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ORIGINAL ARTICLE

Retrospective Cohort Study

C-peptide as a key risk factor for non-alcoholic fatty liver disease in the United States population

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Abstract

AIM

To determine whether fasting C-peptide is an independent predictor for non-alcoholic fatty liver disease (NAFLD) in United States population.

METHODS

Using the National Health and Nutrition Examination Survey (NHANES) 1988-1994, NAFLD participants aged 20 or greater without any other liver diseases were included in this study. Excessive alcohol intake is defined as > 2 drinks per day for males and > 1 drink per day for females. C-peptide and 27 other factors known to be associated with NAFLD (*e.g.*, age, gender, body mass index, waist circumference, race/ethnicity, liver chemistries, and other diabetes tests) were tested in both univariate and multivariate level using logistic regression with a *P*-value 0.05.

RESULTS

Of 18825 participants aged ⩾ 20, 3235 participants (n = 3235) met inclusion criteria. There were 23 factors associated with NAFLD by univariate analysis. 9 factors, ranked by the highest change in pseudo R^2, were found to be significant predictors of NAFLD in multivariate model: waist circumference, fasting C-peptide, natural log of alanine aminotransferase (ALT), total protein, being

Mexican American, natural log of glycated hemoglobin, triglyceride level, being non-Hispanic white, and ferritin level.

CONCLUSION

Together with waist circumference and ALT, fasting C-peptide is among three most important predictors of NAFLD in United States population in the NHANES data set. Further study is needed to validate the clinical utility of fasting C-peptide in diagnosis or monitoring insulin resistance in NAFLD patients.

Key words: Insulin resistance; Fatty liver; Hepatosteatosis; Metabolic syndrome; C-peptide

Core tip: Non-alcoholic fatty liver disease (NAFLD) is a growing global epidemic and associated with many conditions and factors, including insulin resistance. However, C-peptide has not been used in practice to assess insulin resistance in NAFLD patients. Using a large national dataset, we demonstrated that three most important risk factors for NAFLD are waist circumference, fasting C-peptide, and alanine aminotransferase, respectively. Such results revealed that C-peptide superior to measurement of fasting insulin levels and can potentially be used for screening or monitoring the degree of insulin resistance in NAFLD.

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INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is a condition in which hepatic steatosis exists in the absence of excessive alcohol consumption. NAFLD is the most common cause of chronic liver disease in the United States with estimated prevalence around 30%-40%[1-3]. Given the epidemic of obesity, NAFLD is increasingly prevalent and challenging[4,5]. NAFLD can progress to more severe liver diseases, such as non-alcoholic steatohepatitis (NASH), cirrhosis, and hepatocellular carcinoma.

Obesity and insulin resistance are among important risk factors for NAFLD[6,7]. Many studies found that indicators of obesity [*i.e.*, body mass index (BMI) and waist circumference] and insulin resistance [*i.e.*, glycated hemoglobin (HbA1c), insulin level, fasting glucose, and diabetes mellitus] are independently associated with NAFLD and/or severity of liver fibrosis in NAFLD[8-11]. C-peptide levels can be used to measure insulin secretion[12]. However, there is limited evidence of the association between NAFLD and C-peptide at the multivariate level[13,14].

Both C-peptide and insulin are produced and released in equimolar amounts. C-peptide can therefore be used to assess endogenous insulin secretion. However, the level of C-peptide and insulin level in blood are typically different deriving from the differences in clearance mechanisms and half-life[15]. In addition to diabetes and insulin resistance, C-peptide has been associated with many risk factors for NAFLD including cardiovascular diseases and metabolic syndrome[16-18].

Therefore, our primary objective was to determine if fasting C-peptide is independently associated with NAFLD using multivariate analysis in the United States general population.

MATERIALS AND METHODS

Study population and study design

The Third National Health and Nutrition Examination Survey (NHANES Ⅲ) is a probability sample of 39695 persons aged 2 mo and older representing the United States population and conducted by the National Center for Health Statistics (NCHS) to evaluate health and nutritional status[19]. The survey collected multiple data sets, including demographic, interviews, physical examinations, and laboratory testing of biologic samples. NHANES Ⅲ was conducted from 1988 to 1994. The NHANES protocol was approved by the NCHS Research Ethics Review Board.

