Interleukin 1 and tumor necrosis factor in obese alcoholics compared with normal-weight patients[1–3]

Daniel Bunout, Carlos Muñoz, Marcelo López, María Pía de la Maza, Liana Schlesinger, Sandra Hirsch, and Margarita Pettermann

ABSTRACT We performed a liver biopsy and measured plasma concentrations of interleukin 1β (IL-1β) and tumor necrosis factor α (TNF-α), and spontaneous and lipopolysaccharide-stimulated in vitro monocyte production of IL-1β and TNF-α in 19 obese and 17 age-matched, normal-weight alcoholics admitted for treatment of their alcoholism. Nine healthy normal-weight subjects acted as control subjects. Five obese and no normal-weight alcoholics had cirrhosis in their liver biopsy (Fisher's exact test: $P = 0.031$). A histologic score (derived from the sum of fat, necrosis, fibrosis, and inflammation in the biopsy) correlated with body mass index and the percentage body fat, calculated by using the sum of four skinfold-thickness measures. Plasma concentrations and spontaneous in vitro monocyte production of IL-1β and TNF-α were below detection limits. No significant differences were observed between normal-weight and obese alcoholics with or without cirrhosis and normal control subjects in lipopolysaccharide-stimulated monocyte production of IL-1β (6.5 ± 0.8, 10.1 ± 2.7, 7.9 ± 1.6, and 5.28 ± 4.24 μg/L, respectively) or TNF-α (2.8 ± 0.4, 3.7 ± 1.0, 3.0 ± 0.44, and 1.97 ± 1.01 μg/L, respectively). However, a positive correlation was found between IL-1β production and body mass index ($r = 0.333$, $P = 0.047$), percentage body fat ($r = 0.412$, $P = 0.013$), abdominal circumference ($r = 0.416$, $P = 0.012$), and total histologic score ($r = 0.331$, $P = 0.049$). A multiple-regression model accepted abdominal circumference as the only independent predictor of IL-1β production. TNF-α did not correlate with any of the above-mentioned indexes. We conclude that obese alcoholics have a higher frequency of histologic liver damage and that IL-1β production by stimulated monocytes is related to abdominal fat accumulation. *Am J Clin Nutr* 1996;63:373–6.

KEY WORDS Obesity, alcoholism, alcoholic liver disease

INTRODUCTION

We have reported a high frequency of hepatic histologic alterations in alcoholics without clinical stigmata of liver damage who underwent routine liver biopsies. More importantly, overweight alcoholics have a higher prevalence of liver damage than their normal-weight counterparts (1, 2). Because nonalcoholic obese subjects may have hepatic lesions indistinguishable from those caused by alcohol abuse (3), we postulated that overweight may be a risk factor for alcoholic liver disease.

These clinical observations have not shed light on the possible mechanisms that explain the higher susceptibility of overweight alcoholics to develop liver damage. Cytokines have been related to the pathogenesis of alcoholic liver disease (4). Subjects with alcoholic hepatitis have higher plasma concentrations of interleukin 1 (IL-1), interleukin 8 (IL-8), and tumor necrosis factor (TNF). Peripheral monocytes extracted from these individuals produce higher amounts of these cytokines when stimulated with bacterial lipopolysaccharide (5, 6). Although alcohol itself inhibits cytokine secretion by monocytes (7), some authors have postulated that alcoholics have increased intestinal permeability allowing bacterial and endotoxin absorption, with subsequent stimulation of cytokine secretion (8).

The aim of the present study was to measure plasma concentrations of IL-1β and TNF-α and their secretion by monocytes in culture in a group of overweight alcoholics. Their values were compared with those of matched normal-weight alcoholic patients and normal control subjects.

SUBJECTS AND METHODS

Subjects

Every male, obese, alcoholic patient (defined as having a body mass index, in kg/m^2, ≥ 28) admitted to the alcoholism ward for treatment of his addiction was considered eligible for the study. Inclusion criteria were a history of alcoholism for > 5 y, < 1 wk of abstinence from alcohol ingestion, an age of < 50 y, absence of clinical stigmata of alcoholic liver disease, and informed consent by signature. An age-matched, normal-weight alcoholic (defined as having a weight between 80% and 100% of normal) was assigned to every obese alcoholic in the study.

