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Nonalcoholic fatty liver disease in asymptomatic obese women[(a)]

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ABSTRACT

Objective. To determine the clinical characteristics of NAFLD in asymptomatic obese women. **Methods.** A total of 457 asymptomatic obese women were enrolled in a cross-sectional study and allocated into groups with and without NAFLD. Irrespective of ALT levels, diagnosis of NAFLD was established by ultrasonographic findings; irrespective of fibrosis, NASH was defined by hepatic histological changes. **Results.** One hundred ninety five (42.7%) women had elevated ALT levels. Diagnosis of NAFLD was established in 228 (49.9%) women; among women with NAFLD, 34 (14.9%) have ALT levels within the normal range. On the other hand, based on the healthy range for ALT levels (19 UI/L), 336 (73.5%) women had elevated ALT, but only 2 (0.9%) women with NAFLD exhibited ALT levels within normal healthy values. Furthermore, 93 (41%) women who had AST/ALT levels ^{3} 1 underwent liver biopsy; of these, 90 (96.8%) had diagnosis of NASH and 3 (3.2%) of hepatic cirrhosis. Women with NAFLD were more obese and have higher fasting plasma glucose, triglycerides, ALT, and AST levels than obese women without NAFLD. Seventy six (16.6%) women had diagnosis of diabetes; of these 47 (61.8) in the NAFLD group. **Conclusions.** Results of this study support the statement that women with NAFLD have an adverse metabolic profile. Furthermore, our results show that hyperglycemia, hypertriglyceridemia and markers of liver injury such as AST/ALT ≥ 1 may be useful for early recognition of NAFLD.

Key words. Obesity. Steatosis. NAFLD. Diabetes. NASH.

INTRODUCTION

The Nonalcoholic fatty liver disease (NAFLD), a clinical condition characterized by histological features that resemble those of alcohol-induced liver injury, occurs in individuals who do not consume alcohol and is involved in the development of chronic liver disease.[1]

Individuals with NAFLD commonly are asymptomatic or have minimal clinical symptoms; thus, diagnosis of NAFLD is suggested by elevation of aminotransferase levels or changes in the hepatic echogenicity induced by liver fatty infiltration.[2] Obesity and their commonly associated metabolic disorders such as hyperglycemia and hypertriglyceridemia are well-known risk factors for NAFLD.[3,4] The prevalence of NAFLD reach 14-21%,[5] but it is as high as 90%-95% in obese persons and up to 70% in diabetic patients.[6] Irrespective of age, gender, and body mass index (BMI), it has been noted that liver fat content also is significantly increased in the subjects with metabolic syndrome.[7]

Hepatic ultrasonographic changes of NAFLD appear when steatosis involve 15% to 20% of the liver; however ultrasound is unable to distinguish nonalcoholic steatohepatitis (NASH) from other forms of NAFLD, distinction that has important prognostic implications.[8,9] Clinically, the main features that distinguish cirrhosis caused by NAFLD from cirrhosis caused by alcohol ingestion is that patients, who have NAFLD-related cirrhosis, frequently exhibited

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serious end organ complications of metabolic syndrome, particularly those related with type 2 diabetes.[10]

The NAFLD comprises a wide spectrum of histological categories; steatosis alone (type 1), steatosis plus inflammation (type 2), steatosis plus hepatocyte injury (balonization) (type 3), and steatosis plus sinusoidal fibrosis and polymorphonuclear cell infiltrates with or without Mallory-Denk bodies (type 4). Types 3 and 4 are considered as NASH.[11] Furthermore, the NAFLD activity score (NAS), which includes features of active injury such as steatosis, lobular inflammation, and ballooning, is a useful tool for assessing severity of disease.[12]

In this study, we determine the clinical characteristics of NAFLD in asymptomatic obese women.

METHODS

With the approval by the Mexican Social Security Institute Research Committee, and after obtaining the subject informed consent, a cross-sectional study was carried out from December 2007 to May 2009.

Obese women (defined by BMI ≥ 30 kg/m^2) aged 20 to 65 years were enrolled and allocated into the following groups: a) asymptomatic obese women without NAFLD, and b) asymptomatic obese women with diagnosis of NAFLD.

The sampling strategy was based on advertising to general population of Durango, city in northern Mexico, to invite obese women to participate in the study. Durango city has approximately 800,000 inhabitants and the prevalence of obesity is 27.2%.[13]

Alcohol consumption ≥ 30 g per week, viral hepatitis, medical treatment with drugs that promote cholestasis or liver injury, and previous diagnosis of chronic liver disease were exclusion criteria.

