Available online at www.sciencedirect.com

SCIENCE DIRECT

Metabolism Clinical and Experimental 54 (2005) 445–452

Metabolism
Clinical and Experimental

www.elsevier.com/locate/metabol

Different tumor necrosis factor-α–associated leptin expression in rats with dimethylnitrosamine and bile duct ligation–induced liver cirrhosis

Shih Yi Lin[a,b], Wen Yin Chen[c], Yung Tsung Chiu[c], Wen Jane Lee[c], Hurng Sheng Wu[d], Wayne Huey-Herng Sheu[a,b,c,*]

[a]*Division of Endocrinology and Metabolism, Taichung Veterans General Hospital, Taichung 407, Taiwan*
[b]*Institute of Clinical Medicine, School of Medicine, National Yang Ming University, Taipei 112, Taiwan*
[c]*Department of Medical Education and Research, Taichung Veterans General Hospital, Taichung 407, Taiwan*
[d]*Department of Surgery, Show-Chwan Memorial Hospital, Changhua 500, Taiwan*

Received 11 February 2004; accepted 25 October 2004

Abstract

Although serum leptin concentrations are reported by several studies to increase in patients with liver cirrhosis, the mechanisms underpinning this increase remain unclear. Circulating tumor necrosis factor α (TNF-α) concentrations are also recognized to increase in liver cirrhosis. Furthermore, TNF-α administration to rodents results in increased expression and secretion of leptin from adipose tissue in a manner dependent on type 1 TNF-α receptor (TNF-RI). The present study was undertaken to examine adipose leptin expression and to explore potential relationships between leptin expression and TNF-α in subjects with liver cirrhosis. Liver cirrhosis was induced in male Sprague-Dawley rats by dimethylnitrosamine (DMN) administration or by common bile duct ligation (BDL). Ad libitum and pair-fed animals constituted controls. Serum leptin and TNF-α concentrations were determined by immunoassay. Gene expression was determined by the reverse transcription–polymerase chain reaction, and protein levels were measured by Western blotting. Serum leptin values after adjustment of body fat mass in DMN-treated rats were significantly higher than in pair-fed or ad libitum groups. Leptin mRNA and protein levels in epididymal fat in DMN rats increased by 1.8-fold and 2.3-fold, respectively, as compared with ad libitum controls, and by 4-fold and 6-fold, respectively, as compared with the pair-fed group. Epididymal TNF-α and membranous TNF-RI (mTNF-RI) concentrations were both 2.3 times higher in DMN rats than in ad libitum controls but did not differ between ad libitum and pair-fed groups. Adipose leptin protein levels correlated directly with TNF-α and mTNF-RI concentrations in combined DMN, ad libitum, and pair-fed rats ($r = 0.64$ and $r = 0.49$, respectively; $P < .05$). In BDL-treated rats, however, serum and adipose leptin concentrations were identical to those in ad libitum controls despite 2.1-fold and 2.4-fold increase in epididymal TNF-α and mTNF-RI, respectively. TNF-α administration to fasting control animals increased serum and adipose leptin concentrations significantly. The observed TNF-α–associated leptin up-regulation in DMN–induced, but not in BDL–induced, cirrhotic rats is consistent with distinctly different roles for TNF-α in rats with nonbiliary, as opposed to biliary, cirrhosis.

1. Introduction

Malnutrition is frequently observed in patients with liver cirrhosis, with the potential for deterioration of clinical functions and resultant poor prognosis [1]. However, the mechanisms underlying the energy imbalance in this condition are not clear [2]. Leptin, a 16-kDa protein product of the ob gene of adipose tissue, is postulated to regulate energy balance by suppressing appetite and increasing energy expenditure [3,4]. Serum leptin concentrations were found by several studies to be elevated in cirrhotic patients and to correlate with energy expenditure rates [5-7]. It was, therefore, suggested that leptin functions in the development of anorexia or the physical wasting associated with cirrhosis [5]. Although the causes(s) of cirrhotic hyperleptinemia are currently unexplained, decreased clearance or induction of leptin release by the secretogogues is proposed to contribute this phenomenon [7].

Tumor necrosis factor α (TNF-α) is a pleiotropic hormone with numerous immunologic and metabolic actions mediated by 2 distinct cell-surface receptors, termed TNF-RI

* Corresponding author. Department of Medical Education and Research, Taichung Veterans General Hospital, Taichung, Taiwan.
Tel.: +886 4 235 92525x4000; fax: +886 4 235 02942.
E-mail address: whhsheu@vghtc.vghtc.gov.tw (W.H.-H. Sheu).

0026-0495/$ – see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2004.10.011

and TNF-RII [8]. Administration of TNF-α was recently reported to stimulate leptin production and secretion from adipose tissue through TNF-RI of adipocytes [9-12]. This TNF-α–induced hyperleptinemia was therefore proposed to function in the negative energy balance and weight loss associated with inflammatory disorders. Serum leptin concentrations of patients with congestive heart failure or chronic obstructive pulmonary disease, both of which are characterized by cachexia and increased inflammation, are reported to correlate with circulating soluble TNF-α receptor levels [13,14]. By contrast, other findings have cast doubt on a linkage between leptin concentrations and cachexia. For example, circulating leptin levels in patients with inflammatory bowel disease, human immunodeficiency virus infection, or cancer were found to be decreased despite increased TNF-α system activity [15,16].

