Serum levels of mac-2 binding protein are associated with diabetic microangiopathy and macroangiopathy in people with type 2 diabetes

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ABSTRACT

Introduction Non-alcoholic fatty liver disease is reportedly associated with type 2 diabetes and progressive liver fibrosis, as evaluated by transient elastography, and has been linked with micro- and macroangiopathy in people with type 2 diabetes. The purpose of this cross-sectional study was to investigate the association between serum mac-2 binding protein glycosylation isomer (M2BPGi) levels and diabetic complications in people with type 2 diabetes.

Research design and methods Serum M2BPGi levels were measured in terms of cut-off index (C.O.I.) units. Urinary albumin excretion (UAE) was calculated and nephropathy was graded as normalalbuminuria, microalbuminuria, or macroalbuminuria. Retinopathy was divided into three groups: non-diabetic retinopathy (NoDR), non-proliferative-diabetic retinopathy (NPDR), or proliferative-diabetic retinopathy (PDR).

Results The mean age for the 363 studied subjects (212 males) was 66.4±10.6 years, the median serum M2BPGi level was 0.77 (0.57–1.04) C.O.I., and the median UAE was 22 (9–82.1) mg/g creatinine. M2BPGi levels in microalbuminuria (0.83 (0.61 to 1.18) C.O.I.) and macroalbuminuria (0.88 (0.67 to 1.22) C.O.I.) cases were higher than those in normalalbuminuria cases (0.71 (0.54 to 0.92) C.O.I.). M2BPGi levels in NPDR (0.93 (0.68 to 1.28) C.O.I.) and PDR (0.95 (0.71 to 1.31) C.O.I.) cases were higher than in cases with NoDR (0.73 (0.56 to 0.99) C.O.I.). Furthermore, M2BPGi levels in subjects with a history of cardiovascular diseases were higher than in cases with no such history (0.82 (0.65 to 1.22) vs 0.76 (0.55 to 1.03) C.O.I., p=0.019). The logarithm of M2BPGi+1 was associated with the logarithm of UAE values after adjusting for covariates (standardized β=0.107, p=0.031).

Conclusions This study reveals a close association between serum M2BPGi levels and diabetic microangiopathy and macroangiopathy in people with type 2 diabetes. The results also show that liver fibrosis, evaluated by M2BPGi, is independently associated with an increased risk of albuminuria.

INTRODUCTION

The number of people afflicted with type 2 diabetes is on the rise, and diabetic complications, such as microangiopathy and macroangiopathy, are important health problems associated with this disease.1,2 Type 2 diabetes is reportedly associated with non-alcoholic fatty liver disease (NAFLD),3–5 which includes a range of liver conditions from simple steatosis to fibrosis and cirrhosis.6 It has been reported that 56% of people with type 2 diabetes have NAFLD, 37% have liver fibrosis, and 17% have severe liver fibrosis.7 There is an association between NAFLD and microangiopathy8,9 and macroangiopathy10 in patients with diabetes. The gold standard for diagnosing liver fibrosis remains liver biopsy. However, liver biopsy is highly invasive. Recently, mac-2 binding protein glycosylation isomer (M2BPGi) has been reported...
as a non-invasive new serological glyco biomarker for liver fibrosis.11–13

Chronic inflammation and oxidative stress are common pathways in the development of liver fibrosis and diabetes.14,15 There is an association between progressive liver fibrosis, evaluated by transient elastography, and microangiopathy and macroangiopathy in patients with type 2 diabetes.16–18 Thus, it is possible that M2BPGi levels are associated with diabetic complications, particularly microangiopathy and macroangiopathy. However, such an association has not been investigated in previous studies. Therefore, in this cross-sectional study of patients with type 2 diabetes, we investigated these associations.

MATERIALS AND METHODS
Participants and study design
The KAMOGAWA cohort study has been performed by us since 2014 on patients with diabetes.19 Medical data were collected after obtaining informed consent of individuals whose identity was kept secret and compiled into a database. In this cross-sectional study, we selected data of people with type 2 diabetes who attended the outpatient clinic at the Kameoka Municipal Hospital (Kameoka, Japan) or the Kyoto Prefectural University of Medicine (Kyoto, Japan) from January 2016 to May 2018. Data pertaining to the medical history of the participants and their usage of medications were collected. In this study, we only included those people who had their serum levels of M2BPGi checked and excluded those who did not have their urine albumin excretion (UAE) checked.

