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Measurement of the coronary flow velocity reserve in patients with non-alcoholic fatty liver disease

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Background/aims: *Endothelial dysfunction is an early and reversible feature in the pathogenesis of atherosclerosis. Coronary flow velocity reserve is a noninvasive test showing endothelial function of epicardial coronary arteries and coronary microcirculatory function. This study was designed to evaluate the carotid intima-media thickness and myocardial microvascular circulation in patients with non-alcoholic fatty liver disease.* ***Materials and Methods:*** *Twenty-four patients with non-alcoholic fatty liver disease and 28 healthy subjects were studied. According to the pathology of liver biopsies, patients with non-alcoholic fatty liver disease were divided into non-alcoholic fatty liver and non-alcoholic steatohepatitis groups. Coronary diastolic peak flow velocities were measured at baseline, and then dipyridamole infusion was measured by transthoracic Doppler echocardiography. The ratio of hyperemic to baseline diastolic peak velocities was calculated and the intima-media thicknesss of the carotid arteries were measured.* ***Results:*** *Baseline average diastolic peak and diastolic mean flow velocities were similar between non-alcoholic fatty liver disease patients and healthy subjects. However, hyperemic average diastolic peak and diastolic mean flow velocities were significantly lower in the patient groups compared to those in the controls (p=0.005 and p=0.002). Coronary flow velocity reserve was 1.65±0.36 and 2.67±0.81 in patients and healthy subjects, respectively (p<0.001). The intima-media thickness was similar between the patients with non-alcoholic fatty liver disease and healthy subjects. The comparison of patients with non-alcoholic fatty liver and non-alcoholic steatohepatitis within the non-alcoholic fatty liver disease group with respect to coronary flow velocity reserve and intima-media thickness yielded no statistical differences.* ***Conclusions:*** *The present study showed that coronary flow velocity reserve, which establishes coronary microvascular and endothelial functions noninvasively, is significantly impaired in patients with non-alcoholic fatty liver disease. The impaired coronary flow velocity reserve-like early atherosclerotic changes may have value in the prediction of coronary artery disease in patients with non-alcoholic fatty liver disease.*

Key words: Endothelial dysfunction, coronary flow velocity reserve, metabolic syndrome, transthoracic Doppler ultrasonography, intima-media thickness, non-alcoholic fatty liver disease

Alkol dışı yağlı karaciğer hastalarında koroner akım hızı rezervinin ölçümü

Giriş ve Amaç: *Endotel disfonksiyonu; ateroskleroz patogenezinde erken ve geri dönüşümlü bir aşamadır. Koroner akım hızı rezervi, epikardiyal koroner arterlerin endotel fonksiyonlarını ve koroner mikrosirkülatuvar dolaşım fonksiyonlarını gösteren noninvaziv bir testtir. Bu çalışma, alkol dışı yağlı karaciğer hastalarında miyokardial mikrovasküler dolaşımı ve karotis arter intima-media kalınlığını değerlendirmek amacıyla düzenlendi.* ***Gereç ve Yöntem:*** *Alkol dışı yağlı karaciğer hastalığı olan 24 hasta ile sağlıklı kontrol 28 gönüllü çalışmaya alındı. Hastalar karaciğer biyopsi bulgularına göre, alkol dışı yağlı karaciğer ve alkol dışı steatohepatit hastaları olmak üzere iki gruba ayrıldı. Koroner diastolik pik akım hızı, bazal ve dipiridamol infüzyonu sonrası (hiperemik) transtorasik Doppler ultrasonografi ile ölçüldü. Hiperemik diastolik pik akım hızının bazale oranı hesaplandı ve karotis arterlerin intima-media kalınlığı ölçüldü.* ***Bulgular:*** *Bazal ortalama diastolik zirve ve diastolik ortalama akım hızları alkol dışı yağlı karaciğer hastalığı olan bireylerde, sağlıklı kontrol grubu ile benzer bulundu. Ancak, hiperemik ortalama diastolik pik ve diastolik ortalama akım hızları karşılaştırıldığında, alkol dışı yağlı karaciğer hastaları grubunda sağlıklı kontrollerden daha düşük bulundu (sırasıyla p=0.005 ve p=0.002). Koroner akım hızı rezervi hastalar ve sağlıklı kontrollerde sırasıyla 1.65±0,36 ve 2.67±0.81 (p<0.001) saptandı. Koroner arter intima-media kalınlıkları ise, her iki grupta benzerdi. Alkol dışı yağlı karaciğer hastaları kendi içlerinde alkol dışı yağlı karaciğer ve alkol dışı steatohepatit olarak karşılaştırıldığında, koroner akım hızı rezervi ve karotis intima-media kalınlıkları açısından faklılıkları olmadığı görüldü.* ***Sonuç:*** *Çalışmamız alkol dışı yağlı karaciğer hastalığında önemli ölçüde bozulmuş olan koroner mikrovasküler dolaşım ve endotel disfonksiyonunu noninvaziv olarak koroner akım hızı rezervi ölçümü yoluyla gösteren ilk çalışmadır. Bozulmuş koroner akım hızı rezervi, erken evre ateroskleroz bulgusu olup, alkol dışı yağlı karaciğer hastalığında koroner hastalığının öngörülmesinde önemli bir bulgu olabilir.*

