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Gut-flora metabolites is not associated with synchronous carotid artery plaque and non-alcoholic fatty liver disease in asymptomatic adults
A STROBE-compliant article

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Abstract

Synchronous non-alcoholic fatty liver disease (NAFLD) and carotid artery plaque formation increase the risk of mortality in patients with cardiovascular disease (CVD). Metabolic status and host gut flora are associated with NAFLD and CVD, but the risk factors require further evaluation.

To evaluate the risk factors associated with NAFLD and CVD, including gut-flora-related examinations.

This cross-sectional study included 235 subjects aged over 40 years who underwent abdominal ultrasound examination and carotid artery ultrasound examination on the same day or within 12 months of abdominal ultrasound between January 2018 and December 2019. All subjects underwent blood tests, including endotoxin and trimethylamine-N-oxide.

The synchronous NAFLD and carotid artery plaque subjects had a higher proportion of men and increased age compared with those without NAFLD and no carotid artery plaque. The synchronous NAFLD and carotid artery plaque group had increased body mass index (BMI), blood pressure, hemoglobin A1C (5.71% vs 5.42%), triglyceride (TG) (164.61 mg/dL vs 102.61 mg/dL), and low-density lipoprotein (135.27 mg/dL vs 121.42 mg/dL). In multiple logistic regression analysis, increased BMI, mean systolic blood pressure, and $TG > 110$ mg/dL were independent risk factors for synchronous NAFLD and carotid artery plaque formation. Endotoxin and trimethylamine-N-oxide levels were not significantly different between the 2 groups.

Host metabolic status, such as elevated BMI, TG, and systolic blood pressure, are associated with synchronous NAFLD and carotid artery plaque in asymptomatic adults. Aggressive TG control, blood pressure control, and weight reduction are indicated in patients with NAFLD.

Abbreviations: BMI = body mass index, CIs = confidence intervals, CVAs = cerebrovascular accidents, CVD = cardiovascular disease, DM = diabetes mellitus, H pylori = Helicobacter pylori, HbA1c = hemoglobin A1C, LAL = limulus amebocyte lysate, LDL = low-density lipoprotein, LPS = lipopolysaccharide, NAFLD = non-alcoholic fatty liver disease, ORs = odds ratios, SBP = systolic blood pressure, TG = triglyceride, TMA = trimethylamine, TMAO = trimethylamine-N-oxide, TML = trimethyllysine.

Keywords: blood pressure, body mass index, carotid stenosis, non-alcoholic fatty liver disease, triglycerides

1. Introduction

Cardiovascular disease (CVD) and cerebrovascular accidents (CVAs) are leading health problems and important causes of mortality worldwide.[1] The majority of CVD and CVAs are attributable to atherosclerosis, which is characterized by hardening of the arteries and narrowing of the lumen. The pathogenesis of atherosclerosis involves blood vessel inflammation, injury response, degeneration, and thrombosis, and these conditions lead to thickening of the vascular intima and trigger CVD or CVAs.[2,3] The risk factors for atherosclerotic plaque formation include diabetes mellitus (DM), hyperlipidemia, hypertension, sex, habitual smoking, and family history.[4] Nonalcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver disease worldwide and is characterized by an excessive accumulation of fat in the liver parenchyma of patients who consume little to no alcohol.[5] The worldwide prevalence of NAFLD in the general population is 15% to 40% in Western countries, and has been reported to be 11.3% in China, 27% in Hong Kong, and 15% to 45% in South Korea, Japan, and Taiwan.[6–10] Several studies have suggested that metabolites, exercise, and food components play important roles in NAFLD progression.[11]