Every subject had a complete clinical examination and an anthropometric assessment in which weight, height, four skinfold thicknesses, and waist and hip circumferences were measured by using standardized techniques (9). A fasting blood sample was obtained for routine laboratory tests, hepatitis B and C virus screening, and cytokine measurements. A liver biopsy was performed with a Menghini needle (B Braun Melsungen AG, Melsungen, Germany).

Body mass index was calculated as weight/(height)^2 and percentage body fat was calculated by using the formulas of Durnin

[1] From the Institute of Nutrition and Food Technology and Faculty of Medicine, Central Campus, University of Chile, Santiago.

[2] Supported by Fondecyt grant 1059–91.

[3] Address reprint requests to D Bunout, INTA, University of Chile, PO Box 138–11, Santiago, Chile.

Received May 18, 1995.

Accepted for publication October 15, 1995.

and Womersley (10). Liver biopsies were read by one pathologist, who quantified the presence of fat, necrosis, fibrosis, and inflammation, features that were scored from 0 to 3 according to previously published criteria (2). The sum of fat, necrosis, inflammation, and fibrosis scores was the total histologic score. Cirrhosis or alcoholic hepatitis were diagnosed according to standard criteria (11). The pathologist had a variation of < 10% when scoring the same histologic sample repeatedly.

Nine normal-weight male laboratory workers aged 38.1 ± 6.4 y volunteered to act as control subjects for cytokine measurements. These subjects all had an alcohol intake < 30 g/d. The study conformed with the ethical guidelines of the Helsinki Declaration, revised in 1983.

Laboratory procedures

Packed red cell volume, prothrombin time, and fasting concentrations of blood glucose, serum albumin, serum bilirubin, alkaline phosphatase, aspartate aminotransferase, and alanine aminotransferase were measured using standard automated methods. Abdominal ultrasound examination was performed in all patients to look for focal hepatic lesions or ascites.

Blood mononuclear cells were used for the induction of IL-1β and TNF-α. Blood mononuclear cells were isolated by Histopaque gradient (Sigma Diagnostics, St Louis) and washed twice with phosphate-buffered saline, and the cells (2.5×10^9/L) were resuspended in RPMI 1640 culture medium supplemented with gentamicin (Elkins-Sinn, Inc, Cherry Hill, NJ), 0.2% heat-inactivated normal AB plasma, and 1 mg indomethacin/L (Sigma). Two hundred microliters of cell suspension was dispensed in 96-well flat-bottom microtiter plates in the absence (spontaneous cytokine release) or in the presence of endotoxin [1 mg lipopolysaccharide (LPS)/L originating from *Escherichia coli* 055:B5; Sigma L-2880]. Cultures were incubated for 18 h at 37 °C in 5% CO_2 and 95% air. Supernates were collected and kept at −70 °C until assayed for cytokines. In vitro production of IL-1β and TNF-α was measured by using a specific commercial enzyme-linked immunoabsorbent assay (R&D Systems, Minneapolis). Plasma was collected, treated by using the method of Cannon et al (12), and kept at −20 °C until assayed for IL-1β and TNF-α. Circulating cytokines were determined with the same enzyme-linked-immunoabsorbent-assay procedure used above.

Statistical analysis

Results are expressed as mean ± SEM. Fisher's exact test was used to compare frequencies, Student's *t* test was used to compare means of two samples, one-way analysis of variance (ANOVA) was used to compare means of more than two samples, Pearson's coefficient was used for correlations, and forward stepwise models were used for multiple regression. SYSTAT (Systat Inc, Evanston, IL) was used for the analyses.

RESULTS

Twenty-one male, obese alcoholics were considered eligible for the study and two declined to participate. Of the 19 age-matched normal-weight alcoholics, 2 were excluded from the study: 1 because of a positive hepatitis C virus antibody and 1 because of failure to obtain hepatic tissue during the liver biopsy. Demographic, anthropometric, and routine laboratory indexes are shown in **Table 1**. The mean total bilirubin was higher among obese subjects, although their values were within normal limits. Ultrasound did not show focal hepatic lesions or ascites in any patient.