Biochemical analyses and definitions
The formula used for calculating the body mass index (BMI) was: BMI=weight (kg)/[height (m)]2. Diagnosis of type 2 diabetes was performed based on the Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus.20 The study cohort was divided into non-smokers and current-smokers, based on their declarations in a self-administered questionnaire. Similarly, data of alcohol consumption per week over the past 1 month were collected from a self-administered questionnaire. Similarly, data of alcohol consumption per week over the past 1 month were collected. Regular exercisers were defined as people who performed any type of sports activity at least once a week.21 Participants were categorized into non-exercisers or regular exercisers based on their declaration in the questionnaire. Also, data of the activities undertaken by the participants were collected. Regular exercisers were defined as people who performed any type of sports activity at least once a week.22 Venous blood was collected from the participants and high-density lipoprotein (HDL) cholesterol, triglycerides, uric acid, creatinine (Cr), aspartate aminotransferase (AST), alanine transaminase (ALT), and platelets were evaluated. Serum M2BPGi levels were evaluated by the M2BPGi reagent and expressed as cut-off index (C.O.I.) units.23 High-performance liquid chromatography was used to determine hemoglobin A1c levels. The estimated glomerular filtration rate (eGFR) was calculated by the equation of the Japanese Society of Nephrology: eGFR (mL/min/1.73 m2)=194 × serum Cr1.094 × age−0.287 (if women, ×0.739).24 An immuno turbidimetric method was used for UAE evaluation: UAE (mg/g Cr)=the urinary albumin concentration (mg/L)/the urinary Cr concentration (g/L). The mean value was calculated using three UAE values. The fibrosis-4 (FIB4) index was calculated by age (years)×AST (IU/L)/platelets (109/L)×ALT (IU/L).25 NAFLD fibrosis score was calculated by −1.675 + (0.037×Age (years)) + (0.99×AST/ALT) + (1.13×IFG/diabetes [yes=1, no=0]) + (0.99×AST/ALT) – (0.013×platelet [109/L]) – (0.66×albumin (g/dL)).26

In this study, diabetic microangiopathy was defined as the presence of diabetic nephropathy and/or retinopathy. The nephropathy stage was defined as follows: UAE less than 30 mg/g Cr was normoalbuminuria, 30–300 mg/g Cr was microalbuminuria, and more than 300 mg/g Cr was macroalbuminuria.27 Retinopathy was divided into three groups: no diabetic-retinopathy (NoDR), non-proliferative diabetic retinopathy (NPDR), and proliferative diabetic retinopathy (PDR).28 We defined diabetic macroangiopathy as a history of cardiovascular disease (CVD), such as coronary heart disease, cerebral hemorrhage, or ischemic stroke, which was gathered from the medical records.29 Acute myocardial infarction, unstable angina, and silent myocardial infarction, but not stable angina pectoris, were included as coronary heart diseases.29 Patients with an eGFR <60 mL/min/1.73 m2 were defined as having chronic kidney disease (CKD). After at least a 5 min rest in a quiet space, blood pressures were evaluated twice using a HEM-906 device (Omron Healthcare, Lake Forest, Illinois, USA). We used an average of two values for this study.

Medication data were also collected. Specifically, medications for diabetes, including sodium-glucose cotransporter two inhibitors, glucagon-like peptide-1 receptor agonists, and insulin; medications for hypertension, including reninangiotensin-aldosterone system (RAAS) inhibitors; and medications for dyslipidemia, including statins were collected.

Statistical analyses
JMP V.13.2 software (SAS Institute, Cary, North Carolina, USA) was used for statistical analyses and GraphPad Prism V.8.4.2 software (GraphPad Software, La Jolla, California, USA) was used for creation figures. Means, medians, and frequencies of variables were calculated. Continuous variables are shown as means±SD or medians (IQR). Categorized variables are shown as a number. P<0.05 was set as statistically significant.

Because UAE, duration of diabetes, triglycerides, and M2BPGi were skewed variables, logarithmic transformation was executed before doing correlation, and multiple or logistic regression analyses. Pearson’s correlation was calculated to evaluate relationships between logarithms (M2BPGi+1) and other variables. Differences in serum levels of M2BPGi among groups were evaluated by Kruskal-Wallis and Steel-Dwass tests.