Our previous study demonstrated that hyperglycemia combined with *Helicobacter pylori* (*H pylori*) infection might increase the risk of synchronous colorectal adenoma and carotid artery plaque.[12,13] Additionally, we demonstrated a correlation between CVD and NAFLD, in that when an NAFLD patient had atherosclerosis, the prevalence of CVD events was approximately 2-fold higher than that in the general population. Combined *H pylori* infection and NAFLD have also been shown to increase carotid artery plaque formation.[14] These findings suggest that the human gut microbiota may play a role in the development of atherosclerosis and affect NAFLD formation. Recent studies have also found changes in the host gut microbiota in patients with colon cancer, ulcerative colitis, obesity, coronary heart disease, delirium, and senile dementia.[15,16] The gut microbiota may influence host immunity and physiology by producing several types of bacterial products and metabolites, such as trimethylamine-N-oxide (TMAO), endotoxins, and short-chain fatty acids. Furthermore, previous studies have demonstrated a connection between the gut microbiota and atherosclerosis through the metabolite TMAO. TMAO enhances foam cell formation and has the potential to promote atherosclerosis, myocardial infarction, and stroke. Endotoxemia has also been shown to be associated with carotid atherosclerosis and CVD.[17–20] However, some previous studies found no connection between a higher level of TMAO and carotid artery disease.[21]

Previous studies have shown controversial results regarding the link between atherosclerosis and gut flora dependent metabolites. Since some studies noted some connection between CVD, NAFLD, carotid atherosclerosis, gut microbiota, and gut-flora dependent metabolites and some presented controversial results, the relationship between these indicators, events, and diagnosis remains unclear. Therefore, in the current study, we aimed to explore the association between host metabolic status, gut-flora dependent metabolites, and synchronous carotid artery disease and NAFLD in asymptomatic adults.

2. Methods

2.1. Subjects and study design

This was a cross-sectional observation study that involved healthy men and women aged 40 years or older who participated in a comprehensive health-screening examination in the MacKay Memorial Hospital, Taiwan, from January 2018 until December 2019. Asymptomatic individuals who had undergone abdominal ultrasound examination as part of a health check-up were enrolled for analysis. A carotid artery ultrasound survey was arranged on the same day or within 12 months of colonoscopy when the participants accepted annual physical check-ups. As our objective was to evaluate the association between serum endotoxin and TMAO with carotid artery plaque and non-alcoholic fatty liver disease, we excluded patients who met the following criteria: previously proven acute myocardial infarction or stroke; incapacitated or could not undergo carotid artery ultrasound examination; lacked data regarding abdominal ultrasound or carotid artery ultrasound examination, or basic blood tests samples; alcoholic drinking habit or other secondary fat accumulation as a result of steatogenic medication or hereditary disorders; other severe concomitant disease, such as infections, connective tissue disease, malignancies, heart failure, and liver or kidney disease; or positive for hepatitis B surface antigen or hepatitis C antibodies. After excluding 276 subjects, a total of 235 study participants (174 men and 61 women) were enrolled for further study.

2.2. Clinical data collection and questionnaire

Clinical data including fasting plasma glucose AC, hemoglobin A1C (HbA1c), triglyceride (TG), and low-density lipoprotein (LDL) levels were obtained from participants on the same health check-up day as when the abdominal ultrasound examination was performed. These data were reported by the central lab of our hospital which has standardized protocols with routine quality assurance evaluation. Carotid artery ultrasound examination data were collected on the same day, or within 12 months of the endoscopy examination for the participants who accepted an annual healthy examination. Baseline characteristics (age, height, weight, personal medical history, current medicine use, family history of first degree-relatives, and smoking) were obtained from a questionnaire completed at the time of the examination. The study was approved by the MacKay Memorial Hospital Institutional Review Board (18MMHIS185).

2.3. Endotoxin and TMAO

Pierce limulus amebocyte lysate (LAL) chromogenic endotoxin quantitation kit (Thermo Scientific; Waltham, US) was used for the in vitro quantitative detection of human plasma endotoxin concentration. Briefly, purified extract of *Escherichia coli* (0113: H10) was used as a control standard endotoxin. PM (100 μL) extract was mixed with LAL reagent (100 μL) followed by incubation at 37°C for 60 minutes. Then substrate solution was added to the incubated fraction and vortexed for 10 minutes to ensure complete mixing. Presence of endotoxins was confirmed by developing yellow color and mixture was measured spectrophotometrically (PerkinElmer EnSpire 2300) at 405 to 410 nm. Concentration of endotoxin was calculated from the standard curve prepared using the series of different endotoxin standard concentration, that is, 1.0, 0.5, 0.25, 0.1 EU/mL.[22]

