

Serum Leptin Levels in Thai Patients with Nonalcoholic Fatty Liver Disease at Pramongkutklao Hospital

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ABSTRACT

Background: Leptin is a peptide hormone that mainly regulate food intake and energy expenditure of human body. A close correlation between serum leptin levels and the percentage of body fat stores is well established. Nonalcoholic fatty liver disease (NAFLD) is a common disorder which causes serum liver enzyme elevation.

Objective: Serum leptin levels were investigated in patient with NAFLD to determine a possible role in the pathogenesis.

Patients and Methods: Thirty-one patients (20 men, 11 women) with NAFLD at Pramongkutklao hospital diagnosed by a detailed clinical and laboratory evaluation (biochemical and serological tests of all other causes of hepatitis) combined with the ultrasonographic imaging of the liver which showed the typical finding of fatty liver. Thirty one healthy adults enrolled in the study as control with matched for gender, age and body mass index (BMI). Fasting blood samples were obtained, and serum leptin levels were measured by radioimmunoassay. BMI, fasting blood glucose, liver function test, lipid profile and insulin resistance were also detected.

Results: Mean serum leptin levels were significantly higher in both men and women with NAFLD than those in the control group (men 7.6 ± 2.9 vs 6.1 ± 1.4 , $p = 0.049$; women 18.4 ± 9.4 vs 8.4 ± 2.1 , $p = 0.005$). There was a significant correlation between serum leptin and BMI in women ($r = 0.87$, $p = 0.000$), fasting blood glucose in men ($r = 0.52$, $p = 0.018$). Of the predisposing factors for NAFLD, overweight was observed in 29% of patients, obesity was observed in 71% and hyperlipidemia in 45%. There was no close correlation between serum leptin and serum transaminases, gamma glutamyltranspeptidase, triglyceride level or insulin resistance.

Conclusions: The serum leptin levels were significantly higher in patients with NAFLD and was not explained simply by gender, obesity or insulin resistance. Therefore, elevated serum leptin levels may reflect a pathogenic role in hepatic steatosis or steatohepatitis.

Key words : leptin, nonalcoholic, fatty liver

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BACKGROUND

Leptin is a recently isolated protein encoded by the *ob* gene, which is an adipocyte-specific gene⁽¹⁾. It was suggested that leptin regulates body weight by decreasing food intake and increasing energy expenditure⁽²⁾. It is mainly effective through the central hypothalamic mechanism. Although little has been uncovered about leptin and its regulation in humans, it seems that it plays an essential role in the pathophysiology of obesity⁽³⁾. Leptin is expressed exclusively in adipose tissue, and the serum leptin level correlates well, and to a close degree, with the percentage of body fat and body mass index (BMI)^(4,5). Moreover, serum leptin levels are higher in women than in men, secondary to body fat composition and sex hormones⁽⁶⁾. Nonalcoholic fatty liver disease (NAFLD) and Nonalcoholic steatohepatitis (NASH) is found more frequently in patients with obesity, type 2 diabetes mellitus, and hyperlipidemia⁽⁷⁾. While once thought to be a benign condition, there is increasing evidence that NASH can lead to progressive fibrosis and eventually cirrhosis⁽⁸⁻¹⁰⁾. The pathogenesis of NAFLD or NASH remains unclear. Recent experiments showed that leptin promotes insulin resistance, elevates circulating insulin level⁽¹¹⁾, and augments inflammatory and profibrogenic responses in the murine liver exposed to hepatotoxic chemicals⁽¹²⁾. These data, in conjunction with the observation that leptin is expressed and synthesized by the activated hepatic stellate cells⁽¹³⁾, support the notion that leptin might play a role in the pathogenesis of human hepatic steatosis and steatohepatitis. Chitturi *et al.*⁽¹⁴⁾ have recently shown that serum leptin levels were significantly higher in the subjects with NASH as compared with controls. Furthermore, they showed that serum leptin was independently associated with the degree of hepatic steatosis but not hepatic inflammation or hepatic fibrosis. However, Alba *et al.* showed that the association between hepatic steatosis and serum leptin could not be confirmed and leptin is associated with more advanced hepatic fibrosis in NAFLD⁽¹⁵⁾. By conflicting results regarding the role of leptin in the pathogenesis of NAFLD or NASH and no study about leptin and NAFLD in Thailand, we conducted the following studies to further elucidate the correlation of serum leptin levels and NAFLD in Thai patients.

