Inverse Association of Serum Adipsin with the Remission of Nonalcoholic Fatty-Liver Disease: A 3-Year Community-Based Cohort Study

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Keywords

Prospective cohort study · Metabolic disease · Adipokine · Adipose tissue · Complement factor

Abstract

Purposes: Adipokine alterations contribute to the development and remission of nonalcoholic fatty-liver disease (NAFLD). Adipsin is one of the most abundant adipokines and is almost exclusively produced by adipocytes. However, data on adipsin in human NAFLD are limited and controversial. We performed this study to investigate the association between adipsin and the remission of NAFLD in middle-aged and elderly Chinese adults. **Methods:** Whether adipsin is associated with the remission of NAFLD in a 3-year community-based prospective cohort study was investigated. Baseline levels of adipsin were measured in serum samples collected from 908 NAFLD participants. NAFLD was diagnosed using abdominal ultrasonography. Logistic regression analysis and a multiple stepwise logistic regression model including different variables were conducted to evaluate the association between serum adipsin levels and the

remission of NAFLD. **Results:** During a mean follow-up of 3.14 ± 0.36 years, 247 (27.20%) participants with NAFLD at baseline were in remission. At baseline, serum adipsin concentration was positively correlated with body mass index ($r: 0.39, p < 0.001$), insulin ($r: 0.31, p < 0.001$), and homeostasis model assessment of insulin resistance ($r: 0.31, p < 0.001$) and was inversely associated with NAFLD remission with a fully adjusted odds ratio (OR) of 0.28 (0.16–0.48) (p trend < 0.001). In a multiple stepwise logistic regression model, circulating adipsin independently predicted NAFLD remission (OR: 0.284, 95% confidence interval [CI]: 0.172–0.471, p for trend < 0.001). The area under the receiver operating characteristic curve was 0.751 (95% CI: 0.717–0.785) ($p < 0.001$) for the prediction model of NAFLD remission. **Conclusions:** We provide evidence for an association between serum adipsin levels and the remission of NAFLD in a community-based prospective cohort study. Serum adipsin can be a potential biomarker for predicting NAFLD remission.

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Introduction

Nonalcoholic fatty-liver disease (NAFLD) has been a major cause of chronic liver disease worldwide, with an estimated overall global prevalence of 25.24% [1]. NAFLD is characterized by the presence of hepatic steatosis (the presence of $\geq 5\%$ hepatic steatosis) on liver histology in individuals with no history of significant alcohol consumption and no other known secondary causes of hepatic fat accumulation [2]. As a spectrum of disease states, NAFLD starts as simple liver steatosis (SS), which may progress to nonalcoholic steatohepatitis (NASH), advanced fibrosis, and even hepatocellular carcinoma [3]. NAFLD is a dynamic condition, and early-stage NAFLD, for example, simple steatosis, can remit to a normal liver, but once it develops to NASH, the risk leading to liver cancer or death caused by NAFLD increases dramatically [4]. Hence, it is important to identify circulating factors associated with NAFLD remission, which can be used as biomarkers for NAFLD prognosis.

Adipose tissue, as an active endocrine organ and the main site for the storage of excess energy in the form of triglycerides (TGs), participates in energy metabolism [5, 6]. Adipose tissue secretes a number of adipokines, many of which are involved in energy homeostasis and inflammation. Dysregulated production of adipokines profoundly affects the function of different organs and contributes to the development of various metabolic diseases, including insulin resistance (IR), dyslipidemia, and NAFLD [7]. Adipsin is one of the most abundant adipokines mainly produced by adipose tissue as the first adipokine described [8]. Although the physiological effect of adipsin remains largely unknown, studies have found that this adipokine can stimulate synthesis of TGs which contributes to development of NAFLD [9–11]. Adipsin is also known as adipocyte complement factor D, which catalyzes the rate-limiting step in the alternative pathway of complement activation [12, 13]. Growing evidence suggests the presence of a connection between the complement system and development of obesity, inflammation, and IR [11, 14, 15]. Rensen et al. [16] reported that widespread activation of the complement system in NAFLD is increased with disease severity [17].

Population-based studies have demonstrated that circulating adipsin levels are significantly elevated in obese individuals and postmenopausal women [18–20]. It was reported that higher adipsin concentrations were independently associated with greater odds of obesity. However, human studies on the association between adipsin levels and NAFLD are limited and have yielded conflict-

ing results. Similar adipsin levels were observed between NAFLD participants and controls, and no difference was found between SS and NASH in a pediatric population [21, 22]. In our previous case-control study including 211 Chinese participants, we observed that circulating adipsin levels were independently positively associated with the occurrence of NAFLD [23]. To further evaluate the association between adipsin levels and NAFLD disease remission, we evaluated serum adipsin levels in 908 NAFLD participants with a 3-year prospective follow-up in middle-aged and elderly Chinese adults.