Data about family history as well as diagnosis of diabetes, hypertension and hypertriglyceridemia were recorded.

Liver biopsy using tru-cut needle ultrasound-guided, was offered to women with aspartate aminotransferase (AST)/alanine aminotransferase/(ALT) levels ≥ 1. Biopsy specimens were stained using hematoxilin and eosin, Masson's trichrome, and Perls stains.[5]

Definitions

Irrespective of ALT levels, diagnosis of NAFLD was established by the presence of hepatic ultrasonographic findings such as hepatorenal echo contrast, bright liver, deep attenuation, and blurred vessels.[14,15] In general, ultrasonography has a sensitivity of 89% and specificity of 93% for detecting steatosis, and sensitivity and specificity of 77% and 89% for detecting fibrosis.[4]

Irrespective of fibrosis, NASH was defined by hepatic histological changes characterized by macrovesicular steatosis; ballooning, inflammation, and Mallory-Denk bodies.[16] The NAS was estimated by the sum of scores for steatosis (0-3), lobular inflammation (0-3) and ballooning (0-2). The NAS value = 5 suggests diagnosis of NASH.[12]

Family history of diabetes (FHD), obesity, and hypertriglyceridemia was defined by the presence of type 2 diabetes, obesity, and hypertension in at least one first degree relative.

Diabetes was defined according criteria of the American Diabetes Association.[17]

Hypertriglyceridemia was defined by serum triglycerides levels ≥ 150 mg/dL.[18]

Measurements

In the standing position, weight and height were measured with the women in light clothing using a fixed scale with stadimeter (Tanita TBF-215, Tokyo, Japan). The precision of weight and height measurements was 0.1 kg and 0.01 m. BMI was calculated as weight (kilograms) divided by height (meters) squared. Total body fat was measured by bioelectric impedance using a body composition analyzer (Tanita TBF-215, Tokyo, Japan) with 0.1 percent increment.

Abdominal ultrasonography was performed using ultrasound scanner (General Electric, USA). Ultrasonographic evaluations were performed by two independent experts, who were blinded regard results of laboratory.

Assays

A venous whole blood sample was collected after 8-10 hours of fasting. Plasma glucose was assessed by glucose-oxidase method; the inter and intrassay variations were 2.1, and 1.5%. Total-cholesterol (inter- and intrassay variations of 3.0 and 2.5%) and serum triglycerides (inter- and intrassay variations of 3.5, and 3.0%) were determined by enzymatic methods. AST and ALT levels were determined by UV kinetic methods (Erlic, Tlalnepantla, Estado de Mexico, Mex.). Normal reference values of AST and ALT levels were of 38 and 40 UI/L.[19] In addition, data were re-addressed using the healthy range for ALT levels (19 UI/L for women).[20]

All measurements were performed in an Express 500 clinical chemistry autoanalyzer (Ciba Corning, Diagnostic Corp., Overling, Ohio).

Statistical analysis

Numerical values are reported as mean ± standard deviation, and categorical variables are expressed as proportions.

For bivariate analysis, Student's t test (or alternately Mann-Whitney U test for skewed data) and chi-squared test were used for numerical and categorical data, respectively.

Sensitivity, specificity, positive predictive value, and negative predictive value of ALT for diagnosis of NAFLD were estimated.[21]

A p value < 0.05 defined statistical significance. Data were analyzed by using the statistical package SPSS for Windows 15.0.

RESULTS

A total of 457 obese women with average age and BMI of 45.0 ± 10.7 years and 35.5 ± 5.0 kg/m^2 were enrolled.

One hundred ninety five (42.7%) women had elevated ALT levels. Diagnosis of NAFLD was established in 228 (49.9%) women; among women with NAFLD, 34 (14.9%) have ALT levels within the normal range (sensitivity 85%, specificity 99.6%, positive predictive value 99.5% and negative predictive value 87%).

On the other hand, based on the healthy range for ALT levels, a total of 336 (73.5%) women had elevated ALT, but only 2 (0.9%) women with NAFLD exhibited ALT levels within normal healthy values (sensitivity 99.1%, specificity 52%, positive predictive value 67.3%, and negative predictive value 98.3%).

Ninety three (40.8%) women had AST/ALT levels ≥ 1; all of them accepted underwent liver biopsy.

