Association between remnant lipoprotein cholesterol levels and risk of non-alcoholic fatty liver disease in non-obese populations: a Chinese longitudinal prospective cohort study

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ABSTRACT

Objectives The association between remnant lipoprotein cholesterol (RLP-C) levels and the incidence of non-alcoholic fatty liver disease (NAFLD) is unclear, especially in non-obese populations.

Setting We used data from a health assessment database. The assessment was conducted at the Wenzhou Medical Center from January 2010 to December 2014. The patients were divided into low, middle and high RLP-C groups according to tertiles of RLP-C, and baseline metabolic parameters were compared among the three groups. Kaplan-Meier analysis and Cox proportional hazards regression were used to evaluate the relationship between RLP-C and NAFLD incidence. Additionally, sex-specific associations between RLP-C and NAFLD were examined.

Participants 16,173 non-obese participants from the longitudinal healthcare database.

Outcome measure NAFLD was diagnosed using abdominal ultrasonography and clinical history.

Results Participants with higher RLP-C levels tended to have higher blood pressure, liver metabolic index and lipid metabolism index than those with middle or low RLP-C (p<0.001). During the 5-year follow-up period, 2,322 (14.4%) participants developed NAFLD. Participants with high and middle RLP-C levels were at a higher risk of developing NAFLD, even after adjusting for age, sex, body mass index and main metabolic parameters (HR 1.6, 95% CI 1.3, 1.9, p<0.001; and HR 1.3, 95% CI 1.1, 1.6, p=0.01, respectively). The effect was consistent in subgroups of different ages, systolic blood pressures and alanine aminotransferase levels, except for sex and direct bilirubin (DBIL). These correlations, beyond traditional cardiometabolic risk factors, were stronger in males than females (HR 1.3 (1.1, 1.6) and HR 1.7 (1.4, 2.0), p for interaction 0.014 for females and males, respectively).

Conclusions In non-obese populations, higher RLP-C levels indicated a worse cardiovascular metabolic index. RLP-C was associated with the incidence of NAFLD, independent of the traditional risk factors of metabolism. This correlation was more substantial in the male and low DBIL subgroups.

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STRENGTHS AND LIMITATIONS OF THIS STUDY

- The sample size of our study was large, and the results were representative.
- Our analysis was based on longitudinal data, which made the results more convincing.
- Although we adjusted for several variables in our analysis, as a post-hoc analysis, unidentified confounders, such as smoking, alcohol consumption and other lifestyle factors, were not included.

BACKGROUND

Non-alcoholic fatty liver disease (NAFLD) is a group of conditions associated with liver metabolic dysfunction. NAFLD is the leading cause of cirrhosis and hepatocellular carcinoma, with a prevalence of 25%.1 In recent years, viral hepatitis-related mortality has decreased; however, NAFLD-related mortality continues to increase.2 Previous studies have shown that type 2 diabetes mellitus, obesity, dyslipidaemia and age are the main risk factors of NAFLD.3 4 About 25% of the population with NAFLD are lean or non-obese individuals.5 Hepatic lipid accumulation, insulin resistance, visceral fat, metabolic dysfunction, sarcopenia and genetic predisposition may be underlying mechanisms of NAFLD in non-obese individuals.6 Among these, lipid accumulation in the liver constitutes a vital process of both obese and non-obese NAFLD.7 8

While less than 10% of the population with NAFLD develops liver-related complications, identifying individuals at high risk of developing NAFLD poses a significant challenge. Remnant lipoprotein cholesterol (RLP-C), a modern index of lipid metabolism with triglyceride (TG)-enriched precursors of low-density lipoprotein (LDL), has been shown to be associated with an increased incidence...
of coronary heart disease (CHD). An observational study found that RLP-C was more predictive of myocardial infarction than any other lipid particles. Furthermore, an association between RLP-C and NAFLD has been recognised, and in adolescents, this association was found to be beyond that of the traditional risk factors of adiposity and insulin resistance. In the general population, it was found that patients with elevated RLP-C had a higher risk of NAFLD incidence after adjusting for potential confounders (OR 1.77 per SD increase, 95% CI: 1.64–1.91, p for trend<0.001). However, this was a cross-sectional study, and long-term follow-up data exploring the relationship between RLP-C and newly diagnosed NAFLD are still lacking. In addition, most studies were conducted in the high metabolic risk or general populations, whereas studies in non-obese patients are limited.

We investigated the correlation between RLP-C levels and NAFLD in non-obese individuals. Additionally, due to sex differences in prevalence, risk factors, fibrosis degree and NAFLD clinical outcomes, we further explored the sex differences in the prediction of NAFLD development.

