Lean non-alcoholic fatty liver disease (Lean-NAFLD) : a major cause of cryptogenic liver disease

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Abstract

Aims : **Non-alcoholic fatty liver disease (NAFLD) is strongly associated to obesity and type 2 diabetes, but may occur in the absence of these factors. Based on a large series of liver biopsies, we have evaluated the clinical, biochemical, metabolic and pathological characteristics of a new entity, which we refer to as "lean-NAFLD".**

Methods : **Among 1,777 patients, who underwent liver biopsy for chronic liver disease, Lean-NAFLD, defined as patients with NAFLD without obesity (BMI < 30 kg/m²) and without diabetes was found in 50 of them (2.8%), being the most frequent cause (38%) of cryptogenic liver disease. Thirty-one patients from the Lean-NAFLD group were compared to 48 Obese-NAFLD patients diagnosed during the same period and 8 healthy control patients. Insulin resistance was determined using the homeostasis model assessment method.**

Results : **In the Lean-NAFLD group as compared to the obese-NAFLD group, patients were younger : median 40 vs. 49 years, p = 0.047, with male predominance : 71 vs. 46%, p = 0.037. Fasting glucose and HbA1c were lower, as was insulin sensitivity : 1.7 vs. 3.0, p = 0.049. Blood pressure was significantly lower (p = 0.001) while triglycerides and HDL-cholesterol were similar. Although there was less inflammation (p = 0.038) and fibrosis (p = 0.029), non-alcoholic steatohepatitis and fibrosis were present in 61% and 55% of the Lean-NAFLD group, respectively. Compared to healthy controls, Lean-NAFLD were less insulin sensitive, with a insulin sensitivity index of 59 vs. 110 (p = 0.015), and more hypertriglyceridemic (p = 0.003).**

Conclusions : **Lean-NAFLD is a new unrecognized clinico-pathological entity, a frequent cause of cryptogenic liver disease.** (Acta gastroenterol. belg., **2011**, 74, **389-394**).

Key words : obesity, diabetes, non-alcoholic fatty liver disease.

Abbreviations : NAFLD : non-alcoholic fatty liver disease ; NASH : non-alcoholic steatohepatitis ; BMI : body mass index ; AST : aspartate aminotransferase ; ALT : alanine ; γGT : aminotransferase gamma-glutamyl transterase ; HDL : high-density lipoprotein ; LDL : low-density lipoprotein ; HbA1C : glycated hemoglobin, HOMA : homeostasis model assessment.

Introduction

Non-alcoholic fatty liver disease (NAFLD) is characterized by the presence of steatosis with or without hepatitis, fibrosis or cirrhosis on liver biopsy in the absence of excessive alcohol intake (1-3). It is the most common liver disease in western countries (1) with a prevalence of 30% in the US population (4).

The pathogenesis of NAFLD is multifactorial. Insulin resistance plays a major role (5), resulting in accumulation of fat in the liver ; chronic oxidative stress with cytokine involvement may cause hepatocellular apoptosis and/or necrosis, inflammation and fibrosis (1,5-11). More recently, the important role of visceral fat and its infiltration with macrophages responsible for adipokine release has been emphasized (10,11). Although NAFLD is typically associated with abdominal obesity, diabetes type 2 and metabolic syndrome (2,12-16), it has also been observed in individuals without this cluster of risk factors, and insulin resistance may be present in subjects with normoglycemia and normal weight (5,12,18). In recent studies from Asia, NAFLD was observed in 15% of apparently healthy individuals with normal weight and no diabetes (19,20). In those studies, NAFLD was diagnosed based on elevated transaminase levels and bright liver at ultrasound ; however, it is well established that liver histology remains the gold standard for diagnostic purposes, as it is the only tool able to grade steatosis and fibrosis and to diagnose steatohepatitis (NASH) (21). Cryptogenic liver disease characterizes patients with chronic liver tests abnormalities without identification of an etiology. We hypothesized that NAFLD in lean patients could represent an important part of this group. To date, there has been no clinico-pathological description of this new entity for which we have coined the term "lean non-alcoholic fatty liver disease (Lean-NAFLD).

The aim of this study was to characterize clinical, biochemical, metabolic and pathological features of Lean-NAFLD.

