Nonalcoholic fatty liver disease in Japanese junior high school students: its prevalence and relationship to lifestyle habits

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obesity. Additionally, one obese student with severe steatosis and liver dysfunction was diagnosed as having nonalcoholic steatohepatitis (NASH).

Conclusions: Approximately 4% of junior high school students had NAFLD that was primarily associated with obesity and reduced daily physical activity. Serum ALT measurement during school check-ups is recommended for early detection of young adolescent NAFLD/NASH.

Introduction

Due to increasing sedentary lifestyles and the rising prevalence of obesity, nonalcoholic fatty liver disease (NAFLD) has become a common cause of chronic liver disease. NAFLD encompasses a spectrum of histological findings that range from macrovesicular steatosis alone (simple steatosis) to macrovesicular steatosis with hepatocyte ballooning and/or lobular inflammation (steatohepatitis). Nonalcoholic steatohepatitis (NASH) is the severe and progressive form of NAFLD and may develop into cirrhosis, hepatocellular carcinoma, and ultimately death.[1-3] Based on current health screening data, the prevalence of NAFLD in Japanese adults is estimated to be around 10%.[1]

Of great recent concern is the fact that NAFLD/NASH exists even in children and adolescents and that pediatric NASH can also progress to cirrhosis. In 1995, Tominaga *et al.* reported that 2.6% of Japanese children aged 4 to 12 years had NAFLD.[4] Furthermore, Kinugasa *et al.* described 7 Japanese obese children aged between 9 and 15 years having hepatic steatosis with various degrees of lobular inflammation and/or portal fibrosis and one having cirrhosis.[5] Therefore, early detection of NAFLD/NASH and appropriate disease management are now important in the pediatric population as well.

According to data from the Japanese Ministry of Education, Culture, Sports, Science, and Technology, the prevalence of obesity among Japanese junior high school students was double in 2003 compared to 1977 (from 5% to 10%). Given the strong association of NAFLD with obesity, NAFLD is suspected to affect a substantial, but as yet unidentified, proportion of junior high school students. As such, this population-based cross-sectional study was planned to evaluate the prevalence of NAFLD in young adolescents, along with any lifestyle habits associated with its development.

Materials and Methods

Participants

The study population consisted of all students attending a public junior high school in a village located in southern Nagano prefecture. Real-time liver function tests and abdominal ultrasonography (US) were performed in 2004 and 2007 after explaining the significance and protocol of the study to all students and their parents and obtaining written informed consent. This study was approved by the ethics committee of Shinshu University School of Medicine and Showa Inan General Hospital and adheres to the principles of the Declaration of Helsinki.

Data Collection

Anthropometric, biochemical, and ultrasonographic examinations were carried out on the same school-day morning in June 2004 and July 2007 after an overnight fast. Body height and weight were measured with subjects barefoot and in light clothing by a school nurse. Body mass index (BMI) and age-gender-adjusted Japanese standardized weight index for height (JSI) were used as anthropometric parameters. JSI was calculated as [(body weight - standard body weight) / standard body weight] x 100 (%), where the standard body weight for each case's age, sex, and height was determined

from data on 700,000 Japanese children aged 5 to 17 years in 1990. The JSI is considered to be more suitable for evaluation of the physiques of children and adolescents than BMI in Japan. According to the JSI, subject weight status was classified as lean (≤ -20% of JSI), moderately lean (-19.9% to -10.1%), normal (-10% to +10%), overweight (+10.1% to +19.9%), or obese (≥ +20%).

Venous blood samples were drawn just after an anthropometric examination. Serum levels of alanine aminotransferase (ALT), γ-glutamyltransferase (γGT), triglycerides (TG), and high-density-lipoprotein cholesterol (HDL-C) were determined using standard automated analyzers. Normal ranges for serum ALT and γGT levels were set as 0-30 U/L each, in accordance with previous studies in pediatric populations.[6-8]

Real-time abdominal US was performed by two experienced ultrasonographers (KH and CI) using a LOGIQ book equipped with a 4.0 MHz convex-type transducer (GE Yokogawa Medical Systems, Tokyo, Japan). Representative US images of each student were kept in the LOGIQ book and evaluated afterwards in a blinded manner by three independent hepatologists (GT, NT, and MK). The degrees of hepatorenal contrast, profound attenuation of the diaphragm, and blurring of the vascular wall were each scored as 0 (absent), 1 (present), or 2 (marked).[4,9-11] The sum of these scores from each diagnostician ranged from 0 to 6, and a mean total score of ≥ 1 was judged as the

presence of hepatic steatosis. Total scores of 1-2 and $\geq$ 3 were classified as having mild steatosis and moderate-to-severe steatosis, respectively.

