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Association between non-alcoholic fatty liver disease and arterial stiffness in the non-obese, non-hypertensive, and non-diabetic young and middle-aged Chinese population

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Abstract: Background and objective: Non-alcoholic fatty liver disease (NAFLD) is associated with arterial stiffness in the general population. Age, obesity, hypertension, and diabetics are risk factors for arterial stiffness. In this study, we aimed to investigate the association between NAFLD and arterial stiffness as measured by brachial-ankle pulse wave velocity (baPWV) in the non-obese, non-hypertensive, and non-diabetic young and middle-aged Chinese population. Methods: A cross-sectional study with 1296 non-obese, non-hypertensive, and non-diabetic young and middle-aged (20–65 years) subjects undergoing routine medical check-ups in the International Health Care Center of the Second Affiliated Hospital of School of Medicine of Zhejiang University was carried out. Fatty liver was diagnosed by ultrasonography, and baPWV was measured using an automatic waveform analyzer. The subjects were classified into two groups according to the presence of NAFLD, and divided into a further two groups according to their baPWV. Results: The overall incidence of NAFLD was 19.0%, and NAFLD patients had a significantly higher level of baPWV than the controls ((1321±158) cm/s vs. (1244±154) cm/s; P<0.001). The incidence of NAFLD was clearly higher in the increased baPWV group than in the normal baPWV group (29.3% vs. 16.9%; P<0.001), and the incidence increased in line with the increase of baPWV quartiles in the normal range as well as with the severity of arterial stiffness (both P for trend <0.001). Multiple linear logistic regression analysis showed that the presence of NAFLD was positively and independently associated with baPWV. Conclusions: Our results suggest that the presence of NAFLD is associated with arterial stiffness as measured by baPWV in the non-obese, non-hypertensive, and non-diabetic young and middle-aged Chinese population.

Key words: Non-alcoholic fatty liver disease, Arterial stiffness, Brachial-ankle pulse wave velocity, Risk factor
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1 Introduction

Non-alcoholic fatty liver disease (NAFLD) is characterized by significant fat accumulation in hepatocytes after excluding excessive alcohol intake and other causes of liver disease. NAFLD is the most common cause of chronic liver disease worldwide, and its global incidence is estimated to be 14% to 40% in the general population, 50% to 90% in the obese population, and 34% to 74% in the diabetic population (Bellentani *et al.*, 2000; Angulo, 2007; Tolman *et al*., 2007). However, the incidence of NAFLD among non-obese, non-hypertensive or non-diabetic individuals is also considerable. NAFLD has long been regarded as a benign manifestation with rare or no clinical significance; however, it does indeed have clinical implications because of its potential to progress to advanced cirrhosis and hepatic failure, and increased risk for cardiovascular disease

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(CVD) (Targher *et al.*, 2010). A population-based cohort study showed that the main cause of death in NAFLD patients is CVD and liver-related diseases (Adams *et al.*, 2005).

Arterial stiffness is one of the pathological manifestations of subclinical vascular damage and represents the cumulative effect of a cluster of cardiovascular risk factors on the arterial wall (van Popele *et al.*, 2006). Pulse wave velocity (PWV) can reflect arterial distensibility and the stiffness of both central and peripheral muscular arteries (Asmar *et al*., 1995; Yamashina *et al*., 2002). Brachial-ankle PWV (baPWV) is a non-invasive examination by PWV and serves as a simple index of assessing arterial stiffness and early atherosclerotic change (Yamashina *et al*., 2002). Yamashina *et al*. (2002) recently reported on the validity and reproducibility of baPWV measurements. Previous studies had proved that baPWV was associated with CVD and metabolic syndrome (MS) (Tomiyama *et al*., 2005; Lin *et al*., 2009).