Patients and methods

Between November 1999 and October 2004, 1,777 patients underwent liver biopsy at our center due to altered biochemical liver tests for over 6 months. Cryptogenic liver disease was defined as a chronic (> 6-month) alteration in liver tests in the absence of

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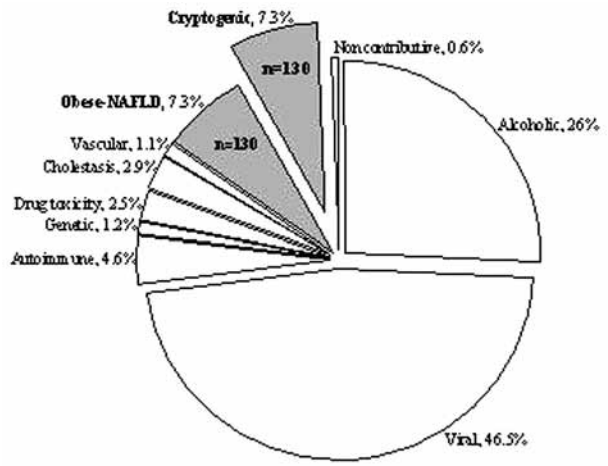

Fig. 1. — Clinico-pathological distribution of chronic liver disease.

any of the following : excessive alcohol intake (> 4 drinks/day in men and > 2 drinks/day in women), body mass index (BMI) ≥ 30 kg/m^2, abnormal fasting plasma glucose (≥ 100 mg/dl) or antidiabetic treatment, use of hepatotoxic drugs or contact with toxic compounds, celiac disease, thyroid dysfunction, transferrin saturation > 50%, α1 antitrypsin or ceruloplasmin deficiency, presence of antinuclear, smooth muscle cell or liver and kidney microsome antibodies, positive serology for hepatitis B or C, or presence of HBs and HBc antibodies in order to exclude occult hepatitis B infection (22).

All patients with a diagnosis of NAFLD, defined as the presence of macrovacuolar steatosis alone or associated with necroinflammatory activity and fibrosis, were included in the study.

NAFLD in patients with obesity (BMI > 30 kg/m^2) and/or diabetes (defined as elevated fasting blood glucose above 126 mg/dl or previously diagnosed type 2 diabetes) was considered as classical Obese-NAFLD (O). NAFLD patients with obesity but with excessive alcohol intake (> 4 drinks/day in men and > 2 drinks/day in women) were classified in classical Obese-NAFLD group (Fig. 1) but were excluded for the analysis (Table 1). Patients with NAFLD without obesity (BMI < 30 kg/m^2) and without abnormal fasting plasma glucose (plasma glucose < 100 mg/dl and no antidiabetic treatment) were considered as Lean-NAFLD (L). Demographic, clinical, biochemical, metabolic and histological characteristics of patients with L were compared to O and to healthy controls (C) matched for age, gender, BMI. Healthy controls had no evidence of NAFLD (based on normal transaminases and absence of fatty liver on ultrasound). No liver biopsy was performed in this group which was recruited among individuals who consulted at the hospital for a regular health check-up.

Patient files were retrospectively analysed for data on age, gender, BMI, blood pressure, waist circumference and number of drinks per day. Biochemical measurements included aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transterase (γGT), total cholesterol, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterol, triglycerides, uric acid, glycated hemoglobin (HbA1c), fasting plasma glucose and insulin. The homeostasis model assessment (HOMA-2 (23)) with measurement of fasting blood glucose and insulin was used to estimate insulin sensitivity and β-cell secretion. This index measures the ability of endogenous insulin to decrease glucose in extracellular fluids by inhibiting glucose release from the liver and stimulating peripheral consumption of glucose.

We used metabolic syndrome criteria from the National Cholesterol Education Program Adult Treatment Panel III (24), updated in 2005 to lower the threshold for glucose levels (25). Metabolic syndrome was defined as the presence of three of the following criteria : 1) fasting glycemia > 100 mg/dl ; 2) triglycerides > 150 mg/dl ; 3) HDL-C < 40 mg/dl in men and < 50 mg/dl in women ; 4) waist circumference > 102 cm in men and > 88 cm in women ; 5) blood pressure > 130/85 mmHg. Only patients with sufficient data were included in the study, leaving us with 31 out of 50 patients for L and 51 out of 130 for O.