Relationships between the logarithm of UAE and logarithm (M2BPGi+1) were determined by multiple regression analyses with the following elements chosen as
independent factors: sex, age, BMI, duration of diabetes, smoking status, systolic blood pressure, hemoglobin A1c, triglycerides, HDL-cholesterol, uric acid, and Cr; and treatment with insulin, RAAS system inhibitors, statins, and platelets.

Last, receiver operator characteristic (ROC) analyses were performed to calculate area under the ROC curve (AUC) of serum levels of M2BPGi for diabetic nephropathy, defined as the presence of microalbuminuria or macroalbuminuria, retinopathy, defined as the presence of NPDR or PDR, and macroangiopathy.

RESULTS

In this study, 365 people with type 2 diabetes were selected. Among these individuals, two for whom there were no UAE data were excluded. Thus, 363 people were enrolled for this study.

Table 1 shows the clinical characteristics data. The mean age was 66.4 (10.6) years, the median serum M2BPGi level was 0.77 (0.57 to 1.04) C.O.I., and the median UAE was 22 (9 to 82.1) mg/g Cr. There were 207 people with normoalbuminuria, 107 people with microalbuminuria, and 49 with macroalbuminuria. Moreover, there were 285 people with NDR, 44 with SDR, and 34 with PDR. Forty-nine people had a history of CVD.

Table 2 reports simple correlations between serum levels of M2BPGi and other factors. Age, duration of diabetes, BMI, systolic blood pressure, Cr level, log UAE, FIB4 index, and NAFLD fibrosis score were positively associated with logarithms (M2BPGi+1), whereas HDL-cholesterol, eGFR, and platelets were negatively associated with logarithms (M2BPGi).

Table 3 reports comparisons of the serum levels of M2BPGi in various groups. Insulin usage, retinopathy stage, nephropathy stage, CKD, and history of CVD were associated with higher M2BPGi levels.

Serum levels of M2BPGi in patients with microalbuminuria and macroalbuminuria were higher than in those with normoalbuminuria (figure 1). In addition, serum levels of M2BPGi in patients with NPDR and PDR were higher than in those with NoDR (figure 1).

The results of multiple linear regression analyses of the log UAE values are reported in table 4. The logarithm values of (M2BPGi+1) (standardized β=0.104, p=0.042), HbA1c (standardized β=0.169, p<0.001), Cr (standardized β=0.312, p<0.001), HDL-cholesterol (standardized β=0.137, p=0.004), systolic blood pressure (standardized β=0.144, p=0.002), RAAS inhibitor usage (standardized β=0.138, p=0.005), and exercise habit (standardized β=−0.112, p=0.017) were associated with log UAE values after adjusting for covariates.

The results of ROC and AUC of M2BPGi for diabetic nephropathy, retinopathy and macroangiopathy are shown in figure 2. The optimal cut-off point of M2BPGi for diabetic nephropathy, retinopathy, and macroangiopathy was 0.95, 0.91, and 0.78, respectively.

DISCUSSION

This study clarifies the relationship between the serum M2BPGi levels and diabetic microangiopathy and macroangiopathy in patients with type 2 diabetes. This is the first study, to our knowledge, to reveal the association between M2BPGi levels and diabetic microangiopathy and macroangiopathy.
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Microangiopathy and macroangiopathy in people with type 2 diabetes.

Possible interpretations for the connection between M2BPGi levels and diabetic microangiopathy and macroangiopathy are as follows. M2BPGi is a marker of liver fibrosis, although the mechanism of the association between M2BPGi and liver fibrosis is not fully understood. Cross-sectional studies and retrospective cohort study reported that there was an association between progressive liver fibrosis, evaluated by transient elastography, and microangiopathy and macroangiopathy in people with type 2 diabetes. In fact, liver fibrosis is a mortality risk from CVD in people with NAFLD.

Both liver fibrosis and diabetic microangiopathy or macroangiopathy are associated with chronic inflammation, including tumor necrosis factor-α (TNF-α), the RAAS, and intercellular adhesion molecule-1. The cytotoxicity induced by TNF-α stimulates apoptosis of glomerular cells and, consequently, results in the progression of albuminuria and CVD. In addition, TNF-α is associated with diabetic retinopathy through