PATIENTS AND METHODS

Patients

A total of 31 patients (20 men, 11 women) with NAFLD and 31 healthy adults as controls were enrolled in the study. NAFLD patients were seen at our gastrointestinal and hepatology clinic with incidentally found liver enzyme elevations. For the diagnosis of NAFLD and to rule out other possible liver diseases, we used the following inclusion criteria for patient enrollment: (1) 18 years of age or above. (2) persistent elevation of aminotransferases to >1.5 times normal for >3 months. (3) upper abdominal ultrasound showed hyperechoic (bright) liver. (4) documented history of zero to minimal alcohol intake (<20 g/day). (5) normal renal function. Exclusion criteria included: (1) known overt diabetes (fasting blood glucose >126 mg/dl on two separate occasions, or therapy with antidiabetic drugs), (2) presence of other forms of liver disease (alcoholic liver disease, primary biliary cirrhosis, biliary obstruction, autoimmune hepatitis, Wilson's disease, chronic viral hepatitis B or C and hereditary hemochromatosis), (3) evidence of decompensated liver disease such as a history of or presence of ascites, bleeding varices, or hepatic encephalopathy, (4) malignancy, (5) presence of secondary causes of fatty liver, such as gastrointestinal bypass surgery or medications (amiodarone, perhexilene maleate, methotrexate, glucocorticoids, estrogens, tamoxifen, nifedipine, diltiazem or chloroquine) that induce steatosis and (6) pregnancy.

Healthy Controls

31 apparently healthy volunteers matched for age, gender, and body mass index (BMI) with normal liver function tests and fasting blood glucose were enrolled as controls. All controls had undergone an upper abdominal ultrasound to exclude hyperechoic (bright) liver.

Clinical Evaluation

A complete physical examination was performed on all subjects. Anthropometric evaluation included measures of BMI. Overweight was defined as BMI >23-25 kg/m² and obesity as BMI >25 kg/m²⁽¹⁶⁾.

Laboratory Studies

Venous blood samples were collected in the morning after overnight fast and 2 ml of serum samples were

stored at -70°C until assayed for leptin. Laboratory studies included serum liver function tests (aminotransferases, total protein, albumin, alkaline phosphatase, gamma glutamyltranspeptidase and total bilirubin); hepatitis B serology (hepatitis B surface antigen, antibody to hepatitis B surface antigen, antibody to hepatitis B core antigen); antibody to hepatitis C virus; autoantibody (ANA); iron profile (serum iron, transferrin saturation); ceruloplasmin (if the patient's age less than 40 years), fasting cholesterol, triglyceride, and glucose. Serum leptin level was measured by radioimmunoassay (Linco Reserch, St. Charles, MO). The limit of detection was 0.1ng/ml, and the intra-and interassay coefficients of variation were 3.9% and 5.2%, respectively. The homeostasis model assessment method (HOMA), a measure of insulin resistance, was calculated in the patient group with this formula:

$$\text{(fasting insulin } [\mu\text{U/ml}] * \text{fasting glucose [mmol/l]}) / 22.5$$

The insulin resistance was defined as HOMA value was more than 2⁽¹⁷⁾.

Hypercholesterolemia was defined as serum cholesterol of >200 mg/dl and hypertriglyceridemia as serum triglyceride level of >200 mg/dl⁽⁸⁾.

Statistical Analysis

Continuous variables were summarized as mean ± SD, and groups compared using unpaired t test and X² test. The correlation between leptin and BMI and biological variables were given by the Pearson corre-

lation coefficient (r).

All analysis were two-tailed and were performed using a computer based statistics (SPSS v.10). A significance level of 5% was used throughout.