TABLE 1
Demographic, anthropometric, and routine laboratory indexes in the study subjects[1]

	Obese alcoholics (n = 19)	Normal-weight alcoholics (n = 17)
Age (y)	42.0 ± 2.2	43.1 ± 3.8
Duration of alcoholism (y)	22.9 ± 1.9	21.7 ± 2.4
Daily alcohol consumption (g)	436.0 ± 38.0	303 ± 76.0
Body mass index (kg/m^2)	30.0 ± 0.5	22.7 ± 1.8[2]
Abdominal circumference (cm)	102.6 ± 1.4	84.0 ± 6.5[2]
Percentage body fat (%)[3]	28.2 ± 1.0	18.9 ± 1.8[2]
Packed red cell volume (%)	45.5 ± 1.0	42.6 ± 3.4
Blood glucose (mmol/L)	5.8 ± 0.8	4.8 ± 0.4
Total bilirubin (μmol/L)	20.4 ± 1.7	13.6 ± 1.7[4]
AST (U/L)[5]	50.8 ± 14.7	43.5 ± 10.5
ALT (U/L)[6]	52.6 ± 18.8	29.7 ± 6.2
Prothrombin time (%)	93.1 ± 2.4	95.4 ± 7.4
Serum albumin (g/L)	43.0 ± 1.0	40.0 ± 3.0

[1] $\bar{x}$ ± SEM.
[2,4] Significantly different from obese alcoholics: [2] $P < 0.01$, [4] $P < 0.05$.
[3] Percentage body fat calculated by using Durnin and Womersley's (10) formulas.
[5] Aspartate aminotransferase (normal values < 40 U/L).
[6] Alanine aminotransferase (normal values < 40 U/L).

Liver biopsy indicated cirrhosis in 5 of the 19 obese subjects (one subject had features of alcoholic hepatitis) and in none of the normal-weight alcoholics (Fisher's exact test: $P = 0.031$). The total histologic score was significantly higher among alcoholics with a body mass index ≥ 28 than in those with a body mass index < 28 (4.2 ± 2.9 compared with 2.6 ± 1.9, $P = 0.048$). Moreover, there was a positive correlation between the histologic score and body mass index ($r = 0.355$, $P = 0.034$), percentage body fat ($r = 0.410$, $P = 0.013$), and abdominal circumference ($r = 0.381$, $P = 0.022$) (**Figure 1**). A multiple-regression model including these last three variables accepted only percentage body fat as an independent predictor of histologic score. No independent predictor among anthropometric variables was obtained when multiple regressions using fat or fibrosis scores as dependent variables were used.

Plasma IL-1β and TNF-α were below detection limits (< 50 ng/L). No spontaneous production of IL-1β and TNF-α monocytes was detected in obese and lean alcoholics. LPS-stimulated monocyte production of IL-1β or TNF-α (**Table 2**) was not significantly different among normal-weight alcoholics, obese alcoholics with or without cirrhosis, and normal control subjects. However, there was a significant correlation among alcoholics between IL-1β produced by stimulated monocytes and body mass index, percentage body fat, abdominal circumference, and total histologic score (**Figure 2**). A multiple-regression model showed that abdominal circumference was the only independent predictor of IL-1β production. TNF-α did not correlate with body fat or histologic score.

DISCUSSION

This paper confirms the higher incidence of liver disease among obese alcoholics when compared with a carefully se-