Arterial stiffness mainly occurs among older people, especially in obese, hypertensive, and diabetic population (Vaitkevicius *et al*., 1993; Henry *et al*., 2003; Zebekakis *et al*., 2005). However, it has been shown that arterial stiffness and atherosclerosis begin in the young population (Strong *et al*., 1999). Recent studies have shown that age and body mass index (BMI) are independent risk factors for the progression of arterial stiffness (Choi *et al*., 2013; Pal and Radavelli-Bagatini, 2013). Two Korean studies reported an independent association between baPWV and NAFLD in general subjects including the elderly (Kim *et al*., 2012; Lee *et al*., 2012). Considering that the incidence of NAFLD decreases with age (Lonardo *et al*., 2006) and increases with BMI (Vernon *et al*., 2011), general subjects may not be representative of the young and middle-aged population which are non-obese, non-hypertensive, and non-diabetic, and thus may not be useful for suggesting the direct association between NAFLD and arterial stiffness. For these reasons, clarifying the association between NAFLD and arterial stiffness in non-obese, non-hypertensive, and non-diabetic young and middle-aged subjects may have significant clinical implications for the prevention and diagnosis of early arterial stiffness as well as early atherosclerotic change by screening NAFLD. The aim of this study was therefore to investigate the association between NAFLD diagnosed by ultrasonography and arterial stiffness as measured by baPWV in this population.

2 Materials and methods

2.1 Study population

The subjects who visited the International Health Care Center of the Second Affiliated Hospital for health check-ups from January 2010 to December 2010 included those undergoing both abdominal ultrasonography and baPWV measurement. Subjects with the following conditions were excluded: (1) age ≥65 or <20 years; (2) BMI ≥28 kg/m^2 or waist circumference (WC) ≥90 cm for men and ≥80 cm for women; (3) a history of diabetes or glycosylated hemoglobin (HbA1c) ≥6.5% or fasting plasma glucose (FPG) ≥6.1 mmol/L; (4) a history of hypertension or systolic blood pressure (SBP) ≥140 mmHg or diastolic blood pressure (DBP) ≥90 mmHg; (5) an alcohol consumption of more than 140 g/week for men and 70 g/week for women; (6) a history of other known causes of chronic liver disease, such as viral hepatitis or autoimmune hepatitis; (7) a history of cancer, cardiovascular, respiratory, renal, or endocrine disease. After exclusion, 1296 subjects (754 men and 542 women, with a mean age of (43.7±7.6) and (42.7±7.8) years, respectively) were included in the final analysis. The subjects were classified into two groups according to the presence of NAFLD (NAFLD vs. non-NAFLD groups). As the cut-off value of baPWV serves to screen subjects at high risk of developing CVDs was 1400 cm/s (Yamashina *et al.*, 2003), the subjects were divided into further two groups according to their baPWV values (group I: baPWV≥1400 cm/s vs. group II: baPWV<1400 cm/s). This study was approved by the Institutional Research Ethics Committee of the Second Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou, China.

2.2 Anthrometric indices

All medical examinations were performed by trained staff using a standardized procedure. Height and body weight were measured using an automatic digital distancer, with the subjects standing barefoot and wearing light clothing. BMI was recorded as body weight in kilograms divided by height in meters

squared. SBP and DBP were measured using an automated sphygmomanometer with the subject in a sitting position after a 10 min rest. WC was measured using a standard tape at the middle of the lowest rib and the superior border of the iliac crest.

2.3 Laboratory measurements

Blood samples were obtained from the antecubital vein in the morning after overnight fasting. Biochemical markers, such as total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), FPG, alanine aminotransferase (ALT), aspartate aminotransferase (AST), C-reactive protein (CRP), white blood cells (WBC), and serum uric acid (sUA), were analyzed using a biochemical auto-analyzer (Olympus AU5400, Shizuoka-ken, Japan). Fasting insulin was measured using a Roche E170 auto-analyzer (Roche, Mannheim, Germany) and HbA1c measured using an Arkray HA8160 auto-analyzer (Arkray, Kyoto, Japan) with a reference value range of 4.4%–6.4%. The homeostatic model assessment was used to estimate the degree of insulin resistance: HOMA-IR= fasting insulin (μIU/ml)×fasting glycemia (mmol/L)/22.5 (Matthews *et al*., 1985).

2.4 Ultrasonography

The diagnosis of fatty liver was based on abdominal ultrasound using a Philips ATL5000 sonography machine (Philips, Tokyo, Japan) with a 3.5-MHz probe. Ultrasonography was performed by trained ultrasonic imaging specialists who were unaware of the aims of the study and blinded to clinical and laboratory data. Fatty liver was diagnosed based on standard criteria, including the evidence of diffuse hyperechogenicity of the liver when compared with the kidney, vascular blurring, and deep attenuation of the ultrasound signal (Saverymuttu *et al*., 1986).

