

A cut-off value of shear wave speed to distinguish nonalcoholic steatohepatitis candidates

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Abstract

To distinguish and characterize nonalcoholic steatohepatitis (NASH) candidates from among medical checkup visitor diagnosed with nonalcoholic fatty liver diseases (mcNAFLDs).

A cut-off value has not been established to differentiate NASH at the earliest stage in NAFLD.

Shear wave speed (SWS) was measured in the livers of 480 mcNAFLDs. NASH candidates were screened out by adopting a statistically defined cut-off value of SWS and were characterized in terms of food preference.

SWS ranged between 1.11 and 2.18 m/s and fit a Gaussian distribution ($r^2 = 0.98$) with an average and SD of 1.324 and 0.0847 m/s, respectively, in 320/160 males/females 64.4 (interquartile range 57.3–69.4) years old. The average plus SD (1.41 m/s) screened out 82 (17.1%) NASH candidates, who were significantly older (66.8 vs. 64.1 years old, $P = 0.001$) and had higher fibrosis 4 index values (1.58 vs. 1.33, $P < 0.0001$) than the remaining mcNAFLDs. The number of patients with a BMI greater than 25 kg/m² was 118 (29.6%) mcNAFLDs and 34 (41.5%) NASH candidates, with a significantly higher frequency in NASH candidates ($P = 0.05$). Obese patients preferentially ate fatty acids in general, while NASH candidates preferred to consume several long-chain unsaturated fatty acids irrespective of their BMI.

These results suggest that NASH candidates who have a longer disease duration and pathological progression can be distinguished from mcNAFLDs by a statistically defined cut-off value of SWS. The defined value indicates that there are different food habitats associated with obesity and NAFLD progression.

Abbreviations: ALP = alkaline phosphatase, ALT = alanine aminotransferase, AST = aspartate aminotransferase, CVR = robust coefficient of variation, FIB4 = fibrosis 4, mcNAFLD = medical checkup visitors diagnosed with nonalcoholic fatty liver diseases, NAFLD = nonalcoholic fatty liver diseases, NASH = nonalcoholic steatohepatitis, Plt = platelet counts, ROI = region-of-interest, SWE = shear wave elastography, SWS = shear wave speed, T2DM = type 2 diabetes mellitus, VTQ = virtual touch quantification.

Keywords: fibrosis stage 1, liver stiffness, long-chain unsaturated fatty acids, medical checkup

1. Introduction

Nonalcoholic fatty liver diseases (NAFLD) is a collective term for fat deposition in the liver without substantial alcohol intake^[1] and is pandemic, especially in developed countries in which a

sedentary life style and high-energy diet are common.^[2] Nonalcoholic steatohepatitis (NASH) is a subgroup of NAFLD with a progressive feature characterized by necroinflammation leading to fibrotic accumulation and eventually to the development of liver cirrhosis.^[3,4] The epidemiology of NAFLD has been reported in many countries and is generally considered to have a close association with metabolic syndrome.^[5,6] By contrast, little is known about NASH in the general population because a histological evaluation is required for NASH diagnosis, which classifies the degree of liver fibrosis in 5 stages from zero, representing the least fiber deposition, to 4 for liver cirrhosis.^[7,8] Data on NASH currently come from patients who visit a hospital and undergo an invasive examination for liver biopsy, which leads to substantial population bias.^[9] Furthermore, the liver biopsy usually samples only 1/50,000 tissues from a single site of the liver, which causes significant sampling bias.^[9–11] Different types of surrogate markers for liver fibrosis show a similar value of area under the receiver-operator characteristic curve, approximately 80%, to deduce the histological fibrous stages, suggesting that the limited accuracy for estimating fibrous stages is inherent in liver biopsy itself.^[12–14] Therefore, it is difficult to obtain information at the early stages of NASH showing F1 fiber accumulation by collecting a liver specimen, especially in a less biased general population, such as voluntary medical checkup visitors.

When we evaluated the usefulness of shear wave speed (SWS) measurements in NASH diagnosis by referencing histological stages, SWS could distinguish F1 and F0 from F2 and higher

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stages with an area under the receiver-operator characteristic curve of 85% but could not distinguish F0 from F1 and higher stages.^[15] To clarify NASH epidemiology and characteristics in the general population, however, it is crucial to distinguish F1 from F0 in a less biased population. In this study, a statistical deviation was employed to set a cut-off value of SWS to differentiate NASH candidates of F1 or higher from F0 instead of referencing histological fibrous stages. The clinical characteristics of the NASH candidates were evaluated to confirm the significance of the cut-off value. This is the first report of an F1 cut-off value of SWS that was statistically defined by introducing SWS measurements in a medical checkup field. The characteristics of NASH candidates in a general population were discussed.