Early gas chromatography–mass spectrometry studies employed a complicated multistep derivatization protocol that first reduced TMAO to the gas trimethylamine (TMA), which was subsequently derivatized with 2,2,2-trichloroethyl chloroformate and detected as N,N-dimethyl-2,2,2-trichloroethyl

carbamate. The coefficient of determination r2 was required to be over 0.98.[23]

2.4. Scanning protocol and definition of carotid artery lesion and NAFLD in ultrasound examination

Ultrasonography of the common carotid artery, carotid bifurcation, and internal carotid artery of the left and right carotid arteries was performed using a 7.5-MHz linear-array transducer (ATL Ultra-Mark IV). On a longitudinal, two-dimensional ultrasound image of the carotid artery, the anterior (near) and posterior (far) walls of the carotid artery were displayed as 2 bright white lines separated by a hypoechogenic space. Frozen images of the arterial wall were saved, and measurements were subsequently performed on the stored digital images. A plaque was defined as a distinct area with an I–M thickness >50% greater than that of neighboring sites.[24] The definition of NAFLD requires that firstly, there is evidence of hepatic steatosis, either by imaging or histology, and secondly, that there are no causes of secondary hepatic fat accumulation, such as significant alcohol consumption, use of steatogenic medication, or hereditary disorders. An experienced gastroenterologist blinded to the study aims performed the abdominal ultrasound using an HD-15 ultrasound system (Philips Medical Systems, Cleveland, OH). Fatty liver diagnosis was made by ultrasound based on standard criteria, including parenchyma brightness, liver-to-kidney contrast, deep beam attenuation, and bright vessel walls.

2.5. Statistical analysis

The following variables were recorded for each subject: age, sex, body mass index (BMI), HbA1c, lipid levels, smoking status, TMAO, and endotoxin level. A t test was applied for continuous variables when the data fit a normal Gaussian distribution, and data for continuous variables were expressed as the mean $\pm$ SD. Categorical variables were tested using the chi-square test or Analysis of Variance test and expressed as numbers (percentage). Unadjusted odds ratios (ORs) with 95% confidence intervals (CIs) were computed for potential predictors of colorectal polyps and adenomas. Multiple variable logistic regression analysis was applied to compute the adjusted OR (95% CI) for predictors of carotid artery plaque and NAFLD. Variables with a P-value <.2 on univariate analysis were selected for multivariate logistic regression. The final model was developed using a stepwise backward approach. All variables with $P < .05$ were considered to be statistically significant and remained in the final model. All analyses were performed using SAS version 9.1 (SAS Institute, Cary, NC).

3. Results

3.1. Demographics of NAFLD and carotid artery plaque statuses

In a total of 235 patients, the NAFLD patients (n = 133) had older mean age and higher levels of BMI, HbA1c, glucose AC, systolic blood pressure (SBP), diastolic blood pressure (DBP), and TG compared with controls (all $P < .05$) (Table 1), as well as the current oral medicine treatment participants, including diabetes, hypertension, and hyperlipidemia. The proportion of participants who were smokers was also higher in the NAFLD group. The TMAO and endotoxin level was not significantly different between the 2 groups.

As to the carotid artery plaque status, patients with carotid artery plaque formation (n = 83) had a higher level of the BMI, SBP, DBP, and LDL than in those without (n = 152) (Table 2). In terms of gut-flora dependent metabolites, the endotoxin and TMAO level were not significantly different between the 2 groups.

Table 1

Demographics of participants with and without NAFLD.