Table 2 Correlations between logarithmic (M2BPGi+1) and other variables

| Variables                              | r     | P value  |
|----------------------------------------|-------|----------|
| Age                                    | 0.240 | <0.001   |
| Logarithmic (duration of diabetes+1)   | 0.149 | 0.005    |
| Body mass index                        | 0.116 | 0.027    |
| Systolic blood pressure                | 0.118 | 0.025    |
| Diastolic blood pressure               | 0.031 | 0.559    |
| Hemoglobin A1c                         | 0.056 | 0.287    |
| Logarithmic triglycerides              | 0.009 | 0.865    |
| HDL-cholesterol                        | −0.160| 0.002    |
| Uric acid                              | 0.102 | 0.054    |
| Creatinine                             | 0.129 | 0.014    |
| eGFR                                   | −0.218| <0.001   |
| Logarithmic UAE                        | 0.216 | <0.001   |
| Alanine transaminase                   | 0.072 | 0.173    |
| Platelets                              | −0.239| <0.001   |
| FIB4 index                             | 0.122 | 0.021    |
| NAFLD fibrosis score                   | 0.341 | <0.001   |

Correlations between M2BPGi and other variables were evaluated by Pearson correlations. eGFR, estimated glomerular filtration rate; FIB4, fibrosis 4; HDL, high-density lipoprotein; M2BPGi, Mac-2 binding protein glycosylation isomer; NAFLD, non-alcoholic fatty liver disease; UAE, urinary albumin excretion.

Table 3 Comparisons of M2BPGi in various groups

| Variables                              | M2BPGi (C.O.I) | P value  |
|----------------------------------------|----------------|----------|
| Sex (male/female)                      | 0.71 (0.54-0.92) | 0.002    |
| Smoking (non-/current-smoker)          | 0.83 (0.61-1.18) | 0.08     |
| Exercise habit (no/yes)                | 0.77 (0.57-1.07) | 0.003    |
| Habit of drinking alcohol (no/yes)     | 0.76 (0.58-1.07) | 0.001    |
| RAAS inhibitor usage (no/yes)          | 0.75 (0.57-1.07) | 0.001    |
| Statins (no/yes)                       | 0.77 (0.57-1.07) | 0.001    |
| Insulin treatment (no/yes)             | 0.75 (0.56-1.00) | 0.015    |
| Nephropathy (normo/micro/macroalbuminuria) | 0.71 (0.54-0.92) | <0.001   |
| Retinopathy (NoDR/NPDR/PDR)            | 0.73 (0.56-0.99) | <0.001   |
| Chronic kidney disease (no/yes)        | 0.73 (0.54-0.99) | <0.001   |
| History of cardiovascular disease (no/yes) | 0.76 (0.55-1.03) | 0.019    |

Differences among the groups were evaluated by Kruskal-Wallis test. *p <0.05 vs. normoalbuminuria by the Steel Dwass test.
†p <0.05 vs. NDR by the Steel Dwass test.
M2BPGi, Mac-2 binding protein glycosylation isomer; NoDR, no diabetic retinopathy; NPDR, non-proliferative diabetic retinopathy; PDR, proliferative diabetic retinopathy; RAAS, renin-angiotensin-aldosterone system.
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reduced adherence of leukocytes to retinal blood vessels, blood-retinal barrier breakdown, and apoptosis of retinal cells. Moreover, activation of the RAAS occurs in liver fibrosis, which promotes inflammation through enhanced production of reactive oxygen species leading to hypertrophy and renal fibrosis. In addition, RAAS inhibitors are protective effectors for diabetic retinopathy. Furthermore, intercellular adhesion molecule-1 is associated with liver fibrosis, and diabetic microangiopathy and macroangiopathy. Thus, M2BPGi, which is a surrogate marker of liver fibrosis, has a close association with diabetic microangiopathy and macroangiopathy.

Previous studies revealed an association between progressive liver fibrosis and diabetic microangiopathy or macroangiopathy in patients with type 2 diabetes. To detect liver fibrosis, a liver biopsy is the gold standard; however, this is a highly invasive procedure. M2BPGi is a non-invasive new serological glycobiomarker for liver fibrosis. In this study, a history of CVD, duration of diabetes, severity of microangiopathy, systolic blood pressure, HDL-cholesterol, and BMI were associated with the serum levels of M2BPGi. These results were similar to those of previous studies. Furthermore, serum levels of M2BPGi are higher in patients with chronic heart failure.