2. Methods

2.1. Medical checkup

Voluntary annual medical checkup visitors who were diagnosed with NAFLD by an ultrasound study that was performed a year ago and by excluding known etiologies of chronic liver diseases, such as positivity for HBsAg, anti-HCV or alcohol intake over 20 g/day, were consecutively enrolled in this study upon obtaining written informed consent. From August 2015 to January 2017, 480 medical checkup visitors diagnosed with nonalcoholic fatty liver diseases (mcNAFLDs) were subjected to the SWS measurement, blood cell counts, and blood biochemical tests measuring alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and γ -glutamyltransferase levels at our affiliated institution of Joetsu Medical Checkup Center. A patient was judged to have type 2 diabetes mellitus (T2DM) based on ongoing medication for glucose intolerance and/or a history of T2DM. This study protocol was approved by the institutional review boards of Uonuma Institute of Community Medicine, Niigata University Medical and Dental Hospital and the affiliated Joetsu Medical Checkup Center and adhered to the Declaration of Helsinki.

2.2. SWS measurement

SWS that is evoked by acoustic radiation force impulse was measured as virtual touch quantification (VTQ; Siemens Healthcare, Erlangen, Germany) or shear wave elastography (SWE; Canon Medical Systems, Yokohama, Japan) using the Aplio 500 ultrasound system version 5 from August 2015 to April 2016; thereafter, version 6 was used. SWS was measured 3 times in each segment (posterior, anterior, medial, and lateral) in a supine position with a transient breath hold at a neutral cycle after 1-night fasting followed by a 30-min or longer rest, and a median value of 12 measurements in each case was calculated as the representative value for the entire liver. A region-of-interest (ROI) was set between 1 and 5 cm beneath the liver capsule. In SWE measurement, ROI was set with a size of approximately 30 mm \times 30 mm square, and 3 measurements were achieved in each ROI by placing an acquisition circle with 2 mm in diameter after confirming a proper propagation of shear wave in “wavefront” style display. To convert the values of VTQ to SWE version 5 and the values of version 5 to version 6, SWSs were measured using 2 different modalities in the same individuals on the same day in 11 and 20 NAFLD cases, respectively, in our affiliated hospitals after obtaining written informed consent to join the study to construct a converting formula. The protocols of the conversion studies

were approved by the institutional review boards of Niigata University and Uonuma Institute of Community Medicine, Niigata University Medical and Dental Hospital. As shown in Supplementary Digital Contents 1A and 1B, <http://links.lww.com/MD/C739>, the VTQ and version 5 and versions 5 and 6 were significantly correlated each other ($P < 0.0001$, $r = 0.93$, and $P < 0.0001$, $r = 0.88$, respectively). For further analyses to infer clinicopathological features in association with SWS, the values of version 5 were converted to those of version 6 by adopting the following formula: $SWE \text{ version } 6 = 0.7943 \times SWE \text{ version } 5 + 0.2203$. When SWS values were compared between 221 and 259 mcNAFLD cases in which versions 5 and 6 were used, respectively, the values were significantly lower in version 6 than in version 5 (Supplementary Digital Content 1C, <http://links.lww.com/MD/C739>, lower panel, 1.46 [interquartile range; 1.40–1.51] vs. 1.29 [1.24–1.34], $P < 0.0001$). Furthermore, a robust coefficient of variation (CVR) was significantly smaller in 259 version 6 measurements than that in 221 version 5 measurements (Supplementary Digital Content 1C, <http://links.lww.com/MD/C739>, upper panel, 5.2 [3.8–7.0] vs. 4.4 [3.2–6], $P < 0.0001$). As shown in Supplementary Digital Content 1D, <http://links.lww.com/MD/C739>, higher CVR values were observed as the SWS value increased; the value peaked at approximately 2 m/s in version 6.

2.3. Blood tests and questionnaires of oral intake and daily activity

Blood cell counts and serum biochemistries were outsourced to a biochemical laboratory (BML, Inc., Tokyo, Japan). HBsAg and anti-HCV were detected using a chemiluminescent enzyme immunoassay. The fibrosis 4 (FIB4) index was originally developed as a noninvasive panel to stage liver disease in subjects with HIV and hepatitis C virus coinfection. It has recently been demonstrated that its performance characteristics for the diagnosis of advanced fibrosis in NAFLD are better than those of other similar panels.^[16] It relies on patient age, AST and ALT levels, and platelet counts (Plt) and is calculated as: $\text{age} \times \text{AST}/\text{Plt} \times \text{ALT}$. GAP-M is a formula developed by aiming to distinguish NASH suspects in NAFLD cases and consisting of 5 factors: γ -glutamyl transpeptidase, ALP, Plt, BMI, and presence/absence of T2DM. It was reported that area under the receiver operating characteristic curve was 86% and 84% in formula-developed and validation cohorts.^[17] GAP-M is calculated as: $\text{exponential}(F)/(1 + \text{exponential}(F))$, where F is $0.027 \times \gamma\text{-glutamyl transpeptidase} + 0.01 \times \text{ALP} - 0.251 \times \text{Plt} + 0.496 \times \text{BMI} + 2.043 \times \text{T2DM}$ (yes, 1; no, 0) – 13.064.