There were 18825 persons aged 20 years or older in NHANES Ⅲ that met inclusion criteria for this study. The exclusion criteria included: (1) Ungradable or missing ultrasound results for hepatic steatosis, (2) excessive alcohol consumption, (3) hepatitis B or hepatitis C infection (4) fasting period outside of 8-24 h (5) incomplete or missing data on physical examination and laboratory testing. Participants were divided into two groups: NAFLD participants (study group) and non-NAFLD participants (control group).

As presented in Table 1, we included 28 factors associated with NAFLD as independent variables in this study: Demographic (*i.e.*, age , gender, race/ethnicity), body measurement (*i.e.*, BMI and waist circumference), general biochemistry tests [*i.e.*, iron, total iron-binding capacity (TIBC), transferrin saturation, ferritin, cholesterol, triglyceride, HDL cholesterol, C-reactive protein, and uric acid], liver chemistry [aspartate aminotransferase (AST), Alanine aminotransferase (ALT), gamma glutamyl transferase (GGT), alkaline phosphatase (ALP), total bilirubin, total protein, and albumin], and diabetes testing profile [*i.e.*, HbA1c, fasting plasma glucose (FPG), fasting C-peptide, and fasting insulin]. Besides demographic variables, the above variables were selected as the risk factors based on the usage in clinical practice and the supporting evidence that demonstrated the association with NAFLD or its commonly accepted risk factors (*i.e.*, obesity, insulin resistance, and liver fibrosis).

Table 1 Baseline characteristics of participants with non-alcoholic fatty liver disease and controls *n* (%)

	NAFLD, *n* = 817	Controls, *n* = 2418
Demographic		
Age (yr)	48.47 ± 15.75	44.36 ± 16.20
Gender (male)	368 (45.04)	1004 (41.52)
Race/ethnicity		
White (non-Hispanic)	308 (37.70)	1046 (43.13)
Black (non-Hispanic)	193 (23.62)	705 (29.16)
Mexican American	280 (34.27)	550 (22.75)
Others	36 (4.41)	120 (4.96)
Body measurement		
Body mass index (kg/m^2)	30.38 ± 6.95	26.56 ± 5.42
Waist circumference (cm)	101.73 ± 16.32	90.84 ± 13.45
Biochemistry tests		
Iron (μg/dL)	75.35 ± 29.71	77.71 ± 32.75
TIBC (μg/dL)	364.79 ± 58.05	359.86 ± 56.59
Transferrin saturation (%)	21.13 ± 8.73	22.09 ± 9.65
Ferritin (ng/mL)	161.75 ± 152.55	110.16 ± 114.71
Cholesterol (mg/dL)	212.23 ± 44.95	202.73 ± 42.19
Triglyceride (mg/dL)	202.91 ± 137.97	136.58 ± 95.79
HDL cholesterol (mg/dL)	46.72 ± 16.80	52.10 ± 15.61
C-reactive protein (mg/dL)[1]	0.56 ± 0.80	0.45 ± 0.65
Uric acid (mg/dL)	5.62 ± 1.52	5.04 ± 1.42
Liver chemistry		
AST (U/L)[1]	24.76 ± 19.62	20.72 ± 14.71
ALT(U/L)[1]	22.78 ± 17.86	15.96 ± 12.14
GGT (U/L)[1]	42.87 ± 66.68	28.14 ± 41.69
ALP (U/L)[1]	93.72 ± 33.61	86.04 ± 36.29
Total bilirubin (mg/dL)	0.55 ± 0.30	0.54 ± 0.28
Total protein (g/dL)	7.49 ± 0.46	7.37 ± 0.45
Albumin (g/dL)	4.11 ± 0.35	4.12 ± 0.36
Diabetes testing profile		
HbA1c (%)[1]	6.02 ± 1.62	5.50 ± 1.09
FPG (mg/dL)[1]	114.50 ± 65.84	97.85 ± 35.68
Fasting C-peptide (pmol/mL)	1.11 ± 0.68	0.69 ± 0.53
Fasting insulin (μU/mL)[1]	21.85 ± 27.82	12.76 ± 19.24

[1]The distribution is positively skewed with skewness > 3. ALP: Alkaline phosphatase; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; FPG: Fasting plasma glucose; GGT: Gamma glutamyl transferase; HbA1c: Glycated hemoglobin; TIBC: Total iron-binding capacity.