Anahtar kelimeler: Endotel disfonksiyonu, koroner akım hızı rezervi, metabolic sendrom, transtorasik Doppler ultrasonografi, intima-media kalınlığı, alkol dışı yağlı karaciğer hastalığı

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INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) describes a wide clinicopathological spectrum ranging from simple steatosis (NAFL) and non-alcoholic steatohepatitis (NASH) to fibrosis and cirrhosis (1-3). This entity was first described by Ludwig in obese, non-diabetic patients who did not use alcohol (4). NAFLD is considered to be one of the components of the metabolic syndrome (MS) associated with mainly insulin resistance (IR) and obesity (4). In fact, NAFLD is considered a hepatic manifestation of MS, which is a well-known precursor of cardiovascular disease (5,6), and thus people with MS carry the risk of coronary heart disease and stroke (7).

The importance of NAFLD and its relationship with MS is now increasingly recognized, as the recent data suggest that NAFLD is linked to increased cardiovascular risk independent of the broad spectrum of risk factors of MS (8-11). Cardiovascular mortality risk was doubled in 129 patients with NAFLD compared with that of the reference population in a study with a mean follow-up of nearly 14 years (12).

The arterial endothelium is a target for the atherosclerotic process. Atherosclerosis is associated with endothelial dysfunction (ED) in the very early stages of the disease process (13,14). The ultrasound examination of the brachial artery is a noninvasive method for the determination of ED. This method has been used commonly in several recent studies. These studies for ED, which are regarded as the precursor for accelerated atherosclerosis, are all based on detection of endothelium-dependent and/or -independent vasodilatation of the brachial and femoral arteries (10,15). The same studies demonstrating increased carotid intima-media thickness (IMT) also support the presence of advanced atherosclerosis in patients with NAFLD (16).

Transthoracic Doppler echocardiography (TTDE) is a cheap, noninvasive and repeatable method to evaluate the structural and functional status of the epicardial coronary arteries (ECA) (17,18). In the past few years, TTDE has attracted attention as a tool for accurate determination of reduced coronary flow velocity reserve (CFVR), the indicator for microvascular hypoperfusion. CFVR is defined as a ratio between the maximal (stimulated) and the baseline (resting) coronary blood flow, and it is important for the understanding of the pathophysiology of coronary circulation. The reduction in CFVR depends not only on severity of stenosis of the ECA, but also on a number of microvascular factors (19,20). Thus, decreased CFVR enables the detection of impaired microvascular vasodilatation in left ventricular hypertrophy, diabetes mellitus, hypercholesterolemia, smoking habit, and MS (19,20). In previous studies, TTDE has been used to show coronary microvascular insufficiency in coronary heart disease, type 2 diabetes mellitus and hemodialysis patients (17,21,22). To date, there have been no studies reporting the detection of coronary microvascular circulation by TTDE in patients with NAFLD. We aimed to investigate the presence of ED in the ECA by TTDE and ultrasound carotid artery IMT in patients with NAFL and NASH, two entities in the clinical spectrum of NAFLD.