Liver biopsies stained with hematoxylin-eosine were reviewed by a pathologist (NN), who was blinded for patient characteristics/category. The grade of macrovesicular steatosis was rated as follows : 1 (10-33%), 2 (34% to 66%) and 3 (> 66%). Picrosirius staining was used to evaluate fibrosis. The grade of necroinflammatory activity and stage of fibrosis were assigned according to the Brunt score (26). Non-alcoholic steatohepatitis (NASH) was defined as the presence of at least grade 1 of necro-inflammatory activity based on the Brunt score (26) with more than 10% steatosis with lobular inflammation and ballooned hepatocytes.

Statistical analyses

The Fisher exact test or Chi-square test were used to compare proportions. Groups with continuous variables were compared using the non-parametric Mann-Whitney test, since distribution of those variables was abnormal in most cases ; they were expressed as median and interquartile range (IQR). Multivariate analyses were conducted according to the logistic regression model including all parameters significant in univariate analyses. In all cases, a p value equal to or lower than 5% was considered significant. All statistical analyses were carried out with StatView software (Abacus Concepts Inc., Berkeley, CA, USA, 1996), a convivial version of the SAS program.

Table 1. — **Comparison of demographic, clinical, biochemical, metabolic and pathological aspects of the three groups : Obese-NAFLD, Lean-NAFLD and control groups**

	Controls n = 8	**Lean-NAFLD (L) n = 31**	**Obese-NAFLD (O) n = 48**	**P value**	
	Median (IQR) or %	Median (IQR) or %	Median (IQR) or %	C vs L	L vs O
Demographic					
Age (years)	48 (24)	40 (22)	49 (19)	0.69	**0.047**
Male gender (%)	62	71	46	0.68	**0.037**
Clinical					
Weight (kg)	78 (8)	74 (17)	95 (19)	0.60	**< 0.001**
BMI (kg/m2)	25 (4)	26 (4)	34 (5)	0.39	**< 0.001**
Exessive alcohol abuse : no/yes	8/0	31/0	48/0	0.99	0.97
Hypertension (%)	12	17	60	0.99	**0.006**
SBP (mm Hg)	120 (15)	130 (15)	135 (30)	0.48	**0.031**
Biochemical					
AST (IU/L)	19 (3)	48 (40)	58 (30)	**< 0.001**	0.39
ALT (IU/L)	16 (4)	84 (56)	73 (44)	**< 0.001**	0.55
Gamma GT (IU/L)	16 (14)	122 (254)	141 (244)	**< 0.001**	0.57
Plasma glucose (mg/dl)	90 (8)	88 (16)	118 (54)	0.46	**< 0.001**
Plasma insulin (μU/ml)	5 (2)	11 (8)†	18 (21)†	**0.027**	0.12
Hemoglobin A1c (%)	5.4 (0.2)	5.1 (0.3)	6.4 (2.7)	**0.035**	**< 0.001**
HOMA-index resistance	0.9 (0.2)	1.7 (1.1)	3.0 (3.2)	**0.017**	**0.049**
HOMA-insulin sensitivity (%)	110 (20)	59 (23)	33 (34)	**0.015**	0.056
HOMA-β-cell secretion (%)	93 (20)	157 (109)	147 (81)	0.10	0.93
Total cholesterol (mg/dl)	221 (73)	202 (85)	214 (46)	0.78	0.74
LDL-cholesterol (mg/dl)	145 (58)	133 (63)	129 (26)	0.74	0.83
HDL-cholesterol (mg/dl)	60 (14)	44 (20)	48 (19)	**0.030**	0.24
Triglycerides (mg/dl)	76 (28)	144 (72)	177 (148)	**0.003**	0.17
Histology					
Macrosteatosis :					
grade : n : 1/2/3		28/3/2	36/11/1		
% :		90/10/0	75/23/2		0.22
Necroinflammatory activity :					
grade : n° : 0/1/2/3		12/17/2/0	7/29/11/1		
% :		39/55/6/0	15/60/23/2		**0.038**
Fibrosis :					
stage : n° : 0/1/2/3/4		14/11/2/3/1	8/17/5/13/5		
% :		45/36/6/10/3	17/36/10/27/10		**0.029**

Abbreviations : ALT, alanine aminotransferase ; AST, aspartate aminotransferase ; BMI, body mass index (weight expressed in kilograms divided by the square of the height expressed in meters) ; DBP, diastolic blood pressure ; HDL, high-density lipoprotein ; HOMA, homeostasis model assessment ; IQR, interquartile range ; LDL, low-density lipoprotein ; NAFLD, non-alcoholic fatty liver disease ; SBP, systolic blood pressure ; †data available in 6 lean-NAFLD and 12 obese-NAFLD.