**METHODS**

We derived data from the Dryad data repository, which can be accessed at http://datadryad.org/ (doi:10.5061/dryad.1n6c4). This longitudinal study, designed and initiated by the Wenzhou Medical Center of Wenzhou People’s Hospital, included non-obese individuals who underwent health screening at the Wenzhou People’s Hospital from January 2010 to December 2014. This study indicated that LDL cholesterol (LDL-C) levels were positively associated with the risk of NAFLD (diagnosed by ultrasound and accompanied by alcohol consumption (≥140 g/week for men and ≥70 g/week for women) in non-obese (body mass index (BMI) <25 kg/m²) populations.

**Statistical analysis**

The participants were grouped according to RLP-C tertiles. Continuous variables are expressed as mean±SD or median (Q1–Q3) based on the distribution of data. All categorical variables are expressed as frequencies (percentiles). The characteristics of the study population according to the different RLP-C groups were examined using analysis of variance for normally distributed data and the Kruskal-Wallis H test for skewed data. We used the X² or Fisher’s exact tests to compare the categorical variables. Kaplan-Meier analysis was used to calculate the survival rates of patients with newly diagnosed NAFLD during the follow-up period. HRs based on Cox proportional hazards regression and 95% CIs were determined to evaluate the association between the RLP-C groups and the incidence of newly diagnosed NAFLD in three models. Model 1 was not adjusted. Model 2 was adjusted for age, sex and BMI. Model 3 was adjusted for age, sex, BMI, alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), alanine transaminase (ALT), aspartate transaminase (AST), TC, albumin (ALB), direct bilirubin (DBIL), creatinine (CR), blood urea nitrogen (BUN) and uric acid (UA).

Tests for linear trends were conducted using tertiles of RLP-C values as continuous variables and the lowest tertile of RLP-C was set as a reference. The relationship between RLP-C groups and newly diagnosed NAFLD according to various subgroups was assessed by stratified analysis and interaction tests using model 3. All analyses were performed using the statistical software package R (V.4.0; The R Foundation; http://www.R-project.org). Statistical significance was set at two-tailed p<0.05.

**RESULTS**

**Baseline characteristics of the participants**

We included 16173 participants in the analysis, of which 2322 (14.4%) non-obese participants developed NAFLD within the 5-year follow-up period. The baseline characteristics of the participants in the different RLP-C groups are shown in table 1. Participants in the higher RLP-C group were more likely to be older and have higher BMI, blood pressure, ALP, GGT, ALT, AST, ALB, BUN, CR, UA,
TC, TG, LDL-C and glucose levels, and newly diagnosed NAFLD risk, but lower levels of DBIL and HDL-C than those in the lower RLP-C groups.

**Kaplan-Meier curve analysis**

Figure 1 shows the Kaplan-Meier curves for the cumulative incidence of newly diagnosed NAFLD in the different RLP-C groups. The risk of newly diagnosed NAFLD differed significantly between RLP-C levels (p<0.001). As RLP-C levels increased, the probability of newly diagnosed NAFLD gradually increased.

**Relationship between RLP-C levels and newly diagnosed NAFLD**

Table 2 shows the HRs (95% CI) of newly diagnosed NAFLD among participants in the different RLP-C groups. Participants in the high RLP-C group had a significantly elevated risk of NAFLD incidence compared with the other two RLP-C groups, with HRs of 1.5 and 2.1, respectively (p<0.001). After adjusting for potential confounding factors, including age, sex, BMI, ALP, GGT, ALT, AST, TC, ALB, DBIL, CR, BUN and UA, the relationship remained statistically significant (p<0.05), especially in the fully adjusted model 3; the adjusted HRs of the newly diagnosed NAFLD for participants in the middle and high-level RLP-C groups were 1.3 (1.1, 1.6) and 1.6 (1.3, 1.9), respectively. Furthermore, we assessed the association between RLP-C and newly diagnosed NAFLD according to sex, and found that regardless of sex, RLP-C was strongly associated with newly diagnosed NAFLD; the association remained significant (p<0.05), specifically in the fully adjusted model 3. Significant linear trends of RLP-C levels and newly diagnosed NAFLD were found among males, females and all participants (HR 1.2 (1.1, 1.4), p for trend=0.003 for females; HR 1.3 (1.1, 1.4) p for trend<0.001 for males; and HR 1.3 (1.2, 1.4), p for trend<0.001 for all participants).

