were in similar range. For this reason, BMI of fatty liver patients was statistically significant to other three groups. BMI median (27.66) is the highest in fatty liver patients and lowest in Hepatitis B patients' (23.06). Hepatitis B patients' BMI is statistically significant compared with control group. Hepatitis C patients have similar BMI median as control group (24.78 and 24.56 respectively).

Similarly, serum leptin concentration of Hepatitis C patients was not statistically significant compared to control subjects. However, when examining Hepatitis B and fatty liver patients, leptin concentration level was significantly higher than the control

**Table 1.** Median, range and 95% confidence interval (CI) of age, anthropometric measure ments, leptin, liver function test, hematological parameters in liver disease patients comparing with control subjects

| Variables | Chronic liver diseases (n = 60) | | Controls (n = 60) | | p-value |
|---|---|---|---|---|---|
| | Median (range) | 95%CI | Median (range) | 95%CI | |
| Age (years) | 47 (26-69) | 43.54-50 | 39 (30-61) | 37-42 | 0.000* |
| BodyWeight (kg) | 68 (46-121.4) | 60.68-70 | 66.5 (45.5-92) | 64.97-69.53 | 0.861 |
| Height (cm) | 164 (148-186) | 161.5-167 | 166.5 (152.3-180.5) | 164-168 | 0.123 |
| BMI (kg/m$^2$) | 24.77 (16.49-38.86) | 23.63-25.74 | 24.56 (17.77-32.46) | 23.11-25.43 | 0.473 |
| Leptin (ng/ml) | 9.3 (1.1-49.35) | 7.4-11.45 | 5.1 (1.2-56) | 4.09-5.9 | 0.000* |
| AST (IU/ml) | 66 (25-936) | 46.08-78.38 | 31 (12-126) | 28-36 | 0.000* |
| ALT (IU/ml) | 90 (24-1293) | 69.5-103.5 | 38.5 (13-260) | 33-41 | 0.000* |
| ALP (IU/ml) | 109 (55-325) | 100.1-119.3 | 69.5 (41-110) | 66.93-72.07 | 0.000* |
| Hct ($l^{-1}$) | 43 (29-57) | 42-45.6 | 44.6 (36.2-54) | 44.05-45.43 | 0.070 |
| WBC (x $10^9$ $l^{-1}$) | 5900 (2900-12200) | 5600-6400 | 6300 (3800-12100) | 5700-6846 | 0.423 |
| Platelet (x $10^9$ $l^{-1}$) | 198500 (76000-310000) | 179000-228580 | 264000 (145000-379000) | 244078-282461 | 0.000* |

* Significant difference using the Mann-Whitney U-test

AST = Aspartate aminotransferase; ALT = Alanine aminotransferase; ALP = Alkaline phosphatase; Hct = hematocrit; WBC = white blood cell count

**Table 2.** Median, range and 95% confidence interval (CI) of age, anthropometric variables, leptin, liver function tests and hematological measurements in Hepatitis B, Hepatitis C, Fatty liver and Control subjects