Definitions

In this study, NAFLD is defined as: (1) Diagnosed with moderate to severe hepatic steatosis on ultrasound; (2) no history of excessive alcohol intake in the past 12 mo; (3) not infected with hepatitis B or hepatitis C.

To evaluate the presence and extent of hepatic steatosis, readers used five main criteria: (1) Parenchymal brightness, (2) liver to kidney contrast, (3) deep beam attenuation, (4) bright vessel walls, and (5) gallbladder wall definition. Based on the presence or absence of these five criteria, a main finding was categorized as normal, mild, moderate or severe[20]. It is worth nothing that participants aged above 74 were not eligible for ultrasound study in NHANES Ⅲ For this reason, patients age above 74 were excluded from this study.

Excessive alcohol intake is defined as more than 2 drinks per day for men or 1 drink per day for women in the past 12 mo, in which one drink of alcoholic beverage is equivalent to a 12 oz beer, a 5 oz glass of wine, or 1.5 oz of liquor. The average number of drinks per day is calculated from number of drinking days × number of drinks on drinking day/365 d. To qualify as hepatitis viral infection, participants must have tested positive for serum hepatitis B surface antigen or serum hepatitis C antibody HCP (anti-HCV).

Statistical analysis

Statistical analyses were performed using STATA Release 14 (StataCorp LP, TX, United States). Numbers are presented in mean ± SD or number (%). All continuous factors were first tested for skewness; if the distributions were extremely skewed to the right (herein defined as skewness > 3), the factors were log transformed before using them as predictors in regression models. Since the response variable is dichotomous variable (NAFLD or non-NAFLD), logistic model is an appropriate model for determining if predictors are significantly associated with the response variable. As a result, logistic regression was used to determine if NAFLD is associated with any predictor in univariate level. Then, the significant factors from univariate analysis were included as predictors in step-wise logistic regression to determine the significant predictors in multivariate level. The significance level is 0.05.

RESULTS

Out of 18825 participants aged ⩾ 20, there were 3235

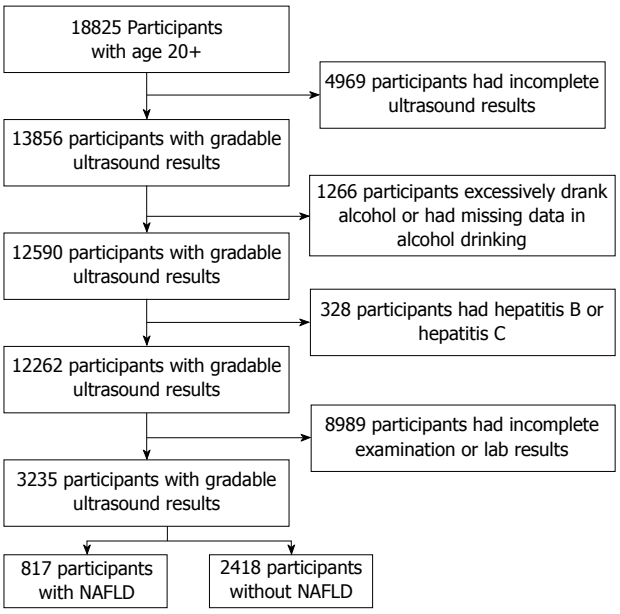

Figure 1 Study design and study population.

participants (n = 3235) that passed the exclusion criteria as shown in Figure 1. Based on ultrasound findings, 817 (25.26%) participants were classified as NAFLD. Baseline characteristics of participants in study group and control group are summarized in Table 1.

For continuous variables, there were 8 factors having skewness greater than 3. Subsequently, the log transformation was applied to these factors, including C-reactive protein, AST, ALT, GGT, alkaline phosphatase, glycated hemoglobin, plasma glucose, and insulin. As shown in Table 2, there are 24 variables significantly associated with NAFLD in univariate level; the P-value of these significant factors mostly below 0.001.

