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Correlational study on the levels of prolactin and non-alcoholic fatty liver disease in type 2 diabetic patients

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1 Correlational study on the levels of prolactin and non-alcoholic fatty liver disease in
2 type 2 diabetic patients

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10 **Abstract:**

11 **Objective:** This research aims to retrospectively probe the association between the
12 prolactin (PRL) and non-alcoholic fatty liver disease (NAFLD), specifically in type 2
13 diabetes mellitus(T2DM). **Methods:** There are 406 patients with T2DM have
14 participated. Based on the ultrasound diagnosis of NAFLD, there are two groups,
15 named T2DM without NAFLD (men: 77 cases, women: 66 cases, respectively) and
16 T2DM with NAFLD group (men: 153 cases, women: 110 cases, respectively).
17 Inter-group comparison was operated separately. Multiple logistic regression analysis
18 was used to research the relevance between PRL and NAFLD. **Results:** The results
19 indicated that both men and women, the levels of PRL in the NAFLD group were
20 significantly lower ($p < 0.01$). In male subjects, the levels of PRL were negatively
21 correlated with hipline, homeostasis model assessment for insulin resistance
22 (C-peptide) and triglyceeide (TG), and inversely related with high density lipoprotein.
23 In female subjects, PRL levels were negatively related with body mass index,
24 diastolic pressure, waistline, hipline and TG ($p < 0.05$ or $p < 0.01$). Logistic regression
25 analysis revealed a negative relationship between PRL and NAFLD (men: $p = 0.031$,
26 women: $p = 0.004$, respectively). As PRL levels increased, NAFLD prevalence
27 decreased in both genders ($p < 0.05$). **Conclusion:** Low levels of PRL in physiological
28 range was a risk factor for NAFLD in T2DM, independent of known metabolic risk
29 factors.

30 **Keywords:** Type 2 diabetes mellitus; Non-alcoholic fatty liver disease; Prolactin;

31 **Introduction**

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32 The liver is an important organ of glycolipid metabolism in the body. When the
33 triglyceride deposition in hepatocytes increases and the content exceeds 5%, and other
34 factors causing liver steatosis (such as drinking alcohol and viral hepatitis, etc) are
35 excluded, it can be diagnosed as NAFLD[1]. In China, with the gradual improvement
36 of living standards, NAFLD has surpassed chronic viral hepatitis and become the
37 primary cause of chronic liver diseases[2]. Nowadays, global incidence of NAFLD is
38 25.2%[3], while the prevalence of NAFLD diagnosed by ultrasound in T2DM patients
39 is 73.7%[4]. T2DM can boost to the development of NAFLD to non-alcoholic
40 steatohepatitis (NASH), even liver fibrosis[1].

41 Risk factors of NAFLD include central obesity, hypertension, hyperlipidemia,
42 T2DM and metabolic syndrome (Met S), etc[5]. Among Met S related diseases, only
43 NAFLD is considered as a strong predictor of Met S, and the incidence of Met S in
44 fatty liver patients is more than 4 times that of non-fatty liver patients [6]. Therefore,
45 NAFLD is considered as the expression of Met S in liver.

46 Prolactin (PRL) is a kind of protein hormone mainly secreted by adenohypophy-
47 sis. Its main physiological function is to stimulate breast development and milk scere-
48 ion [7]. Its receptors are widely distributed in various tissues and organs of the body,
49 including fat, liver, pancreas and so on [8]. PRL can increase the proliferation of β
50 cells, stimulate insulin secretion and participate in the regulation of glucose
51 metabolism [9]. PRL can also inhibit lipolysis and activate adipocyte differentiation
52 by activating peroxisome proliferator-activated receptor γ [10]. Therefore, studies at
53 home and abroad had found that the decrease of serum PRL at physiological level was
54 closely related to the occurrence of T2DM. Wang et al[11] discovered that the PRL
55 levels of patients with T2DM and impaired glucose regulation were significantly
56 lower than that of people with normal glucose metabolism. The researchers further
57 pointed out that the decrease of PRL physiological level was related to the increased
58 risk of T2DM[9]. Manshaei et al[12] also found that the serum PRL concentration of
59 T2DM patients was lower than that of healthy people. Because of the high incidence
60 of NAFLD in T2DM patients, T2DM is also an important part of Met S. The

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relationship among PRL, NAFLD and Met S physiological level has not been explored. The goal of this research is to explore the relationship among PRL, NAFLD and Met S in patients with T2DM.