| Parameters | Hepatitis B (n = 20) | | Hepatitis C (n = 20) | | Fatty Liver (n = 20) | | Control (n = 60) | |
|---|---|---|---|---|---|---|---|---|
| | Median (range) | 95%CI | Median (range) | 95%CI | Median (range) | 95%CI | Median (range) | 95%CI |
| Age (years) | 45[a] (29-63) | 37.71-47.76 | 49[a] (26-65) | 43-59.41 | 49[a] (28-69) | 40-51.76 | 39[b] (30-61) | 37-42 |
| BodyWeight(kg) | 60.5[a] (46-99) | 55.24-69.76 | 67[a] (48-81) | 58-69.57 | 74.5[b] (46-121.4) | 69-87.35 | 66.5[a] (45.5-92) | 64.97-69.53 |
| Height(cm) | 165.25[a] (152-172) | 162.2-167.8 | 163.5[a] (148-186) | 158-168.6 | 162.25[a] (154-181) | 159.2-169.5 | 166.5[a] (152.25-180.5) | 164-168 |
| BMI (kg/m$^2$) | 23.06[a] (16.49-36.36) | 20.48-24.14 | 24.78[a d] (17.01-27.94) | 23.17-25.30 | 27.66[b] (18.9-38.86) | 25.7-31.86 | 24.56[c d] (17.77-32.46) | 23.11-25.43 |
| Leptin(ng/ml) | 7.65[a] (1.1-49.35) | 5.69-11.48 | 7.9[a c] (2.3-34.20) | 3.75-10.75 | 12.6[a] (3.1-39.9) | 8.54-14.05 | 5.1[b c] (1.2-56) | 4.09-5.91 |
| AST (IU/ml) | 82[a] (25-936) | 44-150.9 | 63[a] (26-218) | 42.05-83.41 | 58[a] (29-228) | 39.71-71.53 | 31[b] (12-126) | 28-36 |
| ALT (IU/ml) | 82.5[a] (24-1293) | 61.9-129.1 | 89[a] (25-325) | 44.6-97.1 | 108.5[a] (46-238) | 70.7-145.6 | 38.5[b] (13-260) | 33-41 |
| ALP (IU/ml) | 107[a] (71-294) | 81.1-125.9 | 111.5[a] (55-325) | 90.6-133.5 | 109[a] (63-284) | 89.2-137.6 | 69.5[b] (41-110) | 66.93-72.07 |
| Hct ($l^{-1}$) | 43[a] (31-50) | 42-46 | 42.5[a] (29-57) | 39.52-46 | 44.9[a] (34-50) | 40.9-46.05 | 44.6[a] (36.2-54) | 44.05-45.43 |
| WBC (x $10^9 l^{-1}$) | 5700[a] (4000-12200) | 4646-6768 | 5900[a] (2900-9500) | 5000-6882 | 6120[a] (4400-8600) | 5695-7110 | 6300[a] (3800-12100) | 5700-6846 |
| Paltelet (x $10^9 l^{-1}$) | 199000[a c] (76000-280000) | 171288-255338 | 176000[a] (103000-297000) | 142180-194446 | 243000[b c] (151000-310000) | 204794-249000 | 264000[d] (145000-379000) | 244078-282461 |

[a b c d] Any difference in index along the same horizontal line indicates statistical difference at the P-values < 0.05 using Kruskal- Wallis analysis of variance and multiple comparison

**Table 3.** Correlation coefficients of age, anthropometric parameters, leptin, liver function enzyme measurements and hematological parameters in hepatitis B, hepatitis C and fatty liver patients

| | Leptin | Age | Body weight | Height | BMI | Hct | WBC | Platelet | AST | ALT | ALP |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Age (years) | 0.215 | 1.000 | -0.119 | -0.383** | 0.015 | -0.343* | -0.024 | -0.267 | -0.036 | -0.053 | 0.134 |
| BodyWeight(kg) | 0.055 | -0.119 | 1.000 | 0.372** | 0.846** | 0.441** | 0.025 | 0.059 | -0.090 | 0.017 | -0.015 |
| Height(cm) | -0.192 | -0.383** | 0.372** | 1.000 | -0.077 | 0.420** | -0.269 | -0.037 | -0.165 | -0.207 | -0.123 |
| BMI ($kg/m^2$) | 0.241 | 0.015 | 0.846** | -0.077 | 1.000 | 0.286* | 0.179 | 0.119 | -0.053 | 0.098 | 0.068 |
| Leptin (ng/ml) | 1.000 | 0.215 | 0.055 | -0.192 | 0.241 | -0.111 | 0.166 | 0.103 | 0.109 | 0.207 | 0.151 |
| AST (IU/ml) | 0.109 | -0.036 | -0.090 | -0.165 | -0.053 | -0.260 | 0.007 | -0.108 | 1.000 | 0.781** | 0.284* |
| ALT (IU/ml) | 0.207 | -0.053 | 0.017 | -0.207 | 0.098 | -0.343* | 0.075 | 0.090 | 0.781** | 1.000 | 0.302* |
| ALP (IU/ml) | 0.151 | 0.134 | -0.015 | -0.123 | 0.068 | -0.112 | 0.146 | -0.059 | 0.284* | 0.302* | 1.000 |
| Hct ($l^{-1}$) | -0.111 | -0.343* | 0.441** | 0.420** | 0.286* | 1.000 | 0.140 | 0.101 | -0.260 | -0.343* | -0.112 |
| WBC (x $10^9 l^{-1}$) | 0.166 | -0.024 | 0.025 | -0.269 | 0.179 | 0.140 | 1.000 | 0.318* | 0.007 | 0.075 | 0.146 |
| Platelet (x $10^9 l^{-1}$) | 0.103 | -0.267 | 0.059 | -0.037 | 0.119 | 0.101 | 0.318* | 1.000 | -0.108 | 0.090 | -0.059 |

Significance level: *, $p < 0.05$; **, $p < 0.01$

group. Interestingly, there was no significant difference in serum leptin level among three groups of patients although they were higher than normal subjects. The functions of liver enzymes were not different among the three groups, but the enzymes function levels were too high when they were compared with that of the controls. White blood cell count and hematocrit were in normal range in all groups when amount of platelets in fatty liver patients was unlikely to that of HCV and the control subjects. Fig. 1 also shows median and 95% confidence interval of serum leptin in three patients group and controls.

