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Cross-sectional study of the role of age, gender and ethnicity in the association between visceral adiposity index and nonalcoholic fatty liver disease among U.S. adults (NHANES 2003–2018)

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Cross-sectional study of the role of age, gender and ethnicity in the association between visceral adiposity index and nonalcoholic fatty liver disease among U.S. adults (NHANES 2003–2018)

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Abstract

Objectives: The ability of visceral adiposity index (VAI) to predict the prevalence of nonalcoholic fatty liver disease (NAFLD) has not been fully determined. Here, we aimed to explore the association between VAI and NAFLD in the general U.S. population, and further investigate potential population who could use VAI to predict the prevalence of NAFLD.

Design: Cross-sectional population-based study.

Setting: The National Health and Nutrition Examination Survey (2003–2018).

Participants: A total of 7,545 participants aged 20 years or older who have complete information for NAFLD assessment test were included in this study.

Primary and secondary outcome measures: NAFLD was assessed by the modified fatty liver index for the U.S. population (USFLI) using a cut-off point of 30. Correlation between VAI and NAFLD prediction score was calculated using Spearman correlation analysis. Logistic regression models were further used to estimate ORs and 95% CIs.

Results: Spearman correlation analysis indicated that VAI scores were positively correlated USFLI ($r=0.54$, $P=0.000$ for both genders). In a comparison of highest versus the lowest quartiles of VAI, multivariate logistic regression analysis demonstrated a positive association between VAI and NAFLD (OR=2.57, 95%CI=1.72–3.83 for men and OR=3.42, 95%CI=2.08–5.62 for women). The
stratified analyses indicated that the positive association was observed in man
with age < 55 years and women with age ≥ 40 years, and existed in Hispanic and
non-Hispanic White population but not in non-Hispanic Black population. In
addition, the positive associations were consistently seen in subgroups stratified
by insulin resistance and several metabolic diseases. However, no significant
association was found between VAI and hepatic fibrosis.

**Conclusion:** VAI might be a useful predictive model for NAFLD, but not for
hepatic fibrosis among U.S. adults, and there exist age-gender specific and ethnic
difference.

**Keywords:** Public health, epidemiology, hepatology

**Strengths and limitations of this study:**

✓ This is a large population-based analysis using well-examined nationwide
data, and the findings could be generalized for most U.S. populations.

✓ We provided solid evidence of an independent association between VAI and
NAFLD by performing multiple logistic regression.

✓ We conducted the stratified analyses to identify the appropriate population
who could use VAI to predict the prevalence of NAFLD.

✓ Although we used well-validated NAFLD and fibrosis models, the
information about liver imaging were not available, which meant that NAFLD
and NAFLD related fibrosis may be misclassified.
The cross-sectional nature of our study design meant that we could not investigate the longitudinal dynamic association between progression of NAFLD and changes in VAI levels across several therapeutic interventions.

1. Introduction

With an accelerated pace of nutrition transition, non-alcoholic fatty liver disease (NAFLD) has become an emerging public health issue with high prevalence worldwide, affecting up to one third of the population,[1] and its incidence is expected to rise rapidly in the future alongside increasing rates of obesity.[2] According to epidemiological data, nearly a quarter of NAFLD patients would progress to steatohepatitis with fibrosis, which could lead to serious liver-related complications and death.[3, 4] On the other hand, NAFLD is closely related to higher rates of several cardiometabolic disorders, including diabetes mellitus, metabolic syndrome, and cardiovascular diseases (CVD).[2, 5] Thus, the increasing prevalence of NAFLD is particularly life threatening. It is important to identify modifiable risk factors for NAFLD for reducing the disease burden.

Currently, the pathogenesis of NAFLD has not been completely understood, and increasing evidences suggest that visceral fat accumulation plays a crucial role in the pathogenesis of NAFLD.[6, 7] In recent years, the visceral adiposity index (VAI) based on waist circumference (WC), body mass index (BMI), plasma triglycerides (TG) and high-density lipoprotein cholesterol (HDL-C) has been
proposed as a reliable marker to assess the content and function of visceral fat.[8] Although VAI has been proved to be a powerful indicator of type 2 diabetes mellitus (T2DM),[9] metabolic syndrome (MetS)[10] and cardiovascular events.[11] There are controversial data regarding the association between VAI and NAFLD. A prior study reported that VAI is related with significant fibrosis in NAFLD patients,[12] while other researchers revealed that the association didn’t exist in obese or non-diabetes subjects with NAFLD.[13, 14] In addition to the small sample size, variations in the participants with or without additional metabolic disorders are largely contributed to these conflicting results. According to current evidence, insulin resistance (IR) is related to NAFLD,[15] while VAI is a valuable indicator of IR.[8] As yet it is unknown whether the status of insulin sensitivity is the result of the controversy among NAFLD patients underlying different clinical status. Furthermore, variations in genetic background may be another explanation for these controversial results in NAFLD.[16] Two recent cohort studies with large scale population conducted in China and Japan implied that higher VAI levels are correlated with an increased incidence of NAFLD in Asian population,[17, 18] while similar study has not been done or published in the U.S.. In this study, we intend to investigate whether VAI and NAFLD is significantly associated in the U.S. general population, independent of IR and its related metabolic disorders, using the data from National Health and Nutrition Examination Surveys (NHANES).
2. Methods

The design, implementation, analysis, and reporting of this study were conducted in accordance with the STROBE statement.[19] Page of each checklist item was listed in Supplemental Material.

2.1. Study Population

The NHANES is a multistage, ongoing, complex cross-sectional health examination and survey designed to collect the health data of the U.S. non-institutionalized civilian population. The survey was approved by the National Center for Health Statistics ethics review board, and was conducted by the Centers for Disease Control and Prevention (CDC). All participants provided written informed consent. The data can be freely available from the NHANES website public archive. Information regarding interview processes, examination protocols and sample collection can also be found in the website.[20] Given that the information about fasting plasma glucose (FPG) and insulin were available since 2003, data from 2003-2018 were obtained for analysis.

Of all participants, we initially selected nonpregnant subjects aged 20 years or older. Of these, we excluded individuals missing the information about anthropometric parameters (BMI and WC) and blood pressure. Subsequently, we excluded individuals with the following reasons: excessive alcohol consumption (defined by > 1 drink/day for women or > 2 drinks/day for men), viral hepatitis...
(defined by positive serum hepatitis B or C antibody and/or positive serum hepatitis B surface antigen), and missing laboratory data to rule in or rule out the presence of NAFLD. Finally, 7,545 subjects were included (Fig 1).

2.2. Assessment of VAI

We calculated VAI using the following formulas:[8]

\[
VAI = \frac{WC \text{ [cm]}}{39.68 + (1.88 \times BMI \text{ [kg/m}^2\text{]}))} \times \frac{TG \text{ [mmol/L]}}{1.03} \times \frac{1.31}{HDL \text{ [mmol/L]}} \text{ for males} \tag{1}
\]

\[
VAI = \frac{WC \text{ [cm]}}{36.58 + (1.89 \times BMI \text{ [kg/m}^2\text{]}))} \times \frac{TG \text{ [mmol/L]}}{0.81} \times \frac{1.52}{HDL \text{ [mmol/L]}} \text{ for females} \tag{2}
\]

2.3. Definitions of covariates

Ethnicity was categorized as non-Hispanic white, non-Hispanic black, or Hispanic. High education was defined as completing high school degree or above. Current smokers were defined as the participants smoked at least 100 cigarettes lifetime and now smoke cigarettes every day or some days. Similarly, current drinkers were defined as the participants who had at least 12 alcohol drinks entire life and now drink alcohol every day or some days. Physical activity was defined as engaging in moderate or vigorous exercise on a regular basis (≥20 minutes at a time and at least three times per week).[21]

Regarding the influence of IR and related metabolic complications on the relationship between VAI and NAFLD, the homeostasis model assessment of IR (HOMA-IR) and several diseases were calculated and defined. HOMA-IR was
defined as fasting glucose [mg/dL] \times \text{fasting insulin [μU/ml]}/405. Obese participants were defined as those with BMI ≥30 kg/m² for non-Asians.[22] T2DM was defined based on fasting glucose (≥126 mg/dL) and/or receiving insulin or oral hypoglycemic therapy. Hypertension was defined as systolic pressure ≥140 mm Hg and/or diastolic pressure ≥90 mm Hg, or antihypertensive therapy.[23] MetS was defined according to the National Cholesterol Education Program Adult Treatment Panel III report, as an individual who having three or more of all criteria.[24] CVD were defined as the composite of self-report history of stroke, myocardial infarction, coronary revascularization procedure, angina, and congestive heart failure.[25]