2.5 Brachial-ankle pulse wave velocity

The baPWV was measured using an automatic waveform analyzer (BP-203RPE; Colin Medical Technology, Komaki, Japan) in the supine position after 5 min of bed rest (Yamashina *et al*., 2002). This device simultaneously records volume pulse form, arterial blood pressure electrocardiogram, and phonocardiogram at both the left and right brachia arteries and ankles. The baPWV was calculated by time-phase analysis between brachia and volume waveforms at the ankle. The distance between the brachium and ankle was estimated on the basis of body height. The reference value of baPWV was 1400 cm/s. The average value of baPWV was obtained from all subjects and used for further analysis.

2.6 Definitions

NAFLD was diagnosed by abdominal ultrasonography after excluding alcohol consumption, viral, or autoimmune liver disease (Bedogni *et al*., 2005; Cerda *et al*., 2007). The diagnosis of MS was based on the modified National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATP III) criteria (Fan *et al*., 2007). Therefore, it was defined by the presence of any three or more of the following factors: (1) central obesity: WC ≥90 cm for men, ≥80 cm for women, and/or BMI ≥25 kg/m^2 in both sexes; (2) hypertriglyceridemia: TG ≥1.7 mmol/L; (3) reduced HDL-C: HDL-C <1.03 mmol/L for men and <1.29 mmol/L for women; (4) elevated blood pressure: blood pressure ≥130/85 mmHg; (5) elevated FPG: FPG ≥5.6 mmol/L, or previously diagnosed type 2 diabetes.

2.7 Statistical analysis

Statistical analysis was performed using the SPSS software package version 11.5 (SPSS Inc., Chicago, IL, USA). Continuous variables were expressed as mean value and standard deviation (SD) or median and interquartile range (IQR), and categorical variables were presented as frequency with percentage. Independent two-sample *t*-test or the Mann-Whitney *U* test was used for comparisons of continuous parameters, while the χ^2 test was used for comparisons of categorical variables. Multivariate linear regression was used to assess the association between the presence of NAFLD and baPWV. All statistical tests were two-sided and $P<0.05$ was considered statistically significant.

3 Results

Among the enrolled 1296 subjects, the overall incidence of NAFLD was 19.0% (29.0% in men and 5.0% in women). A total of 48 subjects (3.7%) met the diagnostic criteria for the MS. Table 1 presents the

Table 1 Characteristics of study subjects according to their NAFLD status

Variable	NAFLD (*n*=246)	Non-NAFLD (*n*=1050)	*t*	*P*
Age (year)	43.5±7.1	43.2±7.9	0.546	0.585
Male	219 (89.0%)	535 (51.0%)	118.733[a]	<0.001
Systolic blood pressure (mmHg)	121±10	116±11	6.141	<0.001
Diastolic blood pressure (mmHg)	75±8	71±9	6.524	<0.001
Body weight (kg)	67.6±7.2	58.9±8.5	16.373	<0.001
Body mass index (kg/m^2)	24.13±1.75	21.88±2.18	17.281	<0.001
Waist circumference (cm)	83.5±4.6	77.1±6.1	18.077	<0.001
Triglyceride (mg/dl)	2.29±1.99	1.15±0.75	8.873	<0.001
Total cholesterol (mg/dl)	4.86 (4.29–5.52)	4.50 (4.03–5.04)	6.167[b]	<0.001
HDL-cholesterol (mg/dl)	1.41±0.29	1.60±0.29	−9.379	<0.001
LDL-cholesterol (mg/dl)	3.07±0.76	2.60±0.68	8.975	<0.001
Alanine aminotransferase (U/L)	28±15	16±10	11.677	<0.001
Aspartate aminotransferase (U/L)	22 (17–26)	18 (14–22)	6.758[b]	<0.001
Fasting insulin (μIU/ml)	9.15±4.85	6.00±2.94	9.789	<0.001
Glycosylated hemoglobin (%)	5.65±0.30	5.55±0.29	5.210	<0.001
Fasting plasma glucose (mg/dl)	5.04±0.42	4.85±0.39	6.915	<0.001
HOMA-IR	2.07±1.17	1.30±0.68	9.899	<0.001
C-reaction protein (mg/L)	3.5 (1.6–6.5)	2.9 (1.3–6.2)	1.558[b]	0.119
Serum urine acid (mg/dl)	6.53 (5.80–7.27)	5.15 (4.32–6.19)	12.493[b]	<0.001
White blood cells (×10^9/L)	6.31±1.68	5.64±1.50	5.749	<0.001
baPWV (cm/s)	1321±158	1244±154	7.009	<0.001
Incidence of metabolic syndrome	29 (11.8%)	19 (1.8%)	55.649[a]	<0.001