Energy intake and physical activity in daily life were quantified by means of a brief-type self-administered diet history questionnaire^[18] and an international physical activity questionnaire,^[19] respectively. Response sheets were converted into PDF files and automatically read to digitize the response as a number for each question using Remark Office OMR (Gravic, Inc., Malvern, PA). A digital spreadsheet consisting of the number for each response was sent to a company through a virtual private network connection for the brief-type self-administered diet history questionnaire and analyzed to calculate indices of oral intake of ingredients and nutrients, such as unsaturated long-chain fatty acids. The results were sent back through the same network connection. In terms of physical activity questionnaire, the intensity of each activity was converted to a metabolic equivalent task and then multiplied by the duration of each activity in hours to calculate “exercise.”

2.4. Statistical analyses

Numerical or categorical data were compared between NASH candidates and others using the Mann–Whitney *U* or Chi-square test. Numerical values were compared between liver segments using the Friedman test. The distribution of SWS was fit with nonlinear regression analysis by adapting a Gaussian distribution. A correlation or comparison between two different methods of SWS measurements in the same individuals was evaluated by calculating a Spearman correlation coefficient or using the Wilcoxon matched-pairs signed rank test. A formula converting the SWS values that were measured using different methods was deduced according to a least squares method. All statistical analyses were conducted with GraphPad Prism version 7.0 (GraphPad Software Inc., La Jolla, CA), and two-sided *P* values less than 0.05 were considered statistically significant.

3. Results

3.1. SWS features of NAFLD in a medical checkup population

The median SWS in 480 mcNAFLDs was 1.33 (interquartile range: 1.27–1.39) m/s and was significantly slower than the 1.63

(1.48–1.82) m/s value calculated for the 459 patients who presented with various liver diseases to our affiliated hospital (Fig. 1A, $P < 0.0001$). The average SWS of mcNAFLDs was significantly faster in males than in females (Fig. 1B; 1.34 [1.28–1.39] vs. 1.31 [1.26–1.37] m/s, $P < 0.019$) and in obese patients with a BMI of 25 kg/m² or higher than in the others (Fig. 1C; 1.34 [1.29–1.40] vs. 1.33 ± [1.26–1.39] m/s, $P = 0.018$). When the SWS was compared among the liver segments as shown in Figure 1D, the median values were significantly different between all combinations ($P < 0.0001$, $n = 402$), except for the comparison between the right anterior and posterior segments ($P > 0.99$); however, the CVR did not show a significant difference in any combination between the segments ($P = 0.11$).

3.2. SWS showed a Gaussian distribution in mcNAFLDs

Four hundred eighty median values of SWS in mcNAFLDs ranged from 1.11 m/s to 1.60 m/s except in 4 cases, which showed median values of 1.67, 1.76, 1.76, and 2.18 m/s. As shown in Figure 2A, a nonlinear regression analysis revealed that the scattering fits well on a Gaussian distribution represented by an