2.4. Definitions of NAFLD and liver fibrosis

The definition of NAFLD was based on the US fatty liver index (USFLI).[26] The cut-off of 30 was used to define NAFLD. Furthermore, the presence of fibrosis among individuals with NAFLD was assessed using NAFLD fibrosis score (NFS).[27] Forns index[28] and AST to platelet ratio index (APRI).[29] Significant fibrosis was determined as NFS > 0.676, APRI > 0.7 or highest quartile values of Forns index. All calculation formulas were described as follow:

\[
\text{USFLI} = \frac{1 + \left( 1 + e^{-0.8073 \times \text{non-Hispanic black (yes=1, no=0)} + 0.3458 \times \text{Mexican-American (yes=1, no=0)} +
0.003 \times \text{age} + 0.6151 \times \text{loge (GGT)} + 0.0249 \times \text{WC} + 1.1792 \times \text{loge (insulin)} + 0.8242 \times \text{loge (glucose) - 14.7812} \right)^{-1}}{1 + \left( 1 + e^{-0.8073 \times \text{non-Hispanic black (yes=1, no=0)} + 0.3458 \times \text{Mexican-American (yes=1, no=0)} +
0.003 \times \text{age} + 0.6151 \times \text{loge (GGT)} + 0.0249 \times \text{WC} + 1.1792 \times \text{loge (insulin)} + 0.8242 \times \text{loge (glucose) - 14.7812} \right)^{-1}} \times 100
\]
NFS = -1.675 + (0.037×age) + (0.094×BMI) + (1.13×IFG/Diabetes) + (0.99×AST/ALT) - (0.013×platelet [10⁹/L]) - (0.66×albumin (g/dL))

Forns index = 7.811-3.131 × loge(platelet) + 0.781 × loge(GGT) + 3.467 × loge(age) -0.014× (cholesterol)

APRI =((AST/ULN)/platelet [10⁹/L]) × 100

2.5. Statistical analysis

We summarized weighted median (interquartile range) for continuous variables, and weighted proportions for categorical variables in Table 1. For the full dataset analysis, we created 16-year weights as one-eighth of the value of the fasting subsample weights (WTSAF2YR * 1/8) since this represented the smallest subsample of the study.[30] In view of the calculation of VAI differed between gender, we divided the participants into men and women. The P-value was analyzed according to VAI quartiles using Kruskal–Wallis analysis and Chi-squared tests respectively. Correlation between VAI and NAFLD prediction score was calculated using Spearman correlation analysis in men and women. Compared with participants in the lowest category of VAI, we ran three logistic models to calculate variable-adjusted odds ratios (with 95% confidence intervals) for diagnosed NAFLD. The three models were as follows: Model 1 was unadjusted. Model 2 was adjusted for age, ethnicity, and the survey cycle year. Model 3 was adjusted for all the variables in model 2 plus education level, poverty-to-income ratio, alcohol drinking, smoking, physical activity status,
HOMA-IR, and blood pressure. After testing for multicollinearity, we observed that all models presented were free from collinearity (the variance inflation factor for all variables was < 1.62). Moreover, to further investigate the appropriate population who could use VAI to predict the prevalence of NAFLD, we performed stratified analyses by age, ethnicity, smoking status, IR and presence of several metabolic disorders, using the fully adjusted model (excluding the stratification variable). All data were analyzed with SPSS complex sample module version 21.0 (SPSS Inc., Chicago, IL, USA), and significance was accepted at a two-tailed $P < 0.05$.

2.6. Patient and public involvement

Patients or the public were not involved in the design, or conduct, or reporting, or dissemination plans of our research.

3. Results

3.1. Characteristics of participants classified according to the VAI quartiles

The study analyzed a total of 7,545 participants including 3789 men and 3756 women from the NHANES 2003-2018. Among these participants, 2,793 individuals (1,551 men and 1,242 women) with NAFLD were defined by USFLI (37.0%). The baseline characteristics stratified by VAI quartiles were summarized in Table 1. VAI were categorized by quartiles using the values 0.87,
1.46 and 2.50 in men, and using the values 0.99, 1.63 and 2.65 in women. In both genders, subjects with higher VAI levels were more likely to be older and non-Hispanic White. Likewise, those in the higher quartile of VAI tended to have higher levels of BMI, WC, DBP, FPG, fasting insulin, HOMA-IR, TC and TG, and lower level of HDL-C. Regarding the clinical condition, the proportions of obese, T2DM, hypertension, CVD and MetS were increased with the increase of VAI level. Similarly, subjects with the higher quartile of VAI had more NAFLD burden. The liver fibrosis burden had no significant difference among VAI quartiles.
Table 1. Characteristics of NHANES participants, 2003–2018, by VAI quartiles

|                | Men                          | Women                         | p   |
|----------------|------------------------------|-------------------------------|-----|
|                | Quartile 1 (≤0.87)           | Quartile 2 (0.87-1.45)        |     |
| Age (year)     | 51(36, 64)                  | 53(41, 65)                    |     |
| Ethnicity (%)  |                              |                               |     |
| Hispanic       | 10.4 (12.8, 14.1)            | 12.1 (14.4, 16.8)             |     |
| Whites         | 66.6 (72.4, 81.4)            | 74.6 (82.1, 90.7)             |     |
| Blacks         | 17.2 (20.7, 24.2)            | 16.6 (20.1, 24.6)             |     |
| High education | 83.8 (88.2, 91.6)            | 83.8 (88.2, 91.6)             |     |
| Current smokers| 12.8 (16.5, 20.2)            | 17.0 (21.7, 26.4)             |     |
| Current drinkers| 17.7 (23.4, 34.7)           | 21.3 (28.4, 36.7)             |     |
| Physical activity | 80.2 (74.3, 96.5)        | 71.8 (88.2, 96.8)             |     |
| Obese(%)       | 8.7 (11.0, 17.0)             | 9.4 (13.0, 21.7)              |     |
| CVD(%)         | 9.7 (11.0, 17.0)             | 10.7 (15.0, 22.7)             |     |
| T2DM (%)       | 9.1 (11.0, 20.6)             | 9.8 (15.0, 27.3)              |     |
| MetS(%)        | 14.1 (23.8, 51.9)            | 17.9 (27.3, 56.5)             |     |
| NAFLD(%)       | 14.9 (28.8, 50.3)            | 5.3 (18.6, 34.9)              |     |
| PIR (%)        | 3.5 (12.7, 0.5)              | 3.2 (1.8, 5.0)                |     |
| BMI (kg/m²)    | 24.9 (32.3, 29.3)            | 27.0 (32.4, 30.3)             |     |
| WC (cm)        | 94.8 (86.5, 104.6)           | 100.9 (112.5, 120.6)          |     |
| SBP (mm Hg)    | 120.7 (111.1, 131)           | 121.3 (115.0, 121.3)          |     |
| DBP (mm Hg)    | 70.6 (73.6, 78.4)            | 74.9 (82.2, 97.8)             |     |
| ALT(U/L)       | 21 (17.6, 24.0)              | 26 (20.2, 26.5)               |     |
| AST(U/L)       | 23 (20.2, 27.0)              | 24 (20.2, 27.0)               |     |
| FPG (mg/dL)    | 100 (94.0, 124)              | 106 (96.0, 122)               |     |
| Insulin(µU/ml) | 6.3 (4.2, 9.7)               | 11.4 (7.2, 17.2)              |     |
| HOMA-IR        | 1.6 (1.0, 2.5)               | 3.0 (1.8, 4.8)                |     |
| HDL-C (mg/dL)  | 1.5 (1.3, 1.8)               | 1.2 (1.1, 1.3)                |     |
| TC (mg/dL)     | 4.6 (4.0, 5.3)               | 5.0 (4.5, 5.7)                |     |
| TG (mg/dL)     | 0.7 (0.6, 0.8)               | 0.7 (0.6, 0.8)                |     |
| NGS             | -1.29 (-2.30, -0.23)         | -1.12 (-2.31, -0.02)          |     |
| Forns index    | 4.21 (2.98, 5.43)            | 4.38 (3.02, 5.58)             |     |
| APRI            | 0.28 (0.23, 0.36)            | 0.27 (0.22, 0.35)             |     |

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3.2. Correlations Between VAI and NAFLD related prediction scores

As shown in Table 2, Spearman correlation analysis indicated that VAI scores were positively correlated with USFLI scores (r=0.537, \( P=0.000 \) for men, and r=0.540, \( P=0.000 \) for women). To explore whether the association between VAI scores and NAFLD index was mediated by IR, we also estimate the relationship between HOMA-IR values and NAFLD indices, as well as the relationship between VAI scores and HOMA-IR values. As predicted, HOMA-IR was positively correlated with NAFLD (r=0.895, \( P=0.000 \) for men, and r=0.896, \( P=0.000 \) for women). Moreover, VAI score was positively correlated with HOMA-IR (r=0.479, \( P=0.000 \) for men, and r=0.463, \( P=0.000 \) for women), which indicated that IR might be an important factor connecting VAI scores and NAFLD. However, the coefficients between VAI scores and fibrosis indices had no statistical significance (\( P>0.05 \)), or presented weak correlations (r<0.2).