Table 3 shows the correlation coefficients between various parameters in liver disease patients respectively. Liver function enzymes (AST, ALT, ALP) were strongly correlated to each other ($p < 0.01$). Hematocrit was positively correlated with body weight, height ($p < 0.01$) and BMI ($p < 0.05$) while it has negative correlated with age and Alt ($p < 0.05$). White blood cells and platelets were positively under relation ($p < 0.01$). Unexpectedly, there was no significant correlation between leptin and other parameters such as BMI, liver function enzymes, anthropometric and hematological measurements in liver disease patients.

Table 4 shows prevalence of abnormal liver function test, body mass index (BMI), leptin among different groups. Gender differences were seen in fasting serum levels of leptin in both patients and control subjects. Females had significantly higher levels of leptin compared to males. When the cut-off point of leptin concentration suggested by Ma et al was used which were 5.6 ng/ml for male and 10.8 ng/ml for female, elevated leptin levels were in 80% (16 out of 20) of fatty liver, 65% (13 out of 20) of HBV, 55.0% (11 out of 20) of HCV and 43.3% (26 out of 60) were found in the control subjects[16]. All 20 fatty liver patients had higher ALT concentration than the cut-off point (40 IU/ml) while 95% of HBV and 73.7% of HCV were higher than the standard point. Only 27 out of 60 (45%) in the control group had more than normal concentration. ALP of all 60 control subjects was lower than the cut-off point (126 IU/ml) while 36.8% of fatty liver was higher than standard. Only about 29.4% and 27.8% of HBV and HCV patients had more than 126 IU/ml in ALP. Similarly, AST was higher than the cut-off point (40 IU/ml) in HBV (80%), HCV (73.7%) and fatty liver patients (70%).

## Discussion

The results of the present study demonstrate that in patients with chronic liver diseases, the bio-

**Table 4.** Prevalence of abnormal liver function test, body mass index (BMI), leptin in hepatitis B, hepatitis C, fatty liver patients and control subjects

| Parameters | Hepatitis B | Hepatitis C | Fatty liver | Control |
|---|---|---|---|---|
| AST >40 (IU/ml) | (16/20) 80% | (14/19) 73.7% | (14/20) 70% | (17/60) 28.3% |
| ALT > 40 (IU/ml) | (19/20) 95% | (14/19) 73.7% | (20/20) 100% | (27/60) 45% |
| ALP > 126 (IU/ml) | (5/17) 29.41% | (5/18) 27.8% | (7/19) 36.8% | (0/60) 0% |
| BMI $\geq$ 25 (kg/m$^2$) | (5/20) 25% | (9/20) 45.0% | (15/20) 75% | (27/60) 45% |
| Leptin | (13/20) 65% | (11/20) 55.0% | (16/20) 80% | (26/60) 43.3% |
| M > 5.6 (ng/ml) | | | | |
| F > 10.8 (ng/ml) | | | | |