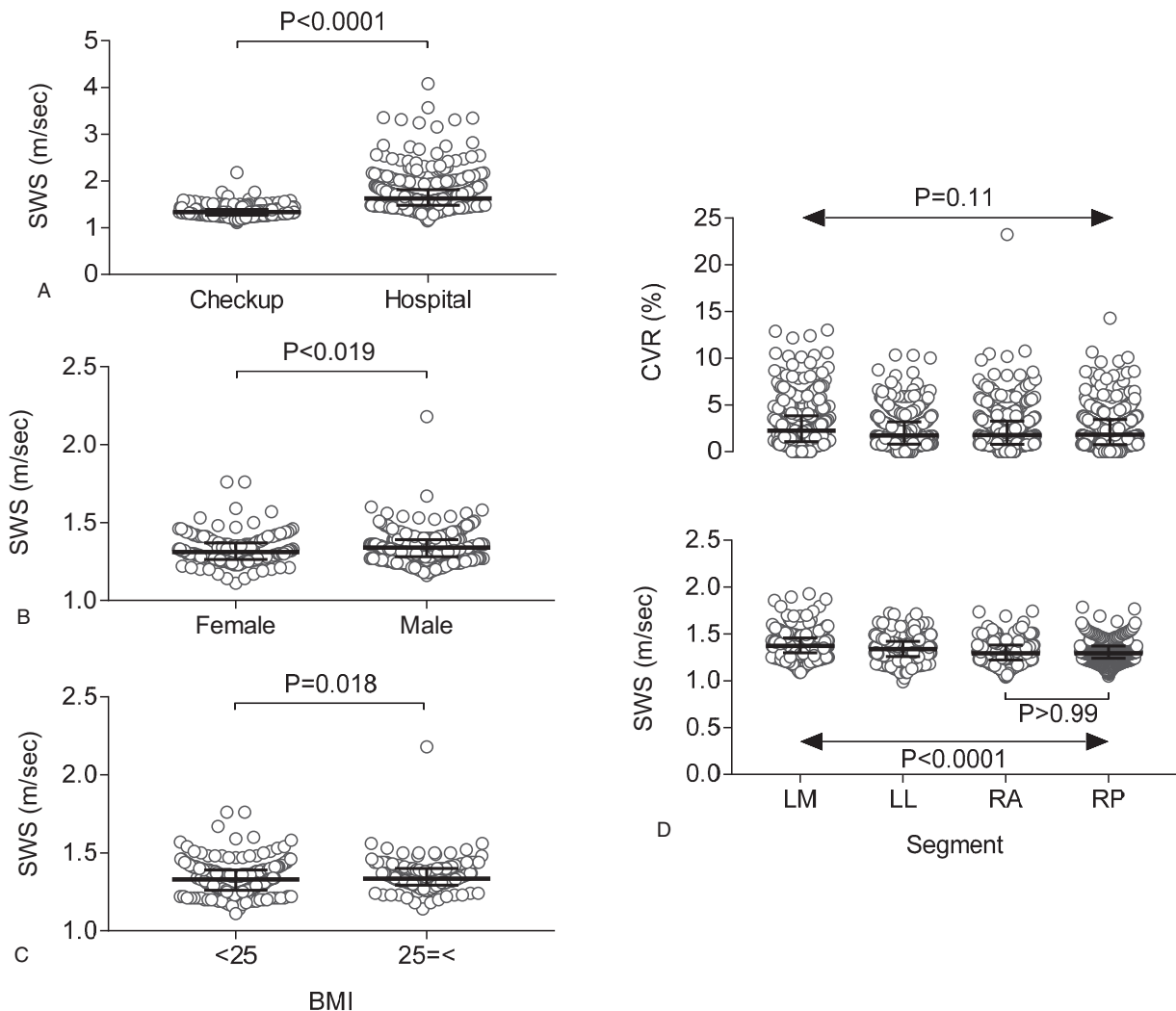


Figure 1. Characteristics of shear wave speed (SWS). The difference of SWS between (A) medical checkup visitors diagnosed with non-alcoholic fatty liver diseases (mcNAFLD) and patients visiting a hospital, (B) male and female in mcNAFLD, and (C) obese and non-obese in mcNAFLD. (D) SWS values of mcNAFLDs were 1.37 (1.30–1.46), 1.34 (1.26–1.42), 1.29 (1.22–1.38), and 1.29 (1.24–1.37) m/sec in the left medial, left lateral, right anterior, and right posterior segments, respectively.