Remark : O-NAFLD was excluded for analysis when exact BMI value was not specify or excessive alcohol intake present. L-NAFLD were excluded when missing values were too numerous, especially exact BMI and missing parameters concerning metabolic syndrome criteria. This explain the gap between the beginning population (Fig. 1, 2) and patients analysed (Table 1).

Results

Pathological characteristics of the entire population, Lean-NAFLD and classical NAFLD groups (Figs. 1 and 2)

Among the 1,777 biopsies performed for chronic liver disease, etiology was established in 1,637 cases (Fig. 1), based on clinical and pathological findings : hepatitis C and B in 826 (46.5%), alcoholic in 462 (26%), NAFLD related to obesity- and/or diabetes (O) in 130 (7.3%), autoimmune in 82 (4.6%), drug toxicity in 45 (2.5%), cholestasis in 52 (2.9%), genetic in 21 (1.2%), vascular in 19 (1.1%). Liver biopsies were not conclusive in 10 cases (0.6%) and cryptogenic liver disease, as defined in Patients and methods, was present in 130 cases (7.3%).

Within the overall and cryptogenic liver disease series (Fig. 2) of 1,777 and 130 patients, 50 of them (2.8 and 38% respectively) met the diagnosis of Lean-NAFLD (L). Other causes of cryptogenic liver disease included normal liver in 26 cases (20%), idiopathic cirrhosis in 16 (12%), non-specific reactive hepatitis in 13 (10%), cholestasis in 10 (7%), toxic hepatitis in 7 (5%), sinusoidal dilatation in 5 (4%), granulomas in 2 (2%) and primary biliary cirrhosis in 1 (1%). The overall NAFLD prevalence in this cohort with chronic liver disease was 10% (130 classical O plus 50 L patients, among a total of 1,777).

Histological comparison by univariate analysis (Table 1) between O and L groups revealed a similar grade of steatosis, but the grade of necroinflammatory activity and stage of fibrosis were less advanced in L ($p = 0.038$ and 0.029, respectively) than in O patients. NASH and fibrosis were present in 61 and 55% of the L group, respectively.

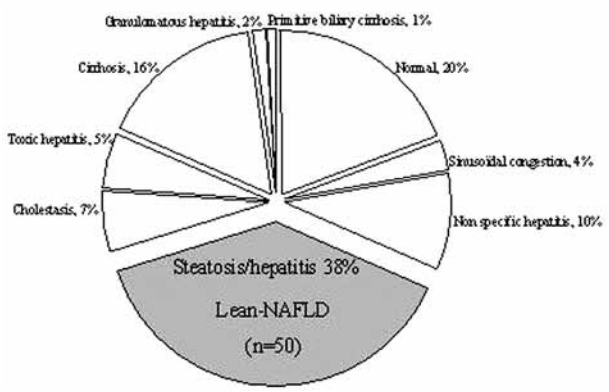

Fig. 2. — Pathological distribution of the cryptogenic liver-disease group.

Demographic, clinical, biochemical and metabolic characteristics of Lean-NAFLD, Obese-NAFLD and healthy controls (Table 1)

Compared to O patients, those with L were younger (40 vs. 49 years, p = 0.047) and there was a male predominance (71 vs. 46%, p = 0.037). BMI, fasting glucose and HbA1c were, by definition, significantly higher in the O group. L patients had higher insulin sensitivity, with a median HOMA insulin resistance index of 1.7 vs. 3.0, p = 0.049 ; and less hypertension : 17 vs. 60%, p = 0.006, while lipids were similar. The significant variables found on the univariate analysis were combined in a multivariate logistic regression, but no predictive model could be found for this study population, probably due to its relatively small population size.