Assessment of lifestyle habits

In the 2007 survey, lifestyle habits were assessed in addition to abdominal US and liver function tests. A 15-item questionnaire focusing on personal dietary and exercise habits was prepared and distributed to all students. All questionnaires were completed and submitted by the students themselves, and were then analyzed for any relationships with NAFLD.

Statistics

Statistical analyses were performed using SPSS software 11.0J for Windows (SPSS Inc., Chicago, IL, USA). Qualitative variables were expressed as a number (percentage) and compared using the χ^2 test. Quantitative data were expressed as mean $\pm$ SD and compared using the two-tailed Student's t-test or one-way analysis of variance. *Post-hoc* comparison was also performed between groups using the Tukey's or Games-Howell's method. Multivariate logistic regression analysis was conducted to find independent predictors of NAFLD. A *P* value of less than 0.05 was considered to be statistically

significant.

Results

Prevalence of obesity and NAFLD

The overall prevalence of obesity was 10.0% and 5.9% in 2004 and 2007, respectively (Table 1). Since all students were presumed to not habitually consume alcohol, the overall prevalence of NAFLD was calculated as 4.4% and 4.5% in 2004 and 2007, respectively (Table 1). Of these, the prevalence of moderate-to-severe steatosis was 0.8% in 2004 and 1.4% in 2007 (Table 1). There was a male preponderance in NAFLD prevalence in the 2004 survey (7.5% in boys vs. 1.6% in girls, $P = 0.022$).

Association between weight status and NAFLD

As shown in Table 2, approximately 90% of students had neither obesity nor NAFLD and 2-3% had both disorders. In the 2007 survey, 4 of 13 NAFLD students were overweight (Table 2). Although a small number of NAFLD students having normal body weight was found (1.2% in 2004 and 1.0% in 2007), the degree of steatosis was very mild in all cases. On the other hand, all students with moderate-to-severe steatosis were obese (Table 2).

Comparison of clinical data between students with and without NAFLD

In both surveys combined, 24 (4.5%) of 537 students were judged as having NAFLD. Several parameters were then compared between students with NAFLD (n = 24) and those without (n = 513) to investigate the clinical features of NAFLD. The prevalence of male gender, obesity, and elevated ALT and γGT levels was significantly higher in the NAFLD group than in the non-NAFLD one (Table 3). JSI, BMI, and serum levels of ALT, γGT, and TG were increased and serum HDL-C levels were decreased in the NAFLD group (Table 3). When these parameters were analyzed in relation to degree of steatosis, BMI and serum levels of ALT, γGT, and TG all increased with the steatosis severity (Fig. 1). Multivariate logistic regression analysis uncovered that the presence of obesity and ALT $\geq$ 30 U/L were identified as independent predictors of NAFLD. The odds ratio was 16.9 for the presence of obesity [95% confidence interval (CI), 6.5-43.9; $P < 0.001$] and 16.6 for ALT $\geq$ 30 U/L (95% CI, 3.1-87.6; $P = 0.001$).

Additionally, when these parameters were compared between boys (n = 275) and girls (n = 262), JSI and serum levels of ALT and γGT were increased in the former group (2.6 $\pm$ 17.2 vs. -1.5 $\pm$ 14.2, $P = 0.003$ for JSI; 15 $\pm$ 6 vs. 12 $\pm$ 6 U/L, $P < 0.001$ for ALT; and 16 $\pm$ 5 vs. 13 $\pm$ 3 U/L, $P < 0.001$ for γGT). The prevalence of elevated ALT and γGT levels tended to be higher in boys, though not significantly (2.5% vs. 1.1% and 2.9% vs. 1.1%, respectively).