As presented in Table 3, the number of significant factors reduced from 24 to 9 in multivariate analysis. The top three factors ranked by the highest change in pseudo R^2 (ΔR^2) are waist circumference (OR = 1.03, ΔR^2 = 2.13%, $P < 0.001$), C-peptide level (OR = 1.82, ΔR^2 = 1.33%, $P < 0.001$), and $\log_e$ of ALT (OR = 1.76, ΔR^2 = 1.16%, $P < 0.001$). The pseudo R^2 of the multivariate model is 16.68%.

DISCUSSION

The most significant NAFLD risk factor in both univariate and multivariate levels is waist circumference. Since waist circumference and BMI are highly interrelated surrogate markers of obesity[21], it is not surprising to see one factor eliminated in multivariate level. Waist circumference-a measure of excess abdominal adiposity-has been identified as an independent risk factor for many obesity-related conditions, such as cardiovascular disease, type 2 diabetes, dyslipidemia, and hypertension, metabolic syndrome, polycystic ovary syndrome[22-27]. The results from this study support that waist circumference is also an independent and probably the most important risk factor for NAFLD in the United States population.

Insulin resistance is another well-known condition commonly found in NAFLD patients[28,29]. Indeed, all diabetes test profiles were positively correlated with NAFLD by univariate analysis. In fact, type 2 diabetes can be diagnosed directly from HbA1c level (⩾ 6.5%) or FPG (⩾ 126 mg/dL)[30], while C-peptide and insulin are not routinely used in clinical practice to diagnose type 2 diabetes. While there are situations where C-peptide or insulin levels are useful-the diagnosis of insulinoma[31], surreptitious use of insulin[32], the diagnosis of type 2 diabetes in the young[33], and the diagnosis of latent autoimmune diabetes in adults[34], direct measurement of insulin levels and not of C-peptide has been used to assess insulin resistance. Previous studies often found that C-peptide levels are raised in patients with NASH[35-37]. However, the application of C-peptide as a biomarker for interventions designed to improve insulin sensitivity remains to be determined. In case of NAFLD, there is only limited evidence; C-peptide was found to be associated with NAFLD in specific groups of population (i.e., obese adolescents and adults, latent autoimmune diabetes, and diabetes patients)[13,14,38]. Our results are the first to show that C-peptide has a role not only as an independent risk factor for NAFLD but can also be useful for screening or monitoring the degree of insulin resistance in NAFLD in the general population. Based on ΔR^2, we conclude that insulin resistance, as indicated by fasting C-peptide, is the second most important condition leading to NAFLD, second only to obesity as diagnosed by waist circumference, and is superior to measurement of fasting insulin levels.

Liver chemistries are used as an indicator of liver inflammation or liver cell damage. Commonly used liver chemistries include AST, ALT, ALP, GGT, total bilirubin, total protein, and albumin. For example, predominance of AST and ALT indicates hepatocellular injury; predominance of ALP and total bilirubin indicates cholestatic injury; an elevated ALP of hepatic origin may be confirmed GGT[39-41]. As shown in Table 2, total protein and the natural log of AST, ALT, ALP, and GGT were positively associated with NAFLD in univariate analysis. However, only total protein and natural log of ALT were positively correlated with NAFLD in multivariate level. AST and ALT are the most widely used liver chemistries. The fact that ALT is included in multivariate model is not unexpected since ALT is generally higher than AST level in NAFLD[40]. On the other hand, total protein is a non-specific marker of health, nutrition and liver synthetic capacity. Due to the fact that total protein consists of albumin and multiple subtypes of globulin, further investigation into the association between NAFLD and each subtype of globulin may provide a clearer explanation of our findings.