Variable	NAFLD status		
	Non-NAFLD (n = 102)	NAFLD (n = 133)	P-value
Age, mean (SD), yr	52.09 (8.30)	53.43 (7.05)	.183
Sex (male %)	61 (60%)	113 (85%)	.000
BMI, mean (SD), kg/m²	22.15 (2.56)	25.52 (2.61)	.000
HbA1c, mean (SD), %	5.44 (0.34)	5.73 (0.78)	.001
Systolic blood pressure, mean (SD), mmHg	116.27 (15.97)	123.50 (13.10)	.000
Diastolic blood pressure, mean (SD) mm Hg	74.15 (10.14)	79.06 (10.70)	.000
Glucose AC, mean (SD), mg/dL	94.03 (9.77)	103.92 (19.58)	.000
Total cholesterol, mean (SD), mg/dL	202.94 (34.71)	205.65 (37.96)	.575
Triglyceride, mean (SD), mg/dL	99.45 (56.57)	160.39 (86.70)	.000
LDL, mean (SD), mg/dL	124.00 (30.78)	131.65 (33.26)	.073
Plasma hs-CRP, mean (SD), mg/L	0.15 (0.38)	0.18 (0.24)	.430
Smoking, no. (%)	20 (20%)	37 (28%)	.000
Anti-platelet agent used, no. (%)	3 (3%)	11 (8%)	.000
Anti-lipid agent used, no. (%)	8 (8%)	18 (14%)	.000
DM control agent used, no. (%)	2 (2%)	11 (8%)	.000
Hypertension control agent used, no. (%)	9 (9%)	30 (23%)	.000
Hb A1c level ≥6.5%, no. (%)	0 (0%)	12 (9%)	.000
TMAO, mean (SD), μM	2.95 (6.73)	2.22 (2.94)	.408
Endotoxin, mean (SD), EU/mL	0.68 (1.18)	0.95 (1.74)	.230

AC = ante cibum; BMI = body mass index; CRP = C-reactive protein; DM = diabetes mellitus; HbA1c = hemoglobin A1C; LDL = low-density lipoprotein; NAFLD = non-alcoholic fatty liver disease; no. = number; SD = standard deviation; TMAO = trimethylamine-N-oxide.

Table 2

Demographics of with and without carotid artery plaque.

Variable	Carotid artery plaque status		*P*-value
	Plaque (−) (n = 152)	Plaque (+) (n = 83)	
Age, mean (SD), year	51.85 (7.78)	54.67 (7.04)	.006
Sex (male %)	106 (70%)	68 (82%)	.042
BMI, mean (SD), kg/m^2	23.59 (2.83)	24.91 (3.34)	.002
HbA1c, mean (SD), %	5.57 (0.65)	5.66 (0.64)	.339
Systolic blood pressure, mean (SD), mm Hg	118.17 (14.24)	124.39 (15.12)	.002
Diastolic blood pressure, mean (SD), mm Hg	75.54 (9.65)	79.47 (12.08)	.007
Glucose AC, mean (SD), mg/dL	99.29 (18.54)	100.25 (13.03)	.675
Total cholesterol, mean (SD), mg/dL	201.36 (33.19)	210.17 (41.58)	.078
Triglyceride, mean (SD), mg/dL	130.51 (82.32)	140.59 (78.35)	.363
LDL, mean (SD), mg/dL	125.29 (29.14)	133.90 (37.07)	.050
Plasma hs-CRP, mean (SD), mg/L	0.17 (0.32)	0.18 (0.28)	.841
Smoking, no. (%)	42 (28%)	15 (18%)	.102
Anti-platelet agent used, no. (%)	7 (5%)	7 (8%)	.236
Anti-lipid agent used, no. (%)	12 (8%)	14 (17%)	.036
DM control agent used, no. (%)	7 (5%)	6 (7%)	.400
Hypertension control agent used, no. (%)	16 (11%)	23 (28%)	.001
Hb A1c level ≥6.5%, no. (%)	7 (5%)	5 (6%)	.637
TMAO, mean (SD), μM	2.48 (5.45)	2.70 (4.30)	.817
Endotoxin, mean (SD), EU/mL	0.90 (1.74)	0.69 (0.97)	.367

AC = ante cibum; BMI = body mass index; CRP = C-reactive protein; DM = diabetes mellitus; HbA1c = hemoglobin A1C; LDL = low-density lipoprotein; NAFLD = non-alcoholic fatty liver disease; no. = number; SD = standard deviation; TMAO = trimethylamine-N-oxide.