The TNF-α system activity is increased in liver cirrhosis and generally thought to be associated with several known cirrhosis-related complications such as hyperdynamic circulation, susceptibility to infection, and hepatic encephalopathy [17-19]. However, the role of the factor in cirrhotic hyperleptinemia is currently unexplored. Based on various findings linking TNF-α with leptin production, we hypothesized that an activated TNF-α system mediates the increased leptin concentrations of liver cirrhosis. To examine leptin expression in liver cirrhosis, 2 established rodent models of this disorder were used. These models were also used to explore potential relationships between leptin expression and components of the TNF-α system.

2. Materials and methods

2.1. Animal models

Experimental models of rat liver cirrhosis are usually of the toxic or biliary type. To ascertain whether hepatic injury affects adipose leptin expression and whether changes in expression are injury type–specific, both the common bile duct ligation (BDL) and the dimethylnitrosamine (DMN) administration models were used. These 2 experimental models, which are characterized by classical cirrhotic alterations, are well established [20,21]. Besides, activations of the TNF-α system are readily observed in both of these models [22,23]. For BDL-induced liver cirrhosis, male Sprague-Dawley rats (purchased from the National Science Council Animal Center, Taipei, Taiwan) weighing 200 to 250 g at the time of cirrhosis induction were selected. The animals were anesthetized with phenobarbital (60 mg/kg body weight intramuscularly), and the common bile duct was exposed and ligated by 2 ligatures with 3-0 silk. The first ligature was made below the junction of the hepatic ducts, and the second ligature was made above the entrance of the pancreatic ducts. The common bile duct was then resected between the 2 ligatures. Benzathine benzylpenicillin was administered postoperatively (50 000 units intramuscularly) for prophylaxis of infection. Vitamin K (8 mg/kg intramuscularly) was given at weekly intervals after surgery. To allow adequate time for recovery, animals were used for experiments 5 to 6 weeks after surgery. To induce liver cirrhosis with DMN, 10 mg/kg of this reagent was dissolved in saline to obtain a 1% solution, which was administered intraperitoneally on 3 consecutive days per week for 3 to 4 weeks. During the induction period, rats were housed in plastic cages and allowed to have free access to food and water. To decrease interobservational variation, rats were divided into 3 to 4 groups before cirrhosis induction and were then induced consecutively at intervals of 1 to 2 weeks. In all experiments, authors adhered to the Guiding Principles for the Care and Use of Laboratory Animals as recommended by the Taiwan Government.

2.2. Experimental procedures

Throughout the cirrhosis induction period, changes in body weight and food intake were recorded weekly. After intraperitoneal anesthesia with sodium phenobarbital, intraarterial catheterization of the left femoral artery was performed in cirrhotic and control animals. Blood samples were withdrawn and immediately subjected to centrifugation, and frozen at −70°C until analysis. In addition, epididymal fat was dissected and weighed, and adipose samples were frozen at −70°C until analysis. To evaluate the anorectic effects of leptin, control rats (~200 g) were injected intraperitoneally once with 3 mg/kg of leptin at 4 PM followed by recording of food intake over the next 18 hours. In addition, leptin measurements were performed on TNF-α treated animals (150 μg/kg) after a 12-hour fast. During the experiment, all animals were still food-deprived, and blood samples and epididymal adipose tissues were collected at 8 hours after cytokine administration.

2.3. Reagents and substrate assays

Injectible human recombinant TNF-α and rat leptin were obtained from PeproTech Inc (London, UK). Blood TNF-α (Biosource, Nivelles, Belgium) and leptin (Crystal Chem Inc, Grove, Ill, USA) concentrations were determined in duplicate with enzyme-linked immunosorbent assay kits. For Western blot analyses, antirat leptin polyclonal antibody was purchased from Santa Cruz Laboratories (Santa Cruz, Calif, USA), TNF-α polyclonal antibody from PeproTech EC Ltd, and TNF-α receptor type 1 (TNF-RI) polyclonal antibody from StressGen Biotechnologies (Victoria, British Columbia, Canada). Serum alanine aminotransferase, alkaline phosphatase, total bilirubin, albumin, glucose, and creatinine concentrations were determined by standard laboratory methods.

2.4. Total body fat assessment

Body composition was assessed in anesthetized rats before sacrifice using an EM-SCAN TOBEC SA-3000 (EM-SCAN Inc, Springfield, Ill, USA). Measurements were based on energy absorption in the presence of a radiofrequency electromagnetic field.