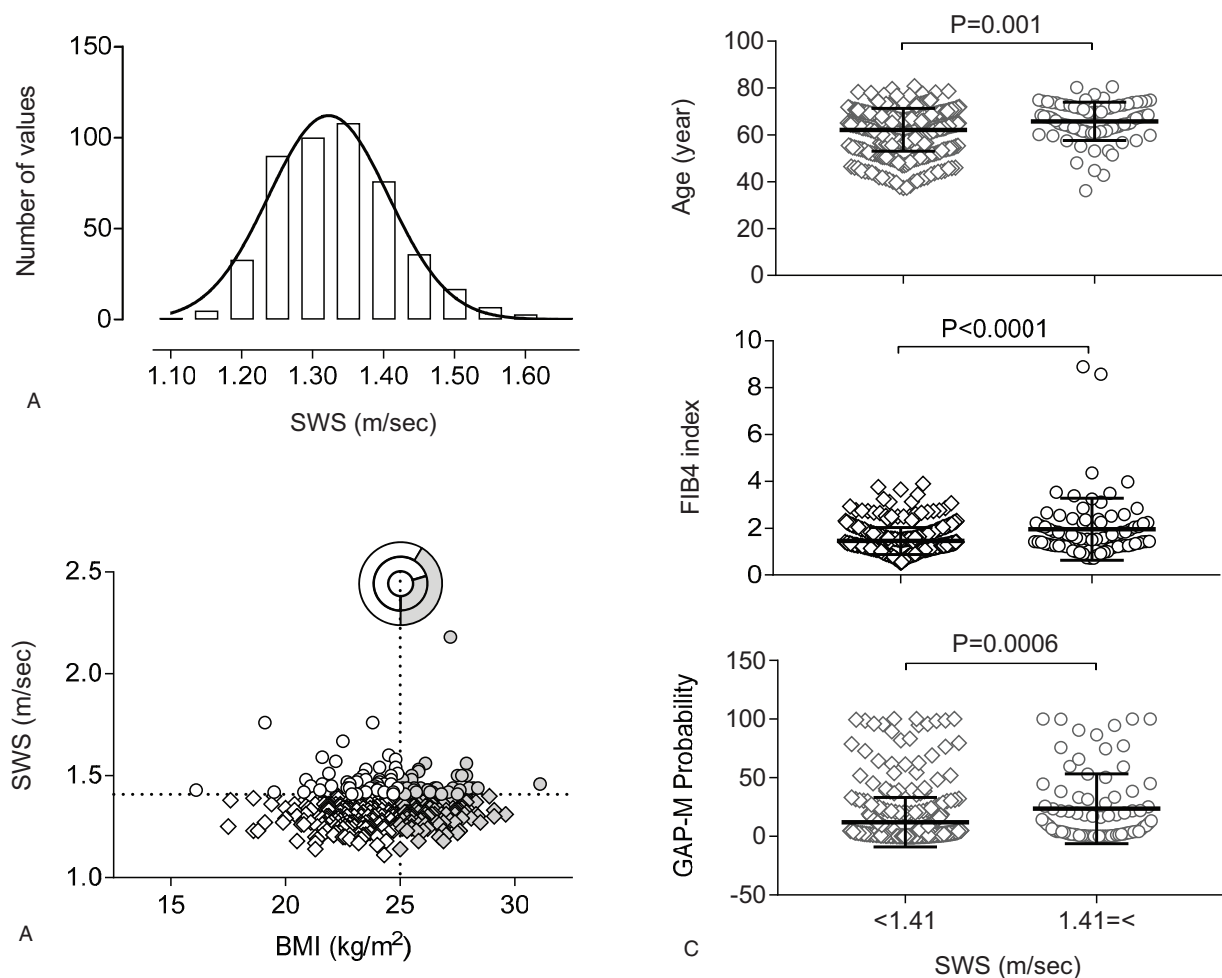


Figure 2. A cut-off value of shear wave speed (SWS) to distinguish nonalcoholic steatohepatitis candidates with mild liver fibrosis in medical checkup visitors diagnosed with non-alcoholic fatty liver diseases (mcNAFLD). The distribution of SWS in mcNAFLDs (A), and the frequencies of obese patients in mcNAFLD (gray belt of the inside doughnut graph) or in nonalcoholic steatohepatitis candidates (gray belt of the outside doughnut graph) (B). (C) Nonalcoholic steatohepatitis candidates were significantly older cases with higher values of FIB4 index and GAP-M probability.

average of 1.324 m/s with a SD of 0.0847 m/s ($r^2=0.98$). A SWS value at the average plus SD was 1.41 m/s and distinguished 82 NASH candidates (17.1%) with a faster SWS in 480 mcNAFLD cases. Consistent with those findings, the average SWS was faster in mcNAFLDs with a BMI of 25 kg/m² or higher than in other patients, and NASH candidates were more frequently observed in the obese than in the nonobese patients (Fig. 2B, 41.5% vs. 29.7%, $P=0.05$).

3.3. Faster SWS values predict a progressed disease

Patients' characteristics were summarized in Table 1. The age of mcNAFLDs with SWS values of 1.41 m/s or faster was 66.8 (61.5–72.5) years old and was significantly older than the age 64.1 (56.5–68.8) years of mcNAFLDs with SWSs slower than 1.41 m/s (Fig. 2C upper panel, $P=0.001$). The patients with faster SWS also showed a significantly higher BMI ($P=0.0099$) and higher value of AST ($P=0.0003$) than those in the other cases. Furthermore, the FIB4 index, a noninvasive fibrotic marker useful to distinguish cirrhotic and near cirrhotic cases in NAFLD, had a significantly higher value of 1.58 (1.25–2.24) for mcNAFLDs with SWSs of 1.41 m/s or faster than for mcNAFLDs

with SWSs of slower than 1.41 m/s (1.33 [1.08–1.71]) (Fig. 2C middle panel, $P<0.0001$). The GAP-M value, a useful indicator for estimating the probability of NASH in NAFLD, was 10.7% (1.9–33.2) in mcNAFLDs with SWSs of 1.41 m/s or faster and was significantly higher than the value of 3.3% (0.88–12.6) in mcNAFLDs with SWSs slower than 1.41 m/s (Fig. 2C lower panel, $P=0.0006$).

3.4. Characteristics of mcNAFLDs with a faster SWS value with respect to daily life activities

Next, energy intake and activity in daily life were compared between mcNAFLDs with SWSs of 1.41 m/s or faster and others. The daily energy intake of mcNAFLDs with faster SWSs was 1814 (1455–2337) kcal and was not significantly different from the 1833 (1527–2226) kcal measured in mcNAFLDs with slower SWSs (Fig. 3AA left panel, $P=0.90$). In the same manner, daily activities, which were calculated as exercise, of mcNAFLDs with faster and slower SWSs were 9.6 (4.0–15.1) and 7.4 (2.7–20.9) and were not significantly different (Fig. 3A right panel, $P=0.62$). Food preference was observed in association with obesity or SWS exacerbation. The amount eaten per day of Western sweets was

Table 1
Patients' characteristics.