Table 2. Spearman correlation between NAFLD index and VAI or insulin sensitivity

| Models                        | Men          |   |   | Women          |   |   |
|-------------------------------|--------------|------------------|------------------|------------------|------------------|------------------|
|                               | VAI          | P    | HOMA-IR       | P    | VAI          | P    | HOMA-IR       | P    |
| NAFLD defined by              |              |      |               |      |              |      |               |      |
| USFLI score                   | 0.537***     | 0.000 | 0.895***      | 0.000 | 0.540***     | 0.000 | 0.896***      | 0.000 |
| VAI                           | -            | 0.479*** | 0.000       | -    | -            | -    | -            | -    |
| Fibrosis defined by           |              |      |               |      |              |      |               |      |
| NFS score                     | 0.093***     | 0.000 | 0.326**       | 0.000 | 0.160**      | 0.000 | 0.355**       | 0.000 |
| Forns index                   | 0.013        | 0.442 | 0.148***      | 0.000 | 0.048**      | 0.003 | 0.120***      | 0.000 |
| APRI score                    | 0.036*       | 0.027 | 0.082***      | 0.000 | -0.041*      | 0.012 | -0.026*       | 0.111 |

VAI, visceral adiposity index; HOMA-IR, homeostasis model assessment of insulin resistance; USFLI, the US fatty liver index; NLFS, the NAFLD liver fat score; HSI, hepatic steatosis index; NFS, NAFLD fibrosis score; APRI, AST to platelet ratio index; *\( P<0.05 \), **\( P<0.01 \), ***\( P<0.001 \).

3.3. The odds ratio of NAFLD across quartiles of VAI
We further conducted logistic regression analyses to calculate the relative risk of NAFLD as predicted by VAI categories, using the lowest VAI level as the reference. As the results shown in Table 3, higher levels of VAI were associated with progressively higher odds ratio of NAFLD in all logistic regression models. For both genders, the positive association persisted in all VAI categories in unadjusted model and model adjusted for age, ethnicity and the survey cycle year. In the most multivariable-adjusted model, although the positive association exist in the third and top quartile of VAI, there was no statistically significance in the second quartile of VAI [OR (95%CI) = 1.35 (0.92-1.99) for men, OR (95%CI) = 1.44 (0.87-2.38) for women]. The results indicated that VAI was positively associated with NAFLD when VAI > 1.46 in men and VAI > 1.63 in women.

Regarding to NAFLD related fibrosis, we found no association between VAI and liver fibrosis in both genders.

3.4. The stratified analyses of VAI and risk of NAFLD

As shown in Table 4, when stratified by age, we found a null association in men with aged ≥ 55y and women aged < 40y. With respect to ethnicity, we found the positive correlation between VAI and NAFLD only in Hispanic and non-Hispanic White population, but not in the non-Hispanic Black population. When stratified by the status of CVD, the positive associations were only seen in participants without CVD. When stratified by somking status, IR and other metabolic disorders, the positive associations exist in almost all evaluated
subgroups. Of note, the positive associations in men with these conditions were more attenuate compared with men without these conditions.

Table 3. Multivariate odds ratio for NAFLD and fibrosis according to VAI levels.

|                  | VAI levels |                  |                  |                  |
|------------------|------------|------------------|------------------|------------------|
|                  |            | Men              | Women            |                  |
|                  |            | Quartile 1       | Quartile 2       | Quartile 3       | Quartile 4       |
| VAI levels       |            | (≤0.87)          | (0.87-1.45)      | (1.46-2.49)      | (>2.49)          |
| Defined by USFLI |            |                  |                  |                  |                  |
| Model 1          | 1.00       | 2.58(2.06-3.25)  | 5.70(4.56-7.12)  | 13.69(10.88-17.22)|                  |
| Model 2          | 1.00       | 2.55(2.01-3.23)  | 5.83(4.61-7.36)  | 14.97(11.73-19.11)|                  |
| Model 3          | 1.00       | 1.35(0.92-1.99)  | 1.67(1.13-2.45)  | 2.57(1.72-3.83)  |                  |
| NAFLD related fibrosis | |                  |                  |                  |                  |
| Defined by NFS   |            |                  |                  |                  |                  |
| Model 1          | 1.00       | 0.60(0.38-0.95)  | 0.67(0.44-1.02)  | 0.56(0.37-0.84)  |                  |
| Model 2          | 1.00       | 0.84(0.50-1.43)  | 1.13(0.69-1.86)  | 1.22(0.75-1.98)  |                  |
| Model 3          | 1.00       | 0.82(0.47-1.43)  | 1.06(0.63-1.79)  | 1.06(0.63-1.79)  |                  |
| By Forns index   |            |                  |                  |                  |                  |
| Model 1          | 1.00       | 0.67(0.44-1.02)  | 0.53(0.35-0.79)  | 0.43(0.29-0.64)  |                  |
| Model 2          | 1.00       | 0.98(0.58-1.65)  | 0.76(0.47-1.25)  | 0.78(0.48-1.26)  |                  |
| Model 3          | 1.00       | 1.02(0.59-1.77)  | 0.84(0.50-1.42)  | 0.82(0.49-1.37)  |                  |
| Defined by APRI  |            |                  |                  |                  |                  |
| Model 1          | 1.00       | 0.36(0.14-0.93)  | 0.42(0.18-0.95)  | 0.51(0.24-1.09)  |                  |
| Model 2          | 1.00       | 0.37(0.14-0.98)  | 0.42(0.18-0.98)  | 0.52(0.24-1.15)  |                  |
| Model 3          | 1.00       | 0.43(0.16-1.18)  | 0.43(0.17-1.07)  | 0.49(0.21-1.15)  |                  |
| Defined by NFS   |            |                  |                  |                  |                  |
| Model 1          | 1.00       | 3.47(2.60-4.62)  | 7.95(6.04-10.47) | 20.14(15.28-26.55)|                  |
| Model 2          | 1.00       | 3.16(2.36-4.23)  | 7.36(5.55-9.77)  | 20.17(15.14-26.87)|                  |
| Model 3          | 1.00       | 1.44(0.87-2.38)  | 2.54(1.56-4.15)  | 3.42(2.08-5.62)  |                  |
| Defined by APRI  |            |                  |                  |                  |                  |
| Model 1          | 1.00       | 0.76(0.23-2.55)  | 0.52(0.16-1.66)  | 0.39(0.12-1.22)  |                  |
| Model 2          | 1.00       | 0.83(0.24-2.85)  | 0.59(0.18-1.95)  | 0.44(0.14-1.46)  |                  |
| Model 3          | 1.00       | 0.53(0.14-1.99)  | 0.35(0.10-1.29)  | 0.30(0.09-1.05)  |                  |

Model 1 is unadjusted; Model 2 is adjusted for age, ethnicity, and the survey cycle year; Model 3 is adjusted for all the variables in model 2 plus education level, poverty-to-income ratio, alcohol drinking, smoking, physical activity status, HOMA-IR, and blood pressure.
Data are expressed as odds ratio and 95% CI adjusted for age (not adjusted in subgroup analysis in age), ethnicity (not adjusted in subgroup analysis in ethnicity), the survey cycle year, education level, poverty-to-income ratio, alcohol drinking, smoking (not adjusted in subgroup analysis in smoking), and physical activity status, HOMA-IR (not adjusted in subgroup analysis in IR, diabetes, and MetS), blood pressure (not adjusted in subgroup analysis in hypertension and MetS).
4. Discussion

In this study, we demonstrated that a positive association between VAI and NAFLD after controlling for several potential confounders, whereas no significant association was found between VAI and NAFLD-related fibrosis. The stratified analyses revealed that the association was independent of smoking, IR and multiple metabolic diseases, but had interaction with age, gender, and ethnicity. The information from stratified analyses indicated that the prediction function of VAI applied to man with age < 55 years and women with age ≥ 40 years, and it was confined to Hispanic and non-Hispanic White population. To our best knowledge, this is the first large population-based study to report a strong association between VAI and the risk of NAFLD in the United States. Furthermore, this is also the first study to investigate the appropriate population who could use VAI to predict the prevalence of NAFLD.

As far as we know, there are only two large scale studies that investigated the VAI in subjects with NAFLD. Xu et al found that VAI was associated with NAFLD in 4,809 Chinese participants after multivariate adjustment.[17] However, these findings were limited to population that in normal-weight from under-developed areas in China. Although Okamura et al confirmed the association in 8,399 Japanese by a nationally representative, population-based cohort study,[18] this study still lacked the data of plasma insulin level and could not evaluate the impact of IR on the relationship between VAI and NAFLD.
However, our study had the unique feature in examining whether the relationship was independent of IR and various metabolic diseases. Moreover, we also explored the function of VAI in predicting the prevalence of NAFLD related fibrosis. Although Petta et al. concluded that VAI was independently associated with significant fibrosis,[12] whereas the previous study performed by Ercin et al concluded that VAI was not associated with hepatic fibrosis in nondiabetic patients with NAFLD.[14] We considered that the discrepant findings of these studies may due to differences in the composition of participants. Different with the study performed by Ercin et al, some participants in the study conducted by Petta et al were patients with hypertension, diabetes, or metabolic syndrome. The correlation between VAI and liver fibrosis in the study done by Petta et al might be affected by these metabolic disorders. Overall, our results are consistent with previous studies and indicate that VAI could be a predictor for NAFLD, but could not predict NAFLD related fibrosis.