Data of variables for NAFLD and non-NAFLD subjects are presented as the mean±SD, median (IQR), or number (percentage). *P* values were calculated using an independent 2-sample *t*-test or the Mann-Whitney *U* test for continuous data and the χ^2 test for categorical data. [a] χ^2 value; [b] *Z* value. NAFLD: non-alcoholic fatty liver disease; HDL: high-density lipoprotein; LDL: low-density lipoprotein; HOMA-IR: homeostatic model assessment of insulin resistance; baPWV: brachial-ankle pulse wave velocity

characteristics of the 1296 subjects according to their NAFLD status. The NAFLD subjects had higher SBP, DBP, body weight, BMI, WC, TC, TG, LDL-C, ALT, AST, FPG, WBC, sUA, fasting insulin, HbA1c, and HOMA-IR, and lower HDL-C than those without NAFLD. It was clear that patients with NAFLD, in comparison with non-NAFLD subjects, showed significantly higher baPWV ((1321±158) cm/s vs. (1244±154) cm/s; $P<0.001$).

Among the 1296 subjects, 215 (16.6%) had increased baPWV. We compared the characteristics of subjects between the increased baPWV group (group I) and the normal baPWV group (group II) (Table 2). The results showed that increased baPWV subjects were older, more likely to be male, and more obese. This group also showed more disordered lipid and metabolic profiles, increased blood pressure, elevated liver enzymes, and a higher incidence of NAFLD (group I vs. group II: 29.3% vs. 16.9%; $P<0.001$).

To assess the incidence of NAFLD in the normal range of baPWV, 1081 (83.4%) subjects in the normal range of baPWV were further categorized into four quartiles (less than 1131 cm/s, 1131–1209 cm/s, 1210–1291 cm/s, and 1292–1400 cm/s). According to baPWV quartiles, the incidence of NAFLD was 9.2%, 12.3%, 20.2%, and 26.0%, respectively. Furthermore, in order to assess the incidence of NAFLD in relation to the severity of arterial stiffness, 1296 subjects were categorized into a further four groups (less than 1400 cm/s, 1400–1600 cm/s, 1600–1800 cm/s, and more than 1800 cm/s). The results showed that the incidence of NAFLD was 16.9%, 28.0%, 35.3%, and 40.0%, respectively. As shown in Fig. 1, the incidence of NAFLD was statistically increased in line with the baPWV quartiles in the normal range, as well as with the severity of arterial stiffness (both *P* for trend <0.001).

Table 2 Characteristics of study subjects according to baPWV

Variable	Group I (n=215)	Group II (n=1081)	t	P
Age (year)	47.6±7.9	42.4±7.4	8.815	<0.001
Male	167 (77.7%)	587 (54.3%)	40.264[a]	<0.001
Systolic blood pressure (mmHg)	125±8	116±11	13.530	<0.001
Diastolic blood pressure (mmHg)	77±7	71±9	11.351	<0.001
Body weight (kg)	62.8±8.0	60.2±9.1	4.376	<0.001
Body mass index (kg/m^2)	22.97±2.21	22.18±2.27	4.687	<0.001
Waist circumference (cm)	80.9±5.7	77.8±6.4	6.442	<0.001
Triglyceride (mg/dl)	1.67±1.35	1.30±1.14	3.737	<0.001
Total cholesterol (mg/dl)	4.73 (4.22–5.40)	4.55 (4.03–5.09)	3.816[b]	<0.001
HDL-cholesterol (mg/dl)	1.52±0.31	1.57±0.30	−2.220	0.001
LDL-cholesterol (mg/dl)	2.88±0.74	2.65±0.71	4.400	<0.001
Alanine aminotransferase (U/L)	22±13	18±12	4.563	<0.001
Aspartate aminotransferase (U/L)	20 (15–25)	19 (15–23)	2.294[b]	0.022
Fasting insulin (μIU/ml)	7.22±4.03	6.47±3.51	2.781	0.013
Glycosylated hemoglobin (%)	5.65±0.31	5.55±0.29	4.507	<0.001
Fasting plasma glucose (mg/dl)	5.00±0.42	4.87±0.40	4.375	<0.001
HOMA-IR	1.62±0.95	1.42±0.82	2.943	0.003
C-reaction protein (mg/L)	3.4 (1.5–6.3)	3.0 (1.3–6.2)	0.952[b]	0.341
Serum urine acid (mg/dl)	5.93 (4.91–6.94)	5.32 (4.38–6.39)	5.093[b]	<0.001
White blood cells (×10^9/L)	5.96±1.56	5.73±1.55	2.002	<0.001
baPWV (cm/s)	1520±107	1207±106	39.535	0.035
Incidence of NAFLD	63 (29.3%)	183 (16.9%)	17.854[a]	<0.001
Incidence of metabolic syndrome	23 (10.7%)	25 (2.3%)	35.352[a]	<0.001