Items	All (n = 480)		A. SWS < 1.41 (n = 398)		B. 1.41 = < SWS (n = 82)		A. vs. B. Provability
	Median	IQR	Median	IQR	Median	IQR	
Age (years)	64.4	(57.3–69.4)	64.1	(56.5–68.8)	66.8	(61.5–72.5)	0.0010
BMI (kg/m ²)	24.0	(22.7–25.5)	23.8	(22.6–25.4)	24.6	(23.2–26.1)	0.0099
BEE (kcal)	1334	(1199–1457)	1336	(1201–1457)	1320	(1185–1458)	0.82
AST (IU/L)	25	(21–31)	24	(21–29)	28.5	(22–35)	0.0003
ALT (IU/L)	25	(19–36)	24	(19–35)	28	(20–43)	0.051
ALP (IU/L)	207	(171–245)	207	(170–244)	208.5	(177–258)	0.40
γ-GTP (IU/L)	32	(22–59)	31	(22–56)	37.5	(24–72)	0.097
Plt (/mm ³)	22.5 × 10 ⁴	(19.6–26.2)	22.6 × 10 ⁴	(19.7–26.4)	22.0 × 10 ⁴	(17.7–25.7)	0.12
Intake (kcal)	1832	(1523–2232)	1833	(1527–2226)	1814	(1455–2337)	0.90
Ex (Mets*hours)	7.97	(2.95–20.38)	7.365	(2.71–20.9)	9.615	(3.97–15.12)	0.61
SWS (m/s)	1.33	(1.27–1.39)	1.31	(1.26–1.36)	1.45	(1.43–1.50)	<0.0001
CVR (%)	4.4	(3.2–6.0)	4.4	(3.2–6.0)	4.3	(3.1–5.6)	0.37
GAP-M (%)	3.9	(1.0–15.3)	3.3	(0.88–12.6)	10.7	(1.9–33.2)	0.0006
FIB4	1.36	(1.10–1.82)	1.33	(1.08–1.71)	1.58	(1.25–2.24)	<0.0001
	Ratio		Ratio		Ratio		
Gender	(male:female) 320:160		(male:female) 264:134		(male:female) 56:26		0.80
T2DM	(No:yes) 401:79		(No:yes) 335:63		(No:yes) 65:17		0.33

ALP = alkaline phosphatase, ALT = alanine aminotransferase, AST = aspartate aminotransferase, BEE = basal energy expenditure, CVR = robust coefficient of variation, Ex = exercise, FIB4 = fibrosis-4 index, GTP = glutamyl transpeptidase, intake = oral intake, IQR = interquartile range, Plt = platelet count, SWS = shear wave speed, T2DM = type 2 diabetes mellitus.

significantly different among 4 groups characterized by stiff liver ($1.41 \text{ m/s} \leq \text{SWS}$) with or without obesity ($\text{BMI} < 25 \text{ kg/m}^2$ [lean] or $25 \text{ kg/m}^2 \leq \text{BMI}$ [obese], respectively) and soft liver ($\text{SWS} < 1.41 \text{ m/s}$) with or without obesity ($P = 0.012$) and was higher in the patients with obesity irrespective of the stiffness, as shown in Figure 3B (left panel). Additionally, the amount of potato consumed per day was significantly different among the 4 groups ($P = 0.033$) and was lower in NASH candidates irrespective of BMI, as shown in Figure 3B (right panel). Consistently, different nutrients were preferentially consumed in the obese patients and NASH candidates (Supplementary Digital Contents 2 and 3, <http://links.lww.com/MD/C739>). Significantly different amounts were eaten among the 4 groups in terms of 22 out of 95 nutrients. Most of them were fatty acids such as dodecanoic acid (Fig. 3C left panel, $P = 0.0078$), and a significant difference was only seen between the lean and obese cases, but not between the cases with soft or stiff liver. In the rest of 73 nutrients, 12 nutrients were preferentially eaten by the obese cases without differences in the amounts that were consumed between the cases with soft or stiff liver. All of them were fatty acids. On the other hand, a significant difference of consumption between the cases with soft or stiff liver was irrespectively seen of BMI only for 4 types of long-chain unsaturated fatty acids; C24M, C184n3, C205n3, and C204n3 (Fig. 3C right panel, $P = 0.012$).

4. Discussion

Hepatocellular carcinoma is a rare cancer for which the risk factors are clearly known, and a methodology has been established to detect this cancer at an early stage.^[20] With this advantage, periodic examination using imaging modalities is useful for identifying hepatocellular carcinoma in high-risk cases. Such cases show substantial fiber accumulation in the liver regardless of the etiology of the chronic liver disorder.^[20] In general, a histological evaluation is employed to classify the liver fibrosis into 1 of 5 stages, from F0 for normal to F4 for cirrhosis.^[7] However, it is impractical to conduct invasive liver biopsies in a large population of NAFLD patients;^[21] the condition is pandemic and is especially prevalent in the developed countries.^[2] Recently, a noninvasive technology was

introduced in the clinic to quantify liver stiffness, which is correlated with the degree of liver fibrosis. SWS, a physical property of elastic materials associated with stiffness, of the liver is a useful surrogate marker for the fibrous stages of the liver.^[13,15,22] We have shown that SWS measurement is useful to distinguish the cases in which the liver is highly potentiated to develop hepatocellular carcinoma.^[23] Unfortunately, however, these findings come from data based on observations in hospitals. As shown in Figure 1A, the distribution of SWS values is tremendously different between a medical checkup field and a hospital. It is a matter of concern, in terms of NAFLD management, in a medical checkup field to distinguish a relatively small number of NASH cases from a large nonalcoholic fatty liver population.