RESULTS

The main predisposing factors for NAFLD were overweight in 6 (19%) patients, obesity in 22 (71%) patients, hyperlipidemia in 14 (45%) patients, and insulin resistance in 19 patients (61%). Only 3 (10%) patients had normal BMI (<23 kg/m²). Therefore, all the patients had one or more predisposing factors. In the control group, 3 (10%) subjects had normal BMI, 10 (32%) subjects were overweight, 18 (58%) subjects were obese and 9 (29%) subjects had hyperlipidemia. Clinical, biochemical characteristics of the 20 men and 11 women with NAFLD are compared (Table 1). Because age-matched controls could not be obtained for 2 men with NAFLD whose age 70 years, the mean age of men with NAFLD was 8 years older than controls (47.4 ± 12.6 years vs 39.4 ± 8.7 years; p = 0.024). Men patients with NAFLD had significantly higher levels of serum cholesterol and serum gamma glutamyltranspeptidase than the controls while women patients with NAFLD had significant higher levels of serum cholesterol than the controls. In 31 NAFLD patients, insulin resistance was found in 19 (61%) cases, 12 men and 7 women.

Figure 1 as expected, serum leptin levels were

Table 1 Characterization of 20 men and 11 women with NAFLD and matched healthy controls

	Men			Women		
	NAFLD (N = 20)	Controls (N = 20)	p*	NAFLD (N = 11)	Controls (N = 11)	p*
Age (y)	47.4 ± 12.6	39.4 ± 8.7	0.024	46.9 ± 9.8	41.1 ± 9.0	0.164
BMI (kg/m ²)	26.7 ± 3.1	25.5 ± 2.1	0.143	29.2 ± 6.0	25.4 ± 2.2	0.069
AST (u/l)	52.0 ± 17.9	29.5 ± 8.4	0.000	58.4 ± 37.0	24.0 ± 3.4	0.012
ALT (u/l)	83.3 ± 31.4	29.9 ± 9.3	0.000	88.4 ± 54.1	23.9 ± 5.8	0.003
FPG (mmol/l)	5.4 ± 0.7	5.2 ± 0.5	0.138	4.9 ± 0.6	5.2 ± 0.7	0.479
GGT (u/l)	77.6 ± 50.2	42.6 ± 10.6	0.006	63.4 ± 60.3	54.3 ± 9.2	0.631
TC (mmol/l)	227.2 ± 34.8	187.9 ± 20.0	0.000	221 ± 44.3	190.5 ± 15.8	0.049
TG (mmol/l)	150.8 ± 69.2	130.1 ± 22.4	0.215	132.9 ± 39.3	127.5 ± 26.8	0.708
Leptin (ng/ml)	7.6 ± 2.9	6.1 ± 1.4	0.049	18.4 ± 9.4	8.4 ± 2.1	0.005

Abbreviations: BMI, body mass index; AST, aspartate aminotransferase; ALT, alanine aminotransferase; FPG, fasting plasma glucose; GGT, gamma glutamyltranspeptidase; TC, total serum cholesterol; TG, total serum triglyceride.

p* values refer to comparisons between patients and matched controls.