In this study, we showed that the cut-off points of diabetic nephropathy, retinopathy, and macroangiopathy were 0.95, 0.91, and 0.78, respectively. These cut-off points were lower than that of chronic hepatitis (M2BPGi≥1.00). M2BPGi, which is easy to measure in clinical practice, could be an indicator to prevent future

### Table 4 Multiple linear regression analyses of UAE logarithms

| Variables                                      | β    | SE     | 95% CI Lower | 95% CI Upper | Standardized β | P value |
|------------------------------------------------|------|--------|--------------|--------------|----------------|---------|
| (Constant)                                     | -4.921 | 1.250  | -7.380       | -2.462       | -               | <0.001  |
| Age                                            | 0.017 | 0.009  | 0.0002       | 0.034        | 0.111           | 0.048   |
| Female                                         | 0.162 | 0.176  | -0.184       | 0.509        | 0.066           | 0.358   |
| Body mass index                                | 0.026 | 0.022  | -0.016       | 0.069        | 0.066           | 0.223   |
| Logarithms (duration of diabetes +1)           | 0.071 | 0.115  | -0.155       | 0.297        | 0.033           | 0.538   |
| HbA1c                                          | 0.215 | 0.062  | 0.093        | 0.336        | 0.169           | <0.001  |
| Creatinine                                     | 0.017 | 0.003  | 0.011        | 0.023        | 0.312           | <0.001  |
| Uric acid                                      | 0.002 | 0.001  | -0.0007      | 0.004        | 0.077           | 0.155   |
| Logarithms triglycerides                       | 0.329 | 0.167  | -0.004       | 0.812        | 0.107           | 0.052   |
| HDL-cholesterol                                | 0.421 | 0.206  | 0.016        | 0.827        | 0.137           | 0.004   |
| Systolic blood pressure                        | 0.012 | 0.004  | 0.004        | 0.021        | 0.144           | 0.002   |
| RAAS inhibitor usage                           | 0.460 | 0.162  | 0.142        | 0.778        | 0.138           | 0.005   |
| Statins usage                                  | 0.024 | 0.165  | -0.301       | 0.349        | 0.007           | 0.885   |
| Insulin treatment                              | 0.224 | 0.189  | -0.148       | 0.596        | 0.057           | 0.238   |
| Exercise habit                                 | -0.371| 0.156  | -0.676       | -0.066       | -0.112          | 0.017   |
| Smoker                                         | 0.352 | 0.218  | -0.076       | 0.780        | 0.076           | 0.107   |
| Platelets                                      | -0.004| 0.013  | -0.031       | 0.022        | -0.016          | 0.742   |
| Logarithms (M2BPGi+1)                          | 0.708 | 0.347  | 0.024        | 1.391        | 0.104           | 0.042   |

R²=0.31.

HDL, high-density lipoprotein; M2BPGi, Mac-2 binding protein glycosylation isomer; RAAS, renin-angiotensin-aldosterone system; UAE, urinary albumin excretion.

Figure 2 ROC curve and AUC. (A) ROC curve and AUC of M2BPGi for diabetic nephropathy. The optimal cut-off point of the M2BPGi for diabetic nephropathy, defined as the presence of microalbuminuria or macroalbuminuria, was 0.95 (AUC 0.62 (95% CI 0.56 to 0.68), sensitivity=0.44, specificity=0.77, P<0.001). (B) ROC curve and AUC of M2BPGi for diabetic retinopathy. The optimal cut-off point of the M2BPGi for diabetic retinopathy, defined as the presence of non-proliferative diabetic retinopathy or proliferative diabetic retinopathy, was 0.91 (AUC 0.64 (95% CI 0.57 to 0.71), sensitivity=0.58, specificity=0.59, P<0.001). (C) ROC curve and AUC of M2BPGi for diabetic macroangiopathy. The optimal cut-off point of the M2BPGi for diabetic macroangiopathy was 0.78 (AUC 0.60 (95% CI 0.52 to 0.68), sensitivity=0.63, specificity=0.54, P<0.001). AUC, area under the ROC curve; M2BPGi, mac-2 binding protein glycosylation isomer; ROC, receiver operating characteristic.
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diabetic microangiopathy or macroangiopathy, and we should pay attention to people with high M2BPGi and improve their metabolic parameters.

The current study has some limitations. First, the study design was cross-sectional. Thus, the causal relationship between the M2BPGi levels and diabetic microangiopathy and macroangiopathy is unclear. Additional prospective studies are needed to establish the association between M2BPGi and the progression or improvement of diabetic microangiopathy or macroangiopathy. Second, we did not perform liver biopsies; thus, we were unable to compare M2BPGi levels with histology data. Third, we did not have liver histology and fibrosis information, nor data on the presence of a hepatitis virus. Such data are important for assessing the degree of liver steatosis and fibrosis and causes of liver diseases. Last, the study population was comprised of only Japanese people; therefore, it is not clear whether the findings of this study can be applied to other ethnic groups.