Although the hallmark of NASH is the presence of active inflammation in the liver,^[24] chronic necroinflammation leads to fiber accumulation irrespective of etiology. Any noninvasive marker that distinguishes F1 from F0 can be a step toward establishing a social system allowing effective NAFLD management. Given the heterogeneous fiber distribution as suggested by Supplementary Digital Content 1D, <http://links.lww.com/MD/C739> and the sampling bias inherent in liver biopsy, it seems difficult to establish a cut-off value to differentiate F1 from F0 by referencing a small needle biopsy specimen taken from a single site. In this report, a cut-off value for the F0–F1 boundary was statistically defined according to a Gaussian distribution of SWS in a medical checkup cohort. Out of 480 mcNAFLD cases, the cut-off value of 1.41 m/s distinguished 82 patients (17.1%), who were significantly older and had higher FIB4 index values and GAP-M probability, suggesting a longer disease duration and higher fiber accumulation. The association between the number of mcNAFLDs with SWSs over the cut-off value and obesity further supports the significance of the value. The results of this study are, however, based on a small number of cases. A limited number of cases may have contributed to an inadequate assessment of the biological variability. Because this is the first report of SWS measurements for a limited number of medical checkup visitors, further study must be conducted to define the cut-off value of the F0–F1 boundary *via* SWS measurements not for hospital patients but for the general population. The clinical

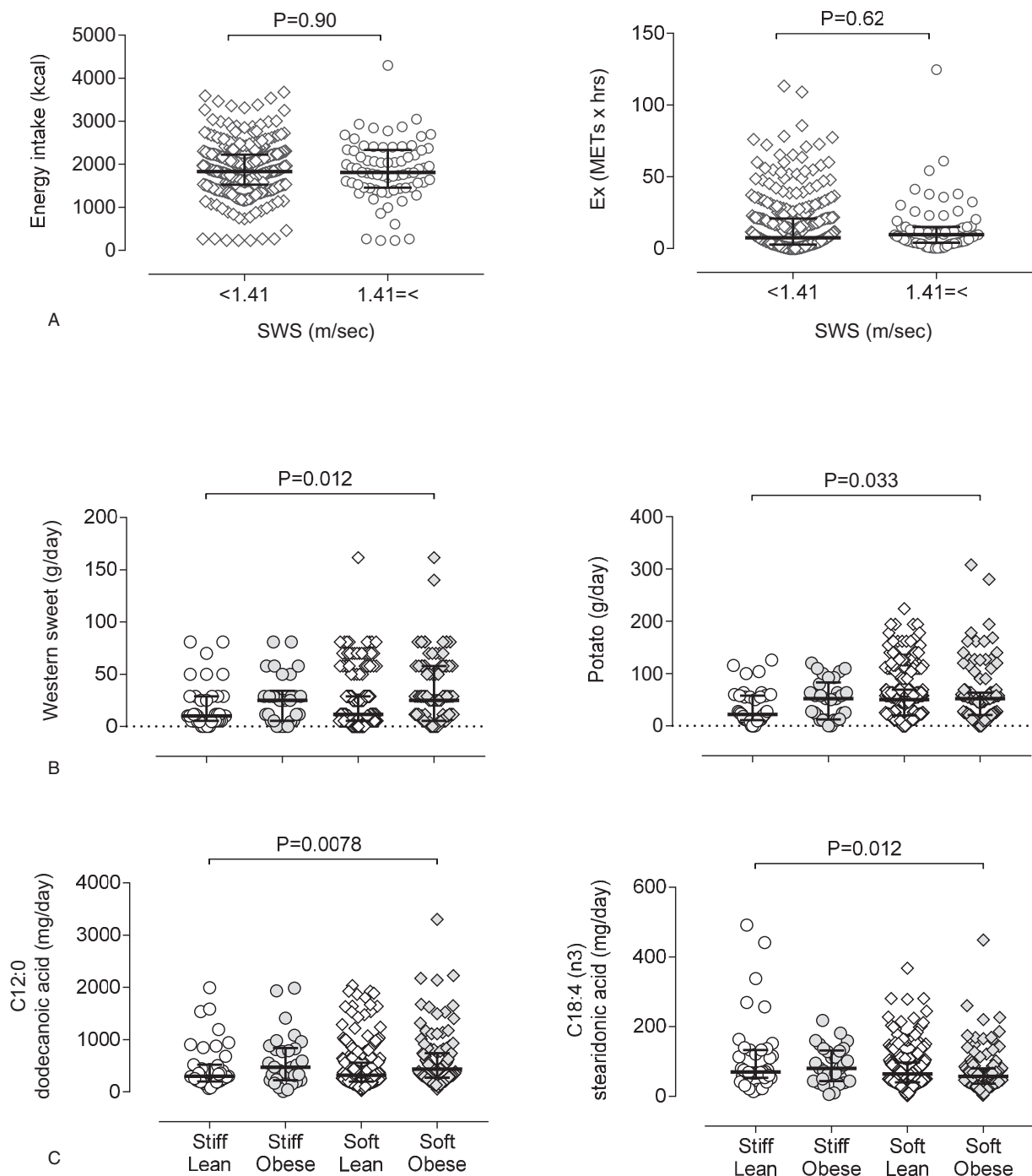


Figure 3. Characteristics of nonalcoholic steatohepatitis (NASH) candidates with a shear wave speed (SWS) of 1.41 m/sec or faster in daily life. Energy intake and daily activity (A), food preference (B) and favorable nutrients (C) were compared among 4 groups characterized by stiff ($1.41 \text{ m/sec} \leq \text{SWS}$, circles) or soft liver (diamonds) with ($25 \text{ kg/m}^2 \leq \text{body mass index (BMI)}$), gray) or without (open) obesity.

relevance to define the cut-off value should be reconfirmed by comparing the prognosis between cases who were distinguished by the value.