2.5. RNA preparation and analyses of gene expression

Total mRNA was extracted using TriZol agents (Life Technologies, Inc, Rockville, Md, USA), and mRNA concentrations were determined by ultraviolet light absorbency at 260 nm. Expression of mRNAs was determined by semiquantitative reverse transcription–polymerase chain reaction (RT-PCR) analysis. Briefly, 1 to 5 μg of total RNA was reverse-transcribed at 42°C for 1 hour using standard reagents which included 50 μL 1× RT buffer, reverse transcriptase, and poly(dT) [12-18] primer. The RT mixtures were incubated at 100°C for 10 minutes to inactivate reverse transcriptase. Each PCR reaction was conducted in a total volume of 50 μL 1× PCR buffer containing 5 μL RT template, 200 nM each of sense and antisense primers, 1 U of Taq polymerase, and 200 μM each of deoxynucleotide triphosphates (dNTPs). The solution was covered with 30 μL mineral oil, and PCR was performed in a DNA Thermal Cycler 480 (Perkin-Elmer Co, Norwalk, Conn, USA). An initial denaturation at 94°C for 5 minutes was followed by cycles of denaturation at 94°C for 30 seconds, annealing at a specific temperature for each product for 1 minute, and elongation at 72°C for 1 minute. PCR cycles for each product were determined in a preliminary run to assure an optimal amount of products. The last cycle was followed by a final extension of 5 minutes at 72°C. The sequences of primers for leptin were 5′-GTGGCTTTGGTCCTATCTGTCCTATG-3′ (sense) and 5′-TCAGGGCTAAGGTCCAACTGTTGAAG-3′ (antisense). A constitutively expressed gene, β-actin, constituted an internal control. Primers for β-actin were 5′-TCCTGTGGCATCCACGAAACT-3′ (sense) and 5′-GGAGCAATGATCTTGATCTTC-3′ (antisense). The primers for leptin and β-actin were designed to allow amplification of 458– and 180–base-pair fragments after 35 PCR cycles at annealing temperatures of 55°C and 58°C, respectively. As references for the quantitation of gene expression, the β-actin housekeeping gene was amplified under conditions similar to those of leptin. To determine the relative quantities of mRNA encoding leptin, 10 μL each of leptin and β-actin PCR products amplified from the same RT template solution were combined and subjected to electrophoresis on 2% agarose gels in Tris-acetate-EDTA buffer for 30 minutes. After staining gel with ethidium bromide for 15 minutes, bands corresponding to leptin and β-actin mRNAs were subjected to densitometry and quantitated using Image Quant Analysis Software. The relative mRNA concentrations of leptin in the original RNA extracts from the various adipose tissue preparations were obtained with normalizing leptin mRNA expression to β-actin mRNA expression.

2.6. Western blot analyses

Extracts for measurement of TNF-α and leptin protein were prepared by thorough homogenization of epididymal fat tissue in ice-cold protein lysis buffer (1% Triton ×100, Tris-HCl, pH 7.6, 150 mM NaCl) supplemented with protease inhibitors (1 mM phenylmethylsulfonylfluoride and Complete, Roche, Switzerland). Extracts were subjected to centrifugation at 2500 *g* for 10 minutes at 4°C. For measurements of plasmalemmal TNF-RI (membranous TNF-RI [mTNF-RI]), fat tissue was first homogenized in 50 mM Tris-HCl, pH 7.5, containing 10 μg/mL leupeptin, 20 mM NaF, 0.5 mM EGTA, 0.1 mM benzamidin, and 0.5 mM EDTA at 4°C. Total membrane fractions were then isolated by centrifugation of homogenates at 146 000 *g* for 75 minutes. Protein concentrations were determined by the BradFord assay (Bio-Rad, Richmond, Calif, USA). Protein samples were diluted with Laemmli buffer (35 mM Tris, 5% glycerol, 2% sodium dodecyl sulfate, 5% mercaptoethanol) and incubated for 5 minutes at 95°C. Samples (100 μg protein) were subjected to electrophoresis on 15%, 7.5%, or 20% sodium dodecyl sulfate/polyacrylamide gels for determinations of TNF-α, TNF-RI, or leptin, respectively, followed by electroblotting onto nitrocellulose membranes. After blocking with 2.5% nonfat dried milk

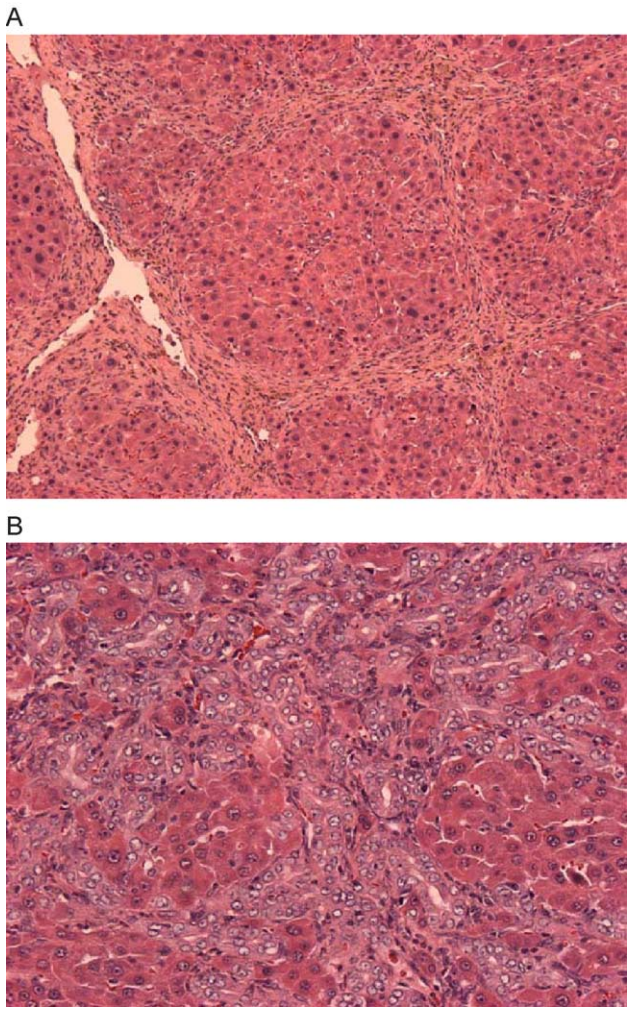

Fig. 1. Hematoxylin and eosin preparations of representative liver samples of (A) DMN-induced cirrhosis with portoportal, centroportal fibrosis, and nodular transformation, and (B) biliary cirrhosis with ductular proliferation, expansion of portal tracts with portoportal and centroportal linkage leading to separation of small nodules of parenchyma.