Ferritin is a protein that mainly stores iron in the body and serum ferritin level is the most accurate blood test to diagnose iron deficiency anemia[42]. Recently, the role of ferritin as a biomarker in inflammatory diseases has been increasingly recognized[43-45]. As an acute phase

Table 2 Univariate analysis for predictors of non-alcoholic fatty liver disease

	Beta	Standard error	Odds ratio	*P* value
Demographic				
Age (yr)	0.0157	0.0025	1.02	< 0.001[a]
Gender (male)	0.1435	0.0815	1.15	0.078
Race/ethnicity				
White (non-Hispanic)	-0.226	0.0831	0.80	0.007[a]
Black (non-Hispanic)	-0.2857	0.0937	0.75	0.002[a]
Mexican American	0.5715	0.0882	1.77	< 0.001[a]
Others	-0.1248	0.1945	0.88	0.521
Body measurement				
Body mass index (kg/m^2)	0.1004	0.0069	1.11	< 0.001[a]
Waist circumference (cm)	0.0506	0.003	1.05	< 0.001[a]
Biochemistry tests				
Iron (μg/dL)	-0.0024	0.0013	1.00	0.069
TIBC (μg/dL)	0.0015	0.0007	1.00	0.032[a]
Transferrin saturation (%)	-0.0111	0.0044	0.99	0.012[a]
Ferritin (ng/mL)	0.0029	0.0003	1.00	< 0.001[a]
Cholesterol (mg/dL)	0.005	0.0009	1.01	< 0.001[a]
Triglyceride (mg/dL)	0.0049	0.0004	1.00	< 0.001[a]
HDL cholesterol (mg/dL)	-0.0261	0.0031	0.97	< 0.001[a]
C-reactive protein (mg/dL)[1]	0.3398	0.0534	1.40	< 0.001[a]
Uric acid (mg/dL)	0.2649	0.0277	1.30	< 0.001[a]
Liver chemistry				
AST (U/L)[1]	1.004	0.1153	1.02	< 0.001[a]
ALT (U/L)[1]	1.0274	0.0777	1.04	< 0.001[a]
GGT (U/L)[1]	0.7441	0.0612	1.01	< 0.001[a]
ALP (U/L)[1]	0.9011	0.1303	1.01	< 0.001[a]
Total bilirubin (mg/dL)	0.0914	0.1395	1.09	0.512
Total protein (g/dL)	0.581	0.0896	1.79	< 0.001[a]
Albumin (g/dL)	-0.0871	0.1141	0.92	0.445
Diabetes testing profile				
HbA1c (%)[1]	2.2201	0.2184	1.34	< 0.001[a]
FPG (mg/dL)[1]	1.3125	0.1428	1.01	< 0.001[a]
Fasting C-peptide (pmol/mL)	1.1976	0.0753	3.31	< 0.001[a]
Fasting insulin (μU/mL)[1]	0.9646	0.059	1.02	< 0.001[a]

[1]Log transformation was applied to this factor before including in regression model. [a]*P* < 0.05. ALP: Alkaline phosphatase; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; FPG: Fasting plasma glucose; GGT: Gamma glutamyl transferase; HbA1c: Glycated hemoglobin; TIBC: Total iron-binding capacity. [a]*P* < 0.05.

Table 3 Risk factors of non-alcoholic fatty liver disease from step-wise logistic regression

	Beta	Standard error	Odds ratio	Change in pseudo R^2	*P* value
Demographic					
Race/ethnicity					
White (non-Hispanic)	0.2969	0.1162	1.35	0.18%	0.011
Mexican American	0.5495	0.1207	1.73	0.57%	0.020
Body measurement					
Waist circumference (cm)	0.0308	0.0035	1.03	2.13%	< 0.001
Biochemistry tests					
Ferritin (ng/mL)	0.0013	0.0004	1.00	0.15%	< 0.001
Triglyceride (mg/dL)	0.0008	0.0004	1.00	0.32%	< 0.001
Liver chemistry					
ALT (U/L)[1]	0.5658	0.0875	1.76	1.16%	< 0.001
Total protein (g/dL)	0.5319	0.1045	1.70	0.72%	< 0.001
Diabetes testing profile					
HbA1c (%)[1]	0.9266	0.2492	2.53	0.38%	< 0.001
Fasting C-peptide (pmol/mL)	0.6009	0.0877	1.82	1.33%	< 0.001

[1]Log transformation was applied to this factor before including in regression model. Note: Pseudo R^2 = 16.68%; constant (Y-intercept) = 0.0000153; change in Pseudo R^2 is an incremental increase in Pseudo R^2 resulting from adding variable to model last. ALT: Alanine aminotransferase; HbA1c: Glycated hemoglobin.

response protein, ferritin concentrations increase during inflammation and may not reflect the size of total body iron stores[44]. Moreover, ferritin was found be associated with histologic severity and advanced fibrosis in patients

with NAFLD[46-48].