3.2. Demographics of synchronous NAFLD and carotid artery plaque

There were 56 patients with synchronous fatty liver and carotid artery plaque and 75 patients with no fatty liver and no carotid artery plaque. Several factors, included aging, sex, BMI, HbA1c, SBP, DBP, TG, and smoking status, were related to synchronous NAFLD and carotid artery plaque (Table 3). However, the gut-flora dependent metabolites (TMAO and endotoxin) and hs-CRP levels were similar between the 2 groups. Univariate logistic regression and multiple logistic regression for predictors of synchronous fatty liver and carotid artery plaque is shown in Table 4. In univariate logistic regression, increased age, BMI, SBP, DBP, HbA1c >5.6%, TG >110 mg/dL, and men were found to be relative risk factors of synchronous fatty liver and carotid artery plaque. Multiple logistic regression demonstrated that only increased BMI, SBP, and TG >110 mg/dL were independent risk factors for participants with synchronous fatty liver and carotid artery plaque, while TMAO and endotoxin levels were not. The synchronous NAFLD and carotid artery plaque group had increased BMI, blood pressure, HbA1c (5.71% vs 5.42%), TG (164.61 mg/dL vs 102.61 mg/dL), and LDL (135.27 mg/dL vs 121.42 mg/dL) Moreover, we found no interaction effect of increased BMI, SBP, and TG >110 mg/dL in synchronous fatty liver and carotid artery plaque formation. While calculating the power retrospectively under the assumption of alpha error 0.05 with our sample sizes and variances, the power of TMAO was 0.79 and the power of endotoxin was 0.05.

4. Discussion

Several previous studies have demonstrated that the relationship between NAFLD and metabolic syndrome is bidirectional. The presence of NAFLD is a strong predictor of metabolic syndrome, such as abdominal obesity, hypertension, atherogenic dyslipidemia, and dysglycemia, and these features are established risk factors for type 2 DM and CVD. Furthermore, liver fat content has also been shown to be significantly increased in participants with dyslipidemia or metabolic syndrome.[25,26] Since there is a clinically significant association between NAFLD and the development of CVD, type 2 DM, and other systemic diseases, it is necessary to identify those subjects at risk of developing NAFLD. A study by Gastaldelli et al[27] demonstrated that NAFLD subjects are more prone to early carotid atherosclerosis, even in the absence of metabolic syndrome. Furthermore, a study by Wong et al[28] demonstrated that the prevalence of NAFLD was 58%, and more severe CAD was predicted in a coronary angiogram. The cohort study described by Moon et al[29] also showed a strong association between NAFLD and carotid artery plaque. Moreover, Musso et al[30] conducted a meta-analysis study and found that patients with NAFLD have a higher risk of mortality and morbidity as a result of CVD (OR, 2.05; 95% CI, 1.8–2.3; P < .0001) than the matched general population. These growing bodies of evidence support the association of NAFLD with cardiovascular events, independent of traditional risk factors. Furthermore, they have provided evidence that future research should not only focus on the hepatic situation but also the need to survey the possibility of CVD in patients with NAFLD. These studies highlight the importance of synchronous fatty liver and carotid artery plaque, as well as the importance of establishing risk factors or biomarkers for this group of patients.

Our previous study revealed the connection between NAFLD and CVD, as well as several noninvasive diagnostic methods for NAFLD.[12] *H pylori* infection combined with NAFLD has been demonstrated to increase the risk of carotid artery plaque formation.[14] Indeed, previous studies have highlighted the association between gut microbiota and human diseases, including enteric infections and malignancy, inflammatory bowel disease, metabolic disease, CVD, and mood disorders.[31] Our previous study also demonstrated that the colorectal adenoma ratio is likely to decrease following *H pylori* eradication.[32] It has been suggested that the gut microbiota contributes to nearly every aspect of the host's growth and development, such that host

Table 3

Demographics of synchronous NAFLD and carotid artery plaque status.