Data of variables for groups I and II subjects are presented as mean±SD, median (IQR), or number (percentage). P values were calculated using an independent 2-sample t-test or the Mann-Whitney U test for continuous data and the χ^2 test for categorical data. [a] χ^2 value; [b] Z value. Group I: baPWV≥1400 cm/s; Group II: baPWV<1400 cm/s. NAFLD: non-alcoholic fatty liver disease; HDL: high-density lipoprotein; LDL: low-density lipoprotein; HOMA-IR: the homeostatic model assessment of insulin resistance; baPWV: brachial-ankle pulse wave velocity

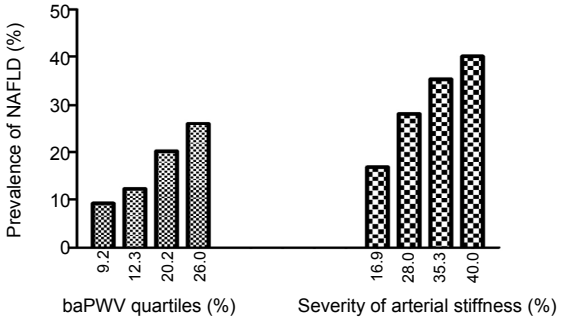

Fig. 1 Incidence of NAFLD associated with baPWV and arterial stiffness

The incidence of NAFLD was increased in line with the increase of baPWV quartiles in the normal range, as well as with the severity of arterial stiffness (both P for trend <0.001)

Multiple linear logistic regression analysis was used to identify variables affecting baPWV (Table 3). Fourteen variables, including age, male gender, WC, BMI, SBP, TG, HDL-C, LDL-C, ALT, CRP, sUA, HbA1c, HOMA-IR, and the presence of NAFLD, were entered into the analysis. Our results showed that age, male gender, BMI, SBP, HOMA-IR, CRP and the presence of NAFLD were positively and independently associated with baPWV.

Table 3 Multiple linear regression analysis to identify variables affecting baPWV

Variable	Standard β	t	P
Age	0.292	11.868	<0.001
Male gender	0.174	4.723	<0.001
Systolic blood pressure	0.375	14.673	<0.001
Body mass index	−0.131	−3.855	<0.001
Waist circumference	0.009	0.246	0.805
Triglyceride	−0.018	−0.636	0.525
HDL-cholesterol	−0.025	−0.955	0.340
LDL-cholesterol	0.012	0.452	0.651
Alanine aminotransferase	0.039	1.478	0.140
Serum urine acid	−0.002	−0.061	0.951
C-reaction protein	0.055	2.408	0.016
HOMA-IR	0.061	2.331	0.020
Glycosylated hemoglobin	0.020	0.815	0.415
NAFLD	0.075	2.765	0.006

β: partial regression coefficient; baPWV: brachial-ankle pulse wave velocity; HDL-C: high-density lipoprotein-cholesterol; LDL-C: low-density lipoprotein-cholesterol; HOMA-IR: homeostatic model assessment of insulin resistance; NAFLD: non-alcoholic fatty liver disease

4 Discussion

Our results show that the presence of NAFLD was independently associated with arterial stiffness in a non-obese, non-hypertensive, and non-diabetic young and middle-aged Chinese population. On the one hand, baPWV was significantly increased in NAFLD patients, and the incidence of NAFLD was statistically increased in line with the baPWV quartiles in the normal range as well as with the severity of arterial stiffness. On the other hand, multivariate linear regression analysis showed that the presence of NAFLD was positively and independently associated with baPWV. Our results are in accordance with those of previous studies which suggest that various non-invasive surrogate markers of early atherosclerosis, such as carotid-femoral PWV, higher carotid intima-media thickness, and reduced flow-mediated dilatation, are associated with the presence of NAFLD (Villanova *et al*., 2005; Targher *et al*., 2006a).