Since VAI is a surrogate marker of both visceral fat distribution and dysfunction, the relationship between VAI and NAFLD could be explained by some potential mechanisms. Among several hypotheses that have been formulated, the ‘portal theory’ describes the directly toxic properties of visceral fat on the liver.[31] The theory proposes that visceral fat releases FFAs via its unique location and enhanced lipolysis, which travel through the portal vein to the liver, with consequent increased accumulation of TG in the liver, promoting the development of hepatic IR and liver steatosis. Thus, IR has been traditionally
considered as a physiological connection between the visceral fat and NAFLD. However, our findings demonstrated that VAI was still associated with higher prevalence of NAFLD in subjects without IR. The results suggest that there are some other mechanisms which directly link the visceral fat to NAFLD along with IR. Of note, in addition to lipotoxicity, there are mounting evidences propose that changes in adipokine expression and secretion also participate in the development of NAFLD, as well as the infiltration of macrophage and T cells in visceral fat.[32, 33] Similar with FFAs, these pro-inflammatory cytokines and adipokines are carried directly to the liver via the portal vein, causing ballooning degeneration of hepatocyte or promoting the transformation of hepatic cells to myo-fibroblastic phenotypes.[34-36] Furthermore, other proposed pathways including endoplasmic reticulum stress, toll-like receptor activation and impaired oxygenation may be also involved in the connection between visceral fat and NAFLD.[37, 38]

Although the present study showed that high VAI was an independent risk factor for the presence of NAFLD, some information in the subgroup analysis also should be worthy of note. First, in the subgroup analysis by ethnicity, we found the positive association between VAI and NAFLD in Hispanic and non-Hispanic White population, but not in non-Hispanic Black population. Although several studies have revealed that prevalence of obesity was higher among Hispanics and non-Hispanic Black than that of non-Hispanic White population,[39, 40] there are known differences in visceral fat distribution across
these ethnic groups. However, non-Hispanic Black had the lowest VAI scores compared with the other two ethnic groups.[41, 42] As the results shown in Table 3, VAI was positively associated with NAFLD when $\text{VAI} > 1.46$ in men and $\text{VAI} > 1.63$ in women. Thus, the ethnic difference might be attribute to lower VAI levels in non-Hispanic Black population. In addition to ethnic disparities, we also found the age-gender specific difference. We found a null association in women with age $< 40\text{y}$, which might be explained by sexual dimorphism and fat distribution. Estrogens could enhance the sympathetic tone differentially to the adipose tissue, favoring lipid accumulation in the subcutaneous depot in premenopausal women, whereas women would shift to accrue more visceral fat after menopause.[43] Different with women, men are susceptible to visceral fat deposition in any stage in life. However, we only found the positive association between VAI and the prevalence of NAFLD in man with age $< 55\text{y}$. According to epidemiological evidence, the prevalence of obese, hypertension, and MetS increased with age, especially in people aged $\geq 50\text{y}$. [44, 45] However, as shown in our subgroup analysis, the status of these metabolic diseases would weaken or abolish the association between VAI and risk of NAFLD in men, but not in women. Thus, the prediction of VAI in older men might be confounded by status of metabolic disease.

This study has several strengths. First, this is a large population-based analysis using well-examined nationwide data, and the findings could be
generalized for most U.S. populations. Second, it is valued because we provided solid evidence of an independent association between VAI and NAFLD by performing multiple logistic regression. Third, we conducted the stratified analyses to identify the appropriate population who could use VAI to predict the prevalence of NAFLD. However, we are also aware of several limitations in our study. First, the cross-sectional nature of the study design meant that we could not investigate the longitudinal dynamic association between progression of NAFLD and changes in VAI levels across several therapeutic interventions, such as lifestyle modification, exercise, and weight control. Second, although we used the validated NAFLD and fibrosis models, the liver radiological or histological information were not available, which meant that NAFLD and NAFLD related fibrosis may be misclassified. Third, estimates across some subgroups should be interpreted with caution due to limited sample size, such as subjects with diabetes or CVD.

5. Conclusions

In conclusion, our study documents that VAI might be a useful predictive model for NAFLD, but not for hepatic fibrosis among U.S. adults, and there exist age-gender specific and ethnic difference. The results reported here has important public health implications in NAFLD screening in the future.

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**Ethical approval:** The survey was approved by the National Center for Health Statistics ethics review board.

**Data Availability:** The data supporting reported results can be freely available from the NHANES website public archive, accessible at NHANES Questionnaires, Data sets and Related Documentation repository (https://wwwn.cdc.gov/nchs/nhanes/Default.aspx).

**Author Contributions:** Conceptualization, Q.L.; methodology, Q.L. and J.W.; software, Q.L.; validation, L.W., J.W. and X.Z.; formal analysis, Q.L. and Y.W.; writing—original draft preparation, Q.L.; writing-review and editing, Q.L., L.W., and X.Z.; funding acquisition, J.W..

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Figure legend:

Fig 1. Flow chart of the participants inclusion and exclusion in this study.
Flow chart of the participants inclusion and exclusion in this study

NHANES 2003-2018
Adults (≥20 years) (n=44790)

Pregnant (n=941)

Subjects with examination (n=38015)

Exclusion
1. Significant alcohol consumption (n=14125)
2. Viral hepatitis (n=6476)
3. Missing ALT/AST/PLA or ALT/AST > 500U/L (n=208)
4. Missing for fasting glucose, lipids, or insulin (n=9661)

Final eligible subjects (n=7545)
Cross-sectional study of the role of age, gender and ethnicity in the association between visceral adiposity index and nonalcoholic fatty liver disease among US adults (NHANES 2003–2018)

Qianwen Li¹, Ling Wang¹, Ling, Jing Wang¹, Yanjie Wang³, and Jian Wu¹*

Supplemental Material:

STROBE Statement—Checklist of items included in this report:

| Item                        | Page |
|-----------------------------|------|
| **No** | **Recommendation** | **Page** |
| **Title and abstract**      | 1    | *(a)* Indicate the study’s design with a commonly used term in the title or the abstract  
                       |      | *(b)* Provide in the abstract an informative and balanced summary of what was done and what was found | 2-3 |
| **Introduction**            |      | **Background/rationale** | 2 | Explain the scientific background and rationale for the investigation being reported | 4-5 |
| **Objectives**              | 3    | State specific objectives, including any prespecified hypotheses | 5 |
| **Methods**                 |      | **Study design** | 4 | Present key elements of study design early in the paper | 5 |
| **Setting**                 | 5    | Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow up, and data collection | 6 |
| **Participants**            | 6    | *(a)* Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up.  
                       |      | *(b)* For matched studies, give matching criteria and number of exposed and unexposed | 6 |
| Variables                        | 7 | Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable | 7-8 |
|---------------------------------|---|--------------------------------------------------------------------------------------------------------------------------------|-----|
| Data sources/                    | 8*| For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group | 6  |
| measurement                      |   |                                                                                                                                |     |
| Bias                             | 9 | Describe any efforts to address potential sources of bias                                                                      | 7-8 |
| Study size                       | 10| Explain how the study size was arrived at                                                                                     | 6   |
| Quantitative                     | 11| Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why    | 7-8 |
| variables                        |   |                                                                                                                                |     |
| Statistical methods              | 12| (a) Describe all statistical methods, including those used to control for confounding                                           | 9-10|
|                                 |   | (b) Describe any methods used to examine subgroups and interactions                                                           |     |
|                                 |   | (c) Explain how missing data were addressed                                                                                  |     |
|                                 |   | (d) If applicable, describe analytical methods taking account of sampling strategy                                              |     |
|                                 |   | (e) Describe any sensitivity analyses                                                                                         |     |
| Results                          |   |                                                                                                                                |     |
| Participants                     | 13*| (a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed. (b) Give reasons for non-participation at each stage (c) Consider use of a flow diagram | 7/10|
| Descriptive data                 | 14*| (a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders (b) Indicate number of participants with missing data for each variable of interest | 10-11|
| Outcome data                     | 15*| Report numbers of outcome events or summary measures                                                                          | 10  |
| Main results | 16 | (a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included.  
(b) Report category boundaries when continuous variables were categorized.  
(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period. |
| Other analyses | 17 | Report other analyses done—eg, analyses of subgroups and interactions, and sensitivity analyses. |
| Discussion | 18 | Summarise key results with reference to study objectives. |
| Limitations | 19 | Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias. |
| Interpretation | 20 | Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence. |
| Generalisability | 21 | Discuss the generalisability (external validity) of the study results. |
| Other information | 22 | Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based. |

*Give such information separately for cases and controls in case-control studies, and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies. An explanation and elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting.*
Cross-sectional study of the role of age, gender and ethnicity in the association between visceral adiposity index and nonalcoholic fatty liver disease among U.S. adults (NHANES 2003–2018)

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Cross-sectional study of the role of age, gender and ethnicity in the association between visceral adiposity index and nonalcoholic fatty liver disease among U.S. adults (NHANES 2003–2018)

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Abstract

Objectives: The association between visceral adiposity index (VAI) and the prevalence of nonalcoholic fatty liver disease (NAFLD) has not been fully determined. Here, we aimed to explore the association between VAI and NAFLD in the general U.S. population, and further investigate whether the association exists population differences.

Design: Cross-sectional population-based study.

Setting: The National Health and Nutrition Examination Survey (2003–2018).

Participants: A total of 7,522 participants aged 20 years or older who have complete information for NAFLD assessment test were included in this study.

Primary and secondary outcome measures: NAFLD was assessed by the modified fatty liver index for the U.S. population (USFLI) using a cut-off point of 30. Correlation between VAI and NAFLD prediction scores was calculated using the partial correlation analysis. Logistic regression models were further used to estimate ORs and 95% CIs.