Participants and Methods

Participants

The current study was based on the Guangzhou Nutrition and Health Study (GNHS), a community-based prospective cohort study in China, which aimed to identify determinants of common chronic diseases. Participants were residents in Guangzhou and were recruited from communities in Guangzhou through advertisements, health talks, and referrals. For the cohort, participants with the following conditions were included: 40–80 years of age at baseline, living in Guangzhou for at least 5 years, and Chinese people and participants with any of the following conditions were excluded: had a history of hospital-confirmed failure(s) of heart, liver, or kidney, cancer, CVD events, metabolic bone diseases, glucocorticoid use (over 3 months) or sexual hormone use (over 6 months), and spine or hip fractures; on a special diet due to a disease or weight control; mental and physical disability; likely to move to another city within 5 years; and did not want to attend any one item of the survey or sample collection. The process and timeline applied in this prospective cohort study are shown in Figure 1. From September 2008 to February 2010, a total of 3,169 eligible Chinese adults of 40–75 years of age were recruited. Of these, 2,510 participants were included in the first follow-up survey between April 2011 and March 2013. An additional 879 participants were recruited between March 2013 and November 2013 to account for participant attrition, resulting in 2,945 participants included in the second follow-up survey at a mean of 3.09 ± 0.41 years between April 2014 and May 2017. In all the follow-up phases, all participants underwent a comprehensive questionnaire survey and body assessment, including physical examination, routine biochemical blood analyses, and hepatitis virus and human immunodeficiency virus tests. Details of the questionnaire data collection have been described previously [24]. Participants with any of the following conditions were excluded from the analysis: participants without NAFLD, excessive alcohol consumption (≥ 140 g/weeks in men or ≥ 70 g/weeks in women), the presence of secondary causes of hepatic fat accumulation (e.g., viral hepatitis, lipodystrophy, parenteral nutrition, Wilson's disease, inborn errors of metabolism, and long-term use of steatogenic medications) [2], women who were pregnant or nursing, and severe diseases (e.g., cancer, stroke, renal dysfunction, and heart failure). Participants who had missing data on the blood biochemical index and abdominal ultrasonographic results were also excluded from the present study. Ultimately, 908 participants with baseline serum adipsin measurements were in-

Fig. 1. Procedure of enrollment of patients in the present study. NAFLD, nonalcoholic fatty-liver disease.

cluded in the analysis and divided into 2 groups as persisting NAFLD and NAFLD remission according to NAFLD status after the 3-year follow-up. Since the first follow-up, we began to conduct abdominal ultrasound examinations in this cohort. Thus, in the present study, our “baseline data” were derived from the first follow-up survey, and the “follow-up data” were based on the second follow-up survey.

The GNHS was registered on ClinicalTrials.gov (registration number NCT03179657) and approved by the Ethics Committee of the School of Public Health at Sun Yat-sen University (approval number ZDGWYL2009-3), which meets the guidelines laid down in the Declaration of Helsinki. Written informed consent was obtained from all participants.

Clinical and Laboratory Assessment

Trained interviewers conducted face-to-face and one-by-one interviews with all participants to complete a detailed survey including questions on demographic characteristics, socioeconomic status, behavior and lifestyle (e.g., alcohol drinking, smoking, and physical activity), histories of chronic diseases, and current medi-

cation use through standardized questionnaires. Participants who drank alcohol once a week or smoked at least one cigarette per day for at least 6 months were identified as current drinkers or smokers.

Height, weight, waist circumference, and hip circumference were measured twice with the participants standing and wearing light daily clothing, and the mean values were calculated. Body mass index (BMI) was calculated as weight (kg) divided by the height squared (m^2), and values greater than 24 kg/m^2 were identified as overweight [25]. The waist-to-hip ratio (WHR) was calculated as the waist circumference divided by the hip circumference. Blood pressure was measured in duplicate on the left arm using an automated sphygmomanometer (HEM 7011; OMRON Corp., Osaka, Japan) and averaged for analyses. The metabolic equivalent intensity was calculated to estimate daily physical activity using a 24-h physical activity questionnaire [26]. Dual-energy X-ray absorptiometry scans (Discovery W; Hologic Inc., Waltham, MA, USA) were used to quantify the fat mass of the trunk region.

Blood samples from all participants were collected via venipuncture after an overnight fast of 8 h or more, and the serum was

collected within 2 h afterward. Serum samples were stored at -80°C until analysis. The baseline biochemical measurements detected enzymatically by a Hitachi 7600-010 automated analyzer (Hitachi, Tokyo, Japan) included TGs, total cholesterol, albumin (Alb), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), uric acid (UA), aspartate aminotransferase (AST), alanine aminotransferase (ALT), fasting glucose, and insulin. The homeostasis model assessment of IR (HOMA-IR) was calculated as fasting glucose (mmol/L) $\times$ fasting insulin ($\mu\text{U/mL}$)/22.5. Adipsin levels were measured by quantitative sandwich enzyme immunoassay using kits obtained from R&D Systems (DFD00, Minneapolis, MN, USA) with a Spark 10M Multimode Reader Platform (Tecan Trading AG, Männedorf, Switzerland).