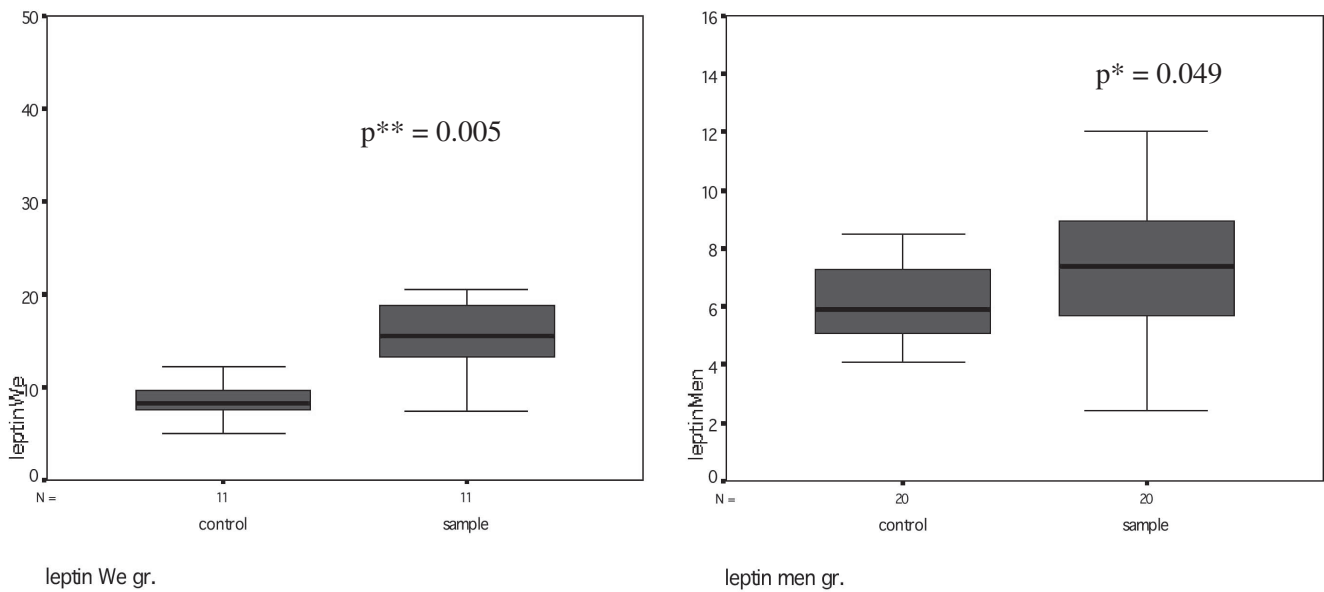


Figure 1 Gender differences in serum leptin levels. $p^* = 0.049$ in men compared with controls; $p^{**} = 0.005$ in women compared with controls.

Table 2 Correlations of serum leptin with biochemical and anthropometric variables

Sex		Age	BMI	FPG	TG	TC	HDL	LDL	AST	ALT	GGT
Men	r	0.17	0.40	0.52	-0.08	-0.48	-0.33	-0.42	0.20	0.17	0.30
	p	0.48	0.08	0.02	0.75	0.03	0.16	0.07	0.39	0.48	0.20
Women	r	-0.57	0.87	-0.64	-0.18	-0.33	0.18	-0.26	-0.03	0.18	-0.26
	p	0.07	0.00	0.03	0.61	0.32	0.60	0.45	0.93	0.73	0.45

Note: Pearson correlation coefficient (r) was used.

significantly higher in women compared with those of men in both NAFLD group and control group (18.4 ± 9.4 vs 7.6 ± 2.9 , $p = 0.003$ and 8.4 ± 2.1 vs 6.1 ± 1.4 , $p = 0.038$). Both men and women in the NAFLD group had higher mean serum leptin levels than did the men and women of the control group (7.6 ± 2.9 vs 6.1 ± 1.4 , $p = 0.049$ and 18.4 ± 9.4 vs 8.4 ± 2.1 , $p = 0.005$; respectively).

Serum leptin levels in NAFLD patients were not significantly different between those with and without insulin resistance (men, 8.37 ± 3.03 ng/ml vs 6.21 ± 2.24 , $p = 0.103$; women, 20.84 ± 11.15 vs 14.18 ± 2.63 , $p = 0.27$)

From Table 2, among serum leptin levels and metabolic and biochemical variables that were positive correlation between leptin levels and BMI in women ($r = 0.87$, $p = 0.00$) and fasting plasma glucose in men ($r = 0.52$, $p = 0.02$). While the negative corre-

lation were found between leptin levels and total cholesterol in men ($r = -0.48$, $p = 0.03$) and fasting plasma glucose in women ($r = -0.64$, $p = 0.03$). There was no correlation between serum leptin and triglyceride, HDL, LDL, serum transaminases, serum gamma glutamyltranspeptidase or insulin resistance.