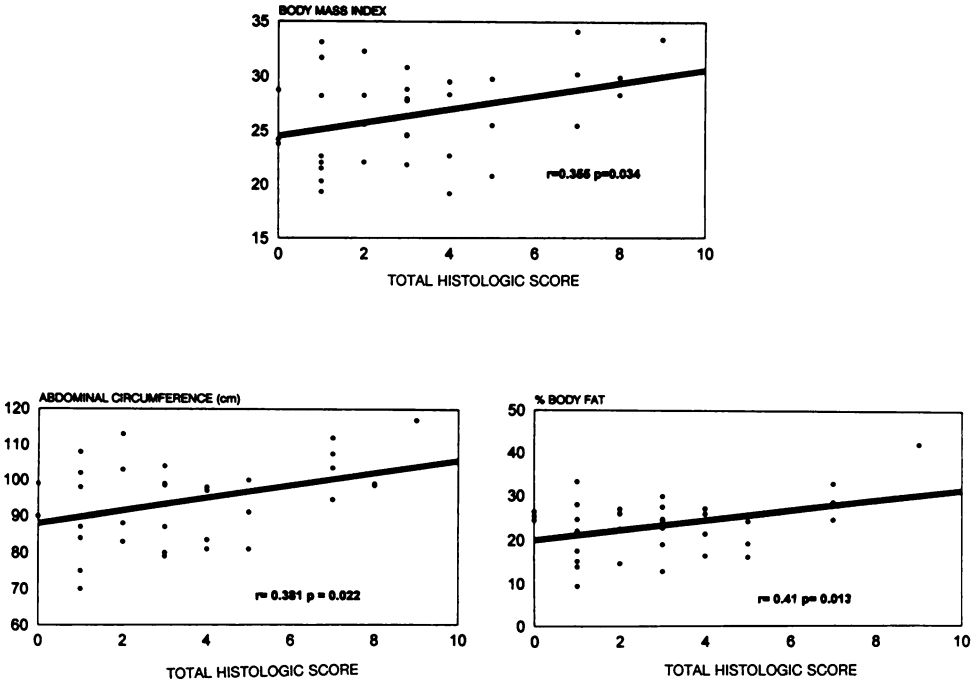

FIGURE 1. Correlation between histologic score and body mass index [wt (in kg)/ht^2 (in m)], percentage body fat (calculated using the sum of four skinfold-thickness measurements), and abdominal circumference in all patients.

lected group of matched normal-weight patients. Also, a positive correlation between IL-1β production and indexes of fat accumulation was observed.

Liver biopsy is an accurate method of diagnosing micronodular cirrhosis, the prominent type of cirrhosis in alcoholic liver disease. There may be a considerable sampling error in the diagnosis of macronodular cirrhosis because when nodules are > 3 mm it is possible that the biopsy needle will fall within a nodule (13).

We did not use food intake records, principally because they are often inaccurate in alcoholic patients. However, it is unlikely that differences in dietary intake could explain the higher frequency of liver disease in obese individuals, because in previous studies we did not find differences in intake between obese and lean alcoholics (1).

The postulated mechanisms of alcoholic liver damage include several hypotheses, such as hypoxic damage due to increased liver oxygen consumption, increased lipid peroxidation, toxic effects of alcohol metabolites, or immunologic damage (14, 15). Cytokines may play a role in the genesis of alcoholic liver disease, acting as pro-inflammatory peptides (16), promoting Ito cell proliferation (17), inducing neutrophil chemotaxis (18), or stimulating metabolism and oxygen consumption (19). Because alcohol does not stimulate cytokine secretion in vitro, other mechanisms such as increased intestinal permeability and absorption of toxic compounds are responsible for the increased monocyte activation observed in alcoholic liver disease (8).

The lack of detection of plasma cytokines does not mean that these substances do not have an important role. In fact these substances act in picomolar concentrations, especially in a paracrine and autocrine manner (4). Assessment of peripheral mononuclear cell activation is a reliable indicator of their in vivo production.

The observed correlation between in vitro monocyte IL-1β production and body fat and especially with abdominal circumference, an indicator of intraabdominal fat accretion (20), must be highlighted. To our knowledge this association has not been reported previously and we have no explanation for it. Individuals with high amounts of abdominal fat could have leakier intestines. Similarly, the accumulation of certain fatty acids in body fat could modify monocyte responses to LPS, resembling the effects of dietary polyunsaturated fatty acids (21).

Because obese patients had more histologic alterations in their liver biopsy than did normal-weight alcoholics and because cytokine secretion correlated with body fat, it is possible that there is an association between increased IL-1β secretion and obesity. However, in this series we found no differences in IL-1 secretion between patients with or without cirrhosis, and the multiple-regression model excluded histologic score as an independent predictor of IL-1β production.