Materials and Methods

Research objects

From November 2018 to December 2019, 656 T2DM patients were selected for hospitalization in our hospital. The diagnosis of T2DM was based on the diagnostic criteria proposed by the WHO Diabetes Expert Committee in 1999. The PRL levels at physiological level are based on the normal reference range of our hospital, that is 2.78-29.20 ng/mL for premenopausal women, 1.79-20.28 ng/mL for menopausal women and 2.12-17.69 ng/mL for men. This study was a retrospective research, so it was exempted from informed consent with the approval of the Ethics Committee of The First Affiliated Hospital of Anhui University of Traditional Chinese Medicine.

Exclus-

ion standard: using drugs that affect PRL (metoclopramide, methyl dopa, opiates, and imetidine) (n=15), pituitary diseases (n=4), the levels of thyroid stimulating hormone, cortisol, estradiol and testosterone are higher than the normal range (30), hyperprolactinemia (n=5), too much drink (n=56), cancer (n=11), gestation (n=5) and suffering from type 1 diabetes (n=7), diabetes mellitus complicated with acute complications (n=25), acute cardiovascular accidents (n=15), severe hepatic and renal insufficiency (n=30), suffering from viral (n=8), alcoholic (n=30), drug-damaged (n=5) and autoimmune liver diseases (n=4). Ultimately, 406 patients with T2DM (230 men and 176 women) were taken into the research.

General clinical message and laboratory test targets

We collected gender, age, menopausal history of women, height, weight, diabetes course, pre-admission hypoglycemic plan (include metformin, insulin and other hypoglycemic drugs such as sulfonylureas, glinides, thiazolidinediones, α -glucosidase inhibitors, glucagon-like peptide-1 receptor agonists, dipeptidyl peptidase 4 inhibitors, sodium-glucose cotransporter protein 2 inhibitors), history of drinking, complicated

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with cancer, other liver diseases history, waistline, hipline, blood pressure data. All patients' morning venous blood samples were collected on the second day after admission and all blood were extracted with a centrifuge. After separation of serum, fasting blood glucose (FBG), blood fat, liver and kidney function were measured using an automatic biochemical analyzer (7600-020; Hitachi). Fasting C-peptide (FCP) was examined using enzyme-linked immunosorbent assay (A2000 Plus; Autolumo). Adopting automated chemiluminescent immunoassay (Siemens Immulite 2000, UK) was to measure PRL. The coefficients of intra-assay and inter-assay variation were between 2.49-3.47% and 2.91-3.14%, respectively. PRL levels are affected by many conditions including various drugs, stress, and exercise, so we drew fasting blood samples on different days when the patients were at rest, and then took the average of two values. High-performance liquid chromatography was used to check glycosylated hemoglobin (HbA1c) (Variant II; Bio-Rad).

Defining, count and group

NAFLD was diagnosed as ultrasound[13] by a senior technician. The ultrasonic diagnosis of fatty liver is as follows: near-field of liver permeate punctiform hyperecho, the composition of the intrahepatic duct is not clearly demonstrated by ultrasonography, and weak echo in the distal echo. Diagnosis of NAFLD is based on following requirements: no drinking history; in addition to other types of liver diseases; unsolvable serum alanine aminotransferase (ALT) or aspartic acid aminotransferase (AST)、 glutamyltransferase (GGT) continued to increase over 6 months[14].