**Subgroup analysis for the risk of NAFLD incidence by RLP-C**

A subgroup analysis was performed to estimate other risk factors that might influence the associations between
As shown in table 3, subgroups including sex (male vs female), age (<45 years vs ≥45 years), systolic blood pressure (SBP) (<140 mm Hg vs ≥140 mm Hg to ≤160 mm Hg vs ≥160 mm Hg), ALT (<16 U/L vs ≥16 U/L) and DBIL (<2.1 µmol/L vs ≥2.1 µmol/L) were analysed. The findings revealed a significant interaction between sex (male vs female; p for interaction=0.0137) and newly diagnosed NAFLD. RLP-C showed better predictive ability for the risk of newly diagnosed NAFLD in males. The HRs for the effect of RLP-C on the risk of newly diagnosed NAFLD in females and males were 1.3 (1.1, 1.6) and 1.7 (1.4, 2.0), respectively. In addition, there was a strong interaction between DBIL (<2.1 µmol/L vs ≥2.1 µmol/L; p for interaction=0.0006) and newly diagnosed NAFLD. The effect of RLP-C on the risk of newly diagnosed NAFLD was more substantial in the low RLP-C group (HR (95% CI): 1.7 (1.5, 2.0), p<0.0001) than in the high RLP-C group (HR (95% CI): 1.2 (0.9, 1.5), p=0.1786). The interaction of other subgroups and RLP-C had no significant effect on the risk of newly diagnosed NAFLD, and the p values of the interaction were all >0.05.

**Table 2** Association between RLP-C and new-onset NAFLD in different models

<table>
<thead>
<tr>
<th>RLP-C groups</th>
<th>Model 1 HR (95% CI) p value</th>
<th>Model 2 HR (95% CI) p value</th>
<th>Model 3 HR (95% CI) p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Middle</td>
<td>1.5 (1.3, 1.8) &lt;0.001</td>
<td>1.3 (1.1, 1.5) 0.011</td>
<td>1.4 (1.0, 1.7) 0.022</td>
</tr>
<tr>
<td>High</td>
<td>1.9 (1.6, 2.3) &lt;0.001</td>
<td>1.4 (1.2, 1.6) &lt;0.001</td>
<td>1.6 (1.2, 2.1) 0.002</td>
</tr>
<tr>
<td>P for trend</td>
<td>1.4 (1.4, 1.5) &lt;0.001</td>
<td>1.2 (1.1, 1.3) &lt;0.001</td>
<td>1.2 (1.1, 1.4) 0.003</td>
</tr>
<tr>
<td>Male</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Middle</td>
<td>1.5 (1.3, 1.8) &lt;0.001</td>
<td>1.3 (1.1, 1.6) &lt;0.001</td>
<td>1.3 (1.0, 1.6) 0.025</td>
</tr>
<tr>
<td>High</td>
<td>2.3 (2.0, 2.7) &lt;0.001</td>
<td>1.7 (1.5, 2.0) &lt;0.001</td>
<td>1.7 (1.3, 2.1) &lt;0.001</td>
</tr>
<tr>
<td>P for trend</td>
<td>1.5 (1.4, 1.5) &lt;0.001</td>
<td>1.3 (1.2, 1.4) &lt;0.001</td>
<td>1.3 (1.1, 1.4) &lt;0.001</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Middle</td>
<td>1.5 (1.3, 1.7) &lt;0.001</td>
<td>1.3 (1.1, 1.5) &lt;0.001</td>
<td>1.3 (1.1, 1.6) 0.001</td>
</tr>
<tr>
<td>High</td>
<td>2.1 (1.9, 2.4) &lt;0.001</td>
<td>1.5 (1.4, 1.7) &lt;0.001</td>
<td>1.6 (1.3, 1.9) &lt;0.001</td>
</tr>
<tr>
<td>P for trend</td>
<td>1.5 (1.4, 1.5) &lt;0.001</td>
<td>1.2 (1.2,1.3) &lt;0.001</td>
<td>1.3 (1.2,1.4) &lt;0.001</td>
</tr>
</tbody>
</table>

Model 1 was not adjusted. Model 2 was adjusted for age, sex and BMI. Model 3 was adjusted for age, sex, BMI, ALP, GGT, ALT, AST, TC, ALB, DBIL, CR, BUN and UA. ALB, albumin; ALP, ALP, alkaline phosphatase; ALT, alanine transaminase; AST, aspartate transaminase; BMI, body mass index; BUN, blood urea nitrogen; CR, creatinine; DBIL, direct bilirubin; GGT, gamma-glutamyl transferase; NAFLD, non-alcoholic fatty liver disease; RLP-C, remnant lipoprotein cholesterol; TC, total cholesterol; UA, uric acid.