The frequency of diabetes was higher in the women with NAFLD than in women without NAFLD. In addition, women with NAFLD were more obese and had higher fasting plasma glucose, triglycerides, ALT, and AST levels than obese women without NAFLD (Table 1).

Women with NAFLD and diabetes were younger more obese, and have higher AST and ALT levels than women without NAFLD (Table 2).

Among women who underwent hepatic biopsy, 90 (96.8%) had diagnosis of NASH and 3 (3.2%) of hepatic cirrhosis. Among women with NASH, 85 (91.4%) had severe NASH, or the most severe and irreversible form of NAFLD, exhibiting steatosis, balonization, inflammation, fibrosis, and necrosis. Only 40% of the women had Mallory-Denk bodies (Table 3). There were not significant differences by age between different stages of fibrosis.

According to NAS 36% of biopsies had a score ≥ 5 and 53% a score > 3 and < 5; however, all of these

Table 1. Characteristics of obese women with and without nonalcoholic steatohepatitis

	Without NAFLD	With NAFLD	p Value
N	229	228	
Obesity duration, years	10.2 ± 8.2	10.6 ± 9.3	0.74
Family history of obesity, n (%)	183 (79.9)	190 (83.3)	0.66
Family history of diabetes, n (%)	212 (92.5)	184 (80.7)	0.0003
Family history of hypertension, n (%)	156 (68)	171 (75)	0.12
Family history of hypertriglyceridemia, n (%)	92 (40.2)	108 (47.3)	0.14
Diabetes, n (%)	29 (12.6)	47 (20.6)	0.03
Hypertension, n (%)	71 (31.0)	91 (39.9)	0.058
Hypertriglyceridemia*, n (%)	65 (28.3)	77 (33.7)	0.25
Age, years	44.7 ± 11.4	45.4 ± 10	0.44
Diabetes duration, years	5.5 ± 3.9	5.1 ± 4.3	0.61
Hypertension duration, years	7.0 ± 7.3	7.4 ± 7.1	0.14
Hypertriglyceridemia duration, years	1.7±1.3	1.7±2.1	0.83
Waist circumference, cm	103.1 ± 14.6	108.4 ± 11.9	0.000
Total body fat, %	43.1 ± 5.6	44.6 ± 8.5	0.02
Fasting glucose, mg/dL	95.0 ± 37.0	103.2 ± 32.2	0.000
Total cholesterol, mg/dL	202.8 ± 63.6	220.4 ± 69.7	0.09
Triglycerides, mg/dL	175.1 ± 155.3	225.4 ± 193	0.000
AST**, U/L	25.9 ± 8.2	46.5 ± 18.2	0.000
ALT**, U/L	22.4 ± 8.3	56.7 ± 23.3	0.000

*Serum triglycerides levels ≥ 150 mg/dL. **Reference value of 40 and 38 U/L for AST and ALT levels.

Table 2. Characteristics of diabetic obese women with and without nonalcoholic steatohepatitis, n = 76.

N	Without NAFLD N = 29	With NAFLD N = 47	p Value
Age, years	52.3 ± 8.8	47.1 ± 7.1	0.009
Hypertension, n (%)	17 (58.6)	25 (53.2)	0.822
Hypertriglyceridemia,* n (%)	18 (62.0)	27 (57.4)	0.874
Weight, kg	90.3 ± 14	86.8 ± 15.5	0.322
Body Mass Index, kg/m^2	36.7 ± 5.0	36 ± 5.2	0.553
Waist circumference, cm	106.4 ± 22.4	109.7 ± 11.7	0.471
Total body fat, %	42.6 ± 9.3	43.1 ± 6.3	0.789
Fasting glucose, mg/dL	127.1 ± 64.9	120.8 ± 49.9	0.663
Total cholesterol, mg/dL	209.1 ± 58.2	232.3 ± 56.1	0.103
Triglycerides, mg/dL	211.7 ± 112.1	268.3 ± 198.2	0.124
AST,** U/L	22.7 ± 5.2	48.5 ± 16.7	0.000
ALT,** U/L	20.9 ± 6.9	53.6 ± 19.4	0.000

*Serum triglycerides levels = 150 mg/dL. **Reference value of 40 and 38 U/L for AST and ALT levels.

Table 3. Hepatic histological changes in women with nonalcoholic steatohepatitis who underwent hepatic biopsy n = 93.