Compared to controls, patients with L had significantly increased transaminase levels and were less insulin-sensitive, with fasting insulin levels more than twice those of controls : 5 vs. 11 μU/ml, p = 0.027, and a low insulin sensitivity index of 59 vs. 110 % (p = 0.015). L patients were hypertriglyceridemic (p = 0.003) and HDL-cholesterol was significantly lower (p = 0.030). Only lower insulin sensitivity was associated with the L versus the controls : OR : 0.96 (0.92-1.00), p = 0.047, in the multivariate analysis.

Discussion

In the present study, we identified a group of patients with NAFLD, whom we refer to as Lean-NAFLD and who do not have classical risk factors for NAFLD, i.e. obesity and /or diabetes. This group is characterized pathologically by more than 10 % steatosis, together with NASH and fibrosis in 61 and 55 % of cases, respectively. This new entity represents 2.8 % of our entire series of patients biopsied for chronic liver disease and appears to be the most frequent cause (39%) of cryptogenic liver disease, especially in a middle-aged male population. The latter prevalence is probably underestimated, since this new clinico-pathological entity is not yet largely known to the medical community and only a minority of patients had undergone liver biopsy, an invasive diagnostic method. Moreover, a fraction of this population may have normal transaminases, as this has been well documented in classical NAFLD (27) and these patients were not submitted to liver biopsy. In addition, it is well known (28) that severity of steatosis, inflammation, hepatocyte ballooning and Mallory's hyaline improve when cirrhosis develops in the setting of NAFLD, so that a certain number of patients among the 16 with cirrhosis within the cryptogenic liver disease group might also belong to the Lean-NAFLD group. L patients were younger and more frequently male. The only independent factor associated with L compared to controls was lower insulin sensitivity.

This suggests that insulin sensitivity should be systematically evaluated in patients with cryptogenic liver disease.

The O group is highly prevalent in general and obese populations (30% and 60%, respectively) and represented, in our series, 7% (compared to 10% in the literature) of the liver biopsy indications for chronic liver tests abnormalities (1,29).

NAFLD in lean persons was initially described by Bacon *et al*. in 1994 in the US without a demographic definition of this population (18), later by Marchesini *et al*. in Italy (5), and more recently by Korean (30-33) and Chinese (19,20) authors. In Kim's study (20), the prevalence of NAFLD based on echography was 16.1% in the

Table 2. — **Published series of non-obese NAFLD patients**

	All NAFLD (n)	*Lean NAFLD n(%)*	*Age (years)*	*Male gender (%)*	*BMI (kg/m²)*	*Fasting glycemia (mg/dl)*	*Metabolic evaluation*	*Histological diagnosis*
Bacon *et al*. (18)	33	20 (61%)	NA	NA	NA	NA	NA	Yes
Lee *et al*. (30)	47	25	NA	NA	NA	NA	Oral glucose tolerance test	No
Marchesini *et al*. (5)		30	41 (24-64)	80%	27 (23-29)	94 (79-108)	Euglycemic clamp	No
Kim *et al*. (20)	180	74 (41%)	53 ± 10	65%	25.4 ± 1.3	95 ± 10	HOMA	No
Park *et al*. (33)		120	43 ± 1	100%	23,4 ± 0,1	94 ± 1	HOMA	No
Cho *et al*. (34)	65	65	NA	NA	NA	NA	HOMA	No
Jun *et al*. (35)	408	408	NA	NA	NA	NA	HOMA	No
Vos *et al*.	79	31 (39%)	40 (22)	71%	26 (4)	88 (16)	HOMA	Yes

NA, not available ; HOMA, homeostasis model assessment ; BMI, body mass index ; NAFLD, non-alcoholic fatty liver disease.

normal-weight adult group and 34.4% in the overweight group. However, those studies lacked histological confirmation of NAFLD (Table 2), and liver imaging is not accurate enough to diagnose these entities (34).