Results: IR, inflammation and WC adjusted-partial correlation analysis indicated that VAI scores were positively correlated with USFLI ($r=0.404$ for men, and $r=0.395$ for women, $p<0.001$). In a comparison of the highest versus the lowest quartiles of VAI, multivariable logistic regression analysis demonstrated a positive association between VAI and NAFLD [OR (95%CI) = 1.97(1.12-3.47)
for men, OR (95%CI) = 4.03(1.98-8.20) for women]. The stratified analyses revealed that the positive association exists age-gender specific and ethnic differences. As for the impact of metabolic disorders, our results revealed that the association was independent of IR and diabetes, but it would be confounded by other metabolic disorders. However, no significant association was found between VAI and hepatic fibrosis.

**Conclusion:** VAI is positively associated with the prevalence of NAFLD, but not hepatic fibrosis among U.S. adults, and the association exists age-gender specific and ethnic differences. The results reported here have important public health implications in NAFLD screening in the future.

**Keywords:** Public health, epidemiology, hepatology

**Strengths and limitations of this study:**

- The quality and scale of the NHANES database and the rigor of its measures ensure the statistical power and reliability of our results.
- The strict exclusion criteria ensure the homogeneity of the study population.
- Multiple potential confounders were well controlled in the study.
- Although we used well-validated NAFLD and fibrosis models, there is chance for misclassifying in some cases due to lacking the information of image and histology of the liver.
- The cross-sectional nature of this study limits the assessment of causality.
1. Introduction

With an accelerated pace of nutrition transition, non-alcoholic fatty liver disease (NAFLD) has become an emerging public health issue with high prevalence worldwide, affecting up to one-third of the population,[1] and its incidence is expected to rise rapidly in the future alongside increasing rates of obesity.[2] According to epidemiological data, nearly a quarter of NAFLD patients would progress to steatohepatitis with fibrosis, which could lead to serious liver-related complications and death.[3, 4] On the other hand, NAFLD is closely related to higher rates of several cardiometabolic disorders, including diabetes mellitus, metabolic syndrome, and cardiovascular diseases (CVD).[2, 5] Thus, the increasing prevalence of NAFLD is particularly life-threatening. It is important to identify modifiable risk factors for NAFLD for reducing the disease burden.

Currently, the pathogenesis of NAFLD has not been completely understood, and increasing evidence suggest that visceral fat accumulation plays a crucial role in the pathogenesis of NAFLD.[6, 7] In recent years, the visceral adiposity index (VAI) based on waist circumference (WC), body mass index (BMI), plasma triglycerides (TG) and high-density lipoprotein cholesterol (HDL-C) has been proposed as a reliable marker to assess the content and function of visceral fat.[8] Although VAI has been proved to be a powerful indicator of type 2 diabetes mellitus (T2DM).[9] metabolic syndrome (MetS)[10] and cardiovascular
There are controversial data regarding the association between VAI and NAFLD. A prior study reported that VAI was related to significant fibrosis in NAFLD patients,[12] while other researchers revealed that the association didn’t exist in obese or non-diabetes subjects with NAFLD.[13, 14] In addition to the small sample size, variations in the participants with or without additional metabolic disorders are largely contributed to these conflicting results. According to current evidence, insulin resistance (IR) is related to NAFLD,[15] while VAI is a valuable indicator of IR.[8] As yet it is unknown whether the status of insulin sensitivity is the result of the controversy among NAFLD patients underlying different clinical statuses. Furthermore, variations in genetic background may be another explanation for these controversial results in NAFLD.[16] Two recent cohort studies with large scale population conducted in China and Japan implied that higher VAI levels are correlated with an increased incidence of NAFLD in Asian population,[17, 18] while a similar study has not been done or published in the U.S.. In this study, we intend to investigate whether VAI is significantly associated with the prevalence of NAFLD in the U.S. general population, independent of IR and its related metabolic disorders, using the data from National Health and Nutrition Examination Surveys (NHANES).

2. Methods

The design, implementation, analysis, and reporting of this study were conducted in accordance with the STROBE statement.[19]
2.1. Study Population

The NHANES is a multistage, ongoing, complex cross-sectional health examination and survey designed to collect the health data of the U.S. non-institutionalized civilian population. The survey was approved by the National Center for Health Statistics ethics review board, and was conducted by the Centers for Disease Control and Prevention (CDC). All participants provided written informed consent. The data can be freely available from the NHANES website public archive. Information regarding interview processes, examination protocols and sample collection can also be found on the website.[20] Given that the information about fasting plasma glucose (FPG) and insulin were available since 2003, data from 2003-2018 were obtained for analysis.

Of all participants, we initially selected nonpregnant subjects aged 20 years or older. Then, we excluded individuals missing the information about anthropometric parameters (BMI and WC) and blood pressure. Subsequently, we excluded individuals with the following reasons: excessive alcohol consumption (defined by > 1 drink/day for women or > 2 drinks/day for men), viral hepatitis (defined by positive serum hepatitis B or C antibody and/or positive serum hepatitis B surface antigen), and missing laboratory data to rule in or rule out the presence of NAFLD. Given the unique condition of puerperium women, we also excluded women who were at 0-12 weeks postpartum[21]. Finally, 7,522 subjects were included (Fig 1).
2.2. Assessment of VAI

We calculated VAI using the following formulas:[8]

\[
VAI = \frac{WC \text{ [cm]}}{(39.68 + (1.88 \times BMI \text{ [kg/m}^2\text{]}))} \times \frac{\text{TG [mmol/L]}}{1.03} \times \left(\frac{1.31}{\text{HDL [mmol/L]}}\right) \text{ for males (1)}
\]

\[
VAI = \frac{WC \text{ [cm]}}{(36.58 + (1.89 \times BMI \text{ [kg/m}^2\text{]}))} \times \frac{\text{TG [mmol/L]}}{0.81} \times \left(\frac{1.52}{\text{HDL [mmol/L]}}\right) \text{ for females (2)}
\]

2.3. Definitions of covariates

Ethnicity was categorized as non-Hispanic white, non-Hispanic black, or Hispanic. High education was defined as completing a high school degree or above. Current smokers were defined as the participants who smoked at least 100 cigarettes lifetime and now smoke cigarettes every day or some days. Similarly, current drinkers were defined as the participants who had at least 12 alcohol drinks their entire life and now drink alcohol every day or some days. Physical activity was defined as engaging in moderate or vigorous exercise regularly (≥20 minutes at a time and at least three times per week).[22] The poverty income ratio was calculated by dividing family income by the poverty guidelines specific to the survey year.[23]

Regarding the influence of IR, inflammation and related metabolic complications on the relationship between visceral fat and NAFLD, the homeostasis model assessment of IR (HOMA-IR), inflammation [C-reactive protein (CRP)] and several diseases were calculated and defined. HOMA-IR was defined as fasting glucose [mg/dL] * fasting insulin [μU/ml]/405.[24]
Overweight and obese participants were defined as those with BMI $\geq 25 \text{ kg/m}^2$ for non-Asians.[25] T2DM was defined based on fasting glucose ($\geq 126 \text{ mg/dL}$) and/or receiving insulin or oral hypoglycemic therapy. Hypertension was defined as systolic pressure $\geq 140 \text{ mm Hg}$ and/or diastolic pressure $\geq 90 \text{ mm Hg}$, or antihypertensive therapy.[26] Mets was defined according to the National Cholesterol Education Program Adult Treatment Panel III report, as an individual who has three or more of all criteria.[27] CVD was defined as the composite of self-report history of stroke, myocardial infarction, coronary revascularization procedure, angina, and congestive heart failure.[28]

2.4. Definitions of NAFLD and liver fibrosis

The definition of NAFLD was based on the US fatty liver index (USFLI) which was calculated by gamma-glutamyl transferase (GGT) [U/L], WC[cm], fasting glucose [mg/dL] and fasting insulin [pmol/L].[29] The cut-off of 30 was used to define NAFLD. Furthermore, the presence of fibrosis among individuals with NAFLD was assessed using NAFLD fibrosis score (NFS), FIB-4 and AST to platelet ratio index (APRI). [30-32] The cut-off of 0.676, 2.67 and 1.0 were used to define NAFLD significant fibrosis respectively was determined. All calculation formulas were described as follow:

$$\text{USFLI} = \frac{(e^{-0.8073 \times \text{non-Hispanic black (yes=1, no=0)} + 0.3458 \times \text{Mexican-American (yes=1, no=0)} + 0.0093 \times \text{age} + 0.6151 \times \text{loge (GGT)} + 0.0249 \times \text{WC} + 1.1792 \times \text{loge (insulin)} + 0.8242 \times \text{loge (glucose)} - 14.7812)}{(1 + e^{-0.8073 \times \text{non-Hispanic black (yes=1, no=0)} + 0.3458 \times \text{Mexican-American (yes=1, no=0)} + 0.0093 \times \text{age} + 0.6151 \times \text{loge (GGT)} + 0.0249 \times \text{WC} + 1.1792 \times \text{loge (insulin)} + 0.8242 \times \text{loge (glucose)} - 14.7812}) \times 100 \quad (3)$$
NFS = -1.675 + (0.037×age) + (0.094×BMI) + (1.13×IFG/Diabetes) + (0.99×AST/ALT) - (0.013×platelet[10^9/L]) - (0.66×albumin [g/dL])