Diagnosis of Met S conforms to the standard which is put forward in the ninth edition of internal medicine in China [15], and the diagnostic standard is the following three or more items: ① central obesity and/or abdominal obesity: waistline is 90cm for men and 85cm for women; ② Hyperglycemia: FBG > 6.1mmol/L or blood glucose 2 hours after sugar load >7.8mmol/L and/or those are confirmed as diabetes and treated with hypoglycemic therapy; ③ Hypertension: blood pressure exceeds 130/85 mmHg and/or those are diagnosed as hypertension and treated with an antihypertensive therapy; ④ fasting triglyceride (TG) surpasses 1.7mmol/l; ⑤ fasting high density lipoprotein (HDL). belows 1.04mmol/l. Body mass index (BMI) was computed by

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121 dividing the body weight (kg) by the square of the height (m²). homeostasis model
122 assessment for insulin resistance (C-peptide) (HOMA-IR (CP)) was reckoned by FCP
123 substitute for fasting insulin, HOMA-IR (CP) = 1.5 + FBG (mmol/L) × FCP (pmol/L) /
124 2800. HOMA-β (CP-DM) = 0.27 × FCP (pmol/L) / (FBG (mmol /L) - 3.5)[16].

125 In conformity with ultrasonic diagnosis, patients with T2DM who met the
126 inclusion criteria were segmented into without NAFLD group (men: 77 cases,
127 women: 66 cases, respectively) and with NAFLD group (men: 153 cases, women: 110
128 cases, respectively).

129 **Patient and Public Involvement**

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

132 **Statistical analysis**

133 SPSS21.0 statistical software was used for the data analysis and the
134 Kolmogorov-Smirnov normality of all data were tested. The measured data of the
135 normal distribution was represented by mean ± SD. Comparisons were conducted
136 between two groups and the comparing process was finished by making full use of
137 independent T test. Measurement data for non-normal distributions were expressed as
138 medians (interquartile intervals). Under this situation, two groups were compared by
139 using the Mann-Whitney rank sum test. Counting data was shown by the number of
140 cases, the Chi-square test was adopted to demonstrate the differences within two or
141 more groups. Spearman correlation analysis compared the relationship between PRL
142 levels and other variables. The links among PRL, NAFLD and Met S were analyzed
143 by logistic regression. P < 0.05 or P < 0.01 represented the obvious differences in
144 statistics.

145 **Results**

146 **1. Comparison of general message and laboratory test targets in each group**

147 The ultrasonic diagnostic rate of NAFLD was 263 cases (153 plus 110 cases)
148 (64.8%) (Table 1). Men cases with NAFLD group had younger age, and higher BMI,
149 waistline, hip line, diastolic pressure (DBP), GGT, FBG, TG, total cholesterol

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150 (TC)、 low density lipoprotein (LDL)、 HOMA-IR (CP)、 HbA1C、 the incidence of
 151 Met S. Women patients with NAFLD group also had higher BMI、 ALT、 GGT、 TG、
 152 HOMA-IR (CP) 、 HbA1C 、 the incidence of Met S. While HDL and PRL were
 153 markedly reduced in the cases merged-NAFLD than without NAFLD in both genders
 154 ($p<0.05$ or $p<0.01$). In terms of medication history, there was no statistical difference
 155 between the two groups of male and female patients in the treatment of hypoglycemic
 156 programs, which could exclude the influence of hypoglycemic drugs on the study.