Table 1
Biochemical characterization of DMN-treated rats, bile duct ligation BDL-induced cirrhotic rats, and ad libitum controls

	Ad libitum (n = 12)	BDL (n = 12)	DMN (n = 12)
Creatinine (mg/dL)	0.61 ± 0.03	0.43 ± 0.04*	0.50 ± 0.03*
AST (U/L)	99 ± 9	801 ± 110*	249 ± 32*
ALT (U/L)	44 ± 5	189 ± 20*	131 ± 15*
Alk-P (U/L)	276 ± 14	575 ± 34*	658 ± 13*
Albumin (g/dL)	3.53 ± 0.07	2.73 ± 0.05*	2.89 ± 0.12*
Total bilirubin (mg/dL)	0.03 ± 0.01	7.69 ± 0.37*	1.23 ± 0.84*

Values are expressed as mean ± SEM. ALT indicates alanine aminotransferase; Alk-P, alkaline phosphatase.
* $P < .01$ versus ad libitum control.

in Tris-saline buffer containing 0.05% Tween 20, membranes were immunoblotted with TNF-α, leptin, or TNF-RI antibodies diluted 1:5000, 1:150, or 1:2000, respectively. After washing, blotted membranes were stained with horseradish peroxidase–labeled antirabbit or antigoat IgG secondary antibodies (Amersham Co, Arlington Heights, Ill, USA) diluted 1:200. Specific protein bands were visualized by enhanced chemiluminescence (Amersham Co, Arlington Heights, Ill, USA).

2.7. Statistical analyses

All data were expressed as mean values ± SEM. Statistical differences were determined by the Mann-Whitney *U* nonparametric test (comparisons between 2 groups) or the Kruskal-Willis 1-way analysis (comparison among more than 2 groups). The relationship between 2 variables was determined by the Spearman rank-order correlation. Results were considered statistically significant at $P < .05$. All data were analyzed by SPSS software (Statistical Package for the Social Sciences, version 6.0 for Windows, SPSS Inc, Chicago, Ill, USA).

3. Results

3.1. General effects of BDL and DMN treatments

Macroscopic and histological liver examinations were performed to confirm the presence of liver cirrhosis in both BDL- or DMN-treated rats (Fig. 1). In addition, serum albumin, total bilirubin, alkaline phosphatase, and alanine

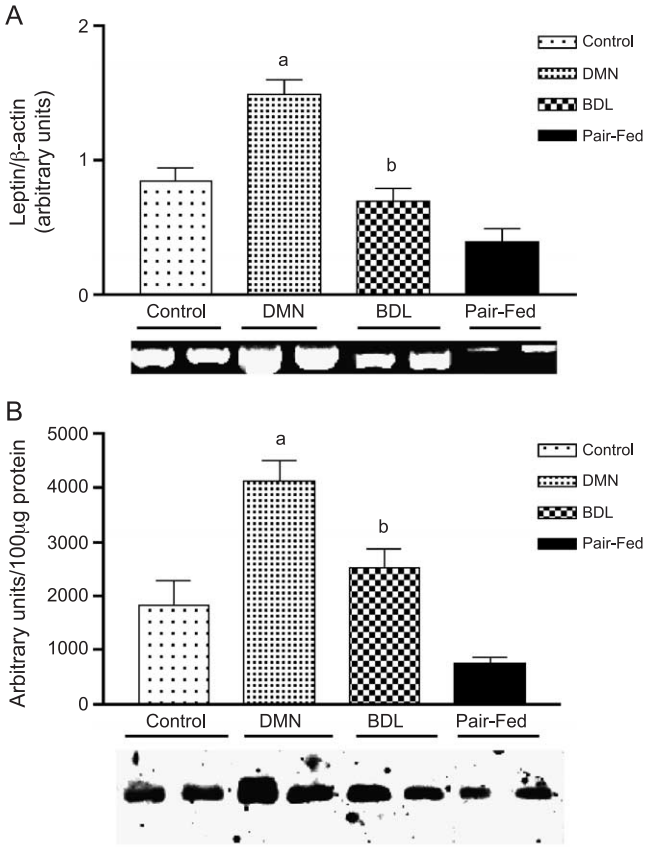

Fig. 2. Adipose tissue leptin mRNA (A) and protein (B) levels in DMN-treated rats, BDL-induced cirrhotic rats, and ad libitum, pair-fed controls. ([a]$P < .05$ vs ad libitum and pair-fed; [b]$P < .05$ vs pair-fed).

and aspartate transferase concentrations were found to be altered significantly after induction of cirrhosis by either method (Table 1). Circulating liver enzyme and bilirubin concentrations were higher in BDL-treated than in DMN-treated rats. Food intake was significantly reduced (50% decrease) at the start of the second week in DMN-induced cirrhotic rats, but except during the first week, no differences in food intake between BDL rats and normal ad libitum controls were observed throughout the induction period (Table 2). In DMN-treated rats body weight, fat mass and liver weight were lower than in the ad libitum controls (Table 2). In BDL-treated rats, body weight and fat mass were significantly lower as compared with ad libitum

Table 2
Anthropometric parameters and serum leptin and TNF-α levels in DMN-treated rats, BDL-induced cirrhotic rats, and ad-libitum, pair-fed controls

	Ad libitum (n = 12)	BDL (n = 12)	DMN (n = 12)	Pair-fed (n = 12)
Body weight (g)	468.1 ± 9.7	438.1 ± 15.0*	297.3 ± 14.7*	356.0 ± 18.2*
Average intake (g/wk)	210 ± 8	193 ± 16	102 ± 24*	101 ± 16*
Liver weight (g)	18.1 ± 0.9	32.9 ± 1.6*	10.1 ± 1.2*	Not determined
Fat mass (g)	104.2 ± 3.2	73.5 ± 5.1*	51.0 ± 3.0*	64.4 ± 3.8*
Leptin (pg/mL)	2681 ± 386	2010 ± 223	3663 ± 614	1116 ± 68*
Leptin/fat mass (pg/mL · g)	27.6 ± 3.3	28.0 ± 2.5	74.6 ± 13.6*	17.7 ± 1.9*
TNF-α (pg/mL)	<0.7	11.1 ± 4.1*	7.5 ± 2.0*	<0.7

Values are expressed as mean ± SEM.
* $P < .01$ versus ad libitum control.