The difference in age and the male predominance in our L population were interesting features. It is a disease of men in their forties (Table 1), whereas O-NAFLD is a disease of women in their fifties, possibly explained by hormonal changes. Several studies (35,36) have shown the impact of menopausal hormone deficiency on the development of metabolic syndrome, with an increase in associated abdominal fat and waist circumference, a reduction in insulin sensitivity and dyslipidemia, thus explaining the higher incidence of NAFLD in menopausal women at a later age. Another point of interest is the genetic predisposition of NAFLD : a PNPLA3 gene variant has shown a strong association with liver steatosis, hepatic inflammation and fibrosis progression in obese people (37). It remains to be determined if lean individuals harbor indeed this gene variant or another polymorphism, for example within the apolipoprotein C3 (APOC3) gene which has been linked to NAFLD in lean Indian men (38).

The limitations of our study are the small number of patients, especially in the control group, and the absence of systematic measurement of waist circumference because of the retrospective nature of the study.

In conclusion, lean NAFLD represents an important fraction of the NAFLD population and establishes a diagnosis in an substantial part of cryptogenic cirrhotic patients.

References

1. CLARK J.M., BRANCATI F.L., DIEHL A.M. Nonalcoholic fatty liver disease. *Gastroenterology*, 2002, **122** : 1649-1657.
2. POWELL E.E., COOKSLEY W.G., HANSON R., SEARLE J., HALLIDAY J.W., POWELL L.W. The natural history of nonalcoholic steatohepatitis : a follow-up study of forty-two patients for up to 21 years. *Hepatology*, 1990, **11** : 74-80.
3. ADLER M., SCHAFFNER F. Fatty liver hepatitis and cirrhosis in obese patients. *Am. J. Med.*, 1979, **67** : 811-816.
4. BROWNING J., SZCZEPANIAK L., DOBBINS R., NUREMBERG P., HORTON J., COHEN J., GRUNDY S.M., HOBBS H.H. Prevalence of hepatic steatosis in an urban population in the United States : impact of ethnicity. *Hepatology*, 2004, **40** : 1387-1395.
5. MARCHESINI G., BRIZI M., MORSELLI-LABATE A.M., BIANCHI G., BUGIANESI E., MCCULLOUGH A.J., FORLANI G., MELCHIONDA N. Association of nonalcoholic fatty liver disease to insulin resistance. *Am. J. Med.*, 1999, **107** : 450-455.
6. CANKURTARAN M., TAYFUR O., YAVUZ B.B., GEYIK S., AKHAN O., ARSLAN S. Insulin resistance and metabolic syndrome in patients with NAFLD but without diabetes : effect of a 6 month regime intervention. *Acta Gastroenterol. Belg.*, 2007, **70** : 253-259.
7. CHITTURI S., ABEYGUNASEKERA S., FARRELL G.C., HOLMES-WALKER J., HUI J.M., FUNG C., KARIM R., LIN R., SAMARASINGHE D., LIDDLE C., WELTMAN M., GEORGE J. NASH and insulin resistance : insulin hypersecretion and specific association with the insulin resistance syndrome. *Hepatology*, 2002, **35** : 373-379.
8. PAGANO G., PACINI G., MUSSO G., GAMBINO R., MECCA F., DEPETRIS N., CASSADER M., DAVID E., CAVALLO-PERIN P., RIZZETTO M. Nonalcoholic steatohepatitis, insulin resistance, and metabolic syndrome : further evidence for an etiologic association. *Hepatology*, 2002, **35** : 367-372.
9. HUI J.M., HODGE A., FARRELL G.C., KENCH J.G., KRIKETOS A., GEORGE J. Beyond insulin resistance in NASH : TNF-alpha or adiponectin ? *Hepatology*, 2004, **40** : 46-54.
10. ARVANITI V.A., THOMOPOULOS K.C., TSAMANDASS A., MAKRI M., PSYROGIANNIS A., VAFIADIS G., ASSIMAKOPOULOS S.F., LABROPOUPOU-KARATZA C. Serum adiponectin levels in different types of non alcoholic liver disease. Correlation with steatosis, necroinflammation and fibrosis. *Acta Gastroenterol. Belg.*, 2008, **71** : 355-360.
11. VAN DER POORTEN D., MILNER K.L., HUI J., HODGE A., TRENELL M.I., KENCH J.G., LONDON R., PEDUTO T., CHISHOLM D.J., GEORGE J. Visceral fat : a key mediator of steatohepatitis in metabolic liver disease. *Hepatology*, 2008, **48** : 449-57.