Evaluation of NAFLD

NAFLD was diagnosed by abdominal ultrasonography following the standard criteria issued by the Fatty Liver Disease Study Group of the Chinese Liver Disease Association [25, 26]. The remission of NAFLD was defined as the presence of NAFLD at baseline and the absence at the follow-up. Ultrasound analyses were performed by 2 experienced technicians who were blinded to the participants' data on a Doppler sonography machine (Sonoscape SSI-5500, Shenzhen, China) with a 3.5-MHz probe. Between-operator reliability evaluation for ultrasonography was assessed in 100 participants, showing very good precision (Spearman's $r = 0.911$, kappa = 0.875, consistency rate = 93%, $p < 0.001$). We estimated the validity evaluation of NAFLD in 34 participants who further underwent CT scanning, with the radiologists blinded to the ultrasound results. There was good agreement in diagnosing NAFLD between the 2 diagnostic methods (Spearman's $r = 0.905$, kappa = 0.691, consistency rate = 85%, $p < 0.001$). The same experienced technician performed ultrasonography at the baseline and follow-up exams. Follow-up evaluations of hepatic ultrasonography were similarly conducted following the standard protocol.

Statistical Analysis

Continuous variables were reported as the means and standard deviations for normally distributed variables or as medians and interquartile ranges for non-normally distributed variables. Categorical variables were summarized as numbers and percentages. The adipsin level was treated as a categorical variable by quartiles. Baseline demographic and laboratory characteristics of the participants according to the status of NAFLD remission or quartiles of adipsin levels were compared using the independent samples t test, one-way ANOVA test, Kruskal-Wallis, and χ^2 test, where appropriate. Tukey post hoc tests were used to follow one-way ANOVA to further define differences. The correlation of serum adipsin levels with other demographic and laboratory characteristics at baseline was evaluated by Spearman's correlation analysis and partial correlation analysis. The multivariable logistic regression models were adjusted for metabolic risk factors. We adjusted for sex and age in model 1. To investigate the independent associations, we further adjusted for BMI, physical activity (metabolic equivalent), current smoking and drinking, hypertension, and diabetes in model 2 plus HOMA-IR, Alb, TGs, LDL-C/HDL-C ratio, UA, and AST/ALT ratio in model 3. All 3 models were applied to the subgroup analysis as described above. Furthermore, multiple stepwise logistic regression analyses were performed to determine the independent predictors and the model for predicting NAFLD

remission. We evaluated the prediction accuracy of the model built by multiple stepwise logistic regression analysis with area under the receiver operating characteristic curve (AUROC) with 95% confidence interval (CI). Sensitivity, specificity, negative likelihood ratio, and positive likelihood ratio of this model were calculated. To test for selection bias, NAFLD participants included in the present study were compared to those who failed to complete the follow-up survey or were lost to the follow-up. All statistical procedures were performed using SPSS Statistics software (version 22.0; SPSS Inc., Chicago, IL, USA). A significant difference was considered when a 2-tailed p value was < 0.05 .

Results

Baseline Characteristics of the Participants

As shown in Figure 1, 908 participants with baseline serum adipsin measurements were ultimately included in the analysis. Except for LDL-C and ALT, no significant differences were observed in major demographic, anthropometric, or laboratory variables when NAFLD participants were included in the present study compared to those who were lost to the follow-up. The mean follow-up time was 3.14 ± 0.36 years. The remission rate of NAFLD during this period was 27.20% (247/908). The baseline characteristics of the NAFLD participants are shown in Table 1. At baseline, participants with subsequently persisting NAFLD had a median age of 59.96 years with 31.6% men, and those with NAFLD remission had a median age of 59.50 years with 31.2% men. The baseline levels of BMI, WHR, systolic blood pressure, diastolic blood pressure, fasting glucose, insulin, HOMA-IR, TGs, UA, ALT, and trunk fat percentage were significantly lower, while the HDL-C level was significantly higher in participants with NAFLD remission (all $p < 0.05$). There was no significant difference between participants with persisting NAFLD and NAFLD remission for the use of antihypertensive agents, hypoglycemic agents, and lipid-lowering agents.

Adipsin Levels of NAFLD Participants with or without NAFLD Remission at the Follow-Up

As shown in Figure 2a, baseline adipsin levels in participants with persisting NAFLD (3,565.52, 3,203.18–4,020.48 ng/mL) were higher than those in remission (3,251.33, 2,956.20–3,628.15 ng/mL) ($p < 0.001$). Meanwhile, the results were consistent in subgroups when stratified by age, sex, BMI, HOMA-IR, TGs, and UA (all p values < 0.01) (Fig. 2).