	Stages				
	0	1	2	3	4
Steatosis	7 (7.5)	54 (58)	22 (23.6)	10 (10.7)	
Inflammation	8 (8.6)	51 (54.8)	28 (30)	6 (6.4)	
Fibrosis	14 (15)	50 (53.7)	21 (22.5)	5 (5.3)	3 (3.2)
Balonization	4 (4.3)	40 (43)	47 (50.5)	2 (2.1)	
Mallory's Hyaline	56 (60)	25 (26.8)	12 (12.9)	0	
Necrosis	15 (16)	50 (53.7)	24 (25.8)	4 (4.3)	

Values are n (%).

biopsies showed histological activity; only 8% of biopsies had score ≤ 3 of NAS.

DISCUSSION

Our results show that obese women with NAFLD have higher frequency of diabetes, fasting plasma glucose, and triglycerides levels than obese women without NAFLD; this finding supports the statement that women with NAFLD have an adverse metabolic profile.

Among obese individuals, the prevalence of NAFLD range from 50% to 90%[4,22,23] and in diabetic patients from 34 to 74%. In the subjects with obesity and type 2 diabetes, NAFLD is a common finding.[24] In our population, the prevalence of NAFLD reach 49.9%, higher than that in previous reports;[25] on the other hand, the frequency of risk factors such as type 2 diabetes; hypertension, and hypertriglyceridemia was similar to the observed in US population.[26]

In this study, 14.9% of the women with normal ALT levels had NAFLD; on this regard, in the Dallas Heart Study[27] and Dyonisios study,[25] 79% and 55% of the subjects with normal ALT exhibited NAFLD. These findings suggest that liver enzymes have an elevated rate of false negative results in the diagnosis of NAFLD. Using the healthy range for ALT levels increase the sensitivity and negative predictive value but decrease specificity and predictive positive value; these finding suggest that healthy range for ALT levels are more appropriate as a screening tool for NAFLD. However, using the appropriate methodological design study, the best cutoff point of ALT for recognizing NAFLD should be established in a Receiver Operating Characteristic scatter plot.

In agree with other studies,[27] the hepatic histological changes of obese women with NAFLD were compatible with a severe and progressive form of NASH. Furthermore, in agree with the report by Marchesini,[28] the hepatic disease was more severe in the presence of type 2 diabetes. However, the presence of moderate or severe fibrosis was lower than the reported in other populations, in which 30 to 40% of the obese patients with NAFLD have advanced fibrosis and 10-15% cirrhosis.[4]

According NAS, 89% of the women in this study have NASH; taking into account that NAS is a tool for assessing disease severity[12] we hypothesized

that the target population had a severe hepatic disease.

Some clinical parameters such as duration of obesity and diabetes, the presence of AST/ALT ≥ 1, and hyperglycemia may be useful for predicting NAFLD.[29,30] In addition, we have previously showed that triglycerides levels ≥ 300 mg/dL are associated to NAFLD (unpublished data). However, because NAFLD and NASH represent advanced stages of hepatic steatosis that are associated with metabolic diseases; and a high proportion of individuals with fatty liver do not show laboratory abnormalities,[31] based on the results of this study, we develop an algorithm to facilitate recognition of NAFLD, Figure 1. The algorithm suggests that in diabetic obese women aged < 40 years, triglycerides and ALT levels should be assessed; if triglycerides are ≥ 300 mg/dL and healthy ALT levels < 20 U/L, hepatic ultrasonography is mandatory; in the presence of hepatic steatosis and AST/ALT levels ≥ 1, hepatic biopsy should be offered. Although our results suggest that the algorithm facilitates recognition of NASH, further research is required to validate it.