157 Table 1 Comparison of general material and biochemical indexes of each group

	Men			Women		
	T2DM without NAFLD	T2DM with NAFLD	P value	T2DM without NAFLD	T2DM with NAFLD	P value
N	77	153		66	110	
Age(years)	63(54-63)	54(48-62)	0.000	65(57-71)	61(55-69)	0.077
Metabolic syndrome(%)	64.9	85.6	0.000	59.100	80.000	0.003
Menopause(%)	NA	NA		99.100	83.600	0.117
Diabetes course(years)	10(3-15)	8(3-12)	0.280	10(5-20)	10(4-15)	0.070
BMI(kg/m ²)	24.90±2.97	27.18±2.94	0.000	24.54±3.35	26.33±3.55	0.000
Systolic pressure(mmHg)	130(125-146)	132(121-145)	0.880	130(124-151)	130(123-144)	0.233
Diastolic pressure(mmHg)	81.48±9.59	85.80±9.94	0.002	80.48±8.59	79.07±8.16	0.277
Waistline(cm)	90.71±8.02	96.29±8.45	0.000	89.02±9.07	91.38±9.41	0.103
Hipline(cm)	96.64±6.77	100.66±6.18	0.000	97.00±6.52	97.73±7.81	0.526
ALT(U/L)	19(13-28)	21(15-32)	0.082	15(12-21)	19(14-33)	0.000
AST(U/L)	18(15-23)	19(15-23)	0.881	17(15-20)	18(15-25)	0.094
GGT (U/L)	24(17-36)	35(23-56)	0.000	19(14-28)	25(19-35)	0.000
FBG (mmol/L)	6.81(5.41-9.49)	7.80(6.21-11.0)	0.002	6.61(5.48-9.34)	7.89(6.05-10.96)	0.050
TG (mmol/L)	1.20(0.79-1.75)	2.01(1.42-3.27)	0.000	1.23(0.93-1.50)	1.81(1.19-2.35)	0.000
TC(mmol/L)	4.32±0.92	4.83±1.10	0.001	4.77±1.24	5.02±1.11	0.158
HDL (mmol/L)	1.04(0.96-1.18)	0.97(0.82-1.11)	0.004	1.23(1.05-1.47)	1.10(0.99-1.28)	0.002
LDL (mmol/L)	2.45±0.78	2.78±0.85	0.004	2.84±1.05	2.98±0.88	0.373
HOMA-IR(CP)	2.90(2.46-3.97)	3.99(3.18-5.20)	0.000	2.97(2.54-3.68)	3.68(2.91-4.41)	0.001
HOMA-β(CP-DM)	46.94(25.29-88.92)	44.33(27.17-83.92)	0.686	38.55(22.52-80.19)	48.27(25.02-73.90)	0.553
HbA1C (%)	7.5(6.7-9.1)	8.3(7.0-9.7)	0.043	7.7(6.7-9.3)	8.5(7.4-9.9)	0.020
PRL(ng/mL)	10.36(9.35-14.72)	9.56(7.81-12.60)	0.001	12.97(10.03-16.58)	10.38(8.43-14.27)	0.001
Hypoglycemic plan						
Metformin	26(33.8%)	62(40.5%)		17(25.8%)	43(39.1%)	
Other hypoglycemic drugs	16(20.8%)	44(28.8%)	0.083	23(34.8%)	27(24.5%)	0.150
Insulin	35(45.4%)	47(30.7%)		26(39.4%)	40(36.4%)	

158 Note: NAFLD, Nonalcoholic fatty liver disease; T2DM, Type 2 diabetes mellitus; BMI, Body mass index; ALT, Alanine aminotransferase; AST, Aspartic acid

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159 aminotransferase; GGT, Glutamyltransferase; FBG, Fasting blood glucose; TG, Triglyceride; TC, Total cholesterol; HDL, High density lipoprotein; LDL, Low
 160 density lipoprotein; HOMA-IR(CP), homeostasis model assessment for insulin resistance(C-peptide); HOMA-β(CP-DM): homeostasis model assessment for
 161 beta(C-peptide- diabetes mellitus); HbA1c, Glycosylated hemoglobin; PRL, prolactin. The measured data of the normal distribution was represented by mean±SD.
 162 Measurement data for non-normal distributions were expressed as medians (interquartile intervals).¹

163 Because women's serum PRL is affected by menopause or not, we analyzed the
 164 metabolic status and PRL levels of female patients with or without NAFLD before
 165 and after menopause (Table 2). Premenopausal women with NAFLD had higher BMI,
 166 FBG、TG、HbA1C and the incidence of Met S. Postmenopausal women with
 167 NAFLD had higher BMI、ALT、GGT、TG、HOMA-IR (CP) and the incidence of
 168 Met S, while HDL and PRL were markedly reduced in the cases merged-NAFLD than
 169 without NAFLD (p<0.05 or p<0.01).