Age is an independent risk factor for arterial stiffness (Choi *et al*., 2013). However, the incidence of arterial stiffness is also high in the young population (Strong *et al*., 1999). In addition to the known metabolic factors, such as obesity, hypertension, and diabetes, there are other factors that affect the formation of arterial stiffness. Considering that NAFLD is the aggregation of multiple risk factors in liver, in order to exclude the confounding factors, such as age, obesity, hypertension, and diabetes, we chose non-obese, non-hypertensive, and non-diabetic young and middle-aged subjects as a research population to investigate the relationship between NAFLD and arterial stiffness; the results in this population demonstrated that the presence of NAFLD is positively and independently associated with arterial stiffness, an observation with significance in clinical practice.

The possible biological mechanisms linking NAFLD and arterial stiffness remain little known. Several mechanisms have been suggested, such as an increase in whole-body insulin resistance and abnormal secretion of adipose cytokines, including decreased adiponectin and increased leptin levels (Singhal *et al*., 2002; Angulo *et al*., 2004; Machado and Cortez-Pinto, 2005; Mahmud and Feely, 2005; Targher *et al*., 2006b; Targher and Arcaro, 2007). Overall, insulin resistance and adipose cytokines could explain the association between NAFLD and arterial stiffness. However, when we choose a population without obesity, hypertension, or diabetes as research material, the result still shows that NAFLD has a close relationship with arterial stiffness, suggesting that there are other mechanisms linking them. Our results showed that WBC was significantly higher in NAFLD patients than in non-NAFLD subjects, which indicated that inflammation may be associated with NAFLD. In addition, we found that CRP was positively and independently associated with baPWV, which also indicated that inflammation may correlate with arterial stiffness. Therefore, we infer that inflammation may play a major role in the association between NAFLD and arterial stiffness in this population. Reactive oxygen related to hepatic steatosis stimulates fatty acid oxidation, which, accompanying liver cell injury and inflammatory cytokine release, promotes pre-inflammation (Park *et al*., 2004). NAFLD can be seen as a kind of inflammation disorder characterized by low levels of systemic inflammation. Many studies have reported that various inflammatory markers, such as interleukin-6, high-sensitivity CRP (hs-CRP), tumor necrosis factor-α, and WBC count, are associated with NAFLD (Bahcecioglu *et al*., 2005; Dogru *et al*., 2008; Kogiso *et al*., 2009; Lee *et al*., 2010). Chronic low-grade inflammation plays a crucial role in reducing endothelial production of nitric oxide (NO) and impairing NO-mediated vasodilation; further, increasing the binding of LDL-C to endothelium, which is subsequently oxidized within the artery wall, leads to uptake by macrophages to form foam cells (Stopeck *et al*., 1993; Villanova *et al.*, 2005). These reactions may alter arterial elastic properties and cause endothelial dysfunction, leading to arterial stiffness.

Previous studies have shown that increased body weight is a major factor for inflammation. Chen *et al.* (2012) showed that central obesity was an important determinant for increased hs-CRP in patients with NAFLD. Weng *et al.* (2010) showed increased BMI to be an independent factor for increased hs-CRP in hypertensive patients. In our study, we found that body weight and BMI have no correlation with CRP in the non-obese, non-hypertensive, non-diabetic young and middle-aged Chinese population (data not shown). This could be due to the fact that in subjects without obesity, hypertension or diabetes, mutually adjusting for all of the metabolic variables, may

attenuate the correlation between body weight and CRP. The effect of body weight on increased CRP in this population merits further investigation by prospective studies.