MATERIALS AND METHODS

Patients

Twenty-four patients who were referred to the Gastroenterohepatology Department of Internal Medicine and diagnosed as having NAFLD upon liver biopsies were enrolled in this study. All the patients had high levels of aminotransferases for at least six months without any underlying etiologic factor (viral, autoimmune, metabolic etc.), and none had a history of alcohol consumption. According to the pathology of liver biopsies, patients were divided in NAFL (n=12, 5 males; mean age 48.4±7.6 years) or NASH (n=12, 6 males; mean age 46.5±11 years) groups. Histological findings were evaluated by a single pathologist according to the Clinical NASH Research Network's Pathology Committee Scoring System (23). This scoring system designates a NAFLD activity score (NAS) of 0-2 as not diagnostic of steatohepatitis and a NAS ≥5 as diagnosis of steatohepatitis. NAS of 3 and 4 are divided almost evenly among the three diagnostic categories (not NASH, borderline, and NASH). In this study, 12 patients with NAS ≥3 were considered as having NASH, while 12 patients with NAS ≤2 were considered as having NAFL. The control subjects were recruited from among hospital staff and friends. The control subjects were age- and sex-matched and all were nonsmokers, normotensive and nondiabetic with no other discernible risk factor for the development of impaired CFVR; 28 healthy subjects (HS) (17 males; mean age 43.1±7 years) were enrolled in the study. Patients with established cardiovascular disease, overt clinical evidence of atherosclerotic

cardiovascular disease, other chronic diseases that could accelerate atherosclerosis such as chronic renal failure and uncontrolled diabetes mellitus and hypertension, or severe disorders such as cancer were excluded from the study. Venous blood samples for biochemical analyses were drawn after an overnight fast between 8:00 pm and 8:00 am. All biochemical analyses including glucose, creatinine, total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol and plasma triglyceride concentrations, and serum aminotransferases levels were performed by an oxidase-based technique on Roche/Hitachi Modular System (Japan) in the Central Biochemistry Laboratory. IR was determined by homeostasis model assessment (HOMA) (fasting insulin [μU/ml]×fasting glucose [mmol/L]/22.5) (24). Patients with HOMA values >1.64 were considered insulin-resistant. Ethical approval for the study was obtained from the Institutional Ethical Committee, and each subject was informed and provided a written consent to participate in the study. The study was performed according to the Declaration of Helsinki.

Echocardiographic Examinations

Echocardiographic examination was performed using a Vivid 7 echocardiography device (General Electric, USA) using 2.5-MHz transducers. M-Mode and two-dimensional measurements were performed in accordance with methods recommended by the American Society of Echocardiography (25,26).

Carotid IMT Measurements

The carotid arteries were evaluated with the Vivid 7 echocardiography device (General Electric, USA) using a 10 MHz linear probe. The acquired images were recorded for playback analysis and were later measured off-line. The common carotid artery, the carotid bulb, and internal and external carotid arteries were visualized on both sides. The IMTs of the carotid arteries were measured in the distal common carotid artery at a level of 15-20 mm proximal to the carotid bulb. The two bright echogenic lines in the arterial wall were identified as the intima and the media. Three measurements were made for each side of the body; separate means were calculated and recorded as the right and left IMT. None of the patients had stenosis, atheroma plaque or local thickening in excess of 2 mm in the carotid arteries. The intra-observer coefficient of variation for carotid IMT was 2.5%.

Coronary Flow Velocity Measurements

The coronary flow velocity recordings were performed by a single investigator (H.O.) who was blinded to the two groups. CFVR recordings were performed with the Vivid 7 echocardiography device (General Electric, USA) using a middle range frequency (3-8 MHz) broadband transducer.

Coronary flow velocity reserve (CFVR) recordings were performed in the left anterior descending coronary artery (LAD) by TTDE, as described previously (27). The acoustic window was around the midclavicular line in the fourth and fifth intercostal spaces in the left lateral decubitus position. The left ventricle was imaged in the long-axis cross-section and the ultrasound beam was inclined laterally. The coronary blood in the mid-to-distal LAD was searched by color Doppler flow mapping guidance with the optimal velocity range (+12 - +15 cm/sec). Then, the sample volume (1.5 or 2.0 mm wide) was positioned on the color signal in the LAD artery. Variables of LAD artery velocity were measured using fast Fourier transformation analysis. After baseline recordings of flows, dipyridamole (Persantin, Boehringer Ingelheim, 0.56 mg/kg) was infused over a 4-minute (min) period. An additional infusion of dipyridamole (0.28 mg/kg over a 2-min period) was used providing that the heart rate did not exceed a 10% increase from the baseline. Two minutes after the end of the infusion, hyperemic spectral profiles in the LAD artery were recorded. All images were recorded for playback analysis and were later measured off-line. Average peak diastolic velocity (APDV) and average mean diastolic velocity (AMDV) were measured at baseline and under hyperemic conditions. CFVR was defined as the ratio of APDV at hyperemia: APDV at baseline. The intra-observer variability of CFVR measurement was 3.4% in the current study.