All participants were divided into 4 groups according to the quartiles of serum adipsin levels. Online supple-

Table 1. Baseline characteristics by the remission of NAFLD

Variables	Total (n = 908)	Persisting NAFLD (n = 661)	NAFLD remission (n = 247)	p value (persisting vs. NAFLD remission)
Age, years [§]	59.83 (56.58–63.83)	59.96 (56.60–63.48)	59.50 (56.58–64.58)	0.916
Sex (males), [†] n (%)	286 (31.5)	209 (31.6)	77 (31.2)	0.898
Adipsin, ng/mL [§]	3,469.35 (3,111.51–3,920.78)	3,565.52 (3,203.18–4,020.48)	3,251.33 (2,956.20–3,628.15)	<0.001
BMI, kg/m ^{2†}	24.90±3.05	25.33±3.11	23.77±2.57	<0.001
WHR [†]	0.93±0.063	0.93±0.06	0.91±0.07	<0.001
Trunk fat percentage, n (%) [§]	36.19 (31.22–39.95)	36.54 (31.77–40.45)	35.08 (29.98–38.64)	0.002
SBP, mm/Hg [†]	127.71±18.13	128.68±18.22	125.65±17.66	0.025
DBP, mm/Hg [†]	77.10±10.52	77.94±10.69	75.34±9.99	0.001
Alb, g/L [§]	45.60 (42.13–47.90)	45.60 (42.3–48.0)	45.50 (41.7–47.4)	0.211
Fasting glucose, mmol/L [§]	4.75 (4.39–5.27)	4.80 (4.41–5.35)	4.65 (4.21–5.10)	<0.001
Insulin, µU/mL [§]	9.74 (6.91–13.75)	10.39 (7.52–14.66)	7.90 (5.64–10.34)	<0.001
HOMA-IR [§]	2.08 (1.44–3.07)	2.28 (1.57–3.28)	1.60 (1.11–2.35)	<0.001
TC, mmol/L [§]	5.49 (4.82–6.14)	5.54 (4.85–6.19)	5.45 (4.79–5.99)	0.122
TG, mmol/L [§]	1.38 (1.00–2.00)	1.54 (1.11–2.14)	1.11 (0.84–1.57)	<0.001
HDL-C, mmol/L [§]	1.27 (1.05–1.54)	1.24 (1.02–1.46)	1.40 (1.17–1.73)	<0.001
LDL-C, mmol/L [†]	3.64±0.91	3.66±0.91	3.59±0.90	0.301
UA, µmol/L [§]	343.18 (296.37–401.18)	350.36 (301.00–409.71)	325.26 (288.48–373.52)	<0.001
ALT, U/L [§]	16.00 (12.00–22.00)	17.00 (13.00–23.00)	14.00 (12.00–19.00)	<0.001
AST, U/L [§]	18.00 (16.00–22.00)	18.00 (16.00–22.00)	18.00 (15.00–21.00)	0.486
Physical activities, MET/day [§]	23.21 (19.85–27.66)	23.26 (19.97–27.63)	22.75 (19.75–27.90)	0.886
Current smoking, [†] n (%)	75 (8.3)	51 (7.7)	24 (9.7)	0.330
Current drinking, [†] n (%)	73 (8.0)	57 (8.6)	16 (6.5)	0.290
Hypertension, [†] n (%)	299 (32.9)	230 (34.8)	69 (27.9)	0.142
Diabetes, [†] n (%)	70 (7.7)	54 (8.2)	16 (6.5)	0.566
Dyslipidemia, [†] n (%)	398 (43.8)	310 (46.9)	88 (35.6)	0.001
Use of antihypertensive agents, [†] n/N (%)				
Always	229/299 (76.6)	173/230 (75.2)	56/69 (81.2)	0.426
Usually	13/299 (4.3)	10/230 (4.3)	3/69 (4.3)	
Often	7/299 (2.3)	6/230 (2.6)	1/69 (1.4)	
Sometimes	10/299 (3.3)	6/230 (2.6)	4/69 (5.8)	
Seldom	39/299 (13)	34/230 (14.8)	5/69 (7.2)	
Use of hypoglycemic agents, [†] n/N (%)				
Always	48/70 (68.6)	37/53 (69.8)	11/17 (64.7)	0.748
Usually	1/70 (1.4)	1/53 (1.9)	0	
Often	0	0	0	
Sometimes	0	0	0	
Seldom	21/70 (30.0)	15/53 (28.3)	6/17 (35.3)	
Use of lipid-lowering agents, [†] n/N (%)				
Always	91/398 (22.9)	67/310 (21.6)	24/88 (27.3)	0.393
Usually	7/398 (1.8)	7/310 (2.3)	0	
Often	13/398 (3.3)	11/310 (3.5)	2/88 (2.3)	
Sometimes	22/398 (5.5)	19/310 (6.1)	3/88 (3.4)	
Seldom	265/398 (66.6)	206/310 (66.5)	59/88 (67)	

BMI, body mass index; WHR, waist-to-hip ratio; SBP, systolic blood pressure; DBP, diastolic blood pressure; Alb, albumin; HOMA-IR, homeostasis model assessment of insulin resistance; TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; UA, uric acid; ALT, alanine aminotransferase; AST, aspartate aminotransferase; MET, metabolic equivalent. [†] Categorical variables are shown as numbers and percentages and compared using the χ^2 test. [‡] Normally distributed data were expressed as means and standard deviations and compared using Student's *t* tests. [§] Non-normally distributed data were expressed as the median and 25th–75th interquartile range and compared using the Kruskal-Wallis test.