There is now growing evidence suggesting that NAFLD is not merely a marker of CVD but may also be involved in its pathogenesis. Although it is not fully clarified, prospective studies may be of value in supporting this idea. In a large cohort of type 2 diabetic adults, NAFLD, as diagnosed by ultrasound, is associated with an increased incidence of CVD, independent of traditional CVD risk factors and MS components (Targher *et al.*, 2007). In a study with a follow-up of 8.7 years, compared with the general population, suspected NAFLD had a significantly higher rate of cardiovascular mortality after adjusting for conventional cardiovascular risk factors (Dunn *et al*., 2008). In another study with 13.7 years of follow-up, biopsy-proven NAFLD was associated with higher all-cause death and higher incidence of CVD events (Ekstedt *et al.*, 2006). These results suggest that there may be a potential role of NAFLD in the process of arterial stiffness and atherosclerosis. In the present study, a liver biopsy was not performed and histologic data were not available. Thus, additional studies may be needed to confirm the association between the degree of histologically proven NAFLD and arterial stiffness.

There are some limitations in our study. Firstly, the study subjects who were visitors to a check-up center in a single hospital might be a biased population in terms of having paid more attention to their heath and being wealthier than the common population. Thus, generalization of our results should be made with caution. Secondly, the diagnosis of NAFLD was made by ultrasonography, which is not sensitive enough to detect minimal or mild hepatic fat deposition. However, this method is widely accepted by epidemiologists for diagnosing NAFLD, not only because it is non-invasive and widely available but also because it has reasonable sensitivity and specificity for detecting hepatic steatosis (Saverymuttu *et al.*, 1986). Thirdly, the severity of fatty liver was not graded by B ultrasound, so the correlation between the severity of fatty liver and arterial stiffness could not be analyzed. Further study is needed to explore their relationship using magnetic resonance imaging or computerized tomography to quantity analysis of the steatosis. Finally, as our study is a cross-sectional observational study, we could not explain a causal relationship between the presence of NAFLD and arterial stiffness. A prospective study is needed to illustrate a more precise interrelationship.

5 Conclusions

Our results showed that the presence of NAFLD was independently associated with arterial stiffness in a non-obese, non-hypertensive, and non-diabetic young and middle-aged Chinese population. We infer that inflammation may be a link between the presence of NAFLD and arterial stiffness in this population. Further clarification of the precise relationship may have significant clinical implications for early prevention and diagnosis of arterial stiffness by screening for NAFLD.

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Compliance with ethics guidelines

Xin-yan YU, Yi ZHAO, Xiao-xiao SONG, and Zhen-ya SONG declare that they have no conflict of interest.

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008 (5). Informed consent was obtained from all subjects for being included in the study.

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中文概要：

本文题目： **非肥胖、高血压和糖尿病的中国中青年人群中非酒精性脂肪性肝病和动脉硬度的关系研究**
Association between non-alcoholic fatty liver disease and arterial stiffness in the non-obese, non-hypertensive, and non-diabetic young and middle-aged Chinese population

研究目的： 探讨在非肥胖、高血压和糖尿病的中国中青年群人中非酒精性脂肪性肝病（NAFLD）和动脉硬度的关系。

创新要点： 在非肥胖、高血压和糖尿病的中青年人群中，阐明 NAFLD 和动脉硬度的关系，对预防和诊断早期动脉硬度有重要临床意义。

研究方法： 在非肥胖、高血压和糖尿病的中青年体检人群中进行一项大型横断面研究。用 B 超诊断脂肪肝，用臂踝脉搏波指数（baPWV）测量动脉硬度，根据是否有 NAFLD 和 baPWV 水平分组。

重要结论： NAFLD 组患者的 baPWV 水平明显高于对照组（(1321±158) cm/s vs. (1244±154) cm/s；$P<0.001$），NAFLD 患病率在 baPWV 升高组明显高于 baPWV 正常组（29.3% vs. 16.9%；$P<0.001$），且 NAFLD 患病率随 baPWV 水平和动脉硬度程度的升高而升高（两者趋势 P 值 <0.001）。多因素线性回归分析表明，NAFLD 发生与 baPWV 独立相关。因此，在非肥胖、高血压和糖尿病的中国中青年群人中，NAFLD 发生与动脉硬度密切相关。

关键词组： 非酒精性脂肪性肝病；动脉硬度；臂踝脉搏波指数；危险因素