FIB-4 = Age × AST/ (platelet ×ALT\(^{1/2}\))

APRI = ((AST[U/L]/ULN)/platelet [10^9/L]) × 100

2.5. Statistical analysis

We summarized the weighted median (interquartile range) for continuous variables, and weighted proportions for categorical variables in Table 1. For the full dataset analysis, we created 16-year weights as one-eighth of the value of the fasting subsample weights (WTSAF2YR * 1/8) since this represented the smallest subsample of the study.[33] Given the calculation of VAI differed between gender, we divided the participants into men and women. The \(P\)-value was analyzed according to VAI quartiles using Kruskal–Wallis analysis and Chi-squared tests respectively. Partial correlation analysis was performed to investigate the relationship between VAI and NAFLD prediction models. Nonnormally distributed data were transformed to Gaussian distribution before assessing the partial correlation analysis via Blom’s rank-based inverse normal transformations[34]. In addition, we ran three logistic models to calculate variable-adjusted odds ratios (with 95% confidence intervals) for NAFLD, taking the lowest category of VAI as the reference. The three models were as follows:

Model 1 was unadjusted. Model 2 was adjusted for age, ethnicity, and the survey cycle year. Model 3 was adjusted for all the variables in model 2 plus education.
level, poverty-to-income ratio (PIR), alcohol drinking, smoking, physical activity status, HOMA-IR, CRP, blood pressure and the same variable between VAI and NAFLD prediction scores. After testing for multicollinearity, we observed that all models presented were free from collinearity (the variance inflation factor<1.61). Moreover, to further investigate potential factors that influenced the association between VAI and the prevalence of NAFLD, we performed stratified analyses by age, ethnicity, smoking status, IR and presence of several metabolic disorders, using the fully adjusted model (excluding the stratification variable). All data were analyzed with SPSS complex sample module version 21.0 (SPSS Inc., Chicago, IL, USA), and significance was accepted at a two-tailed p < 0.05.

2.6. Patient and public involvement

Patients or the public were not involved in the design, or conduct, or reporting, or dissemination plans of our research.

3. Results

3.1. Characteristics of participants classified according to the VAI quartiles

The study analyzed a total of 7,522 participants including 3,789 men and 3,733 women from the NHANES 2003-2018. Among these participants, 2,793 individuals (1,551 men and 1,238 women) with NAFLD were defined by USFLI (37.0%). The baseline characteristics stratified by VAI quartiles were
summarized in Table 1. VAI were categorized by quartiles using the values 0.87, 1.46 and 2.49 in men, and using the values 0.99, 1.63 and 2.65 in women. In both genders, subjects with higher VAI levels were more likely to be older and non-Hispanic White. Likewise, those in the higher quartile of VAI tended to have higher levels of BMI, WC, DBP, FPG, fasting insulin, HOMA-IR, TC and TG, and lower level of HDL-C. Regarding the clinical condition, the proportions of obesity, T2DM, hypertension, CVD and MetS were increased with the increase of VAI level. Similarly, subjects with the higher quartile of VAI had more NAFLD burden. The liver fibrosis burden had no significant difference among VAI quartiles.
| Age (year) | Men | Women |
|-----------|-----|-------|
| 51(36, 64) | 53(41, 65) | 53(40, 65) |

| Ethnicity (%) | Men | Women |
|---------------|-----|-------|
| Hispanic | 10.4 | 12.4 |
| Whites | 66.6 | 72.4 |
| Blacks | 17.2 | 10.7 |
| High education (%) | 83.8 | 83.7 |
| Current smokers (%) | 12.8 | 16.5 |
| Current drinkers (%) | 17.7 | 13.6 |
| Physical activity (%) | 80.2 | 74.3 |
| Obesity (%) | 21.6 | 26.8 |
| CVD (%) | 9.7 | 11.0 |
| T2DM (%) | 9.1 | 11.6 |
| Hypertension (%) | 33.4 | 43.4 |
| MetS (%) | 14.1 | 23.8 |
| NAFLD (%) | 14.9 | 28.8 |
| PIR (%) | 3.5(1.9, 5.0) | 3.6(2.0, 5.0) |

| BMI (kg/m²) | Men | Women |
|-------------|-----|-------|
| 24.9(23.2, 29.3) | 27.3(24.8, 30.3) | 29.4(26.2, 32.9) |

| WC (cm) | Men | Women |
|---------|-----|-------|
| 94.9(85.8, 104.6) | 100(92.5, 108.3) | 106(97.2, 110.7) |

| SBP (mm Hg) | Men | Women |
|-------------|-----|-------|
| 120.7(111, 131) | 122.7(112, 132) | 121.3(110, 121) |

| DBP (mm Hg) | Men | Women |
|-------------|-----|-------|
| 70(63, 76) | 71(64, 80) | 73(66, 80) |

| ALT/UL | Men | Women |
|--------|-----|-------|
| 21(17, 26) | 24(19, 30) | 26(20, 34) |

| AST/UL | Men | Women |
|--------|-----|-------|
| 23(20, 27) | 24(20, 27) | 25(21, 30) |

| FPG (mg/dL) | Men | Women |
|-------------|-----|-------|
| 100(94, 108) | 102(96, 110) | 105(97, 116) |

| Insulin (μU/ml) | Men | Women |
|----------------|-----|-------|
| 6.3(4.2, 9.7) | 8.1(5.5, 12.8) | 11.4(7.2, 17.2) |

| HOMA-IR | Men | Women |
|---------|-----|-------|
| 1.6(1.0, 2.5) | 2.1(1.4, 3.4) | 3.0(1.8, 4.8) |

| CRP | Men | Women |
|-----|-----|-------|
| 0.50(0.09, 1.58) | 0.40(0.11, 1.42) | 0.39(0.12, 1.31) |

| HDL-C (mmol/L) | Men | Women |
|----------------|-----|-------|
| 1.5(1.3, 1.8) | 1.3(1.2, 1.4) | 1.1(1.0, 1.2) |

| TC (mmol/L) | Men | Women |
|-------------|-----|-------|
| 4.6(4.0, 5.3) | 4.8(4.2, 5.5) | 4.9(4.2, 5.5) |

| TG (mmol/L) | Men | Women |
|-------------|-----|-------|
| 0.7(0.5, 0.8) | 1.1(0.9, 1.2) | 1.5(1.3, 1.7) |

| USFLI | Men | Women |
|-------|-----|-------|
| 9.8(5.1, 2.7) | 18.0(9.9, 33.6) | 30.2(15.8, 50.0) |

| NFS | Men | Women |
|-----|-----|-------|
| -1.29(-2.30, -0.23) | -1.18(-2.31, 0.02) | -1.00(-2.35, -0.08) |

| FIB-4 | Men | Women |
|-------|-----|-------|
| 1.17(0.74, 1.69) | 1.10(0.75, 1.59) | 1.05(0.74, 1.49) |

| APRI | Men | Women |
|-----|-----|-------|
| 0.28(0.23, 0.36) | 0.28(0.22, 0.35) | 0.29(0.23, 0.38) |

| VAI, visceral adiposity index; BMI, body mass index; WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; ALT, alanine aminotransferase; AST, aspartate aminotransferase; FPG, fasting plasma glucose; CRP, C-reactive protein; HOMA-IR, homeostasis model assessment of insulin resistance; TC, total cholesterol; TG, triglycerides; CVD, cardiovascular disease; USFLI, the US fatty liver index; PIR, poverty-to-income ratio; MetS, Metabolic syndrome; NFS, NAFLD fibrosis score; FIB-4, the fibrosis-4 index ;APRI, AST to platelet ratio index. |
3.2. Correlations Between VAI and NAFLD related prediction scores

By performing the Pearson correlation analysis, we found that the index of IR (HOMA-IR) and inflammation (CRP) were positively correlated with VAI and NAFLD indices respectively, which indicated that IR and inflammation might be the important factors connecting visceral fat and NAFLD (Supplemental Table 1). In addition, the mediating effect of the same variable between VAI and NAFLD indices also should be taken into account (WC for USFLI; BMI for NFS). Therefore, we performed the partial correlation analysis which adjust the influence of these variables to calculate the correlation coefficients between VAI and NAFLD indices. As shown in Table 2, VAI was found to be significantly correlated with USFLI in both genders (r=0.404, p<0.001 for men, and r=0.463, p<0.001 for women), but not liver fibrosis indices.

| Table 2. Partial correlation coefficients between VAI and NAFLD indices |
|---------------------------------------------------------------|
| Models                                              | r     | p     | r     | p     |
| NAFLD defined by                                        |       |       |       |       |
| USFLI score                                           | 0.404*** | <0.001 | 0.395*** | <0.001 |
| Fibrosis defined by                                     |       |       |       |       |
| NFS score                                              | -0.029 | 0.140 | 0.019 | 0.328 |
| FIB-4                                                  | 0.026  | 0.185 | -0.013 | 0.514 |
| APRI score                                             | -0.030 | 0.118 | -0.047 | 0.017 |

VAI, visceral adiposity index; HOMA-IR, homeostasis model assessment of insulin resistance; USFLI, the US fatty liver index; NFS, NAFLD fibrosis score; FIB-4, the fibrosis-4 index; APRI, AST to platelet ratio index; ***p<0.001.