There was no correlation between TNF-α production and body fat or liver damage. Increased production of TNF-α has been reported in patients with cirrhosis and liver failure (22) and a

TABLE 2
Lipopolysaccharide-stimulated interleukin 1β (IL-1β) and tumor necrosis factor α (TNF-α) production by cultured monocytes in alcoholics and control subjects[1]

	IL-1β	TNF-α
	$\mu g/L$	
Normal control subjects (n = 9)	5.28 ± 4.24	1.97 ± 1.01
Normal-weight alcoholics (n = 17)	6.5 ± 0.8	2.8 ± 0.4
Obese alcoholics with cirrhosis (n = 5)	10.1 ± 2.7	3.7 ± 1.0
Obese alcoholics without cirrhosis (n = 14)	7.9 ± 1.6	3.0 ± 0.44

[1] $\bar{x}$ ± SEM. There were no significant differences between groups by one-way ANOVA.

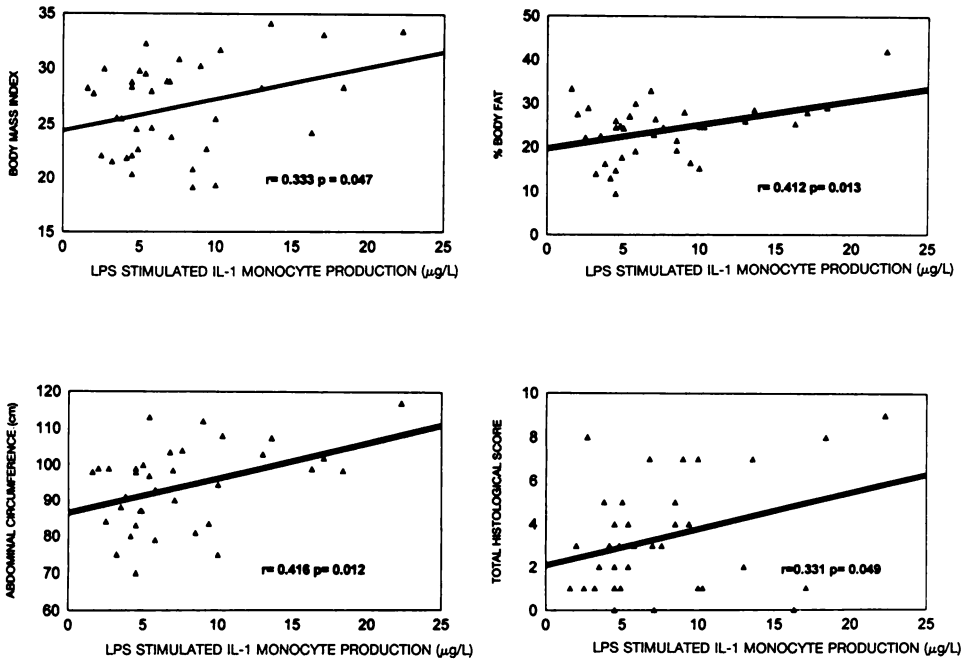

FIGURE 2. Correlation bet2ween lipopolysaccharide (LPS)-stimulated in vitro interleukin 1 (IL-1) monocyte production and body mass index, percentage body fat, abdominal circumference, and total histologic score in all patients.

relation between TNF-α plasma concentrations and the degree of liver failure (23, 24) has been observed. Because our patients had normal liver function, the lack of association between TNF-α and histologic liver alterations in our patients is not surprising.

In summary, there is an association between obesity and alcoholic liver disease. The correlation between IL-1β production by monocytes and fat accretion in alcoholics, although weak, may have a pathogenic role in the higher susceptibility of these patients to the hepatotoxic effects of alcohol.