DISCUSSION

In this study, significant high levels of serum leptin were found in both men and women with NAFLD. Our findings consistent with previous reports suggesting higher levels of leptin in NAFLD patients^(14,15,18). The mechanism by which leptin could promote steatosis remains unknown. Leptin could regulate body weight by decreasing food intake and increasing energy expenditure⁽¹⁹⁾ and, as a result, would correlate with BMI, percentage body fat⁽²⁰⁾ and insulin

activity⁽²¹⁾. It has been suggested that leptin could induce the release of cytokines such as tumor necrosis factor- α , interferon- α , interferon-18, and tumor growth factor α 1 and these could mediate liver steatosis and eventually fibrosis^(12,22). Another mechanism has been suggested that leptin induces insulin resistance and increases fatty acid concentrations in the liver while enhancing lipid peroxidation⁽²³⁾ and promote steatosis. Of note is that a portal perfusion of leptin in rats induced hypertriglyceridemia and contributed to hepatic steatosis by increasing free fatty acids in the liver⁽²⁴⁾.

The majority of our study patients having high levels of serum leptin were overweight or obese. Obesity is closely correlated with elevated levels of serum leptin. However, we found the positive correlation between BMI and serum leptin levels only in the women group. The most likely explanation is that the amount of total body fat and subcutaneous fat are important in determining the serum levels of leptin in obese people⁽²⁵⁾. Because total body fat or abdominal fat distribution were not determined. It is possible that women subjects with NAFLD in our study might have had significantly higher body fat or subcutaneous fat than men subjects to account for the significant positive correlation between BMI in women with NAFLD and serum levels of leptin.

The relationship between serum leptin and insulin resistance is complex. For example, whereas some *in vitro* studies and animal experiments have shown that leptin might promote insulin resistance and hyperinsulinemia^(21,26), recent studies have shown that leptin administration might actually improve the insulin resistance in patients with lipodystrophy (who have low serum leptin levels)⁽²⁷⁾. The insulin resistance now has emerged as an important factor in the pathogenesis of NAFLD^(14,28,29). In the present study, 61% of patients with NAFLD have insulin resistance. However, we failed to show any significant correlation between serum leptin and insulin resistance. We also did not find that serum leptin levels correlated with serum triglyceride, serum transaminases, or serum gamma glutamyltranspeptidase. This might be argues against the potential role played by leptin in the pathogenesis of human NAFLD or may be from the small number of subjects in our study.

A limitation of the present study is that we did not perform liver biopsy. Thus, the possibility that leptin could influence fibrotic and functional severity in NAFLD are not proven. Thus, further studies are

needed to determine more clearly the role of leptin on hepatic injury.

To minimize the risk of misclassification, we obtained a liver ultrasound in all controls and did not find a bright liver in any of them.

In summary, serum leptin levels were significantly high in Thai patients with NAFLD which is consistent with previous reports from other countries^(14,15,18) and there existed positive correlations between serum leptin and BMI in women and fasting plasma glucose in men. These data support the role of leptin in the pathogenesis of human NAFLD.

REFERENCES

1. Zhang Y, Proenca R, Maffei M, *et al.* Positional cloning of the mouse obese gene and its human homologue. *Nature* 1994; 372: 425-32.
2. Friedman JM, Halaas JL. Leptin and the regulation of body weight in mammals. *Nature* 1998; 395: 763-70.
3. Considine RV, Sinha MK, Heiman ML, *et al.* Serum immunoreactive-leptin concentrations in normal weight and obese humans. *N Engl J Med* 1996; 334: 292-5.
4. Mantzoros CS, Moschos S, Avramopoulos I, *et al.* Leptin concentrations in relation to body mass index and the tumor necrosis factor- α system in humans. *J Clin Endocrinol Metab* 1997; 82: 3408-13.
5. Blum WF, Englaro P, Hanitsch S, *et al.* Plasma leptin levels in healthy children and adolescents: dependence on body mass index, body fat mass, gender, pubertal stage, and testosterone. *J Clin Endocrinol Metab* 1997; 82: 2904-10.
6. Hickey MS, Israel RG, Gardiner SN, *et al.* Gender difference in serum leptin levels in humans. *Biochem Mol Med* 1996; 59: 1-6.
7. Shelh SG, Gordon FD, Chopra S. Nonalcoholic steatohepatitis. *Ann Intern Med* 1997; 126: 137-45.
8. Bugianesi E, Leone N, Vanni E, *et al.* Expanding the natural history of nonalcoholic steatohepatitis: from cryptogenic cirrhosis to hepatocellular carcinoma. *Gastroenterology* 2002; 123: 134-40.
9. Caldwell SH, Oelsner DH, Iezzoni JC, *et al.* Cryptogenic cirrhosis: clinical characterization and risk factors for underlying disease. *Hepatology* 1999; 29: 664-9.
10. Matteoni CA, Younossi ZM, Gramlich T, *et al.* Nonalcoholic fatty liver disease: a spectrum of clinical and pathological severity. *Gastroenterology* 1999; 116: 1413-9.
11. Sivits WI, Walsh SA, Morgan DA, *et al.* Effects of leptin on insulin sensitivity in normal rats. *Endocrinology* 1997; 138: 3395-401.
12. Ikejima K, Honda H, Yoshikawa M, *et al.* Leptin augments inflammatory and profibrogenic responses in the murine liver induced by hepatotoxic chemicals. *Hepatology* 2001; 34: 288-97.