Despite these limitations, this study reveals, for the first time, the relationship between M2BPGi levels and diabetic microangiopathy and macroangiopathy in people with type 2 diabetes. This study also shows that M2BPGi is independently associated with an increased risk of albuminuria.

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REFERENCES
1. Haffner SM, Lehto S, Ronnemaa T, et al. Mortality from coronary heart disease in subjects with type 2 diabetes and in nondiabetic subjects with and without prior myocardial infarction. N Engl J Med 1998;339:229–34.
2. Miyake H, Kanazawa I, Sugimoto T. Albuminuria increases all-cause mortality in Japanese patients with type 2 diabetes mellitus. J Clin Med 2018;7:23.
3. Mantovani A, Byrne CD, Bonora E, et al. Nonalcoholic fatty liver disease and risk of incident type 2 diabetes: a meta-analysis. Diabetes Care 2018;41:372–82.
4. Okonura T, Hashimoto M, Muro M, et al. Ectopic fat obesity presents the greatest risk for incident type 2 diabetes: a population-based longitudinal study. Int J Obes 2019;43:139–48.
5. Fukuda T, Hamaguchi M, Kojima T, et al. Transient remission of nonalcoholic fatty liver disease decreases the risk of incident type 2 diabetes mellitus in Japanese men. Eur J Gastroenterol Hepatol 2016;28:1443–9.
6. Matteoni CA, Younossi ZM, Gramlich T, et al. Nonalcoholic fatty liver disease: a spectrum of clinical and pathological severity. Gastroenterology 1999;116:1413–9.
7. Younossi ZM, Golabi P, de Avila L, et al. The global epidemiology of NAFLD and NASH in patients with type 2 diabetes: a systematic review and meta-analysis. J Hepatol 2019;71:793–801.
8. Tripolino C, Itrace C, Cutruzzola A, et al. Hepatic steatosis index is associated with type 1 diabetes complications. Diabetes Metab Syndr Obes 2019;12:405–10.
9. Targer G, Bertolini L, Rodella S, et al. Non-alcoholic fatty liver disease is independently associated with an increased prevalence of chronic kidney disease and proliferative/laser-treated retinopathy in type 2 diabetic patients. Diabetologia 2008;51:44–50.
10. Yan L-H, Mu B, Guan Y, et al. Assessment of the relationship between non-alcoholic fatty liver disease and diabetic complications. J Diabetes Investig 2016;7:889–94.
11. Kuno A, Ikehara Y, Tanaka Y, et al. A serum “sweet-doughnut” protein facilitates fibrosis evaluation and therapy assessment in patients with viral hepatitis. Sci Rep 2013;3:1065.
12. Shirabe K, Bekki Y, Gantumur D, et al. Mac-2 binding protein glycan isomer (M2BPGi) is a new serum biomarker for assessing liver fibrosis: more than a biomarker of liver fibrosis. J Gastroenterol 2018;53:819–28.
13. Abe M, Miyake T, Kuno A, et al. Association between Wisteria floribunda aglutinin-positive Mac-2 binding protein and the fibrosis stage of non-alcoholic fatty liver disease. J Gastroenterol 2015;50:776–84.
14. Diehl AM, Li ZP, Lin HZ, et al. Cytokines and the pathogenesis of non-alcoholic steatohepatitis. Gastroenterology 2015;148:1222–31.
15. Okuno Y, Fukushima A, Hashimoto E, et al. Oxidative stress inhibits healthy adipose expansion through suppression of SREBF1-Mediated lipogenic pathway. Diabetes 2018;67:1113–27.
16. Yeung M-W, Wong GL-H, Choi KC, et al. Advanced liver fibrosis but not steatosis is independently associated with albuminuria in Chinese patients with type 2 diabetes. J Hepatol 2018;69:833–41.
17. Lombrard R, Airaghi L, Targher G, et al. Liver fibrosis by FibroScan® independently of established cardiovascular risk parameters associates with macrovascular and microvascular complications in patients with type 2 diabetes. Liver Int 2020;40:347–54.
18. Kitagawa N, Hashimoto Y, Hamaguchi M, et al. Liver stiffness is associated with progression of albuminuria in adults with type 2 diabetes: nonalcoholic fatty disease cohort study. Can J Diabetes Care 2020;44:428–33.
19. Sakai R, Hashimoto Y, Ushigome E, et al. Late-night-dinner is associated with poor glycemic control in people with type 2 diabetes; the KAMOGAWA-DW cohort study. Endocr J 2018;65:395–402.
20. American Diabetes Association. 2. classification and diagnosis of diabetes: Standards of Medical Care in Diabetes–2018. Diabetes Care 2018;41:13–27.
21. Kaji A, Hashimoto Y, Sakai R, et al. Frequent usage of convenience stores is associated with low diet quality. Nutrients 2019;11:E1212.
Okamura T, Hashimoto Y, Hamaguchi M, et al. Short sleep duration is a risk of incident nonalcoholic fatty liver disease: a population-based longitudinal study. *J Gastrointestin Liver Dis* 2019;28:73–81.