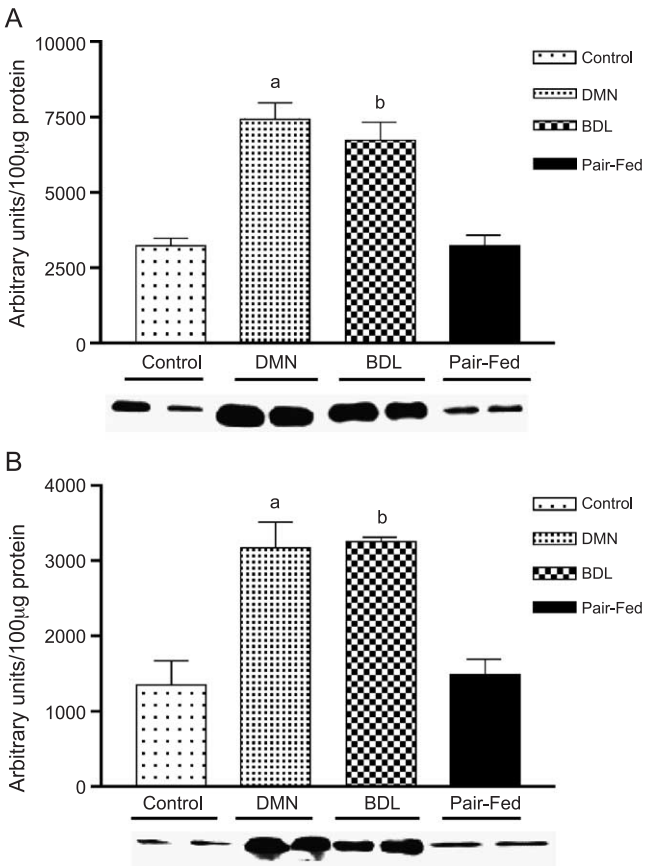

Fig. 3. Adipose TNF-α (A) and mTNF-α receptor I (B) protein levels in DMN-treated rats, BDL-induced cirrhotic rats, and ad libitum, pair-fed controls. ([a]$P < .05$ vs ad libitum and pair-fed; [b]$P < .05$ vs ad libitum and pair-fed).

controls, whereas liver weight was increased in the treated animals because of bile stasis (Table 2).

3.2. Serum leptin and TNF-α in BDL- and DMN-treated rats

A significant correlation between body fat mass and serum leptin values was observed in all combined ad libitum, pair-fed, and cirrhotic rats ($r = 0.69$; $P < .01$). Serum leptin levels in DMN-treated rats were significantly higher than those in the pair-fed group but showed no difference to that in ad libitum groups (Table 2). However, after adjustments for body fat mass, the leptin/fat mass ratio in DMN-treated rats was significantly higher as compared with ad libitum and pair-fed controls (Table 2). In the BDL-treated animals, either with or without adjustment for fat mass, serum leptin concentrations and ad libitum groups were not significantly different.

Serum TNF-α concentrations were significantly elevated in both BDL- and DMN-treated rats but comparable increases in cytokine were not observed in the ad libitum or pair-fed controls (Table 2). A significant negative correlation was observed between serum TNF-α and albumin (parameter of hepatic function) concentrations in all cirrhotic rats ($r = -0.67$; $P < .01$) but not between TNF-α and hepatic enzymes (parameter of hepatic injury).

3.3. Leptin, TNF-α, and membranous TNF-RI expression in epididymal fat

Leptin mRNA and protein levels in adipose tissue of pair-fed controls, similarly to serum leptin concentrations in these animals, decreased by 50% and 42%, respectively, as compared with the ad libitum group (Fig. 2). In DMN-treated rats, the leptin gene and protein expression in epididymal fat were increased by 1.8-fold and 2.3-fold, respectively, as compared with free-fed controls, and by 4-fold and 6-fold, respectively, as compared with the pair-fed group (Fig. 2). By contrast, the leptin values for BDL-treated rats were similar to those of ad libitum controls but higher than those of pair-fed animals (Fig. 2). As compared with ad libitum controls, fat TNF-α and mTNF-RI concentrations were increased by 2.3-fold in DMN-induced cirrhotic rats, and by 2.1-fold and 2.4-fold, respectively, in BDL-treated rats (Fig. 3). However, adipose TNF-α and mTNF-RI levels concentrations in the ad libitum and pair-fed groups were not significantly differently. A statistical correlation of adipose leptin protein concentrations with TNF-α ($r = 0.64$; $P < .01$) and TNF-RI concentrations ($r = 0.49$; $P < .05$) were in combined DMN-treated, free-fed, and pair-fed animals (Fig. 4).