All of the measurements were performed between 8:00 am and 9:00 am, and all of the subjects had abstained from caffeinated drinks for at least 12 hours (h) before the tests.

Statistical Analyses

Data were processed on a personal computer and analyzed using NCSS-PASS-2000 software (Kaysville, Utah, USA). Comparisons of patients and control subjects were made with unpaired t tests or the Mann-Whitney U test, and statistical analysis of each variable across all three groups (NASH, NAFL and HS) was conducted using one-way analysis of variance (ANOVA) with $\alpha = 0.05$. Mul-

tiple comparisons were carried out by Tukey test. Data are expressed as mean ± SE, with significance level of $p<0.05$.

RESULTS

Demographic, vital signs and biochemical data are shown in Table 1. Age, body mass index (BMI), hemoglobin level, serum LDL cholesterol, and triglyceride concentrations showed no difference between the control group and NAFLD patients. While the mean fasting glucose and total cholesterol levels were significantly higher in comparison to the control group (79.8±8.2 vs. 110.4±46, p=0.006 and 203.5±28 vs. 178.1±19.1, p<0.001), serum HDL-cholesterol levels were lower in the NAFLD group (44±13 vs. 45.6±7.2, p=0.04). Among the NAFLD group itself, all demographic and metabolic parameters including aminotransferases levels were similar except the HOMA-IR, which was significantly higher in the patients with NASH than NAFL (p=0.01).

Intima-media thickness (IMT) and CFVR measurements are presented in Table 2. No significant differences were observed between patient and control groups with respect to IMT (0.66±0.15 vs. 0.74±0.25 and 0.75±0.23). While ADPV-basal measurements in the NAFL and NASH groups were similar to those in controls (28.75±9.4 cm/second (sn) vs. 30.0±10.1 and 29.1±7.5 cm/sn, non-significant), ADPV-hyperemia in the NAFL and NASH groups were significantly lower compared to controls (52.0±18.3 and 48.4±18.4 cm/sn vs. 75.5±30.2 cm/sn, p=0.005). AMDV-baseline measurements of patients were not statistically different when compared with controls (controls vs. NAFL and NASH: AMDV-baseline (cm/sn): 23.6±7.9 vs. 24.75±9.4 and 22.9±6.0). However, AMDV-hyperemia measurements of patients were significantly lower than those in controls (controls vs. NAFL and NASH: AMDV-hyperemia (cm/sn): 56.9±22 vs. 35.0±10.1 and 39.1±14.2, p=0.002). CFVR values were significantly lower in NAFL and NASH groups than the

Table 1. Clinical characteristics and laboratory findings of patients with NAFLD and HS.