170 Table 2 Comparison of clinical data of women with and without NAFLD before and
 171 after menopause

	Premenopause			Postmenopause		
	T2DM without NAFLD	T2DM with NAFLD	P value	T2DM without NAFLD	T2DM with NAFLD	P value
N	6	18		60	92	
Age(years)	44.80±3.76	45.20±4.37	0.848	66.15±8.34	64.61±8.16	0.261
Metabolic syndrome(%)	0	77.8	0.001	65	80.4	0.033
Diabetes course(years)	8.180±6.69	4.78±4.12	0.149	12.86±9.02	10.69±6.88	0.116
BMI(kg/m ²)	22.80±3.87	26.70±3.43	0.029	24.71±3.28	26.26±3.59	0.008
Systolic pressure(mmHg)	121.83±7.08	128.50±8.78	0.107	133(127-152)	131(122-145)	0.167
Diastolic pressure(mmHg)	77.50±7.18	84.06±6.78	0.055	80.78±8.71	78.10±8.08	0.054
Waistline(cm)	80.33±12.36	89.06±7.92	0.055	89.88±8.32	91.84±9.65	0.200
Hipline(cm)	94.67±6.83	95.61±7.65	0.791	97.23±6.50	98.14±7.81	0.456
ALT(U/L)	13(11-16)	15(13-45)	0.121	15(12-22)	20(15-33)	0.000
AST(U/L)	16(15-18)	16(13-35)	1.000	17(15-20)	19(16-25)	0.073
GGT (U/L)	20.67±14.28	37.67±31.34	0.217	19(14-29)	26(18-35)	0.002
FBG (mmol/L)	6.61±1.59	10.99±3.10	0.003	7.81±2.97	8.08±2.78	0.566
TG (mmol/L)	1.09(0.63-1.30)	2.02(1.37-2.83)	0.003	1.24(0.93-1.56)	1.74(1.17-2.34)	0.000
TC(mmol/L)	4.77±0.84	5.16±1.46	0.534	4.77±1.28	4.99±1.04	0.229
HDL (mmol/L)	1.23±0.17	1.06±0.21	0.086	1.27±0.29	1.14±0.27	0.005
LDL (mmol/L)	2.92±0.83	2.84±0.86	0.838	2.84±1.08	3.00±0.89	0.301
HOMA-IR(CP)	2.30±0.57	4.87±2.98	0.051	3.28±1.00	3.73±1.43	0.036

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HOMA-β(CP-DM)	25.07(19.86-28.67)	25.99(13.78-56.47)	0.689	47.00(22.63-85.05)	51.60(28.83-75.27)	0.505
HbA1C (%)	7.62±0.89	9.53±1.66	0.014	8.16±1.82	8.53±1.67	0.196
PRL(ng/mL)	18.92±8.57	14.54±4.64	0.122	13.16±3.79	10.88±3.77	0.000

172 Note: NAFLD, Nonalcoholic fatty liver disease; T2DM, Type 2 diabetes mellitus; BMI, Body mass index; ALT, Alanine aminotransferase; AST, Aspartic acid
 173 aminotransferase; GGT, Glutamyltransferase; FBG, Fasting blood glucose; TG, Triglyceride; TC, Total cholesterol; HDL, High density lipoprotein; LDL, Low
 174 density lipoprotein; HOMA-IR(CP), homeostasis model assessment for insulin resistance(C-peptide); HOMA-β(CP-DM): homeostasis model assessment for
 175 beta(C-peptide- diabetes mellitus); HbA1c, Glycosylated hemoglobin; PRL, prolactin. The measured data of the normal distribution was represented by mean±SD.
 176 Measurement data for non-normal distributions were expressed as medians (interquartile intervals).²

177 2. Relationship between PRL levels and related parameters of Met S

178 We further discussed the relationship between PRL levels and related parameters
 179 of Met S (Table 3). We found that in male subjects, the levels of PRL were negatively
 180 correlated with hipline, TG and HOMA-IR (CP), and positively associated with HDL,
 181 in female subjects, PRL levels were negatively related with BMI, DBP, waistline,
 182 hipline and TG ($p < 0.05$ or $p < 0.01$).