12. MARCHESINI G., BRIZI M., BIANCHI G., TOMASSETI S., BUGIANESI E., LENZI M., MCCULLOUGH A.J., NATALE S., FORLANI G., MELCHIONDA N. Nonalcoholic fatty liver disease : a feature of the metabolic syndrome. *Diabetes*, 2001, **50** : 1844-1850.
13. CORTEZ-PINTO H., CAMILO M.E., BAPTISTA A., DE OLIVIERA A.G., DE MOURA M.C. Nonalcoholic fatty liver : another feature of the metabolic syndrome ? *Clin. Nutr.*, 1999, **18** : 353-358.
14. MARCHESINI G., BUGIANESI E., FORLANI G., CERRELLI F., LENZI M., MANINI R., NATALE S., VANNI E., VILLANOVA N., MELCHIONDA N., RIZZETTO M. Nonalcoholic fatty liver, steatohepatitis, and the metabolic syndrome. *Hepatology*, 2003, **37** : 917-923.
15. VERRIJKEN A., FRANCQUE S., VAN GAAL L. The metabolic syndrome and the liver. *Acta Gastroenterol. Belg.*, 2008, **71** : 48-59.
16. CANBAKAN B., SENTURK H., TAHAN V., HATEMI I., BALCI H., TOPTAS T., SONSUZ A., VELET M., AYDIN S., DIRICAN A., OZGULLE S., OZBAY G. Clinical, biochemical and histological correlations in a group of non-drinker subjects with non-alcoholic fatty liver disease. *Acta Gastroenterol. Belg.*, 2007, **70** : 277-284.
17. WANLESS I.R., LENTZ J.S. Fatty liver hepatitis (steatohepatitis) and obesity : an autopsy study with analysis of risk factors. *Hepatology*, 1990, **12** : 1106-1110.
18. BACON B.R., FARAHVASH M.J., JANNEY C.G.,NEUSCHWANDER-TETRI B.A. Nonalcoholic steatohepatitis : an expanded clinical entity. *Gastroenterology*, 1994, **107** : 1103-1109.
19. GAO A.B., XIAO Q., LI L.Y., GAO Y., ZHANG H.N., MIAO J. Epidemiological survey of the association between non alcoholic fatty liver disease and metabolic syndrome in civil servants of Chongqing city. *Zhonghua Yu Fang Yi Xue Za Zhi*, 2008, **42** : 107-110.
20. KIM H.J., KIM H.J., LEE K.E., KIM D.J., KIM S.K., AHN C.W., LIM S.K., KIM K.R., LEE H.C., HUH K.B., CHA B.S. Metabolic significance of nonalcoholic fatty liver disease in nonobese, nondiabetic adults. *Arch. Intern. Med.*, 2004, **164** : 2169-2175.
21. SAADEH S., YOUNOSSI Z.M., REMER E.M., GRAMLICH T., ONG J.P., HURLEY M., MULLEN K.D., COOPER J.N., SHERIDAN M.J. The utility of radiological imaging in nonalcoholic fatty liver disease. *Gastroenterology*, 2002, **123** : 745-750.
22. RAIMONDO G., ALLAIN J.P., BRUNETTO M.R., BUENDIA M.A., CHEN D.S., COLOMBO M., CRAXÌ A., DONATO F., FERRARI C., GAETA G.B., GERLICH W.H., LEVRERO M., LOCARNINI S., MICHALAK T., MONDELLI M.U., PAWLOTSKY J.M., POLLICINO T., PRATI D., PUOTI M., SAMUEL D., SHOUVAL D., SMEDILE A., SQUADRITO G., TRÉPO C., VILLA E., WILL H., ZANETTI A.R., ZOULIM F. Statements from the Taormina expert meeting on occult hepatitis B virus infection. *J. Hepatol.*, 2008, **49** : 652-657.
23. LEVY J.C., MATTHEWS D.R., HERMANS M.P. Correct Homeostasis Model Assessment (HOMA) : evaluation uses the computer program. *Diabetes Care*, 1998, **21** : 2191-2192.
24. EXPERT PANEL ON DETECTION EVALUATION AND TREATMENT OF HIGH BLOOD CHOLESTEROL IN ADULTS. Executive summary of the third report of National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III). *JAMA*, 2001, **285** : 2486-2497.
25. GRUNDY S.M., CLEEMAN J.I., DANIELS S.R., DONATO S.R., ECKEL R.H., FRANKLIN B.A., GORDON DJ., KRAUSS R.M., SAVAGE P.J., SMITH S.C. JR., SPERTUS J.A., COSTA F. Diagnosis and management of the metabolic syndrome : an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. *Circulation*, 2005, **112** : 2735-2752.
26. BRUNT E.M., JANNEY C.G., DI BISCEGLIE A.M., NEUSCHWANDER-TETRI B.A., BACON B.R. Nonalcoholic steatohepatitis : a proposal for grading and staging the histological lesions. *Am. J. Gastroenterol.*, 1999, **94** : 2467-2474.
27. USLAN I., ACARTURK G., KARACA E., ALBAYRAK R., YUKSEL S., COLBAY M., KARAMAN O., AKCAN Y. The effects of weight loss on normal transaminase levels in obese patients. *Am. J. Med. Sci.*, 2007, **334** : 327-330.