	NAFL Patients (n=12)	NASH Patients (n=12)	NAFLD Patients (n=24)	Healthy Subjects (HS) (n=28)	*P* values
Age (years)	46.0±8.3 (33-65)	46.5±11.3 (20-60)	46.2±9.7 (20-65)	43.1±7.0 (30-57)	#♦Φξ> 0.005
Height (cm)	163.8±10.3 (140-178)	164.7±12.1 (150-192)	164.3±11 (140-192)	166.7±7.9 (154-189)	#♦Φξ> 0.005
Weight (kg)	72.5±12.3 (49-90)	78.4±16.7 (58-115)	75.5±14.7 (49-115)	74.0±11.8 (54-96)	#♦Φξ> 0.005
Body mass index (kg/mg^2)	27.1±3.4 (24-36.1)	28.6±5.0 (21.3-41)	28.0±4.3 (21.3-41)	26.5±3.5 (18.1-33.1)	#♦Φξ> 0.005
Hemoglobin (gr/dl)	14.0±0.8 (13.2-15.6)	14.3±1.0 (12.7-16.3)	14.2±0.98 (12.7-16.3)	13.7±1.6 (11.5-16.3)	#♦Φξ> 0.005
Glucose (mg/dl)	112±65 (79-284)	108.8±27.4 (62-146)	110±46.0 (62-284)	79.8±8.2 (67-89)	**ξ= 0.006**
Total Cholesterol (mg/dl)	215.4±20.2 (117-250)	194.6±30.4 (156-247)	203.5±28.0 (156-250)	178.1±19.1 (149-217)	**ξ<0.0001**
HDL-Cholesterol (mg/dl)	48.3±14.7 (31-70)	39±8.0 (24-57)	44.0±13.0 (24-70)	45.6±7.2 (39-61)	**ξ=0.04**
LDL-Cholesterol	128.1±13.6 (106-155)	129±28 (91-176)	128.6±22.4 (91-176)	113.7±19.8 (72-143)	#♦Φξ> 0.005
Triglyceride	131±70 (66-276)	136.7±54 (53-208)	134.3±60 (53-276)	120.6±33.6 (54-194)	#♦Φξ> 0.005
AST (IU/L)	43.1±13.9 (25-69)	48.8±11.8 (29-68)	46±12.8 (25-68)	22±9.0 (11-32)	**ξ<0.005**
ALT (IU/L)	73.4±25.6 (48-148)	86.3±21.7 (64-133)	79.8±23.6 (48-148)	25±11 (9-34)	**ξ<0.005**
Creatinine	0.80±0.18 (0.60-1.1)	0.75±0.18 (0.50-1.1)	0.77±0.18 (0.50-1.1)	0.81±0.12 (0.6-1.0)	#♦Φξ> 0.005
HOMA-IR	3.51±4.3 (1.07-16.05)	6.24±5.6 (1.68-22.9)	4.93±5.1 (1.07-22.9)	ND	**#= 0.01**

NAFL: Non-alcoholic fatty liver. NASH: Non-alcoholic steatohepatitis. NAFLD: Non-alcoholic fatty liver disease. HDL: High density lipoprotein. LDL: Low density lipoprotein. AST: Aspartate aminotransferase. ALT: Alanine aminotransferase. HOMA: Homeostasis model assesment. ND: Not done. #p compared with NAFL vs. NASH, ᶻp compared with NAFL vs. HS, *p compared with NASH vs. HS, ᵋp compared with NAFLD vs. HS. Results are presented as mean value±SE. Ranges are given in parenthesis.

Table 2. The coronary flow velocity reserve (CFVR) and IMT data of patients with NAFLD and healthy subjects (HS)

	NAFL Patients (n=12)	NASH Patients (n=12)	NAFLD Subjects (HS) Patients (n=28)	*P* value
Baseline APDV (cm/sec)	30.0±10.1	29.1±7.5	28.75±9.4	#♦Φ>0.005
Hyperemic APDV (cm/sec)	52.0±18.3	48.4±18.4	75.5±30.2	Φ♦>0.005
Baseline AMDV (cm/sec)	24.75±9.4	22.9±6.0	23.6±7.9	#♦Φ>0.005
Hyperemic AMDV (cm/sec)	35.0±10.1	39.1±14.2	56.9±22	***=0.002** **Φ=0.002**
CFVR	1.72±0.33	1.65±0.39	2.67±0.81	***<0.001** **Φ<0.001**
IMT (mm)	0.74±0.25	0.75±0.23	0.66±0.15	#♦Φ>0.005

(Results are presented as mean value±SE. APDV: Average peak diastolic velocity; AMDV: Average mean diastolic velocity; CFVR: Coronary flow velocity reserve; IMT: intima-media thickness)

#p compared with NAFL vs. NASH, *p compared with NAFL vs. HS, Φp compared with NASH vs. HS

controls (2.67±0.81 vs. 1.72±0.33 and 1.65±0.39 respectively, p<0.001) (Figure 1).