Fig. 2. Serum adipsin concentrations (ng/mL) in study participants. **a** Serum adipsin levels (ng/mL) in participants with persisting NAFLD and NAFLD remission. **b** Serum adipsin levels (ng/mL) according to age (age <65 and age ≥65). **c** Serum adipsin levels (ng/mL) according to sex. **d** Serum adipsin levels (ng/mL) according to BMI levels (BMI <24 kg/m² and BMI ≥24 kg/m²). **e** Serum adipsin levels (ng/mL) according to HOMA-IR (HOMA-IR <2 and HOMA-IR ≥2). **f** Serum adipsin levels (ng/mL) according

to TGs (TG <1.7 mmol/L and TG ≥1.7 mmol/L). **g** Serum adipsin levels (ng/mL) according to UA (UA <420 μmol/L and UA ≥420 μmol/L). *Student's *t* tests were used. †The box plots display the median values and 25th and 75th percentiles; the whiskers represent 25th percentiles −1.5 × interquartile range and 75th percentiles +1.5 × interquartile range. NAFLD, nonalcoholic fatty-liver disease; BMI, body mass index; HOMA-IR, homeostasis model assessment of insulin resistance; TG, triglyceride; UA, uric acid.

Table 2. Multivariable-adjusted ORs and 95% CI for the remission of NAFLD according to the quartiles of serum adipsin levels[†]

	Quartiles of serum adipsin levels, ng/mL				<i>p</i> for trend
	Q1	Q2	Q3	Q4	
Total (<i>n</i> = 908)	≤3,115.71	3,115.72–3,476.33	3,476.34–3,920.18	≥3,920.19	
Model 1 [‡]	1	0.57 (0.39–0.84)	0.27 (0.18–0.42)	0.17 (0.11–0.27)	<0.001
<i>p</i> values		0.004	<0.001	<0.001	
Model 2 [§]	1	0.66 (0.44–0.98)	0.35 (0.23–0.55)	0.23 (0.14–0.38)	<0.001
<i>p</i> values		0.037	<0.001	<0.001	
Model 3 [¶]	1	0.69 (0.45–1.04)	0.40 (0.25–0.65)	0.27 (0.16–0.46)	<0.001
<i>p</i> values		0.075	<0.001	<0.001	
Female (<i>n</i> = 622)	≤3,090.87	3,090.88–3,442.50	3,442.51–3,865.89	≥3,865.90	
Model 1 [‡]	1	0.57 (0.36–0.89)	0.30 (0.18–0.49)	0.19 (0.11–0.34)	<0.001
		0.014	<0.001	<0.001	
Model 2 [§]	1	0.63 (0.39–1.01)	0.39 (0.23–0.66)	0.27 (0.15–0.51)	<0.001
		0.054	<0.001	<0.001	
Model 3 [¶]	1	0.66 (0.41–1.1)	0.45 (0.26–0.80)	0.30 (0.16–0.59)	0.002
		0.104	0.006	<0.001	
Male (<i>n</i> = 286)	≤3,183.11	3,183.11–3,538.32	3,538.33–4,010.86	≥4,010.87	
Model 1 [‡]	1	0.58 (0.28–1.17)	0.23 (0.10–0.51)	0.13 (0.06–0.31)	<0.001
		0.13	<0.001	<0.001	
Model 2 [§]	1	0.81 (0.37–1.77)	0.30 (0.12–0.71)	0.17 (0.07–0.44)	<0.001
		0.596	0.007	<0.001	
Model 3 [¶]	1	0.93 (0.40–2.15)	0.34 (0.13–0.88)	0.23 (0.09–0.62)	0.006
		0.861	0.026	0.004	
BMI <24 kg/m ² (<i>n</i> = 360)	≤2,945.43	2,945.44–3,252.90	3,252.91–3,604.77	≥3,604.78	
Model 1 [‡]	1	0.57 (0.34–0.96)	0.35 (0.18–0.67)	0.27 (0.13–0.56)	<0.001
		0.034	0.002	<0.001	
Model 2 [§]	1	0.55 (0.32–0.95)	0.30 (0.15–0.60)	0.28 (0.13–0.59)	<0.001
		0.032	0.001	0.001	
Model 3 [¶]	1	0.63 (0.36–1.13)	0.44 (0.21–0.93)	0.31 (0.13–0.72)	0.024
		0.122	0.032	0.006	
BMI ≥24 kg/m ² (<i>n</i> = 548)	≤3,273.63	3,273.64–3,630.70	3,630.71–4,045.73	≥4,045.74	
Model 1 [‡]	1	0.68(0.38–1.24)	0.32 (0.17–0.59)	0.19(0.10–0.36)	<0.001
		0.21	<0.001	<0.001	
Model 2 [§]	1	0.69 (0.38–1.30)	0.32 (0.17–0.60)	0.19 (0.10–0.38)	<0.001
		0.23	<0.001	<0.001	
Model 3 [¶]	1	0.59 (0.30–1.15)	0.30(0.15–0.59)	0.21 (0.10–0.44)	<0.001
		0.119	0.001	<0.001	