Yamasaki K, Tateyama M, Abug S, et al. Elevated serum levels of Wisteria floribunda agglutinin-positive human Mac-2 binding protein predict the development of hepatocellular carcinoma in hepatitis C patients. *Hepatology* 2014;60:1563–70.

Matsu S, Imai E, Horio M, et al. Revised equations for estimated GFR from serum creatinine in Japan. *Am J Kidney Dis* 2009;53:982–92.

Sumida Y, Yonedo M, Hyogo H, et al. Validation of the FIB4 index in a Japanese nonalcoholic fatty liver disease population. *BMC Gastroenterol* 2012;12:2.

Angulo P, Hui JM, Marchesini G, et al. The NAFLD fibrosis score: a noninvasive system that identifies liver fibrosis in patients with NAFLD. *Hepatology* 2007;45:846–54.

Hashimoto Y, Tanaka M, Sennaru T, et al. Heart rate-corrected QT interval is a novel risk marker for the progression of albuminuria in people with Type 2 diabetes. *Diabet Med* 2015;32:1221–6.

Wu H, Hwang D-K, Song X, et al. Association between aqueous cytokines and diabetic retinopathy stage. *J Ophthalmol* 2017;2017:9402198.

Yoshitaka H, Hamaguchi M, Kojima T, et al. Nonoverweight nonalcoholic fatty liver disease and incident cardiovascular disease: a post hoc analysis of a cohort study. *Medicine* 2017;96:e6712.

Ekstedt M, Hagström H, Naur P, et al. Fibrosis stage is the strongest predictor for disease-specific mortality in NAFLD after up to 33 years of follow-up. *Hepatology* 2015;61:1547–54.

Tilg H, Moschen AR. Adipocytokines: mediators linking adipose tissue, inflammation and immunity. *Nat Rev Immunol* 2006;6:772–83.

Van Gaal LF, Mertens IL, De Block CE. Adipocytokines: mediators linking adipose tissue, inflammation and immunity. *Nat Rev Immunol* 2006;6:772–83.

Sumida Y, Yoneda M, Hyogo H, et al. Validation of the FIB4 index in a Japanese nonalcoholic fatty liver disease population. *BMC Gastroenterol* 2012;12:2.

Okamura T, Hashimoto Y, Hamaguchi M, et al. Short sleep duration is a risk of incident nonalcoholic fatty liver disease: a population-based longitudinal study. *J Gastrointestin Liver Dis* 2019;28:73–81.

Yamasaki K, Tateyama M, Abug S, et al. Elevated serum levels of Wisteria floribunda agglutinin-positive human Mac-2 binding protein predict the development of hepatocellular carcinoma in hepatitis C patients. *Hepatology* 2014;60:1563–70.

Matsu S, Imai E, Horio M, et al. Revised equations for estimated GFR from serum creatinine in Japan. *Am J Kidney Dis* 2009;53:982–92.

Sumida Y, Yonedo M, Hyogo H, et al. Validation of the FIB4 index in a Japanese nonalcoholic fatty liver disease population. *BMC Gastroenterol* 2012;12:2.

Angulo P, Hui JM, Marchesini G, et al. The NAFLD fibrosis score: a noninvasive system that identifies liver fibrosis in patients with NAFLD. *Hepatology* 2007;45:846–54.

Hashimoto Y, Tanaka M, Sennaru T, et al. Heart rate-corrected QT interval is a novel risk marker for the progression of albuminuria in people with Type 2 diabetes. *Diabet Med* 2015;32:1221–6.

Wu H, Hwang D-K, Song X, et al. Association between aqueous cytokines and diabetic retinopathy stage. *J Ophthalmol* 2017;2017:9402198.