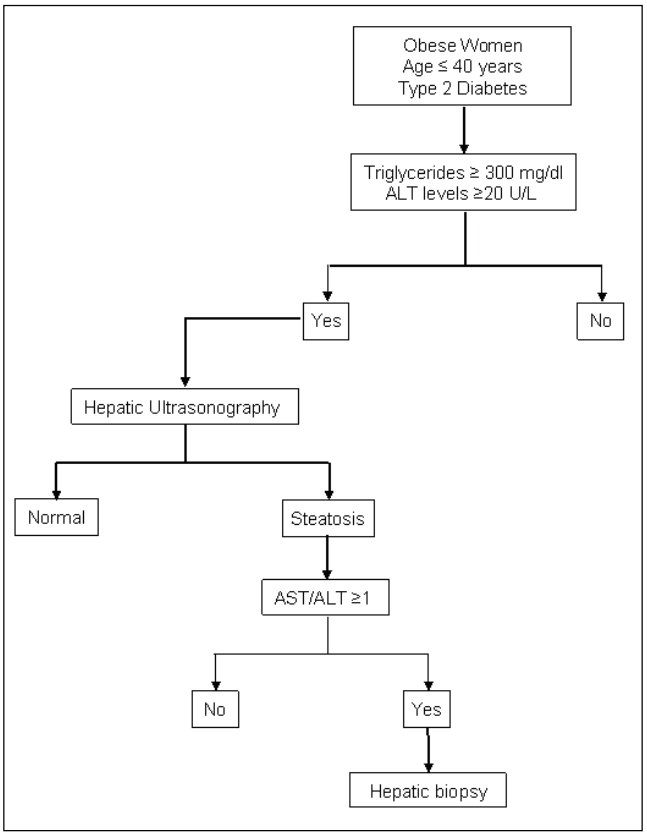

***Figure 1**. Algorithm for the early clinical recognition of NAFLD. In the presence of obesity and diabetes, women aged <40 years would have a determination of triglycerides levels; if triglycerides are ≥ 300 mg/dL and healthy ALT levels ≥ 20 U/L, hepatic ultrasonography is mandatory; in the presence of steatosis and AST/ALT levels ≥ 1, hepatic biopsy should be proposed.*

Some limitations of the present study deserve to be mentioned: first, only obese women were studied; in consequence, our results cannot be applied to men; second, only 41% of obese women underwent hepatic biopsy; so, is probable that the frequency of NASH and cirrhosis have been underestimated; third, Mallory-Denk bodies were estimated using hematoxilin and eosin stain but not more sensitive techniques such as ubiquitin; thus, it is probable that we underestimated the frequency of Mallory-Denk bodies. However, is necessary to keep in mind that Mallory-Denk body is a frequent feature of alcoholic steatohepatitis rather than a feature of NASH. Nonetheless, these limitations do not influence our conclusions.

In conclusion, the present study shows that women with NAFLD are more obese and have higher hyperglycemia, hypertriglyceridemia, and elevated rates of diabetes than obese women without NAFLD. Furthermore, our results show that hyperglycemia, hypertriglyceridemia and markers of liver injury as such AST/ALT ≥ 1 may be useful for early recognition of NAFLD and severe form of NASH.

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REFERENCES

1. Mulhall BP, Ong JP, Younossi ZM. Non-alcoholic fatty liver disease: An overview. *J Gastroenterol Hepatol* 2002; 17: 1136-43.
2. Hamer OW, Aguirre D, Casola G, Sirlin CV. Imaging features of perivascular fatty infiltration of the liver. *Radiology* 2005; 237: 159-69.
3. Adams LA, Lymp JF, Sauver J, et al. The natural history of nonalcoholic fatty liver disease: A population-based cohort study. *Gastroenterology* 2005; 129: 113-21.
4. Angulo P. Nonalcoholic fatty liver disease. *N Engl J Med* 2002; 346: 1221-31.
5. Bellentani S, Marino M. Epidemiology and natural history of non-alcoholic fatty liver disease (NAFLD). *Ann Hepatol* 2009; 8: S4-S8.
6. Bloomgarden ZT. Second World Congress on the Insulin Resistance Syndrome: Insulin resistance syndrome and nonal-