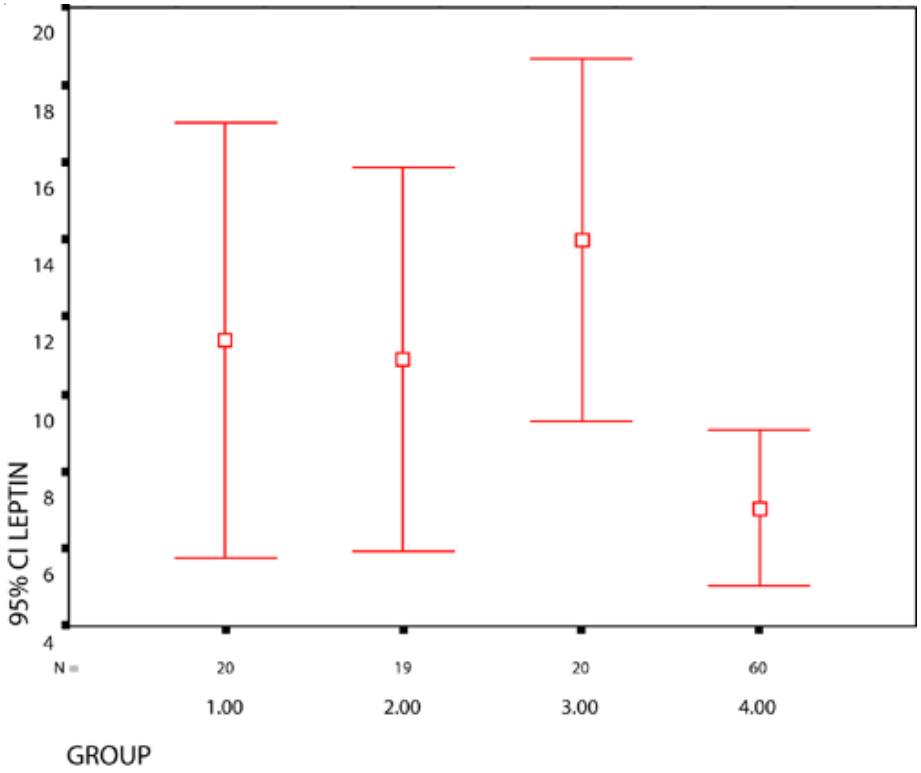


**Fig. 1** The mean and 95%CI of leptin concentration in difference groups

chemical correlation among serum leptin level, gender, BMI and age are well preserved. The liver disease patients were first compared with control subjects in terms of age, anthropometric measurements, leptin, liver function test, hematological parameters.

**Age:** Interestingly, liver disease patients were commonly found in old age. The median of patients' age was 8 years older than that of the control subjects. According to a recent study, chronic hepatitis C virus is most prevalent among people between 30 to 49 years of age. The authors observed that among patients whose age at infection and duration of HCV infection were similar (i.e., with no influence of co-founding factors). On the other hand, these findings are not related to either HCV genotype or to modality of infection. From a 5-year observational study in Greece, anti-HCV was increasing with age and peaking in the 41-60 years age group for males and in 61-90 years age group for females[17].

Similarly, old age was found in non-alcoholic fatty liver patients. It agrees with Sligte et al that NASH is diagnosed mostly in adults from 40 to 60 years of

age[18]. From the study of Koulentaki et al**,** HBV had a lower carrier rate under the age of 20 years and peak carrier rates in middle age groups[17]. Moreover, the present study showed the relationship between serum leptin and age seemed to be linear in a large population which means the older the age is, the higher the serum leptin concentration[12]. However, due to the limited number of cases, it cannot find the exact linear equation for this case. This result is different from Isidori et at, that there is an inverse relationship between leptin and age in both obese and nonobese[19]. According to their study, BMI-adjusted leptin levels were progressively lower with increasing age in women, with a consistent fall after menopause; in men, leptin levels also tended to be lower in subjects more than 50 years of age, but the reduction was not significant.

**Gender:** In the present study, most subjects were males since liver disease is more common among men, with 70% of those infected individuals as the proportion of hepatitis patients in Thailand. The gender, racial, and ethnic differences in HCV prevalence likely represent differences in the prevalence of risk factors for hepatitis C as well as the degree of endemicity in their country of origin. From the research of Koulentaki et al, HbcAb with exposures to HBV virus had the same rate in both sexes whereas anti-HCV also had no significant difference between sexes (male 4.9%, female 4.6%)[17]. Despite the fact that about 75% of NASH patients are men in the present study, there was no accountable difference between control and patient groups since the control subjects were men who were in the military.

The results show that females seem to have higher leptin levels than males, even when fat mass or BMI has been adjusted. This might be due either to their different body fat distribution, or the inducing effects of estrogen and progesterone combined with the suppressive effect of androgens or leptin. Since it is easy to measure height and weight, many investigators have relied on BMI to assess level of adiposity. Even though BMI has been shown to be highly correlated with percent of body fat, a wide range of body compositions exist at any given BMI. In addition, body composition differs by gender, i.e., women tend to have much less lean mass and a little more fat mass than men at the same BMI. It might be one of the reasons why there are gender differences in leptin concentration. Rosenbaum et al mentioned that lower fat-free mass and blood volume might explain the higher leptin concentrations in women but did not use their data to directly test for an association[20]. To the extent that the androgen association with leptin is causal, it argues that the role of lean mass may be more than just a surrogate for blood volume.