NAFLD, nonalcoholic fatty-liver disease; BMI, body mass index; Q, quartile; OR, odds ratio; CI, confidence interval; Alb, albumin; HOMA-IR, homeostasis model assessment of insulin resistance; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; UA, uric acid; ALT, alanine aminotransferase; AST, aspartate aminotransferase; MET, metabolic equivalent. [†] Logistic regression analysis was used. [‡] Model 1: adjusted for age and sex. Adjusted for age only when stratified the population by sex. [§] Model 2: adjusted for variables in model 1 plus BMI, physical activities (MET), current smoking and drinking, hypertension, and diabetes. Adjusted for variables in model 1 plus physical activities (MET), current smoking and drinking, hypertension, and diabetes when stratified the population by BMI. [¶] Model 3: adjusted for variables in model 2 plus HOMA-IR, Alb, TG, LDL-C/HDL-C ratio, UA, and AST/ALT ratio.

mentary Table 1 (for all online suppl. material, see www.karger.com/doi/10.1159/000520368) shows that the higher the quartile grade at baseline, the fewer participants remitted to normal. Remission rates reduced from

41.3% to 29.6%, 17.4%, and 11.7% in participants from quartile 1 (Q1) to quartile 4 (Q4) of adipsin levels, respectively (*p* trend < 0.001). In addition, participants in the highest quartile had higher age, BMI, WHR, systolic

Table 3. Multiple stepwise logistic regression analyses of factors predicting remission NAFLD during the follow-up[†]

Variables	Partial regression coefficient	Standard error	Wald χ^2	OR (95% CI)	p value
Adipsin	–	–	29.08	–	<0.001
Q1	–	–	–	–	–
Q2	–0.42	0.21	4.15	0.66 (0.44–0.98)	0.042
Q3	–0.91	0.23	15.54	0.40 (0.26–0.63)	<0.001
Q4	–1.26	0.26	23.80	0.28 (0.17–0.47)	<0.001
BMI, kg/m ²	–0.12	0.03	12.78	0.89 (0.84–0.95)	<0.001
TGs, mmol/L	–0.55	0.11	23.99	0.58 (0.46–0.72)	<0.001
AST/ALT ratio	0.57	0.20	8.41	1.77 (1.20–2.60)	0.004
Constant	2.58	0.87	8.78	13.23	0.003

Variables in original model were baseline age, sex, BMI, physical activity (METs/day), HOMA-IR, fasting glucose, AST/ALT ratio, TC, LDL-C/HDL-C ratio, Trunk fat percentage (%), UA, Alb, and adipsin levels in quartiles. OR, odds ratio; CI, confidence interval; Q, quartile; BMI, body mass index; Alb, albumin; HOMA-IR, homeostasis model assessment of insulin resistance; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; UA, uric acid; ALT, alanine aminotransferase; AST, aspartate aminotransferase; MET, metabolic equivalent.

blood pressure, diastolic blood pressure, fasting glucose, TGs, insulin, HOMA-IR, UA, ALT, trunk fat percentage, hypertension, and diabetes prevalence and lower levels of Alb and HDL-C than those in the lowest quartile (all $p < 0.05$).

Serum Adipsin Level Is Positively Correlated with BMI, Insulin, and HOMA-IR

The correlation between baseline serum adipsin levels and clinical parameters was analyzed. Correlation coefficients are shown in online supplementary Table 2. Significant positive correlations were observed between adipsin levels and BMI ($r: 0.38, p < 0.001$), insulin ($r: 0.30, p < 0.001$), and HOMA-IR ($r: 0.31, p < 0.0001$). After adjusting for age and sex, positive correlations of serum adipsin levels with BMI, insulin, and HOMA-IR were still observed (all $p < 0.001$).