Other factors significantly associated with NAFLD include race/ethnicity (non-Hispanic white and Mexican American), and triglyceride level. Race/ethnicity were often found associated with obesity-related diseases in United States based population[49,50] Triglyceride is an important biomarker of cardiovascular disease risk[51], another condition highly interrelated with NAFLD.

There are several limitations in this study. First, the diagnosis of NAFLD in this study is based on the hepatic ultrasound results although liver biopsy remains the gold standard for the diagnosis of NAFLD. Second, the statistical analysis used is logistic regression. Since the relationship among these factors are complex, inter-related, and non-linear, linearity assumptions embedded in logistic regression may not be able to address all aspects of NAFLD. Furthermore, given a pseudo R^2 of 16.68%, only 16.68% of variation can be explained by multivariate model in Table 3.

In conclusion, NAFLD is associated with many conditions and factors. Three most important factors from multivariate model in this study are waist circumference, fasting C-peptide, and ALT. Further study is needed to validate the clinical utility of C-peptide in diagnosis or monitoring insulin resistance in NAFLD patients.

ARTICLE HIGHLIGHTS

Research background

Non-alcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver disease in the United States. Additionally, NAFLD can progress to more severe liver diseases, such as non-alcoholic steatohepatitis, cirrhosis, and hepatocellular carcinoma. Many factors were found to be independently associated with NAFLD and/or severity of liver fibrosis in NAFLD. Nevertheless, there is limited evidence of the association between NAFLD and C-peptide.

Research motivation

Among many risk factors that are associated with NAFLD, obesity and insulin resistance are probably the most well-known ones. C-peptide levels can be used to measure insulin secretion and a surrogate marker of insulin resistance. However, C-peptide is not routinely used in clinical practice to diagnose type 2 diabetes or monitor insulin resistance status in NAFLD.

Research objectives

The objective of this study was to determine if fasting C-peptide is independently associated with NAFLD using multivariate analysis in the United States general population.

Research methods

Using the National Health and Nutrition Examination Survey 1988-1994, NAFLD participants aged 20 or greater without any other liver diseases were included in this study. The participants with excessive alcohol intake (> 2 drinks per day for males and > 1 drink per day for female) were excluded from the study. C-peptide and 27 other factors known to be associated with NAFLD (*e.g.*, age, gender, body mass index, waist circumference, race/ethnicity, liver chemistries, and other diabetes tests) were selected as predictors in regression model. Univariate logistic regression and multivariate step-wise logistic regression were used to determine if the significant predictors of NAFLD, respectively.

Research results

There were 3235 participants (n = 3235) that passed the exclusion criteria. Based on ultrasound findings, 817 (25.26%) participants were classified as NAFLD. Twenty-four variables were significantly associated with NAFLD in univariate level; the P-value of these significant factors mostly below 0.001. Using multivariate analysis, we found 9 out of 24 factors to be significantly associated with NAFLD. Ranked by $\triangle R^2$, the top three factors ranked are waist circumference (OR = 1.03, $\triangle R^2$ = 2.13%, P < 0.001), C-peptide level (OR = 1.82, $\triangle R^2$ = 1.33%, P < 0.001), and $\log_e$ of ALT (OR = 1.76, $\triangle R^2$ = 1.16%, P < 0.001). The pseudo R^2 of the multivariate model is 16.68%.

Research conclusions

C-peptide is the second most important predictor of NAFLD in United States population after waist circumference.

Research perspectives

Further prospective research is needed to validate the clinical utility of fasting C-peptide in diagnosis or monitoring insulin resistance in NAFLD patients. Moreover, C-peptide should be considered as a potential factor for calculative liver scores to evaluate the fibrosis level.

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