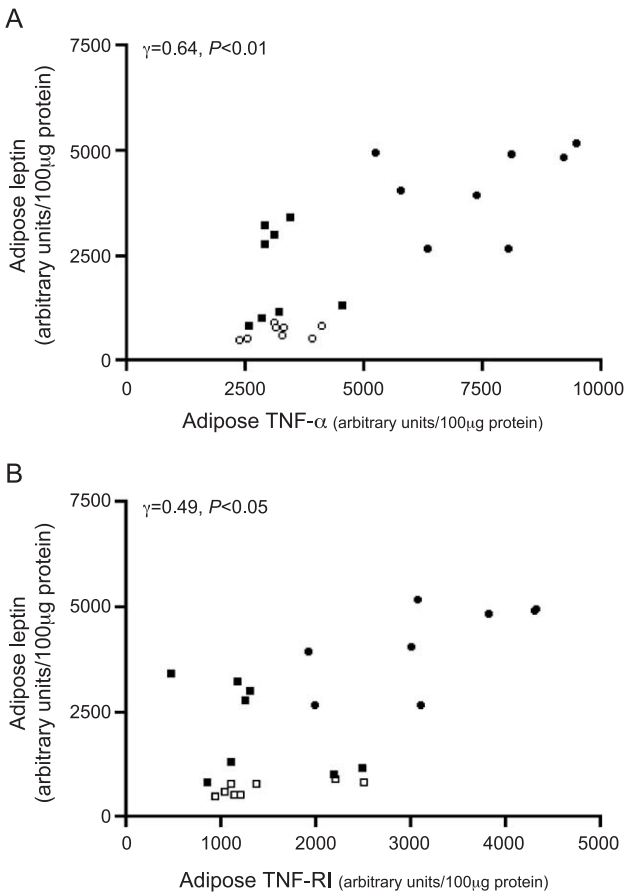

Fig. 4. Relationship of adipose TNF-α (A) and mTNF-RI (B) levels with adipose leptin levels. ■ Indicates ad libitum controls; ○, □, pair-fed controls; ●, DMN-induced cirrhotic rats.

3.4. Effects of leptin on food intake and effects of TNF-α injection on leptin concentrations

Food intake by leptin-treated control rats (n = 6) was approximately 20% to 25% lower than by vehicle-injection groups (n = 6) (20.4 ± 0.6 vs 25.4 ± 0.5 g; $P < .05$). At 8 hours after TNF-α injection, both serum and adipose leptin levels were increased significantly as compared with that in the vehicle control group (1090 ± 60 vs 350 ± 30 pg/mL; 266 ± 20 vs 130 ± 16 pg/mg protein; respectively; $P < .05$).

4. Discussion

In DMN-induced cirrhotic rats described in this study, serum leptin concentrations and body fat mass were found to correlate directly as in other cirrhotic subjects [24,25]. Furthermore, despite lower body fat mass, DMN-treated animals were found to have higher circulating leptin values in association with increased adipose leptin expression relative as compared with ad libitum controls. This finding implicates that factors other than adiposity itself are responsible in the leptin up-regulation in DMN-induced cirrhotic animals. Because administration of TNF-α resulted a substantial increase in circulating and adipose leptin values in normal rats and fat leptin concentrations correlated with adipose TNF-α and TNF-RI concentrations levels in cirrhotic animals, it appears reasonable to propose that TNF-α plays a role in this phenomenon in DMN-induced cirrhosis as reported in other inflammatory diseases [9,10,26]. This TNF-α–leptin linkage is also supported by the recent report that activated TNF-α system correlates with circulating leptin levels in cirrhotic patients [27]. It should be noted, however, that direct evidence for a cause-and-effect relationship between TNF-α and leptin expression was not proved in this study, and the possibilities remain that factors other than or in addition to TNF-α are responsible for the observed up-regulation. For example, interleukin-1, another cytokine elevated in liver cirrhosis or glucocorticoids subject to activation by TNF-α may be capable of inducing leptin synthesis [9,28]. Studies with specific anti–TNF-α antibodies or pharmacological agents that block TNF-α production may provide better evidence for a direct role of TNF-α in leptin up-regulation.

Increased endogenous TNF-α in advanced liver disease is generally proposed to be the consequence of chronic liver failure, which is associated with endotoxin-dependent macrophage stimulation and decreased cytokine clearance [29]. Interestingly, this study also for the first time demonstrates that adipose TNF-α concentrations are increased in liver cirrhosis, as is the case for several rodent models of obesity [30]. This finding is not surprising, however, because activated macrophages can circulate to several organs and thereby increase adipose TNF-α in liver cirrhosis. Endotoxin was recently reported to increase TNF-α release from isolated adipocytes [31,32]. As endotoxemia is frequently observed in cirrhosis, adipocytes may represent another potential sources for increased adipose TNF-α. The chemical DMN has been shown to stimulate TNF-α production in macrophages [33]. However, the increase in fat TNF-α observed in cirrhotic rats in this study seems less likely owing to chemical induction because the cytokine was elevated in both DMN- and BDL-treated animals.

The parallel increases in circulating and adipose leptin concentrations seen in DMN-induced cirrhotic rats were not observed in BDL-treated animals, although TNF-α system activity was increased in both groups. This finding is compatible with the observations that patients with biliary cirrhosis exhibit decreased serum leptin concentrations, whereas patients with other types of liver cirrhosis display increased serum leptin concentrations [5-7,10,34]. Significant reductions in circulating and adipose leptin levels have been observed in rodents after acute obstruction of the common bile duct [35]. However, intraduodenal infusion of bile acids restores adipose leptin concentrations to normal [36]. It has been suggested that bile acids in the intestinal lumen alter adipose leptin expression through the effects of neuroendocrine mediators. In addition, biliary cirrhosis is known to be associated with fat malabsorption, which of itself may decrease adipose and serum leptin concentrations [37,38]. A variety of factors may therefore contribute to the low circulating and tissue leptin levels observed in BDL-induced cirrhotic rats.