coholic fatty liver disease. *Diabetes Care* 2005; 28: 1518-23.
7. Matteoni C, Younossi Z, Gramlich T, Boparai N, Liu YC, McCullough A. Nonalcoholic fatty liver disease: A spectrum of clinical and pathologic severity. *Gastroenterology* 1999; 116: 1413-9.
8. Charatcharoenwitthaya P. Lindor K. Role of radiologic modalities in the management of non-alcoholic steatohepatitis. *Clin Liv Dis* 2007; 11: 37-54.
9. Saadeh S, Younossi Z, Remer EM, et al. The utility of radiological imaging in nonalcoholic fatty liver disease. *Gastroenterology* 2002; 123: 750-4.
10. Abdelmalek MF, Diehl AM. Nonalcoholic fatty liver disease as a complication of insulin resistance. *Med Clin N Am* 2007; 91: 1125-49.
11. McCullough AJ. The clinical features, diagnosis and natural history of nonalcoholic fatty liver disease. *Clin Liver Dis* 2004; 8: 521-33.
12. Kleiner DE, Brunt EM, Van Natta M, et al. Design and validation of a histological scoring system for non-alcoholic fatty liver disease. *Hepatology* 2005; 41: 1313-21.
13. Olaiz G, Rojas R, Barquera S, Shamah T, Aguilar C, Cravioto P, López P, Hernández M, Tapia R, Sepúlveda J. Encuesta Nacional de Salud 2000. Tomo 2. La salud de los adultos. Cuernavaca, Morelos, México. Instituto Nacional de Salud Pública, 2003.
14. Nakao K, Nakata K, Ohtsubo N, et al. Association between nonalcoholic fatty liver, markers of obesity, and serum leptin level in young adults. *Am J Gastroenterol* 2002; 97: 1796-801.
15. Hamaguchi M, Kojima T, Itoh Y, et al. The severity of ultrasonographic findings in nonalcoholic fatty liver disease reflects the metabolic syndrome and visceral fat accumulation. *Am J Gastroenterol* 2007; 102: 2708-15.
16. Brunt EM, Janney CG, Di Bisceglie AM, Neuschwander-Tetri BA, Bacon BR. Nonalcoholic steatohepatitis: A proposal for grading and staging the histological lesions. *Am J Gastroenterol* 1999; 94: 2467-74.
17. American Diabetes Association. Diagnosis and Classification of diabetes mellitus. *Diabetes Care* 2005; 28 (Suppl 1): S37-S42.
18. Executive summary of the third report of the national cholesterol education program expert panel on detection, evaluation, and treatment of high blood cholesterol in adults. *JAMA* 2001; 285: 2486-97.
19. Clark JM, Brancati FL, Diehl AM. The prevalence and etiology of elevated aminotransferase levels in the United States. *Am J Gastroenterol* 2003; 98: 960-7.
20. Prati D, Taioli E, Zanella A, Della Torre E, Butelli S, Del Vecchio E, Vianello L, Zanuso F, Mozzi F, Milani S, Conte D, Colombo M, Sirchia G. Updated definitions of healthy ranges for serum alanine aminotransferase levels. *Ann Intern Med* 2002; 137: 1-10.
21. Kramer MS (ed). Clinical Epidemiology and Biostatistics. A Primer for investigators and Decision-Makers. Berlin: Springer-Verlag Press, 1988: 201-210.
22. Ruhl CE, Evaerhart JE. Determinants of the association of overweight with elevated serum alanine aminotransferase activity in the United States. *Gastroenterology* 2003; 124: 71-9.
23. Serfaty L, Lemoine M. Definition and natural history of metabolic steatosis: clinical aspects of NAFLD, NASH and cirrhosis. *Diabetes & Metabolism* 2008; 34: 634-7.
24. Tolman KG, Fonseca V, Dalpiaz A, Tan MH. Spectrum of liver disease in type 2 diabetes and management of patients with diabetes and liver disease. *Diabetes Care* 2007; 30: 734-43.
25. Bedogni G, Miglioli L, Masutti F, et al. incidence of natural course of fatty liver in the general population: The dyonisos study. *Hepatology* 2007; 46: 1387-91.
26. Bellentani S, Marino M. Epidemiology and natural history of non-alcoholic fatty liver disease (NAFLD). *Ann Hepatol* 2009; 8: S4-S8.
27. Browning JD, Szczepaniak LS, Dobbins R, et al. Prevalence of hepatic steatosis in an urban population in the United States: Impact of ethnicity. *Hepatology* 2004; 40: 1387-95.
28. Marchesini G, Marzocchi R, Agostini F, Bugianesi E. Nonalcoholic fatty liver disease and the metabolic syndrome. *Curr Opin Lipidol* 2005; 16: 421-7.
29. Paradis V, Bedossa P. Definition and natural history of metabolic steatosis: histology and cellular aspects. *Diabetes & Metabolism* 2008; 34: 638-42.
30. Gholam PM, Flancbaum L, Machan JT, Charney DA, Kotler DP. Nonalcoholic fatty liver disease in severely obese subjects. *Am J Gastroenterol* 2007; 102: 399-408.
31. Argo CK, Northup PG, Al-Osaimi AMS, Caldwell SH. Systematic review of risk factors for fibrosis progression in nonalcoholic steatohepatitis. *J Hepatol* 2009; 51: 371-9.