3.3. The odds ratio of NAFLD across quartiles of VAI

We further conducted logistic regression analyses to calculate the odds ratio...
and 95%CI to assess the association between VAI and NAFLD, using the lowest VAI level as the reference. As the results shown in Table 3, the positive association between VAI and NAFLD persisted in all VAI categories in unadjusted model and model adjusted for age, ethnicity and the survey cycle year. In the most multivariable-adjusted model, although the positive associations were weakened in both genders, the associations remained statistically significant in the top quartile of VAI [OR (95%CI) = 1.97(1.12-3.47) for men, OR (95%CI) = 4.03(1.98-8.20) for women]. Regarding NAFLD-related fibrosis, we found no association between VAI and liver fibrosis in both genders.

3.4. The stratified analyses of VAI and risk of NAFLD

To investigate the effect of confounding factors, the ORs comparing the highest versus the lowest quartile of VAI were calculated in all subgroups. As shown in Fig 2, when stratified by age, we found a positive association between VAI and NAFLD in men with age<55y, and women aged 40-64y. With respect to ethnicity, we found a positive correlation between VAI and NAFLD only in Hispanic and non-Hispanic White population, but not in the non-Hispanic Black population in both genders. In addition, the positive associations were consistently seen in all evaluated subgroups when stratified by the status of IR and diabetes. When stratified by the smoking status and other metabolic disorders (hypertension, CVD and MetS), the positive associations were only persisted in individuals without these conditions. Of note, the stronger positive
associations were found in individuals with normal BMI, while the associations were weakened in overweight and obese people. Considering the different prevalence of obesity during the period of more than 10 years, we also tested the consistency of our results by stratifying the data release year (before 2010 and after 2010). Similar to the subgroup of obesity, the positive association was weakened in recent years.

Table 3. The multivariable odds ratio for NAFLD and fibrosis according to VAI levels.

| VAI levels | Men | NAFLD Defined by USFLI | NAFLD related fibrosis Defined by NFS | By FIB-4 | Defined by APRI |
|------------|-----|------------------------|--------------------------------------|---------|---------------|
|            |     | Model 1                | Model 1                              | Model 1 | Model 1       |
|            |     | 1.00                   | 1.00                                 | 1.00    | 1.00          |
|            |     | (0.87)                 | (0.87-1.45)                          | (0.97)  | (0.97-1.77)   |
|            |     | NaFLD Defined by USFLI | Model 2                              | Model 2 | Model 2       |
|            |     | 2.58(2.06-3.25)        | 0.60(0.38-0.95)                      | 0.96(0.67-1.40) | 0.95(0.51-1.77) |
|            |     | (0.57-4.67)            | (0.38-0.95)                          | (1.00)  | (0.97)        |
|            |     | (0.87-4.67)            | (3.85-0.78)                          | (3.02-1.98) | (3.02-1.98)   |
|            |     | (1.46-4.29)            | (1.37-1.83)                          | (1.37-1.83) | (1.37-1.83)   |
|            |     | (2.49)                 | (3.41-5.45)                          | (3.21-5.30) | (3.21-5.30)   |
|            |     | (2.49)                 | (3.41-5.45)                          | (3.21-5.30) | (3.21-5.30)   |
|            |     | (2.49)                 | (3.41-5.45)                          | (3.21-5.30) | (3.21-5.30)   |

Model 1 is unadjusted; Model 2 is adjusted for age, ethnicity, and the survey cycle year; Model 3 is adjusted for all the variables in model 2 plus education level, PIR, alcohol drinking, smoking, physical activity status, HOMA-IR, CRP, blood pressure and the same variable between VAI and NAFLD indices (WC for USFLI; BMI for NFS).
4. Discussion

In this study, we found a positive association between VAI and NAFLD after controlling for several potential confounders, whereas no significant association was found between VAI and NAFLD-related fibrosis. The stratified analyses revealed that the positive association between VAI and NAFLD exists age-gender specific and ethnic differences. In addition, as for the impact of metabolic disorders, our results revealed that the association was independent of IR and diabetes, but it would be confounded by other metabolic disorders, such as hypertension, CVD and MetS. To our best knowledge, this is the first large population-based study to report a strong association between VAI and the risk of NAFLD in the United States. Furthermore, this is also the first study that reveals the role of age, gender, ethnicity and multiple metabolic disorders in the association between VAI and NAFLD.

As far as we know, there are only two large-scale studies that investigated the VAI in subjects with NAFLD. Xu et al found that VAI was associated with NAFLD in 4,809 Chinese participants after multivariate adjustment.[17] However, these findings were limited to the population that is normal-weight from under-developed areas in China. Although Okamura et al confirmed the association in 8,399 Japanese by a nationally representative, population-based cohort study,[18] this study still lacked the data of plasma insulin level and could not evaluate the impact of IR on the relationship between VAI and NAFLD.
However, our study had the unique feature in examining whether the relationship was independent of IR and various metabolic diseases. Moreover, we also explored the association between VAI and the prevalence of NAFLD-related fibrosis. Although Petta et al. concluded that VAI was independently associated with significant fibrosis,[12] whereas the previous study performed by Ercin et al concluded that VAI was not associated with hepatic fibrosis in non-diabetic patients with NAFLD.[14] We considered that the discrepant findings of these studies may due to differences in the composition of participants. Different from the study performed by Ercin et al, some participants in the study conducted by Petta et al were patients with hypertension, diabetes, or metabolic syndrome. The correlation between VAI and liver fibrosis in the study done by Petta et al might be affected by these metabolic disorders. Overall, our results are consistent with previous studies and indicate that VAI is an independent risk factor for NAFLD, but not NAFLD-related fibrosis.

Since VAI is a surrogate marker of both visceral fat distribution and dysfunction, the relationship between VAI and NAFLD could be explained by some potential mechanisms. Among several hypotheses that have been formulated, the ‘portal theory’ describes the directly toxic properties of visceral fat on the liver.[35] The theory proposes that visceral fat releases FFAs via its unique location and enhanced lipolysis, which travel through the portal vein to the liver, with consequently increased accumulation of TG in the liver, promoting the development of hepatic IR and liver steatosis. Thus, IR has been traditionally
considered as a physiological connection between visceral fat and NAFLD. However, our findings demonstrated that VAI was still associated with a higher prevalence of NAFLD in subjects without IR. The results suggest that there are some other mechanisms that directly link the visceral fat to NAFLD along with IR. Of note, in addition to lipotoxicity, there are mounting evidence propose that changes in adipokine expression and secretion also participate in the development of NAFLD, as well as the infiltration of macrophage and T cells in visceral fat.[36, 37] Similar to FFAs, these pro-inflammatory cytokines and adipokines are carried directly to the liver via the portal vein, causing ballooning degeneration of hepatocytes or promoting the transformation of hepatic cells to myofibroblastic phenotypes.[38-40] Furthermore, other proposed pathways including endoplasmic reticulum stress, toll-like receptor activation and impaired oxygenation may be also involved in the connection between visceral fat and NAFLD.[41, 42]

Although the present study showed that high VAI was an independent risk factor for the presence of NAFLD, some information in the subgroup analysis also should be worthy of note. First, in the subgroup analysis by ethnicity, we found a positive association between VAI and NAFLD in the Hispanic and non-Hispanic White population, but not in the non-Hispanic Black population. On the one hand, the formula of VAI which was evaluated based on a Caucasian population might have limitations regarding the non-Hispanic Black population. On the other hand, the ethnic difference might be attributed to lower VAI levels
in non-Hispanic Black population. As the results are shown in Table 3, VAI was only positively associated with NAFLD in the top quartile in men and higher quartiles in women. In addition to ethnic disparities, we also found the age-gender specific difference. We found a null association in women with age<40y, which might be explained by sexual dimorphism and fat distribution. Estrogens could enhance the sympathetic tone differentially to the adipose tissue, favoring lipid accumulation in the subcutaneous depot in premenopausal women, whereas women would shift to accrue more visceral fat after menopause.[43] Different from women, men are susceptible to visceral fat deposition in any stage of life. However, we did not find a valid association between VAI and the prevalence of NAFLD in people with age≥65y. According to epidemiological evidence, the prevalence of obesity, hypertension, and MetS increased with age.[44, 45] However, as shown in our subgroup analysis, the status of these metabolic diseases would weaken or abolish the association between VAI and the risk of NAFLD in both genders. Thus, the association of VAI and NAFLD in older people might be confounded by the status of metabolic disease. Moreover, we also found that the effect of VAI was highlighted in normal-weight people compared to the overweight or obese ones. As reported, non-obese NAFLD affects about one-third of the persons with NAFLD in the U.S.[46], and these individuals probably could not get as much attention from doctors as obese ones. The association reported here has important clinical and public health implications in NAFLD screening in the future.
This study has several strengths. First, this is a large population-based analysis using well-examined nationwide data, and the findings could be generalized for most U.S. population. Second, it is valued because we provided solid evidence of an independent association between VAI and NAFLD by performing multiple logistic regression and the stratified analyses. However, we are also aware of several limitations in our study. First, the cross-sectional nature of the study design meant that we could not investigate the longitudinal dynamic association between the progression of NAFLD and changes in VAI levels across several therapeutic interventions, such as lifestyle modification, exercise, and weight control. Second, although we used well-validated NAFLD and fibrosis models, there is chance for misclassifying in some cases due to lacking the information of image and histology of the liver. Third, estimates across some subgroups should be interpreted with caution due to limited sample sizes, such as subjects with diabetes or CVD.