Comparison of lifestyle habits between students with and without NAFLD

To explore lifestyle habits associated with NAFLD development, questionnaires were distributed to all students in 2007 and compared between cases with NAFLD (n = 13) and those without (n = 275). The clinical features of the students examined in 2007 are shown in Table 4. The ratios of students skipping breakfast (≥ 2 times/week) and always drinking more than half of the broth that comes with noodles were significantly higher in NAFLD students than in non-NAFLD ones (Table 5). The ratios of students often commuting to and from school by car were also significantly higher in NAFLD students (Table 5). As expected, the ratios of students liking exercise and doing sports outside of school were significantly lower in the NAFLD group (Table 5). There were no gender differences in the ratios of students having such lifestyle habits.

A similar comparison was performed between obese students with NAFLD (n = 5) and those without (n = 12). Although there were no remarkable differences in dietary habits, obese NAFLD students showed a higher tendency to commute to and from school by car (80.0% vs. 8.3%, $P = 0.010$) and a lower tendency to do sports outside of school (0% vs. 58.3%, $P = 0.041$) than obese non-NAFLD students. Furthermore, when lifestyle habits were compared between non-obese students with NAFLD (n = 8) and

those without (n = 263), the former group had a higher ratio of drinking more than half of the broth that comes with noodles (87.5% vs. 50.6%, $P = 0.042$) and a lower ratio of doing sports outside of school (0% vs. 31.9%, $P = 0.048$).

Overall, the absence of outside-of-school sports was detected in all students with NAFLD, regardless of obesity, suggesting an important association between the development of NAFLD and reduced physical activity in junior high school students.

Follow-up of junior high school students with NAFLD

The 11 junior high school students with NAFLD found in 2004 were advised to come to our hospital for treatment and monitoring. Of these, only 2 obese students with severe steatosis and elevated ALT and γGT levels received further examination. They were negative for hepatitis B virus surface antigen, anti-hepatitis C virus antibody, and anti-nuclear antibody in sera, had normal levels of serum ceruloplasmin, and had no history of regular intake of drugs, which indicated that their liver dysfunction stemmed from NAFLD. One student reduced dietary calorie intake and started daily walking, and marked improvement of hepatic steatosis and normalization of serum ALT levels were observed 1 year later. On the other hand, the other student continued to gain weight despite repeated lifestyle instruction, experienced worsened serum ALT levels, and was

later diagnosed as having NASH by liver biopsy (Fig. 2). This student is now undergoing treatment for NASH.

Discussion

As far as we know, this is the first epidemiological study on the prevalence and clinical features of NAFLD in Japanese junior high school students. In addition, this study explored for the first time the relationship between the development of young adolescent NAFLD and lifestyle habits. Our results demonstrated that NAFLD in junior high school students occurs in approximately 1 out of 20 students and is primarily associated with obesity and decreased daily physical activity.

Very few population-based epidemiological studies have been conducted on pediatric NAFLD to date. Tominaga *et al.* reported that the recent prevalence of NAFLD in Japanese children aged 11 to 15 years was 5.2%.[9] Our findings are similar to these observations.

In this study, the presence of obesity and ALT $\geq$ 30 U/L were identified as independent predictors of NAFLD; anthropometric indices, such as JSI and BMI, and serum ALT levels all increased with the degree of steatosis. Therefore, inclusion of serum ALT level determination in addition to anthropometric examination may be useful for detecting students with a risk of NAFLD/NASH in annual school-based health checkups. Alavian *et al.* also described elevation of serum ALT levels to be a superior predictor of NAFLD over several metabolic variables, such as BMI, waist

circumference, and serum concentrations of lipid profiles and insulin, in a cross-sectional study of 966 Iranian children aged 7 to 18 years.[12] Further large-scale population-based prospective studies are needed to verify the usefulness of serum ALT measurement for early detection of childhood NAFLD.

The most remarkable finding in this study was that the ratios of students commuting to and from school by car and not doing sports outside of school were significantly higher in students with NAFLD compared with those without. Such tendencies were also detected in non-obese NAFLD students, suggesting that decreased daily physical activity contributes, at least in part, to the occurrence of NAFLD independently of the presence of obesity. This observation is supported by the evidence that regular aerobic exercise reduces hepatic lipids in obese individuals even in the absence of body weight reduction.[13] Thus, strategies to increase daily physical activity might prevent the development of NAFLD/NASH in junior high school students.

Concerning diet, we found a significant relationship between skipping breakfast and the presence of NAFLD. This observation is partially consistent with a previous study that showed skipping breakfast to be strongly associated with childhood obesity.[14] The dietary habit of always drinking most of the broth that comes with noodles might reflect an excess intake of salt, fat, and calories.