28. ADAMS L.A., SANDERSON S., LINDOR K.D., ANGULO P. The histological course of nonalcoholic fatty liver disease : a longitudinal study of 103 patients with sequential liver biopsies. *J. Hepatol*., 2005, **42** : 132-138.
29. TORRES D.M., HARRISON S.A. Diagnosis and therapy of nonalcoholic steatohepatitis. *Gastroenterology*, 2008, **134** : 1682-1698.
30. LEE J.H., RHEE P.L., LEE J.K., LEE K.T., KIM J.J., KOH K.C., PAIK S.W., RHEE J.C., CHOI K.W. Role of hyperinsulinemia and glucose intolerance in the pathogenesis of nonalcoholic fatty liver in patients with normal body weight. *Korean J. Intern. Med*., 1998, **13** : 12-14.
31. PARK S.H., KIM B.I., YUN J.W., KIM J.W., PARK D.I., CHO Y.K., SUNG I.K., PARK C.Y., SOHN C.I., JEON W.K., KIM H., RHEE E.J., LEE W.Y., KIM S.W. Insulin resistance and C-reactive protein as independent risk factors for non-alcoholic fatty liver disease in non-obese asian men. *J. Gastroenterol. Hepatol*., 2004, **19** : 694-698.
32. CHO Y.K., LEE W.Y., OH S.Y., PARK J.H., KIM H.J., PARK D.I., SOHN C.I., JEON W.K., KIM B.I., KIM S.W., OH K.W., YUN E.J., OH E.S. Factors affecting the serum levels of adipokines in Korean male patients with nonalcoholic fatty liver disease. *Hepatogastroenterology*, 2007, **54** : 1512-1516.
33. JUN D.W., HAN J.H., KIM S.H., JANG E.C., KIM N.I., LEE J.S., SONG M.H., KIM S.H., JO Y.J., PARK Y.S. Association between low thigh fat and non-alcoholic fatty liver disease. *J. Gastroenterol. Hepatol*., 2008, **23** : 888-893.
34. SAVERYMUTTU S.H., JOSEPH A.E., MAXWELL J.D. Ultrasound scanning in the detection of hepatic fibrosis and steatosis. *Br. Med. J*., 1986, **292** : 13-15.
35. TCHERNOF A., POEHLMAN E.T., DESPRES J.P. Body fat distribution, the menopause transition, and hormone remplacement therapy. *Diabetes Metab*., 2000, **26** : 12-20.
36. CARR M.C. The emergence of the metabolic syndrome with menopause. *J. Clin. Endocrinol. Metab*., 2003, **88** : 2404-2411.
37. HOTTA K., YONEDA M., HYOGO H., OCHI H., MIZUSAWA S., UENO T., CHAYAMA K., NAKAJIMA A., NAKAO K., SEKINE A. Association of the rs738409 polymorphism in PNPLA3 with liver damage and the development of nonalcoholic fatty liver disease. *BMC Med. Genet*., 2010, **11** : 172-180.
38. CHITTURI S., WONG V.W., FARRELL G. Nonalcoholic fatty liver in Asia : Firmly entrenched and rapidly gaining ground. *J. Gastroenterol. Hepatol*., 2011, **26** : 163-172.