5. Conclusions

In conclusion, our study documents that VAI might be a useful indicator for NAFLD, but not for hepatic fibrosis among U.S. adults, and there exists age-gender specific and ethnic differences. The results reported here have important public health implications in NAFLD screening in the future.

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**Ethical approval:** The survey was approved by the National Center for Health Statistics ethics review board (NCHS). The NCHS IRB/ERC Protocol number in this survey covers 98-12, 2005-06, 2011-17 and 2018-01.

**Data Availability:** The data supporting reported results can be freely available from the NHANES website public archive, accessible at NHANES Questionnaires, Data sets and Related Documentation repository (https://wwwn.cdc.gov/nchs/nhanes/Default.aspx).

**Author Contributions:** Q.L. and X.Z. conceived and designed the study. Q.L. and J.W. performed the database search and checked the results against the inclusion and exclusion criteria. Q.L. and Y.W. analyzed the data. Q.L. wrote the initial draft of the paper. L.W., J.W. and X.Z. reviewed and edited the manuscript. All authors have read and approved the final version.

**Conflicts of interest:** The authors declare no conflict of interest.
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Figure legend:

Fig 1. Flow chart of the participants inclusion and exclusion in this study.

Fig 2. The association between VAI and the risk of NAFLD stratified by age, ethnicity, smoking, IR and other related metabolic diseases.
NHANES 2003-2018
Adults (≥20 years) (n=44,790)

Pregnant (n=941)

Subjects with examination (n=38,015)

Exclusion
1. Significant alcohol consumption (n=14,125)
2. Viral hepatitis (n=6,476)
3. Missing ALT/AST/PLA or ALT/AST > 500U/L (n=208)
4. Missing for fasting glucose, lipids, or insulin (n=9,661)
5. Women within 12 weeks after delivery (n=23)

Final eligible subjects (n=7,522)

Flow chart of the participants inclusion and exclusion in this study
The association between VAI and the risk of NAFLD stratified by age, ethnicity, smoking, IR and other related metabolic diseases

| Subgroup                     | Men OR (95% CI) | Women OR (95% CI) |
|------------------------------|-----------------|-------------------|
| Age <40                      | 4.76 (1.68, 13.50) | 2.50 (0.78, 8.03) |
| Age (40-54)                  | 4.87 (1.14, 20.83) | 5.16 (1.75, 15.22) |
| Age (55-64)                  | 3.88 (1.08, 13.91) | 6.53 (1.26, 33.87) |
| Age (65+)                    | 0.82 (0.40, 1.70)  | 1.58 (0.45, 5.55)  |
| Hispanic                     | 3.55 (1.19, 10.58) | 4.06 (0.26, 13.07) |
| Whites                       | 2.31 (1.20, 4.45)  | 2.91 (1.05, 8.08)  |
| Blacks                       | 0.80 (0.14, 4.57)  | 2.75 (0.52, 14.51) |
| Nonsmoker                    | 1.90 (1.03, 3.52)  | 4.77 (2.25, 10.12) |
| Current smoker               | 3.02 (0.55, 16.60) | 1.12 (0.06, 20.71) |
| HOMA-IR<3                    | 2.77 (1.41, 5.43)  | 4.21 (1.47, 12.00) |
| HOMA-IR\geq3                | 3.67 (1.66, 8.13)  | 3.65 (1.84, 7.25)  |
| BMI <25                      | 3.94 (2.66, 6.33)  | 8.04 (1.33, 48.62) |
| BMI \geq25                  | 1.55 (0.89, 2.70)  | 3.70 (1.39, 7.24)  |
| Diabetes (no)                | 7.98 (5.06, 12.27) | 9.09 (5.25, 15.43) |
| Diabetes (yes)               | 6.27 (2.99, 13.16) | 5.89 (2.33, 14.91) |
| Hypertension (no)            | 2.44 (1.04, 5.72)  | 10.50 (2.97, 37.12) |
| Hypertension (yes)           | 1.69 (0.78, 3.68)  | 2.41 (0.93, 6.24)  |
| MetS (no)                    | 2.44 (1.24, 4.82)  | 4.54 (1.24, 16.66) |
| MetS (yes)                   | 1.62 (1.22, 2.15)  | 2.05 (0.83, 5.04)  |
| CVD (no)                     | 2.28 (1.19, 4.38)  | 4.30 (1.98, 9.34)  |
| CVD (yes)                    | 1.20 (0.35, 4.12)  | 3.09 (0.33, 29.08) |
| Before 2010y                 | 2.65 (1.37, 5.13)  | 6.34 (2.88, 13.93) |
| After 2010y                  | 1.58 (1.00, 2.50)  | 4.62 (1.64, 13.02) |
Cross-sectional study of the role of age, gender and ethnicity in the association between visceral adiposity index and nonalcoholic fatty liver disease among US adults (NHANES 2003–2018)

Qianwen Li¹, Ling Wang¹ ², Jing Wang¹, Yanjie Wang³, and Jian Wu¹*

Supplemental Material:

Supplemental Table 1. The mediating effect of HOMA-IR/ CRP on the associations between VAI and NAFLD indices

| Models                  | Men          |           |          | Women          |           |
|-------------------------|--------------|-----------|----------|----------------|-----------|
|                         |  HOMA-IR     |       p   |  CRP     |   p            |  HOMA-IR  |       P  |  CRP    |     P   |
| VAI                     | 0.475***     | <0.001   | 0.058*** | <0.001        | 0.459     | <0.001   | 0.174   | <0.001 |
| USFLI defined by        |              |          |          |                |           |          |         |        |
| NAFLD                   |              |          |          |                |           |          |         |        |
| NFS                     | 0.903***     | <0.001   | 0.301*** | <0.001        | 0.904***  | <0.001   | 0.388***| <0.001 |
| FIB-4                   | 0.002        | 0.925    | 0.089*** | <0.001        | -0.044**  | 0.007    | 0.027***| 0.164  |
| APRI                    | 0.080***     | <0.001   | -0.019   | 0.319         | -0.002    | 0.886    | 0.103   | <0.001 |

CRP, C-reactive protein; HOMA-IR, homeostasis model assessment of insulin resistance; VAI, visceral adiposity index; USFLI, the US fatty liver index; NFS, NAFLD fibrosis score; FIB-4, the fibrosis-4 index; APRI, AST to platelet ratio index; **p<0.05, ***p<0.001.
Cross-sectional study of the role of age, gender and ethnicity in the association between visceral adiposity index and nonalcoholic fatty liver disease among US adults (NHANES 2003–2018)

Qianwen Li¹, Ling Wang¹², Jing Wang¹, Yanjie Wang³, and Jian Wu¹*

STROBE Statement—Checklist of items included in this report:

| Item                          | Page |
|-------------------------------|------|
| Title and abstract            |      |
| No                            | Recommendation                              |
| 1                             | (a) Indicate the study’s design with a commonly used term in the title or the abstract  |
|                               | (b) Provide in the abstract an informative and balanced summary of what was done and what was found |
| Introduction                  |      |
| Background/rationale          |      |
| 2                             | Explain the scientific background and rationale for the investigation being reported |
| Objectives                    |      |
| 3                             | State specific objectives, including any prespecified hypotheses |
|Methods                        |      |
| Study design                  |      |
| 4                             | Present key elements of study design early in the paper |
| Setting                       |      |
| 5                             | Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow up, and data collection |
| Participants                  |      |
| 6                             | (a) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up. |
|                               | (b) For matched studies, give matching criteria and number of exposed and unexposed |
| Variables                     |      |
| 7                             | Clearly define all outcomes, exposures, predictors, potential confounders, and effect |
| Data sources/measurement | 8* | For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group | 6 |
| Bias | 9 | Describe any efforts to address potential sources of bias | 7-8 |
| Study size | 10 | Explain how the study size was arrived at | 6 |
| Quantitative variables | 11 | Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why | 7-8 |
| Statistical methods | 12 | (a) Describe all statistical methods, including those used to control for confounding (b) Describe any methods used to examine subgroups and interactions (c) Explain how missing data were addressed (d) If applicable, describe analytical methods taking account of sampling strategy (e) Describe any sensitivity analyses | 9-10 |
| Results | | | |
| Participants | 13* | (a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed. (b) Give reasons for non-participation at each stage (c) Consider use of a flow diagram | 10 |
| Descriptive data | 14* | (a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders (b) Indicate number of participants with missing data for each variable of interest | 10-11 |
| Outcome data | 15* | Report numbers of outcome events or summary measures | 10 |
| Main results | 16 | (a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders | 12-15 |
| Section               | Recommendation |
|-----------------------|----------------|
| Other analyses        | Report other analyses done—eg, analyses of subgroups and interactions, and sensitivity analyses | 14-15 |
| Discussion            |                 |
| Key results           | Summarise key results with reference to study objectives | 16 |
| Limitations           | Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias | 20 |
| Interpretation        | Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence | 16-20 |
| Generalisability      | Discuss the generalisability (external validity) of the study results | 18-19 |
| Other information     |                 |
| Funding               | Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based | 21 |