There were some NAFLD students without apparent obesity. Since all non-obese NAFLD students had normal concentrations of serum ALT and γGT and mild fatty deposition in the liver, it is inconclusive whether the clinical course of this type of NAFLD is similar to that of typical obesity-related NAFLD. Long-term longitudinal studies are required in such populations to address this issue.

It is well-known that gender differences exist in NAFLD.[9,15] In 537 students in the 2004 and 2007 surveys, the prevalence of NAFLD, JSI, and serum ALT and γGT levels were higher in boys. Such differences might result from the difference of susceptibility to fat accumulation in the body and insulin resistance.[16] Although gender differences in lifestyle habits could not be found in the present study, further large-scale assessment would enable us to clarify the relationship between gender differences in NAFLD prevalence and lifestyle habits.

Although the natural history of pediatric NAFLD has not been fully explored, some NAFLD children cases can develop into advanced fibrosis and cirrhosis. Considering that a high proportion of obese children are likely to become obese adults,[17] early detection of pediatric NAFLD and establishment of appropriate interventions may lead to a decreased incidence of adult NASH. To better predict pediatric NAFLD, we recommend the addition of serum ALT measurement to

school-based health screening. More importantly, it remains difficult to convince NAFLD students to receive further examination and follow-up because of child and parent schedules and the lack of any apparent symptoms. To solve this problem, we believe that establishment of a comprehensive community-based network system to screen for pediatric NAFLD and related lifestyle disorders[18] will be needed in the future.

Acknowledgments

We thank our nursing, nutrition, and laboratory staff for their skilled work, Mr. Takahiro Rokuhara for preparing US instruments, and Mr. Trevor Ralph for his editorial assistance.

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Figure Legends

Fig. 1. Comparison of clinical parameters by degree of steatosis

Data were obtained in 2004 and 2007 and compared among students with absent steatosis (white bars, n = 513), mild steatosis (gray bars, n = 18), and moderate-to-severe steatosis (black bars, n = 6). Data are expressed as mean ± SD. Statistical analysis was conducted using the Tukey's or Games-Howell's analysis. **, $P<0.01$; *, $P<0.05$; JSI, age-gender-adjusted Japanese standardized weight index for height; BMI, body mass index; ALT, alanine aminotransferase; γGT, γ-glutamyltransferase; TG, triglycerides; HDL-C, high-density-lipoprotein cholesterol.

Fig. 2. Histological findings of a liver sample obtained from an obese NAFLD student

Severe macrovesicular steatosis, hepatocyte ballooning, and pericellular/perivenular fibrosis were evident. The NAFLD activity score was 7. (**A**) Azan-Mallory staining (original magnification, x140); (**B**) Hematoxylin and eosin staining (original magnification, x300).

Table 1. Prevalence of obesity and NAFLD

Year	2004			2007		
	Boys (n = 120)	Girls (n = 129)	Overall (n = 249)	Boys (n = 155)	Girls (n = 133)	Overall (n = 288)
Weight status						
Lean	2 (1.7%)	2 (1.5%)	4 (1.6%)	0 (0%)	13 (9.8%)	13 (4.5%)
Moderately lean	9 (7.5%)	21 (16.3%)	30 (12.0%)	23 (14.8%)	38 (28.6%)	61 (21.2%)
Normal	87 (72.5%)	81 (62.8%)	168 (67.5%)	102 (65.8%)	61 (45.9%)	163 (56.6%)
Overweight	9 (7.5%)	13 (10.1%)	22 (8.8%)	19 (12.3%)	15 (11.3%)	34 (11.8%)
Obese	13 (10.8%)	12 (9.3%)	25 (10.0%)	11 (7.1%)	6 (4.5%)	17 (5.9%)
JSI (%)	2.9 ± 15.1	1.9 ± 14.3	2.4 ± 14.7	2.4 ± 18.7	- 4.7 ± 13.4	- 0.9 ± 16.8
BMI (kg/m^2)	19.7 ± 3.0	19.9 ± 3.0	19.8 ± 3.0	19.2 ± 2.7	19.2 ± 2.8	19.2 ± 2.7
Hepatic steatosis						
Absent	111 (92.5%)	127 (98.4%)	238 (95.6%)	147 (94.8%)	128 (96.2%)	275 (95.5%)
Present (NAFLD)	9 (7.5%)	2 (1.6%)	11 (4.4%)	8 (5.2%)	5 (3.8%)	13 (4.5%)
Mild	8 (6.7%)	1 (0.8%)	9 (3.6%)	4 (2.6%)	5 (3.8%)	9 (3.1%)
Moderate-to-severe	1 (0.8%)	1 (0.8%)	2 (0.8%)	4 (2.6%)	0 (0%)	4 (1.4%)