Variable	Synchronous fatty liver and carotid artery plaque (n = 56)	No fatty liver and No carotid artery plaque (n = 75)	P-value
Age, mean (SD), year	54.21 (6.30)	50.81 (7.94)	.009
Sex (male %)	50 (89%)	43 (57%)	.000
BMI, mean (SD), kg/m²	26.09 (2.93)	22.04 (2.49)	.000
HbA$_{1c}$, mean (SD), %	5.71 (0.75)	5.40 (0.34)	.002
Systolic blood pressure, mean (SD), mm Hg	124.68 (11.64)	113.57 (12.95)	.000
Diastolic blood pressure, mean (SD), mm Hg	79.98 (12.65)	72.61 (9.43)	.000
Glucose AC, mean (SD), mg/dL	102.70 (14.12)	93.61 (10.17)	.000
Total cholesterol, mean (SD), mg/dL	209.80 (42.43)	200.03 (32.14)	.137
Triglyceride, mean (SD), mg/dL	164.61 (82.32)	102.61 (62.57)	.000
LDL, mean (SD), mg/dL	135.27 (38.23)	121.42 (28.88)	.020
Plasma hs-CRP, mean (SD), mg/L	0.21 (0.32)	0.17 (0.44)	.585
Smoking, no. (%)	10 (18%)	15 (20%)	.000
Anti-platelet agent used, no. (%)	6 (11%)	2 (3%)	.000
Anti-lipid agent used, no. (%)	8 (14%)	2 (3%)	.000
DM control agent used, no. (%)	4 (7%)	0 (0%)	.000
Hypertension control agent used, no. (%)	16 (29%)	2 (3%)	.000
Hb A$_{1c}$ level ≥6.5%, no. (%)	5 (9%)	0 (0%	.000
TMAO, mean (SD), μM	1.98 (1.22)	2.58 (6.81)	.640
Endotoxin, mean (SD), EU/mL	0.64 (0.87)	0.64 (1.20)	.987

AC = ante cibum; BMI = body mass index; CRP = C-reactive protein; DM = diabetes mellitus; HbA1c = hemoglobin A1C; LDL = low-density lipoprotein; NAFLD = non-alcoholic fatty liver disease; no. = number; SD = standard deviation; TMAO = trimethylamine-N-oxide.

disease and organ dysfunction may result when an imbalance exists in either the composition, number, or habitat of the gut microbiota.[31] A further possible mechanism of this condition might relate to the role of microbiota in gut inflammation, as it has been shown that when toll-like receptors are stimulated by bacterial products, such as endotoxin, IL-23 increases, and acts on downstream cells, including lymphocytes. Endotoxin is a complex that contains lipopolysaccharide, a cell wall component of gram-negative bacteria that elicits strong immune responses in humans and may function as a copromoter that triggers the formation of carotid artery plaque.[33] However, in the current study, the endotoxin level was not significantly different between participants with and without carotid artery plaque and NAFLD. There are several possible explanations for this observation. First, endotoxin levels might be affected by a high-fat diet.[34] Second, the endotoxin level measured by the LAL assay may be inaccurate due to technical difficulties with the assay and the need to collect samples under lipopolysaccharide-free conditions, as well as its lack of sensitivity.[35]

Recent studies have shown that the direct connection between the gut microbiota and atherosclerosis, at least in part through the metabolite TMAO and elevated TMAO levels, might predict the incident risk of thrombotic events in human subjects.[18,36] However, a study by Skagen et al[21] found that serum γ-butyrobetain and carnitine, but not TMAO or trimethyllysine, were increased in patients with carotid atherosclerosis. TMAO has been proposed to be produced in several steps. The dietary intake of carnitine or phosphatidylcholine leads to its conversion to trimethylamine (TMA) by the intestinal microbiota, which is subsequently oxidized to TMAO by flavins containing mono-oxygenase in the liver.[37] Since γ-butyrobetain is produced endogenously from trimethyllysine and partly converted to TMA in the colon, it has been suggested that γ-butyrobetain could mediate some of its effects through TMA, and subsequently TMAO.[37] Both microbiota-dependent and endogenous pathways could potentially affect the formation of carotid atherosclerosis. The recent study conducted by Koay et al[38] showed

that both "healthy" and "unhealthy" diets could cause an increase in the plasma level of TMAO and no direct association of plasma TMAO and the extent of atherosclerosis, both in mice and humans. According to the author's opinion, this result might be related to the fact that different diets induce changes in the gut microbiome, and the gut itself is a site of significant oxidative production of TMAO. Thus, the TMAO level might not be a straight marker of carotid atherosclerosis.