183 Table 3 Relationship between PRL levels and related parameters of Met S

	Men		Women	
	r	P value	r	P value
BMI	-0.092	0.166	-0.192	0.011
Systolic pressure	0.046	0.492	-0.045	0.552
Diastolic pressure	-0.125	0.059	-0.220	0.003
Waistline	-0.056	0.398	-0.152	0.044
Hipline	-0.141	0.032	-0.157	0.037
FBG	-0.109	0.098	-0.034	0.654
TG	-0.252	0.000	-0.258	0.001
TC	-0.096	0.146	-0.061	0.421
HDL	0.147	0.025	0.065	0.390
LDL	-0.042	0.528	-0.110	0.146
HOMA-IR(CP)	-0.141	0.032	-0.049	0.519
HOMA-β(CP-DM)	0.019	0.772	-0.044	0.562
HbA1C	-0.091	0.168	0.057	0.450

184 Note: Met S, metabolic syndrome; BMI, Body mass index; FBG, Fasting blood glucose; TG, Triglyceride; TC, Total cholesterol; HDL, High density
 185 lipoprotein; LDL, Low density lipoprotein; HOMA-IR(CP), homeostasis model assessment for insulin resistance(C-peptide); HOMA-β(CP-DM): homeostasis
 186 model assessment for beta(C-peptide- diabetes mellitus); HbA1c, Glycosylated hemoglobin; PRL, prolactin.; PRL, prolactin.

187 3. Multiple factors logistic regression analysis of serum PRL levels and NAFLD 188 risk

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189 The risk factors for NAFLD were assessed using multiple logistic regression
 190 analysis, which included age, BMI, menopause, TG, LDL as well as HOMA-IR (CP),
 191 HbA1C and PRL levels as variables. We found that PRL levels were independently
 192 negative associated with NAFLD in both men and women (odds ratio (OR): 0.891,
 193 95% confidence interval (CI): 0.803-0.989, $p=0.031$, for men; OR: 0.874, 95% (CI):
 194 0.797-0.957, $p=0.004$, for women). Other risk factors included age, BMI, LDL and
 195 HOMA-IR (CP) for men, and TG for women (Table 4).

196 Table 4 Multivariate logistic regression analysis of serum PRL levels and NAFLD risk

	Men			Women		
	β	OR(95%CI)	p value	β	OR(95%CI)	p value
Age	-0.045	0.956(0.924-0.989)	0.010	-0.044	0.957(0.912-1.004)	0.070
BMI	0.255	1.291(1.122-1.484)	0.000	0.090	1.094(0.97-1.224)	0.120
Menopause				0.213	1.237(0.281-5.441)	0.778
TG	0.176	1.193(0.959-1.483)	0.113	0.981	2.666(1.404-5.064)	0.003
LDL	0.493	1.637(1.046-2.561)	0.031	-0.121	0.886(0.596-1.318)	0.550
HOMA-IR(CP)	0.360	1.134(1.062-1.936)	0.019	0.215	1.240(0.859-1.788)	0.250
HbA1C	0.057	1.059(0.872-1.287)	0.564	0.047	1.048(0.840-1.308)	0.676
PRL	-0.115	0.891(0.803-0.989)	0.031	-0.135	0.874(0.797-0.957)	0.004

197 Note: The risk factors for NAFLD were assessed using multiple logistic regression analysis in men and women. The ORs with corresponding 95% CIs were
 198 adjusted for age, BMI, menopause, TG, LDL as well as HOMA-IR (CP), HbA1C and PRL levels as variables. NAFLD, Nonalcoholic fatty liver disease; BMI, Body mass
 199 index; FBG, Fasting blood glucose; TG, Triglyceride; LDL, Low density lipoprotein; HOMA-IR (CP), homeostasis model assessment for insulin
 200 resistance (C-peptide); HbA1c, Glycosylated hemoglobin; PRL, prolactin. OR: Odds ratio; CI: Confidence

201 4. Relationship between PRL levels and the prevalence of NAFLD and Met S

202 According to the quartile of PRL levels, the subjects were divided into four groups:
 203 T1 < 8.29 (n= 57 cases)、 8.29 ≤ T2 < 9.93 (n= 58 cases)、 9.93 ≤ T3 < 12.68 (n=
 204 57 cases)、 T4 ≥ 12.68 (n= 58 cases) ng/mL in men (n= 230 cases) and T1 < 8.95 (n=
 205 44 cases)、 8.95 ≤ T2 < 11.32 (n= 44 cases)、 11.32 ≤ T3 < 14.95 (n= 44 cases)、 T4 ≥
 206 14.95 (n= 44 cases) ng/mL in women (n= 176 cases). Chi-square test was used to
 207 compare the prevalence and composition ratio among different groups. The
 208 prevalence of NAFLD had a decreasing trend with the rise of the quartile of PRL in
 209 both genders (T1: 84.2%, T2: 63.8%, T3: 59.6%, T4: 58.6%, $p=0.012$ in men; T1:
 210 79.5%, T2:
 211 65.9%, T3: 54.5%, T4: 50%, $p=0.013$ in women). However, the prevalence rates of
 212 Met S were T1: 86%, T2: 79.3%, T3: 77.2%, T4: 72.4%, $p=0.354$ in men; T1: 84.1%,