DISCUSSION

Non-alcoholic fatty liver disease (NAFLD) is a clinicopathologic diagnosis that is closely correlated to visceral obesity, dyslipidemia, IR, and type 2 diabetes mellitus, which suggests NAFLD as another feature of MS (28,29). Substantial experimental and clinical data indicate that NAFLD is like the hepatic expression of MS (26), and MS is also a strong predictor of NAFLD (30). Although MS is a well-known precursor of cardiovascular disease, the biological mechanism by which NAFLD promotes atherosclerosis is not well known (5,30). These mechanisms linking NAFLD with coronary vascular diseases are at least partly mediated by the atherogenic abnormalities of MS (obesity, hyperglycemia, hypertriglyceridemia, low HDL-cholesterol, and hypertension) (31). On the other hand, some of the authors have accepted that cardiovascular risk is increased independently of MS in NAFLD patients (9). Possible mechanistic pathways include subclinical inflammation, increased oxidative stress, an abnormal adipocytokine profile, and lipid abnormalities (32). Several previous studies have demonstrated associations between NAFLD and IMT and/or plaques of carotid artery and brachial artery flow-mediated dilatation that were used as measures of early atherosclerosis (15,33-36). In the studies mentioned above, ultrasound of brachial and carotid arteries was used to detect ED. Our study is the first in which determination of ED using TTDE was introduced in patients with NAFLD.

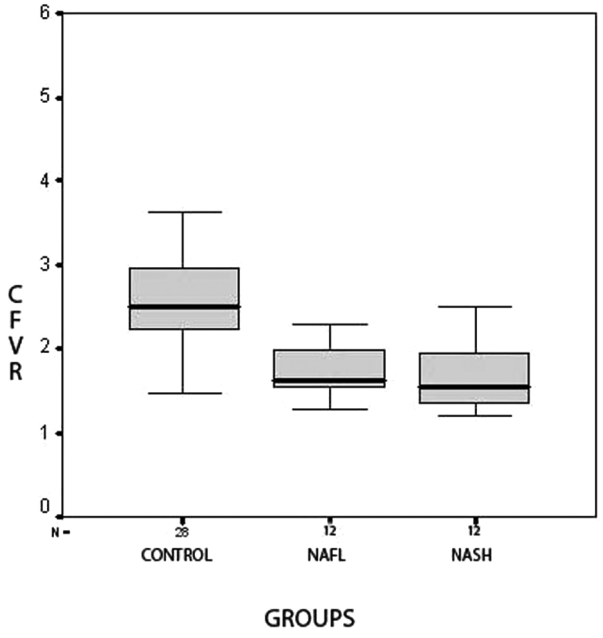

Figure 1. Comparison of coronary flow velocity reserve

Our study was designed to evaluate the endothelial function of ECA by TTDE and carotid artery IMT by ultrasound in patients with NASH and NAFL and to compare the results with HS matched for age and sex. In the NAFLD group, we found decreased CFVR in ECA as a sign of ED, with significant difference, but no difference was determined between patients with NASH and NAFL. In fact, a correlation between the severity of liver histology of NAFLD and early carotid atherosclerosis was reported (34). Recent studies have shown that NAFLD patients have significantly greater carotid IMT than age- and sex-matched patients without

NAFLD, independent of the classical risk factors of MS (37). In this study, carotid artery IMT in the NAFL and NASH groups exhibited no statistical difference. Moreover, the carotid artery IMTs of the NAFLD and the control group were found to be similar. These results may be attributed to the small number of patients in our study group. On the other hand, the lower CFVR in the NAFLD group with respect to the controls confirms the presence of impaired coronary microvascular circulation and suggests TTDE as a more effective tool for determining ED.

Our data showed that CFVR was similar between the NAFL and NASH groups. The pathogenesis of NAFLD was explained by IR-mediated first hit, and IR also contributes to the development of ED. The HOMA-IR score, a quantitative measure for IR, was higher in NASH than in NAFL in our study group (p=0.01). Oxidative stress and excessive reactive oxygen substrate (ROS) formation are held responsible for the second hit in the pathogenesis of progression of NAFL to NASH (38). Increased production of ROS is thought to be one of the key events in the pathogenesis of ED (39). Therefore, it is reasonable to expect that the endothelial function is more deteriorated in NASH than in NAFL, but one recent electron microscopic investigation showed mitochondrial dysfunction in the two NAFLD groups, and the mitochondrial dysfunction, an important determinant of oxidative stress, was not statistically different in NAFL and NASH groups (40). However, the former finding is unreliable due to the insufficient size of the study group. It will not be appropriate to generalize the findings because our study involves a limited number of cases. Therefore, it is necessary to support these findings with such studies that involve a much greater number of cases.

In conclusion, CFVR, which establishes coronary microvascular and endothelial functions noninvasively, is significantly impaired in patients with NAFLD. The impaired CFVR-like early atherosclerotic changes may have value in the prediction of coronary artery disease in patients with NAFLD.

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