Data were expressed as a number (percentage to students in the same gender or overall in each year) or mean ± SD. Body weight status was classified according to the criteria of the age-gender-adjusted Japanese standardized weight index for height (JSI). BMI, body mass index; NAFLD, nonalcoholic fatty liver disease.

Table 2. Association between weight status and NAFLD

Year	2004			2007		
	Lean-to-normal (n = 202)	Overweight (n = 22)	Obese (n = 25)	Lean-to-normal (n = 237)	Overweight (n = 34)	Obese (n = 17)
Hepatic steatosis						
Absent	199 (79.9%)	22 (8.8%)	17 (6.8%)	234 (81.3%)	30 (10.4%)	11 (3.8%)
Present (NAFLD)	3 (1.2%)	0 (0%)	8 (3.2%)	3 (1.0%)	4 (1.4%)	6 (2.1%)
Mild	3 (1.2%)	0 (0%)	6 (2.4%)	3 (1.0%)	4 (1.4%)	2 (0.7%)
Moderate-to-severe	0 (0%)	0 (0%)	2 (0.8%)	0 (0%)	0 (0%)	4 (1.4%)

Data were expressed as a number (percentage to all students in each year). NAFLD, nonalcoholic fatty liver disease.

Table 3. Comparison of clinical data

NAFLD	(-) (n = 513)	(+) (n = 24)	*P*
Male	258 (50.3%)	17 (70.8%)	**0.049**
Obesity (JSI ≥ 20%)	28 (5.7%)	14 (58.3%)	**<0.001**
ALT ≥ 30 U/L	4 (0.8%)	6 (25.0%)	**<0.001**
γGT ≥ 30 U/L	6 (1.2%)	5 (20.8%)	**<0.001**
JSI (%)	- 0.6 ± 12.9	27.0 ± 38.0	**0.002**
BMI (kg/m^2)	19.3 ± 2.7	23.2 ± 4.6	**<0.001**
ALT (U/L)	13 ± 5	22 ± 15	**0.012**
γGT (U/L)	14 ± 4	20 ± 10	**0.006**
TG (mg/dL)	75 ± 42	99 ± 53	**0.037**
HDL-C (mg/dL)	64 ± 13	57 ± 10	**0.006**

Data were obtained from 249 junior high school students in 2004 and 288 students in 2007 and expressed as a number (percentage) or mean ± SD. *P* values of less than 0.05 are in bold and underlined. NAFLD, nonalcoholic fatty liver disease; JSI, age-gender-adjusted Japanese standardized weight index for height; ALT, alanine aminotransferase; γGT, γ-glutamyltransferase; BMI, body mass index; TG, triglycerides; HDL-C, high-density-lipoprotein cholesterol.

Table 4. Comparison of clinical data of 288 junior high school students examined in 2007

NAFLD	(-) (n = 275)	(+) (n = 13)	*P*
Male	147 (53.5%)	8 (61.5%)	0.568
Obesity (JSI ≥ 20%)	11 (4.0%)	6 (46.2%)	**<0.001**
ALT ≥ 30 U/L	3 (1.1%)	3 (23.1%)	**0.001**
γGT ≥ 30 U/L	4 (1.5%)	3 (23.1%)	**0.002**
JSI (%)	- 2.2 ± 12.2	26.8 ± 49.9	**0.048**
BMI (kg/m^2)	19.0 ± 2.5	22.5 ± 4.9	**0.025**
ALT (U/L)	14 ± 5	21 ± 14	**0.044**
γGT (U/L)	14 ± 4	20 ± 13	**0.032**
TG (mg/dL)	62 ± 29	83 ± 47	0.136
HDL-C (mg/dL)	68 ± 13	60 ± 8	**0.004**

Data were expressed as a number (percentage) or mean ± SD. *P* values of less than 0.05 are in bold and underlined. Abbreviations are the same as those in Table 3.