The exact clinical relevance of the increased fat TNF-α and leptin concentrations in liver cirrhosis is currently unclear. Based on the physiological effects that TNF-α can increase circulating leptin concentrations and leptin suppresses food intake significantly in normal animals, it seems conceivable to speculate that the TNF-α–induced leptin overexpression observed in DMN-treated animals may be responsible for their decreased food intake. However, leptin may also not be the sole mediator of reduced feeding in DMN-induced cirrhotic rats. Grunfeld et al have demonstrated that lipopolysaccharide and its associated cytokines produce anorexia in ob/ob and db/db mice, both of which lack leptin or its receptor [39]. It is therefore possible that increased TNF-α promotes anorexia even in the absence of leptin in inflammatory wasting diseases such as liver cirrhosis. Studies involving induction of cirrhosis in leptin and leptin receptor–deficient or in TNF-α and TNF-α receptor–deficient animals should prove helpful in clarifying the respective roles of leptin and TNF-α in the anorexia and cachexia of liver cirrhosis. TNF-α and leptin have also been shown to modulate insulin action in adipocytes and skeletal muscle [40-42]. As decreased insulin sensitivity is frequently present in liver cirrhosis, TNF-α–dependent leptin up-regulation may be linked to cirrhotic insulin resistance [43]. Leptin was recently reported to enhance the fibrogenic response induced by hepatotoxic chemicals [44]. In the presence of such inappropriate leptin up-regulation in cirrhotic status, exacerbation of disease severity would be anticipated.

In summary, fat and circulating leptin concentrations were found to increase in DMN-treated, but not in BDL-induced, cirrhotic rats despite the presence of enhanced TNF-α system activity in both groups. The TNF-α–associated up-regulation of leptin in DMN-treated, but not in BDL-treated, animals implies distinctly different roles of the cytokine in biliary, as opposed to nonbiliary, cirrhosis. The observed association between TNF-α and leptin in DMN-induced cirrhotic rats is proposed to contribute to the metabolic abnormalities displayed by these animals.

Acknowledgment

This work was supported by grants from National Science Council, Taipei, Taiwan, and from Taichung Veterans General Hospital (grant no. NSC 91-2314-B-075A-009, TCVGH 937303C, TCVGH 937301A, NSC 92-2314-B-075A-004, NSC 93-2314-B-075A-002).