Prospective Analyses of the Association between Baseline Serum Adipsin and Remission of NAFLD at the Follow-Up

Baseline serum adipsin levels were inversely associated with NAFLD remission in participants diagnosed with NAFLD at baseline (all $p < 0.05$ for trend). Table 2 shows the results of logistic regression analysis. The odds ratio (OR) for the remission of NAFLD was 0.17 for participants in the highest quartile compared with those in the lowest quartile (95% CI: 0.11–0.27, p for trend <0.001) in model 1. Higher serum adipsin levels were associated with an increased risk of persisting NAFLD in model 2

(OR: 0.23, 95% CI: 0.14–0.38, p for trend <0.001) and model 3 (OR: 0.27, 95% CI: 0.16–0.46, p for trend <0.001). Analyses showed similar results in subgroups. Serum adipsin levels were also inversely associated with the remission of NAFLD in the quartiles defined sex-specific and BMI-specific.

Serum Adipsin Level Independently Predicts the Remission of NAFLD

We investigated the prediction of NAFLD remission with the observed parameters using a multiple stepwise logistic regression model (Table 3). According to the ORs, 6 variables were significantly and independently related to the remission of NAFLD, including baseline adipsin quartile 2 (Q2), adipsin quartile 3 (Q3), adipsin Q4, BMI, TGs, and AST/ALT ratio (all $p < 0.05$). Adipsin Q2, adipsin Q3, adipsin Q4, BMI, and TGs were inversely correlated, while the ratio was positively correlated with NAFLD remission. Based on the above results, we established and named the model for predicting NAFLD remission the *Adipsin Model for NAFLD Remission*, which is calculated as follows: $e^M / (1 + e^M)$, $M = 2.58 - 0.42 \times \text{adipsin Q2 (vs. Q1)} - 0.91 \times \text{adipsin Q3 (vs. Q1)} - 1.26 \times \text{adipsin Q4 (vs. Q1)} - 0.12 \times \text{BMI (kg/m}^2\text{)} - 0.55 \times \text{TGs (mmol/L)} + 0.57 \times \text{AST/ALT ratio}$.

Evaluation of Serum Adipsin as a Predictor for NAFLD Remission

The receiver operating characteristic curves shown in Figure 3 represent the prediction accuracy of serum

Table 4. Sensitivity, specificity, positive likelihood ratio, and negative likelihood ratio for NAFLD remission prediction with serum adipsin and *Adipsin Model for NAFLD Remission*[†]

Predictor	Cutoff values of the SHBG mode	Sensitivity, %	Specificity, %	Positive likelihood ratio	Negative likelihood ratio	<i>p</i> value
Serum adipsin						
16th percentile		0.883	0.300	0.618	0.035	<0.001
26th percentile		0.709	0.578	0.299	0.168	
39th percentile		0.413	0.811	0.078	0.476	
Adipsin Model for NAFLD Remission						
15th		0.931	0.384	0.358	0.042	<0.001
25th		0.749	0.638	0.478	0.091	
35th		0.575	0.782	0.450	0.093	
45th		0.332	0.890	0.295	0.074	
55th		0.154	0.949	0.146	0.044	
65th		0.061	0.988	0.060	0.011	
75th		0.004	0.998	0.004	0.002	
86th		0.000	0.998	0.000	0.002	

NAFLD, nonalcoholic fatty-liver disease; ROC, receiver operating characteristic. [†]ROC curve analyses were used.

Fig. 3. The ROC curves of serum adipsin and the *Adipsin Model for NAFLD Remission* developed by ROC curve analyses. Prediction of NAFLD remission by serum adipsin (a) and the *Adipsin Model for NAFLD Remission* (b). c AUROC for the model was larger than

that for serum adipsin ($p < 0.001$). $p < 0.001$ for all ROC curves compared with the 0.5 curve. NAFLD, nonalcoholic fatty-liver disease; AUROC, receiver operating characteristic curve; ROC, receiver operating characteristic.

adipsin and the *Adipsin Model for NAFLD Remission*. The AUROC for the model was larger (AUROC: 0.751, 95% CI: 0.717–0.785) ($p < 0.001$) than that for serum adipsin alone (AUROC: 0.673, 95% CI: 0.634–0.712) ($p < 0.001$). The optimal cutoff values of serum adipsin level and the model were located at 25.6th percentile and 25.1th percentile, which corresponded to 70.9% sensitivity and 57.8% specificity for adipsin and 74.9% sensitivity and 64.0% specificity for the model, respectively (Table 4).

Discussion

In this study, using a community-based population with a 3-year prospective follow-up, we showed for the first time the association between serum adipsin levels and the remission of NAFLD in middle-aged and elderly Chinese adults. Our results indicate that adipsin levels are inversely correlated with the remission of NAFLD and support the hypothesis that serum adipsin is an independent predictor for the remission of NAFLD.