In our study, hs-CRP, endotoxin, and TMAO were ineffective in the detection of NAFLD or carotid artery plaque in asymptomatic adults, and we found that elevated BMI, TG > 110 mg/dL, and elevated SBP were related to synchronous NAFLD and carotid artery plaque after univariate logistic regression and multiple logistic regression analysis (Table 4). Indeed, elevated BMI and TG levels were previously thought to be highly related to NAFLD.[39] In Table 2, we show that both SBP and DBP were significantly higher, and LDL was borderline higher in the carotid artery plaque group. Our study showed that both NAFLD and carotid artery plaque subjects had higher SBP (approximately 125 mmHg) than subjects without NAFLD or carotid artery plaque. A similar result was found in synchronous NAFLD and carotid artery plaque than in non-NAFLD and non-carotid artery plaque participants (Table 3). Although the systolic blood pressure was only borderline elevated (approximately 125 mmHg), it was also demonstrated to be linked to NAFLD and carotid artery plaque formation. Indeed, the study by Lemne et al[40] also reported the presence of carotid intima-media thickness and plaque in borderline hypertension. Our study results demonstrated that the risk of synchronous NAFLD and carotid artery plaque was not linked to TMAO and endotoxin increase in relatively healthy adults with a high BMI and TG if the systolic pressure was borderline elevated (approximately 125 mmHg).

The causal factors and mechanisms of NAFLD are not yet completely understood, and the debate on the role of NAFLD as a primary or secondary illness continues. Although our study provides some factors associated with NAFLD according to a

Table 4

Univariate logistic regression and multiple logistic regression for predictors of synchronous NAFLD and carotid artery plaque.

	Univariate logistic regression			Multiple logistic regression		
	OR	S.E.	*P*-value	OR	S.E.	*P*-value
Age	1.06	0.03	.009	1.037	0.081	.639
Male, sex	6.20	3.05	.000	6.761	8.330	.121
BMI	1.88	0.22	.000	1.658	0.409	.040
HbA1c ≥5.6	3.05	0.17	.002	2.183	2.015	.398
Systolic blood pressure, mean	1.08	0.02	.000	1.156	0.085	.047
Diastolic blood pressure, mean	1.08	0.02	.000	0.954	0.086	.597
Smoking	0.87	0.39	.758	0.290	0.339	.289
LDL >100 mg/dL	1.52	0.65	.330	6.076	7.479	.143
Triglyceride >110 mg/dL	5.79	2.25	.000	14.080	15.438	.016
TMAO, mean	1.00	0.19	.987	1.835	0.763	.144
Endotoxin, mean	0.97	0.06	.649	0.757	0.436	.244

BMI = body mass index; HbA1c = hemoglobin A1C; LDL = low-density lipoprotein; NAFLD = non-alcoholic fatty liver disease; OR = odds ratio; S.E. = standard error; TMAO = trimethylamine-N-oxide.

population-based dataset, there are still many hypotheses proposed in the past that we were not able to address in this study, such as the overproduction of reactive oxygen species, the expression of polymorphisms of specific genes such as patatin-like phospholipase domain-containing protein 3 (*PNPLA3*), the adipose tissue expandability hypothesis, environmental factors contributing to ectopic lipid accumulation, behavioral factors affecting the gut microbiota, and the effects of gut microbiota on host metabolism, nutrient absorption, and immune function.[41]

This study has some limitations. First, although blood samples were taken before meals, and fasting samples were used, we still lacked information on vegetarians versus omnivores in our analyses. Second, this study included subjects with relatively high incomes, who may have been healthier and may not be representative of the general population. Third, as a cross-sectional study, we can only show an association between metabolic status and the risk of synchronous NAFLD and carotid artery plaque; thus, a larger population and long-term follow-up are required to make definite conclusions on the impact of metabolic status or gut-flora dependent metabolites on cardiovascular mortality. Further studies are needed to determine the relationships among metabolic status, gut microbiota and their metabolites, and NAFLD or carotid artery plaque. Other gut microbiota-related metabolites, such as serotonin or short-chain fatty acids, might be included in studies on NAFLD or carotid artery plaque formation.

5. Conclusion

Our study demonstrated that elevated BMI, TG over 110 mg/dL, and elevated SBP are associated with synchronous NAFLD and carotid artery plaque in asymptomatic adults. According to this disease connection, we should not only focus on the hepatic situation, but also survey the possibility of CVD in patients with NAFLD. Given the increasing proportion of obesity in general population, as well as the high mortality and morbidity of CVD when carotid artery plaque is found in combination with NAFLD, more aggressive blood pressure and weight reduction, as well as TG control should be considered in clinical practice.

Author contributions

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