REFERENCES

1. Bunout D, Gattas V, Iturriaga H, Perez C, Pereda T, Ugarte G. Nutritional status of alcoholic patients: its possible relationship to alcoholic liver damage. Am J Clin Nutr 1983;38:469–73.
2. Iturriaga H, Bunout D, Hirsch S, Ugarte G. Overweight as a risk factor or a predictive sign of histological liver damage in alcoholics. Am J Clin Nutr 1988;47:235–8.
3. Klain J, Fraser D, Goldstein J, et al. Liver histology abnormalities in the morbidly obese. Hepatology 1989;10:873–6.
4. Andus T, Bauer J, Gerok W. Effects of cytokines on the liver. Hepatology 1991;13:364–75.
5. Tilg H, Wilmer A, Vogel W, et al. Serum levels of cytokines in chronic liver diseases. Gastroenterology 1992;103:264–74.
6. Deviere J, Content J, Denys C, et al. Excessive in vitro bacterial lipopolysaccharide-induced production of monokines in cirrhosis. Hepatology 1990;11:628–34.
7. D'Souza NB, Bagby CJ, Nelson S, Lang CH, Spitzer JJ. Acute alcohol infusion suppresses endotoxin-induced serum tumor necrosis factor. Alcohol Clin Exp Res 1991;13:295–8.
8. Bjarnason I, Ward K, Peters TJ. The leaky gut of alcoholism: possible route of entry for toxic compounds. Lancet 1984;1:179–82.
9. Lohman TG, Roche AF, Martorel E, eds. Anthropometric standardization reference manual. Champaign, IL: Human Kinetics, 1988.
10. Durnin JVGA, Womersley J. Body fat assessed from total body density and its estimation from skinfold thickness: measurements on 481 men and women aged from 16 to 72 years. Br J Nutr 1974;32:77–94.
11. Baptista A, Bianchi L, DeGroote J, et al. Alcoholic liver disease: morphologic manifestations. Lancet 1981;1:707–11.
12. Cannon JG, Van der Meer JWM, Kwiatkowwski D, et al. Interleukin-1 in human plasma: optimization of blood collection, plasma extraction and radioimmunoassay methods. Lymphokine Res 1988;7:457–67.
13. Bianchi L. Hepatic histology. Semin Liver Dis 1986;6:173–276.
14. Lieber CS. Metabolic effects of ethanol and its interaction with other drugs, hepatotoxic agents, vitamins and carcinogens. A 1988 update. Semin Liver Dis 1988;8:47–68.
15. Israel Y, Orrego H, Niemela O. Immune responses to alcohol metabolites: pathogenic and diagnostic implications. Semin Liver Dis 1988;8:81–90.
16. Hill DB, Marsano LS, McClain C. Increased plasma interleukin-8 concentrations in alcoholic hepatitis. Hepatology 1993;18:576–80.
17. Matsuoka M, Pham NT, Tsukamoto H. Differential effects of interleukin-1α, tumor necrosis factor α and transforming growth factor β 1 on cell proliferation and collagen formation by cultured fat storing cells. Liver 1989;9:71–8.
18. Shiratory Y, Takada H, Hikiba Y, et al. Production of chemotactic factor, interleukin-8, from hepatocytes exposed to ethanol. Hepatology 1993;18:1477–82.
19. Roh MS, Moldawer LL, Ekman LG, et al. Stimulatory effect of interleukin-1 on hepatic metabolism. Metabolism 1986;35:419–24.
20. Busetto L, Baggio MB, Zurlo F, Carraro R, Digito M, Enzi G. Assessment of abdominal fat distribution in obese patients: anthropometry versus computerized tomography. 1992;16:731–6.
21. Endres S, Ghorbani R, Kelly V, et al. The effect of dietary supplementation with n−3 polyunsaturated fatty acids on the synthesis of interleukin-1 and tumor necrosis factor by mononuclear cells. N Engl J Med 1989;320:265–71.
22. McClain CJ, Cohen DA. Increased tumor necrosis factor production by monocytes in alcoholic hepatitis. Hepatology 1989;9:349–51.
23. Bird GLA, Sheron N, Goka AKJ, Alexander GJ, Williams RS. Increased plasma tumor necrosis factor in severe alcoholic hepatitis. Ann Intern Med 1990;112:917–20.
24. Felver ME, Mezey E, McGuire M, et al. Plasma tumor necrosis factor predicts decreased long-term survival in severe alcoholic hepatitis. Alcohol Clin Exp Res 1990;14:255–9.