**Leptin and BMI:** In the present study, significantly high levels of serum leptin in liver disease patients were found, which to our surprise, did not correlate with BMI levels in the same group. On the other hand, there was no correlation between the serum leptin level and body mass index (BMI) in all groups. The above results suggest that there was no significant difference between normal and overweight liver disease patients with regard to serum leptin levels. The present findings contrast with previous reports suggesting higher levels of leptin in obese patients[21-23]. On the other hand, obesity is closely correlated with elevated levels of serum leptin; therefore, the majority of NASH patients may be expected to have high levels of serum leptin. The present study follows that expectation. The majority of NASH patients in our case display high levels of serum leptin. In the subjects investigated in the present study, the relationship between leptin and BMI in liver disease patients seems to be linear. But, this case contrasts with a previous report, which claims whether serum leptin elevation is simply a consequence of obesity or whether it is really related to NASH[24]. In the study, serum leptin levels were increased in non-obese NASH patients and that it is impossible to explain serum leptin elevations in terms of obesity because there were only 3 obese patients.

However, neither the pathophysiological significance of this elevation nor the underlying exact mechanisms are clear at present. It has been suggested that hepatic failure in cirrhosis may cause this elevation by simply altering metabolism and clearance of leptin. At this point, the present study confirms that leptin elevation in liver cirrhosis seems to be related to hepatic failure rather than to chronic inflammation or host immune response related to hepatic cell injury. On the other hand, the similar serum leptin levels in chronic viral hepatic patients and controls point out that the increased serum leptin levels in NASH do not result simply from the liver damage. Unfortunately, clinical and histopathological signs suggesting hepatic failure in hepatitis patients are not observed in detail.

Some NASH patients in the present study had very high serum levels. These patients might be grade 2 and 3 steatohepatitis since the higher serum leptin levels were detected in those patients, compared with the grade 1 patients found in a recent study[24]. This

finding suggests that increased serum leptin level may be involved in the pathogenesis of steatohepatitis. Unfortunately, in the present study, the authors could not detect the exact grade of NASH patients from the histological point of view.

In HCV patients, there is a small increase in circulating leptin levels that did not reach statistical significance. This result agrees with a recent study that did not detect an association between circulating leptin levels and in patients with chronic HCV. Recently Hourigan *et al.* suggested that the connection between increased BMI and liver steatosis may contribute to the development of fibrosis in chronic hepatitis C (HCV)[24]. They found that increased BMI, through the presence of steatosis, leads to higher staging. The authors obtained the same results in a group of Italian patients with HCV, finding that the presence of steatosis was associated with higher fibrosis scores and decreased liver function. Furthermore, the authors found that the degree of steatosis in HCV does not seem to depend on leptin level and viral factors, at least as far as HCV viremia and genotype, and HGV co-infection is concerned. Steatosis is detected more frequently in patients infected by genotype 3 than by genotype1[25]. In patients infected with genotype 1, steatosis correlated with serum leptin levels, and in patients infected with genotype 3, steatosis is strongly related to intrahepatic HCV load. In the present study, three patients in HCV had high leptin concentration. It might be that these patients were genotype 1 HCV. The other reason might be the association between BMI, steatosis and liver fibrosis which is partly related to a higher secretion of leptin. Interestingly, Potter et al have observed leptin expression and protein synthesis in activated hepatic stellate cells grow in cell culture, suggesting that this additional source of leptin may explain elevated plasma leptin levels observed in chronic liver disease[24]. High level of leptin concentration is also found in chronic HBV. The high concentration might be related to the changes in metabolism of liver function due to the chronic viral infection. NASH patients have the highest leptin concentration, chronic HBV is moderate and HCV is the lowest when comparing these three groups.

Moreover, the serum leptin level of the liver disease groups and control group differ significantly as confirmed in the number of recent studies. It may be due to a difference in factors such as the levels of cytokines or sex steroids, and or nutrition. Furthermore, it is likely that leptin is cleared in part by the portosystemic circulation through the liver[26].

**Liver enzyme function (ALT, AST and ALP):** As found in recent research, the present study also confirms the higher level of liver enzyme function in liver disease patients. The mechanisms underlying the association of weight and liver enzyme levels could be that increased appetite and changes in food intake with increased intake of dietary fatty acids and carbohydrates in obese subjects. Carbohydrates are metabolized to free fatty acids in the liver, and a high amount of fatty acids results in a fatty liver. AST and ALT values are higher in obese patients, probably because these persons commonly have fatty livers[27]. ALT levels have been noted to decline with weight loss[28]. On the other hand, depending on the physician's point of view, the upper limits of normal for AST and ALT levels could be set higher for more obese persons. Rare individuals have chronically elevated AST levels because of a defect in clearance of the enzyme from the circulation[29]. For both AST and ALT, the average values and upper limits of normal in patients undergoing renal dialysis are about one half of those found in the general population[30]. Mild elevations of ALT or AST in symptomatic patients can be evaluated efficiently by considering alcohol abuse, hepatitis B, hepatitis C and several other possible diagnoses[31].