13. Potter JJ, Womack L, Mezey E, *et al.* Transdifferentiation of rat hepatic stellate cells results in leptin expression. *Biochem Biophys Res Commun* 1998; 244: 178-82.
14. Chitturi S, Farrell G, Frost I, *et al.* serum leptin in NASH correlates with hepatic steatosis but not fibrosis: a manifestation of lipotoxicity? *Hepatology* 2002; 36: 403-9.
15. Alba LM, Petrovic L, Lindor KD, *et al.* Leptin is an independent marker of liver fibrosis in humans with nonalcoholic fatty liver disease. *Hepatology* 2002; 3: 296A (abstract).
16. World Health Organization. The Asia-Pacific perspective: redefining obesity and its treatment. [online]. 2000. [access 2003 January]. Available from URL <http://www.obesityasiapacific.com>.
17. Haffner SM, Miettinen H, Stern MP. The homeostasis model in the San Antonio heart study. *Diabetes Care* 1997; 20: 1087-92.
18. Uygun A, Kadayifci A, Yesilova Z, *et al.* Serum leptin levels in patients with nonalcoholic steatohepatitis. *Am J Gastroenterol* 2000; 95: 3584-9.
19. Ockenga J, Bischoff SC, Tilmann HL, *et al.* Elevated bound leptin correlate with energy expenditure in cirrhotics. *Gastroenterology* 2000; 119: 1656-62.
20. Considine RV, Sinha MK, Heiman ML, *et al.* Serum immunoreactive leptin concentrations in normal-weight and obese humans. *N Engl J Med* 1996; 334: 292-5.
21. Cohen B, Novick D, Rubinstein M. Modulation of insulin by leptin. *Science* 1996; 274: 1185-8.
22. Powell EE, Edwards-Smith CJ, Hay JL, *et al.* Host genetic factors influence disease progression in chronic hepatitis c. *Hepatology* 2000; 31: 828-33.
23. Kaplan LM. Leptin, obesity and liver disease. *Gastroenterology* 1998; 115: 997-1001.
24. Roden M, Anderwald C, Fornsinn C, *et al.* Effects of shortterm leptin exposure on triglyceride deposition in rat liver. *Hepatology* 2000; 32: 1045-9.
25. Van Harmelen V, Reynisdottir S, Eriksson P, *et al.* Leptin secretion from subcutaneous and visceral adipose tissue in women. *Diabetes* 1998; 47: 913-7.
26. Sivitz WL, Walsh SA, Morgan DA, *et al.* Effects of leptin on insulin sensitivity in normal rats. *Endocrinology* 1997; 138: 3395-401.
27. Oral EA, Simha V, Ruiz E, *et al.* Leptin-replacement therapy for lipodystrophy. *N Engl J Med* 2002; 346: 570-8.
28. Pagano G, Pacini G, Musso G, *et al.* Nonalcoholic steatohepatitis, insulin resistance, and metabolic syndrome: further evidence for an etiologic association. *Hepatology* 2002; 35: 367-72.
29. Willner IR, Waters B, Patil SR, *et al.* Ninety patients with nonalcoholic steatohepatitis: insulin resistance, familial tendency, and severity of disease. *Am J Gastroenterol* 2001; 96: 2957-61.