In terms of the technical issues associated with SWS measurements, the range of the values in the individual liver raises the question of how many measurements should be taken and from where in each case. Currently, SWS are recommended to be measured in the right lobe and calculated as a mean or median value surrogating a fibrous stage of the entire liver.^[25–27] It was

reported that 3 measurements are sufficient to calculate reliable value by placing 15 mm or larger acquisition circle in a ROI using supersonic shear imaging.^[28] In these reports, the reliability and accuracy of SWS were evaluated by referencing histological findings in liver biopsy specimen, which was obtained from the right lobe, or by referencing the liver stiffness, which was solely measured in the right lobe using transient elastography. Because it is well known that pathological progression heterogeneously takes place in the liver, it is reasonable to assume that SWS

revealed the higher correlation coefficient in the right lobe than in the left lobe if the referencing value is obtained from the right lobe. In addition, a larger acquisition circle must be effective to compensate variability of SWS and to reduce measurements that are required to calculate a statistically reasonable mean or median value. In other words, a larger acquisition circle diminishes the possibility to evaluate the heterogeneity itself through pathophysiological progression. Because repetitive histological evaluations at multiple sites are practically unacceptable, SWS measurements are the unique technology that enables hepatologists to repeatedly evaluate pathological alteration at multiple sites over the liver. Therefore, we planned to employ a small acquisition circle and measured SWS at 12 sites in both lobes. As shown in Figure 1C, the dispersion was not significantly different between the right and left lobes, although SWS was significantly higher in the left as previously reported, suggesting that the higher SWS in the left lobe may not be simply due to the larger dispersion. Given the noninvasive nature of SWS measurements, they should be taken in both lobes by employing a small acquisition circle to clarify the pathophysiological differences among the segments, as suggested by a diverse progression/alleviation process based on the stream line theory.^[19,29]

There are several instruments available for SWS measurements from different companies and in different versions, and it is not recommended to convert SWS values measured by different technologies. A conversion incapability is, however, unacceptable drawback to the society for the study of liver diseases. On the other hand, it was reported that no statistically significant differences were found in SWS estimates among operators using the same or equivalent systems under the same conditions.^[30,31] Actually, this study clearly revealed the high correlation coefficient between the values obtained using machines from different companies or different versions (Supplementary Digital Contents 1a and 1b, <http://links.lww.com/MD/C739>), although the absolute values are significantly different among measurements (Supplementary Digital Contents 1a and 1c, <http://links.lww.com/MD/C739>). Therefore, it should be practically acceptable to convert SWS estimates between different technologies as long as the measurements are reliably performed in each measurement. In this regard, it is demanded to establish a standard SWS measuring condition and to enforce a regulation that would standardize SWS values to ensure the conversion and enable the implication of liver pathogenesis. It is challenging to regularly verify a unified specification of SWS measurements from both industrial and practical perspectives.

This study revealed that energy intake and daily activity were not significantly different between the candidates with simple steatosis and mild fibrosis in a medical checkup cohort. However, significant differences were found in dietary preference. NASH candidates preferentially ate several long-chain unsaturated fatty acids while avoiding potato. In terms of fatty acids, the results that have been reported seem to be controversial.^[32–34] A rice-fish model that is overfed to reproduce NASH pathology showed fewer long-chain polyunsaturated fatty acids in the liver.^[35] Similarly, a mouse model of NASH revealed the depletion of long-chain polyunsaturated fatty acids in the liver.^[36] However, the results in model animals were for the content in the liver and not for the amount of intake. Furthermore, in animal models, these analyses were performed at the end stage of the disorder, while NASH candidates in our cohort were estimated to be at the beginning of the clinical course. It is possible that the events causing this pathology are dispersed at the end stage of the disease as the level of fat in the liver is gradually reduced with the

development of cirrhosis in NASH. It is not possible to clarify the characteristic features governing NASH onset and progression without identification of NASH at an early stage. SWS measurements at a medical checkup center are a useful strategy to elucidate the pathogenesis of NASH by identifying NASH candidates at an early stage. Because the food and nutrients preferentially taken were deduced in this study based on the questionnaire, which may not reliably reveal actual intake, the impact of nutritional variation on liver fibrosis should be farther evaluated by quantifying the blood concentration of various nutrients, especially fatty acids.

5. Conclusion

Earlier diagnosis makes disease management easier in NAFLD patients to prevent disease progression. This study strongly suggests that SWS measurements in a general population can define a cut-off value and distinguish NASH candidates in the early stages of disease. Analyses of NASH candidates will help us understand NASH pathogenesis. However, it is challenging to ensure consistent results due to the variety of methodologies used for SWS measurements.

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