Table 5. Lifestyle habits of 288 junior high school students examined in 2007

NAFLD	(-) (n = 275)	(+) (n = 13)	*P*
Skipping breakfast (≥ 2 times/week)	26 (9.5%)	4 (30.8%)	**0.036**
Eating quickly	118 (42.9%)	7 (53.8%)	0.444
Drinking more than half of the broth with noodles	140 (50.9%)	11 (84.6%)	**0.018**
Consuming sweetened drinks every day	131 (47.6%)	6 (46.2%)	0.907
Eating junk food (≥ 3 times/week)	209 (76.0%)	8 (61.5%)	0.186
Eating a midnight snack (≥ 3 times/week)	115 (41.8%)	4 (30.8%)	0.423
Watching TV during meals	207 (75.3%)	12 (92.3%)	0.144
Eating all of the fat around meat	160 (58.2%)	9 (69.2%)	0.438
Often consuming mayonnaise	152 (55.3%)	6 (46.2%)	0.509
Eating cakes/sweet rolls (≥ 3 times/week)	129 (46.9%)	5 (38.5%)	0.543
Eating convenience store lunches (≥ once/week)	35 (12.7%)	4 (30.8%)	0.084
Often commuting to and from school by car	67 (24.4%)	7 (53.8%)	**0.026**
Playing computer games (≥ 1 hour/day)	180 (65.5%)	10 (76.9%)	0.305
Liking exercise	217 (78.9%)	8 (61.5%)	**0.045**
Doing sports outside of school	82 (29.8%)	0 (0%)	**0.012**

Data were expressed as a number (percentage). *P* values of less than 0.05 are in bold and underlined. NAFLD, nonalcoholic fatty liver disease.