References

[1] Italian Multicentre Cooperative Project on Nutrition in Liver Cirrhosis. Nutrition status in cirrhosis. J Hepatol 1994;21:317-25.
[2] Muller MJ. Malnutrition in cirrhosis. J Hepatol 1995;23(Suppl 1): 31-45.
[3] Zhang Y, Proenca R, Maffei M, et al. Positional cloning of the mouse obese gene and its human homologue. Nature 1994;372:425-32.
[4] Pelleymounter MA, Cullen MJ, Baker MB, et al. Effects of the obese gene product on body weight regulation in ob/ob mice. Science 1995;269:540-3.
[5] McCullough AJ, Bugianesi E, Marchesini G, et al. Gender-dependent alterations in serum leptin in alcoholic cirrhosis. Gastroenterology 1998;115:947-53.
[6] Ockenga J, Bischoff SC, Tillmann HL, et al. Elevated bound leptin correlates with energy expenditure in cirrhotics. Gastroenterology 2000;119:1656-62.
[7] Henriksen JH, Holst JJ, Moller S, et al. Increased circulating leptin in alcoholic cirrhosis: relation to release and disposal. Hepatology 1999; 29:1818-24.
[8] Tartglia LA, Goeddel DV. Two TNF receptors. Immunol Today 1992;13:151-3.
[9] Sarraf P, Frederich RC, Turner EM, et al. Multiple cytokines and acute inflammation raise mouse leptin levels : potential role in inflammatory anorexia. J Exp Med 1997;185:171-5.
[10] Grunfeld C, Zhao C, Fuller J, et al. Endotoxin and cytokines induce expression of leptin, the ob gene product, in hamster. J Clin Invest 1996;97:2152-7.
[11] Finck BN, Kelley KW, Dantzer R, et al. In vivo and in vitro evidence for the involvement of tumor necrosis factor-α in the induction of leptin by lipopolysaccharide. Endocrinology 1998;139:2278-83.
[12] Finck BN, Johnson RW. Tumor necrosis factor (TNF)-α induces leptin production through the p55 TNF receptor. Am J Physiol Regul Integr Comp Physiol 2000;278:R537-43.
[13] Filippatos GS, Tsilisa K, Venetsanou K, et al. Leptin serum levels in cachetic heart failure patients: relationship with tumor necrosis factor-α system. Int J Cardiol 2000;76:117-22.
[14] Schols AMWJ, Creutzberg EC, Burrman WA, et al. Plasma leptin levels are related to proinflammatory status and dietary intake in patients with chronic obstructive pulmonary disease. Am J Respir Crit Care Med 1999;160:1220-6.
[15] Ballinger A, Kelly P, Hallyburton E, et al. Plasma leptin in chronic inflammatory bowel disease and HIV: implications for the pathogenesis of anorexia and weight loss. Clin Sci 1998;94:479-83.
[16] Mantovani G, Maccio A, Mura L, et al. Serum levels of leptin and proinflammatory cytokines in patients with advanced-stage cancer at different sites. J Mol Med 2000;78:554-61.
[17] Wang SS, Lee FY, Chan CC, et al. Sequential changes in plasma cytokine and endotoxin levels in cirrhotic patients with bacterial infection. Clin Sci 2000;98:419-25.
[18] Odeh M, Sabo E, Srugo I, et al. Serum levels of tumor necrosis factor-alpha correlate with severity of hepatic encephalopathy due to chronic liver failure. Liver Int 2004;24:110-6.
[19] Lee FY, Lu RH, Tsai YT, et al. Plasma interleukin-6 levels in patients with cirrhosis: relationship to endotoxinemia, tumor necrosis factor-α, and hyperdynamic circulation. Scand J Gastroenterol 1996; 31:500-5.
[20] Kountouras J, Billing BH, Scheuer PJ. Prolonged bile duct obstruction: a new experimental model for cirrhosis in the rats. Br J Exp Pathol 1984;65:301-5.
[21] Jenkins SA, Grandison A, Baxter JN, et al. A dimethylnitrosamine induced model of cirrhosis and portal hypertension in the rats. J Hepatol 1985;1:489-99.
[22] Bemelmans MHA, Gouma DJ, Greve JW, et al. Cytokines tumor necrosis factor and interleukin-6 on experimental biliary obstruction in mice. Hepatology 1992;15:1132-6.
[23] Kitamura K, Nakamoto Y, Akiyama M, et al. Pathogenic roles of tumor necrosis factor receptor p55-mediated signals in dimethylnitrosamine-induced murine liver fibrosis. Lab Invest 2002;82:571-83.
[24] Considine RV, Sinha MK, Heimen ML, et al. Serum immunoreactive-leptin concentrations in normal-weight and obese humans. N Engl J Med 1996;334:292-5.
[25] Frederich RC, Hamann A, Anderson S, et al. Leptin levels reflect body lipid content in mice: evidence for diet-induced resistance to leptin action. Nat Med 1995;1:1311-4.
[26] Moshyedi A, Josephs MD, Abdalla EK, et al. Increased leptin expression in mice with bacterial peritonitis is partially regulated by tumor necrosis factor alpha. Infect Immun 1998;66:1800-2.
[27] Lin SY, Wang YY, Sheu WHH. Increased serum leptin concentrations correlate with soluble tumor necrosis factor receptor levels in patients with liver cirrhosis. Clin Endocrinol 2002;57:805-11.
[28] Benigni F, Faggioni R, Sironi M, et al. TNF receptor p55 plays a major role in the centrally mediated increases of serum IL-6 and corticosterone after intracerebroventricular injection of TNF. J Immunol 1996;157:5563-8.
[29] Tilg H, Wilmer A, Vogel W, et al. Serum levels of cytokines in chronic liver diseases. Gastroenterology 1992;103:264-74.
[30] Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis factor-α: direct role in obesity-linked insulin resistance. Science 1993;259:87-91.
[31] Berkowitz DE, Brown D, Lee KM, et al. Endotoxin-induced alteration in the expression of leptin and β_3-adrenergic receptor in adipose tissue. Am J Physiol Endocrinol Metab 1998;37:E992-7.
[32] Sewter CP, Digby JE, Blows F, et al. Regulation of tumor necrosis factor-alpha from human adipose in vitro. J Endocrinol 1999;163:33-8.
[33] Lockwood JF, Myers MJ, Rutherford MS, et al. Transcriptional change in macrophage TNF-α expression following dimethylnitrosamine exposure in vivo. Immunopharmacology 1991;2:27-38.
[34] Ben-Ari Z, Schafer Z, Sulkes J, et al. Alterations in serum leptin in chronic liver disease. Dig Dis Sci 2002;47:183-9.
[35] Rioux KP, Beck PL, Hoppin AG, et al. Differential leptin responses to acute and chronic biliary obstruction in rats. J Hepatol 2000;33: 19-25.
[36] Levy JR, Heuman DM, Pandak WM, et al. Effects of bile acid composition and manipulation of enterohepatic circulation of leptin gene regulation. Metabolism 1998;47:285-91.
[37] Lanspa SJ, Chan ATH, Bell III JS, et al. Pathogenesis of steatorrhea in primary biliary cirrhosis. Hepatology 1985;5:837-42.
[38] Iritani N, Sugimoto T, Fukuda H. Gene expression of leptin, insulin receptors and lipogenic enzymes are coordinately regulated by insulin and dietary fat in rats. J Nutr 2000;130:1183-8.

[39] Faggioni R, Fuller J, Moser A, et al. LPS-induced anorexia in leptin-deficient (ob/ob) and leptin receptor-deficient (db/db) mice. Am J Physiol 1997;273:R181-6.
[40] Lang CH, Dobrescu C, Bagby GJ. Tumor necrosis factor impairs insulin action in peripheral glucose disposal and hepatic glucose output. Endocrinology 1992;130:43-52.
[41] Cohen B, Novick D, Rubinstein M. Modulation of insulin activities by leptin. Science 1996;274:1185-8.
[42] Liu YL, Emilsson V, Cawthorne MA. Leptin inhibits glycogen synthesis in the isolated soleus muscle of obese (ob/ob) mice. FEBS Lett 1997;411:351-5.
[43] Nolte W, Hartmann H, Ramdori G. Glucose metabolism and liver cirrhosis. Exp Clin Endocrinol Diabetes 1995;103:63-74.
[44] Leclercq IA, Farrell GC, Schriemer R, et al. Leptin is essential for the hepatic fibrogenic response to chronic liver injury. J Hepatol 2002;37:206-13.