**DISCUSSION**

In this longitudinal study, we explored the correlation between RLP-C and the incidence of NAFLD in non-obese populations. RLP-C was positively associated with the incidence of NAFLD. After fully adjusting for potential confounders, the risk of newly diagnosed NAFLD increased by 20% for each tertile of increase in RLP-C levels. The association remained consistent in the
Obese and non-obese NAFLD share a common pathophysiology: accumulation of free fatty acids in the liver. Dyslipidaemia plays a crucial role in the progression of hepatic steatosis in non-obese individuals. RLP-C, a TG-rich precursor of LDL, is a novel risk factor for atherosclerotic cardiovascular disease, and the relationship between RLP-C and atherosclerotic cardiovascular disease is well established. Studies from the Jackson Heart and Framingham Offspring Cohort found that RLP-C levels were predictive of CHD in different groups of primary prevention patients. In those with established CHD, RLP-C was associated with cardiovascular events in Chinese patients receiving statin treatment. In recent years, the association between RLP-C levels and NAFLD has been increasingly recognised. RLP-C was first identified by Pastori et al as an independent risk factor for NAFLD development in patients with cardiac metabolic diseases. The association between RLP-C and NAFLD has also been established in adolescents. We observed a similar association in our study. However, the participants in previous studies were either obese or overweight, and we believe our study is the first to explore the correlation between RLP-C and newly diagnosed NAFLD in non-obese participants. Higher RLP-C levels were associated with higher incidence of NAFLD in non-obese participants, even after adjusting for traditional risk factors.

A possible explanation is that visceral fat contributes more to NAFLD than does total body fat. Ha et al measured visceral and subcutaneous fat using single-slice axial CT in 840 patients with different degrees of hepatic steatosis and 1353 propensity score-matched healthy controls. The results showed that, compared with subcutaneous fat, visceral adiposity made non-obese participants more susceptible to NAFLD. Visceral fat activates immune cells, increases insulin resistance and alters adiponectin levels. Through portal circulation, this dysfunction causes the lipotoxic accumulation of fatty acids in the liver, leading to hepatic fat deposition. Asian individuals with a lower absolute BMI have more visceral or ectopic adipose tissue. A cross-sectional study of 2946 patients with type 2 diabetes mellitus found that adverse lipid metabolism was significantly associated with NAFLD risk, and the association was stronger in non-obese patients, which confirms the findings of our study.

Another important finding of our study was that the risk of NAFLD corresponding to RLP-C differed significantly according to sex and DBIL category. Males and participants with lower DBIL levels had a higher risk of developing NAFLD. Similar findings have been reported. Chin et al found that RLP-C in the lowest quartile compared with that in the highest quartile was associated with 85% lower odds of developing NAFLD in males and 55% in females. These results might be due to the different metabolic characteristics of males and females. The Western Australian Pregnancy Cohort (Raine) Study demonstrated associations between hepatic steatosis and BMI, waist circumference, subcutaneous adipose tissue thickness, serum leptin level and insulin resistance score in both males and females. However, an association between increased visceral adipose tissue thickness and decreased serum adiponectin levels was only found in males. Males with NAFLD had greater visceral adipose tissue thickness, more severe metabolic phenotypes, and higher glucose levels and SBP than females with NAFLD. In our study, there were more male participants in the higher RLP-C groups, which may also account for the sex difference. In addition, we found no association between RLP-C levels and NAFLD in the high DBIL subgroup. In vitro and in vivo models, the stress-responsive protein heme oxygenase-1 (HO-1) interrupted the progression of steatohepatitis. As the final product of HO-1, serum bilirubin is thought to have antioxidative and cytoprotective effects. Previous studies have also found an inverse effect of DBIL levels on NAFLD incidence, and participants with higher DBIL levels had lower incidence and degree of NAFLD. Therefore, the correlation between RLP-C and NAFLD may not be as pronounced in the high-DBIL group.

The main limitation of our study is that it was an observational study with a lower standard of evidence than that of experimental studies. Although we adjusted for several variables in our analysis, unidentified confounders, such as age, sex, and other metabolic factors, may still influence the results.
as smoking, alcohol consumption and other lifestyle factors, were not considered. The results of this study cannot be used to demonstrate causality.

CONCLUSION
We investigated the relationship between RLP-C and newly diagnosed NAFLD in non-obese populations. RLP-C level was an independent risk factor for NAFLD incidence after adjusting for conventional risk factors. A higher RLP-C level was associated with a higher risk of NAFLD, especially in men. RLP-C is a simple and readily accessible parameter that can possibly be used to identify the risk of newly diagnosed NAFLD in non-obese individuals.

Contributors YM and HT wrote the manuscript. YM applied for the database and performed the statistical analysis. HT revised the manuscript. YM is responsible for the overall content as guarantor. All authors have confirmed the final version of the paper.

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Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design, conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not required.

Ethics approval This study involves human participants and was approved by the Institutional Review Committee of Beijing Anzhen Hospital (review no: 2022-184X). Participants gave informed consent to participate in the study before taking part.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available in a public, open access repository. The dataset supporting the conclusions of this article is available from the Dryad repository at http://datadryad.org/ with the doi: 10.5061/dryad.1n6c4.

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