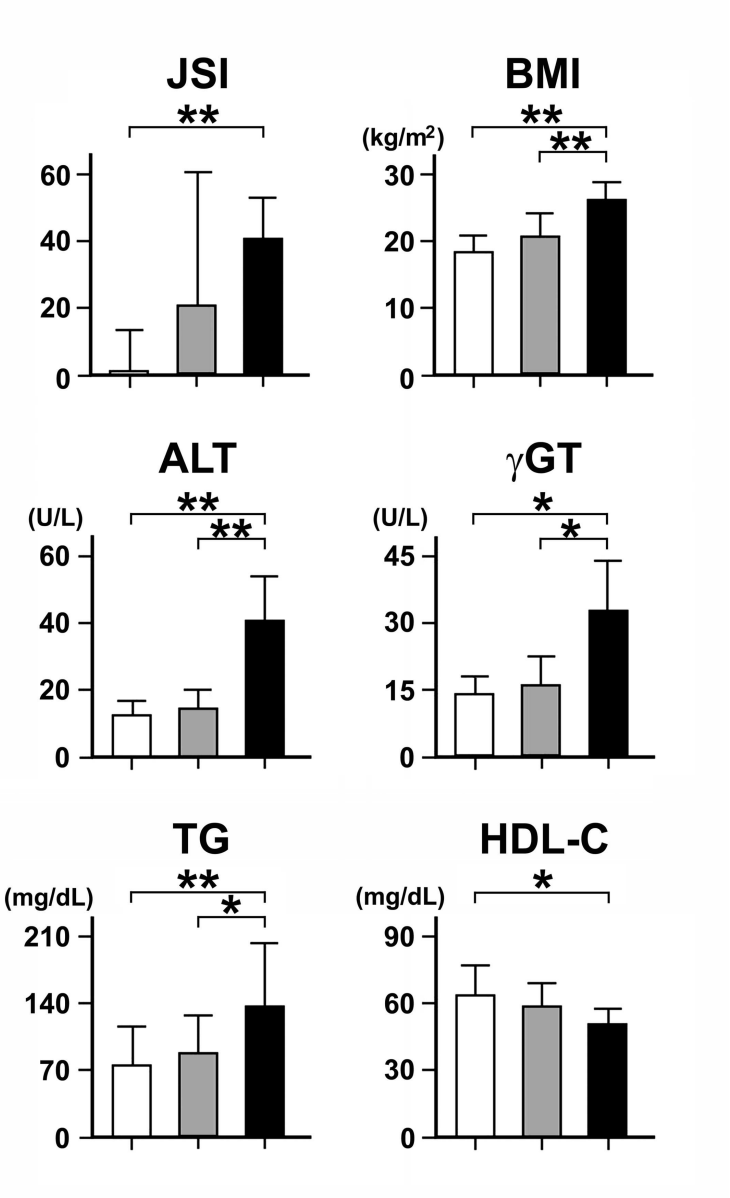

Fig. 1. Comparison of clinical parameters by degree of steatosis

Data were obtained in 2004 and 2007 and compared among students with absent steatosis (white bars, n = 513), mild steatosis (gray bars, n = 18), and moderate-to-severe steatosis (black bars, n = 6). Data are expressed as mean ± SD. Statistical analysis was conducted using the Tukey's or Games-Howell's analysis. **, P<0.01; *, P<0.05; JSI, age-gender-adjusted Japanese standardized weight index for height; BMI, body mass index; ALT, alanine aminotransferase; γGT, γ-glutamyltransferase; TG, triglycerides; HDL-C, high-density-lipoprotein cholesterol.
94x148mm (600 x 600 DPI)

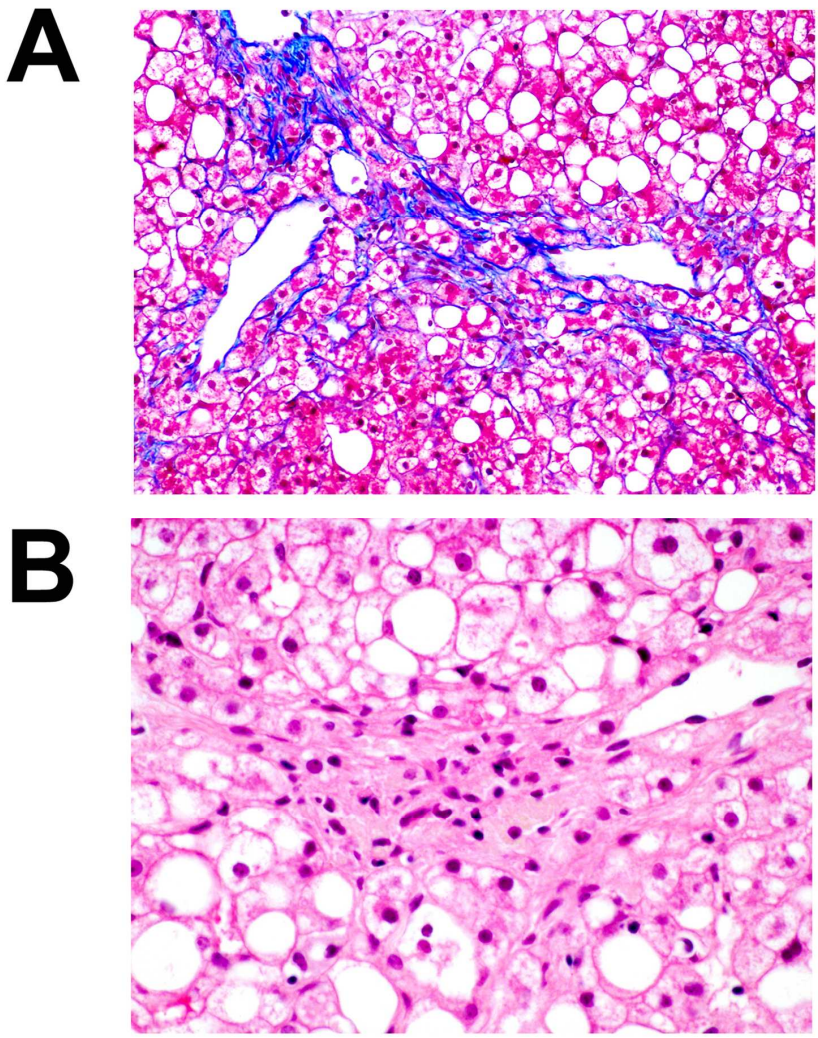

Fig. 2. Histological findings of a liver sample obtained from an obese NAFLD student

Severe macrovesicular steatosis, hepatocyte ballooning, and pericellular/perivenular fibrosis were evident. The NAFLD activity score was 7. **(A)** Azan-Mallory staining (original magnification, x140);**(B)** Hematoxylin and eosin staining (original magnification, x300).
73x89mm (600 x 600 DPI)