Growing evidence suggests a link between metabolic diseases and adipsin. Several human studies have demonstrated that the levels of adipsin are higher in metabolic diseases associated with obesity [18, 20, 27–29]. In a study by Calan et al. [18], circulating adipsin levels were elevated in overweight/obese women compared with lean women, with a positive correlation between adipsin and BMI, HOMA-IR, and TGs in polycystic ovary syndrome women and their controls. It has also been reported that adipsin increases TG synthesis [30, 31]. Our recent study showed a positive association of circulating adipsin levels with the presence of NAFLD in Chinese adults [23]. These research results are coherent with our current results that circulating adipsin levels were positively correlated with BMI and TGs. However, a case-control study conducted by Yilmaz et al. [21] found that serum adipsin levels did not differ between participants with NAFLD and the control group. Moreover, no significant difference in adipsin levels was observed among children with different severities of NAFLD [22]. One study reported that adipsin mRNA expression and circulating adipsin levels significantly decreased in obese mice caused by genetic or metabolic defects [32]. These discrepancies may be due to the different age-groups, relatively small sample sizes, and the limitations of cross-sectional studies in human studies or species differences between humans and mice.

Adipokine alterations, which occur during the expansion of adipose tissue, are involved in the pathogenesis of NAFLD and can promote the development of SS-, NASH-, and NASH-related cirrhosis [33, 34]. NAFLD is the hepatic manifestation of obesity and metabolic syndrome, as well as a dynamic condition in which severe NAFLD can remit to simple steatosis or normal liver. Thus, identifying noninvasive biomarkers to predict the remission of NAFLD to decrease liver disease-related mortality is urgent. In this study, we found that participants with subsequent NAFLD remission had lower serum adipsin levels than those who had persisting NAFLD. Logistic regression analysis revealed that decreased circulating adipsin levels were significantly associated with high odds of NAFLD remission, and the association remained the same in subgroups. Multiple stepwise logistic regression analysis proved that adipsin independently predicted the remission of NAFLD. Our findings highlight that adipsin may act as a detrimental factor during the remission of NAFLD, which could potentially be an indicator of the remission of NAFLD. To the best of our knowledge, our study provided the first clinical evidence on the association between adipsin and NAFLD in a prospective cohort study.

Adipsin is also known as complement factor D, which is a key component of the alternative complement pathway [12]. The immune system plays a significant role in the pathogenesis of NAFLD. Rensen et al. [16] found that most NAFLD participants presented hepatic activated C3 deposition, and participants showing activated C3 deposition displayed increased numbers of apoptotic cells and hepatic inflammatory infiltration, and they were more likely to develop NASH. In the study of Segers et al. [17], alternative pathway activation played an important role in driving hepatic inflammation in NASH. Likewise, Jia et al. [35] reported that increased serum C3 levels were significantly associated with a high risk of NAFLD. Up-regulation of adipsin is related to the progression of steatosis to spontaneous IR, NAFLD, and HCC in mice [36]. In addition to adipocytes, HepG2 cells can also synthesize complement protein D and secrete high levels of factor D into culture medium [37, 38], but this biological effect is not clear.

Our study has limitations that should be taken into consideration. First, up to July 2016, more than one-third of the participants with NAFLD from the baseline survey had not completed the follow-up survey. However, most of the demographic, anthropometric, and biochemical parameters of those participants did not differ from those of the participants who completed the follow-up survey. Therefore, the risk for selection bias may be excluded. In addition, the diagnosis of NAFLD was determined noninvasively, therefore raising concerns about reduced sensitivity and specificity in NAFLD diagnosis compared to liver biopsy. Moreover, there were more than twice as many women as men in the study. To reduce the potential bias, participants were stratified by sex, and the inverse association between adipsin and the remission of NAFLD was still observed in the subgroup analysis.

Conclusions

In conclusion, baseline circulating adipsin levels were lower in NAFLD remission participants in this prospective cohort study performed in the Chinese population. Serum adipsin can be a potential biomarker for predicting NAFLD remission. Although the physiological roles of adipsin in NAFLD have not been elucidated, it is reasonable to speculate that adipsin might play a vital pathophysiological role in NAFLD based on the results of the 3-year community-based cohort study. In the future, prospective studies involving larger sample sizes of multieth-

nic populations of all ages with NAFLD diagnosed and assessed by transient elastography, magnetic resonance spectroscopy, or biopsy are needed.

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Statement of Ethics

The GNHS was registered at ClinicalTrials.gov (Registration Number NCT03179657) and approved by the Ethics Committee of the School of Public Health at Sun Yat-sen University (approval number ZDGWYL2009-3), which meets the guidelines laid down in the Declaration of Helsinki. Written informed consent was obtained from all participants.

Conflict of Interest Statement

The authors declare that there are no conflicts of interest.

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Author Contributions

Wenhua Ling, Yuming Chen, and Lili Yang contributed to study concept and design. Xiaoyun Qian, Yao Liu, Jiewen Xie, Zhongliang Xu, Qian Chen, Yun Qiu, Yujia Zhou, Xu Wang, Yingying Gu, and Jing Luo conducted the study. Yingying Gu and Jing Luo contributed to data collection and analysis. All the authors contributed to critical review and revision of the manuscript for important intellectual content.

Data Availability Statement

Data are available from the corresponding author upon reasonable request.

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