However, slight AST or ALT elevations (within 1.5 times the upper limits of normal) do not necessarily indicate liver disease because the mean values for ALT are very similar from one population to another, but the degree to which the distribution is skewed varies by gender and ethnicity[32].

Abnormally high ALP can have many causes other than liver damage, including bone disease, congestive heart failure, and hyperthyroidism. However, a rise in ALP levels can indicate liver trouble with elevated gamma-glutamyl transferase (GGT) levels[33].

## Conclusion

In summary, the data suggest that leptin level is increased in three groups of liver patients- HBV, HCV, NASH and it is the highest in the NASH group generally. This high leptin concentration inhibits food intake and it may change energy expenditure in different liver patients groups. Consequently, the physiological regulation of leptin might be maintained in relation to body fat, even in chronic viral liver diseases. However, the present study could not figure out the reason why some patients have very high leptin levels and some do not; even in the same disease group. To learn more about those things, prospective longitudinal clinical studies may provide further information in

this regard. Such studies must include standardized histologic definitions of steatosis, accurate measures of host factors such as body composition, insulin level and sufficient sample size to allow more reliable analyses. However, there is no correlation between leptin concentration and age, BMI, ALT, AST, ALP, and haematological parameters among Thai patients. Further studies are needed to determine more clearly the role of leptin on different types of chronic hepatitis patients.

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## ซีรั่มเลปตินในคนไข้ที่เป็นโรคตับอักเสบเรื้อรัง

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*คณะผู้วิจัยทำการศึกษาปริมาณซีรั่มเลปตินในผู้ป่วยที่เป็นโรคไวรัสตับอักเสบเรื้อรังชนิด บี, ชนิด ซี, และโรคตับที่มีการสะสมไขมันที่ไม่ใช่จากการดื่มแอลกอฮอล์ เปรียบเทียบกับคนปกติ โดยคัดเลือกอาสาสมัครกลุ่มละ 20 คน จากโรงพยาบาลเวชศาสตร์เขตร้อน คณะเวชศาสตร์เขตร้อน มหาวิทยาลัยมหิดล คณะผู้วิจัยได้ทำการตรวจหาระดับซีรั่มเลปติน เอนไซม์ที่ใช้ตรวจวัดหน้าที่ของตับ รวมทั้งดัชนีชี้วัดทางโลหิตวิทยา ในอาสาสมัครเหล่านั้น ผลการตรวจพบว่าซีรั่มเลปตินในกลุ่มผู้ป่วยตับอักเสบเหล่านี้มีระดับที่สูงอย่างมีนัยสำคัญทางสถิติเมื่อเปรียบเทียบกับกลุ่มควบคุม ซึ่งอาจจะเนื่องมาจากการที่มีพยาธิสภาพจากการสะสมไขมันที่มากผิดปกติในเซลล์ตับที่เกิดจากโรค ไม่พบว่าระดับซีรั่มเลปตินจะมีความสัมพันธ์กับดัชนีความหนาของร่างกาย เอนไซม์ที่ใช้ตรวจวัดหน้าที่ของตับ (ALT, AST, ALP) และดัชนีชี้วัดทางโลหิตวิทยา แต่พบว่าเอนไซม์ที่ใช้ตรวจวัดหน้าที่ของตับในผู้ป่วยโรคตับจะมีค่าสูงอย่างมีนัยสำคัญทางสถิติเมื่อเปรียบเทียบกับกลุ่มคนปกติ ในขณะที่ปริมาณเม็ดเลือดขาว เกล็ดเลือด และอัตราส่วนเม็ดเลือดแดงอัดแน่นจะต่ำลงในกลุ่มผู้ป่วยที่เป็นโรคตับ ดังนั้นจึงพอสรุปได้ว่าปริมาณซีรั่มเลปตินจะสัมพันธ์กับปริมาณเซลล์ไขมันที่เพิ่มขึ้นในโรคตับ ซึ่งอาจจะมีผลต่อสภาวะพยาธิสภาพของโรคได้*