Yoshitaka H, Hamaguchi M, Kojima T, et al. Nonoverweight nonalcoholic fatty liver disease and incident cardiovascular disease: a post hoc analysis of a cohort study. *Medicine* 2017;96:e6712.

Ekstedt M, Hagström H, Naur P, et al. Fibrosis stage is the strongest predictor for disease-specific mortality in NAFLD after up to 33 years of follow-up. *Hepatology* 2015;61:1547–54.

Tilg H, Moschen AR. Adipocytokines: mediators linking adipose tissue, inflammation and immunity. *Nat Rev Immunol* 2006;6:772–83.

Van Gaal LF, Mertens IL, De Block CE. Mechanisms linking obesity with cardiovascular disease. *Nature* 2006;444:875–80.

Navarro-González JF, Mora-Fernández C, Muros de Fuentes M, et al. Inflammatory molecules and pathways in the pathogenesis of diabetic nephropathy. *Nat Rev Nephrol* 2011;7:327–40.

Navarro JF, Mora C, Gómez M, et al. Influence of renal involvement on peripheral blood mononuclear cell expression behaviour of tumour necrosis factor-α and interleukin-6 in type 2 diabetic patients. *Nephrol Dial Transplant* 2008;23:919–26.

Tang J, Kern TS. Inflammation in diabetic retinopathy. *Prog Retin Eye Res* 2011;30:343–58.

Osterreicher CH, Taura K, De Minicis S, et al. Angiotensin-converting-enzyme 2 inhibits liver fibrosis in mice. *Hepatology* 2009;50:929–38.

Unger T. The role of the renin-angiotensin system in the development of cardiovascular disease. *Am J Cardiol* 2002;89:3–9.

Kästelän T, Tomii M, Gverovč Antunica A, et al. Inflammation and pharmacological treatment in diabetic retinopathy. *Mediators Inflamm* 2013;2013:213130.

Migita K, Horai Y, Kozuru H, et al. Serum cytokine profiles and Mac-2 binding protein glycosylation isomer (M2BPGi) level in patients with autoimmune hepatitis. *Medicine* 2018;97:e13450.

Khalfaoui T, Lizard G, Ouertani-Meddeb A. Adhesion molecules (ICAM-1 and VCAM-1) and diabetic retinopathy in type 2 diabetes. *J Mol Histol* 2008;39:243–9.

Gross MD, Bielinski SJ, Suarez-Lopez JR, et al. Circulating soluble intercellular adhesion molecule 1 and subclinical atherosclerosis: the coronary artery risk development in young adults study. *Clin Chem* 2012;58:411–20.

Sugiura T, Dohi Y, Takase H, et al. Serum levels of Mac-2 binding protein increase with cardiovascular risk and reflect silent atherosclerosis. *Atherosclerosis* 2016;251:192–6.

Ninaga R, Yamamoto H, Yoshii M, et al. Marked elevation of serum M2BP-adiponectin complex in men with coronary artery disease. *Atherosclerosis* 2016;253:70–4.

Arai T, Atsukawa M, Tsubota A, et al. Factors influencing subclinical atherosclerosis in patients with biopsy-proven nonalcoholic fatty liver disease. *PLoS One* 2019;14:e0224184.

Sugiura T, Dohi Y, Takase H, et al. Serum levels of Mac-2 binding protein increase with cardiovascular risk and reflect silent atherosclerosis. *Atherosclerosis* 2016;251:192–6.

Ninaga R, Yamamoto H, Yoshii M, et al. Marked elevation of serum M2BP-adiponectin complex in men with coronary artery disease. *Atherosclerosis* 2016;253:70–4.

Arai T, Atsukawa M, Tsubota A, et al. Factors influencing subclinical atherosclerosis in patients with biopsy-proven nonalcoholic fatty liver disease. *PLoS One* 2019;14:e0224184.

Sugiura T, Dohi Y, Takase H, et al. Factors associated with longitudinal changes in serum concentrations of Mac-2 binding protein: a prospective 3-year observational study. *Nutr Metab Cardiovasc Dis* 2019;29:1337–44.

Okada A, Kanzaki H, Hamatani Y, et al. Increased serum Wisteria floribunda agglutinin positive Mac-2 binding protein (Mac-2 binding protein glycosylation isomer) in chronic heart failure: a pilot study. *Heart Vessels* 2018;33:385–92.