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213 T2: 70.5%, T3: 77.3%, T4: 56.8%, $p=0.031$ in women. Therefore, in female subjects,
214 the prevalence rates of Met S in the fourth quartile groups of PRL were significantly
215 lower than those in the first, second and third group.

216 Discussion

217 At present, due to the rapid increase in the incidence of obesity and obesity-relat-
218 ed diseases, NAFLD has become an important public health problem[17] NAFLD is
219 considered as the manifestation of Met S in liver, especially in T2DM patients [18]. In
220 this study, it was found that the incidence of NAFLD diagnosed by abdominal liver
221 color doppler ultrasound was 64.8%. Compared with non-NAFLD patients, NAFLD
222 patients had higher BMI, TG, GGT, HOMA-IR (CP), HbA1C, incidence of Met S and
223 lower HDL in both genders. Equally, Zhang et al[19] also got similar results. Among
224 which BMI, TG and HDL were the components of Met S. Therefore, cases in T2DM
225 complicated NAFLD were easy to promote the abnormality of metabolic indexes.

226 PRL is considered as a hormone closely related to metabolism [20]. Recent
227 findings have displayed that there had been close association between PRL and
228 T2DM. Across sectional study included 2377 adult community population (excluding
229 hyperprolactinemia), and found that cases with impaired glucose regulation and
230 T2DM had lower PRL levels. Researchers rectified age, sex, BMI and other
231 confounding factors, still discovered that the risk of above-mentioned people with
232 high serum PRL was significantly reduced [11]. Further follow-up of 3.7 years, it
233 was revealed that female cases had a lower risk of T2DM in the highest quartile PRL
234 levels, with a risk ratio of 0.48[9]. Another cross-sectional study also found that the
235 risk of Met S and T2DM in women with lower baseline PRL increased[21]. A large
236 meta-analysis indicated that higher serum PRL levels in normal range were related
237 with low level of T2DM risk[22]. At the same time, Jha et al[23] also found that
238 serum PRL had a significant correlation with liver disease and predicted its mortality.
239 In adipose tissue, PRL intervention can reduce the production of malonyl coenzyme A
240 in human primary adipocytes, thus inhibiting the restart of triglyceride synthesis [24].
241 PRL receptor can also directly inhibit the expression of fatty acid synthetase and fatty
242 acid synthesis in 3T3L1 cells[25]. PRL reduced the accumulation of triglyceride in

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243 liver through PRL receptor, thus improving liver steatosis [10]. These results indicate
244 that higher PRL levels had positive protective effect on glucose and lipid metabolism.

245 Considering that PRL secretion may be different due to gender, we studied male
246 and female subjects separately. We found that compared with non-NAFLD patients,
247 the PRL value of NAFLD patients was lower in both genders. The age, BMI, TG,
248 LDL, HOMA-IR (CP), HbA1C were corrected, at the same time, female subjects cor-
249 rected menopause factors, the study suggested that PRL levels had negative relation
250 to the risk of NAFLD. In line with the quartile of PRL, the incidence of NAFLD
251 showed a generally downturn with the increase of PRL levels in both genders. Zhang
252 et al[26] noted that PRL increased by one standard deviation, the risk of male NAFLD
253 patients decreased by 12.3%, and that of female patients decreased by 21.4%. PRL
254 was proved to be a protective factor, which affected the existence and progress of
255 NAFLD. In another study, Zhang et al[19] also found that the PRL levels of NAFLD
256 patients diagnosed by ultrasound were significantly lower than that of non-NAFLD
257 patients, whether male or female. In addition, with the increase of PRL quartile, the
258 incidence of NAFLD decreased. All subjects were corrected for age, sex, BMI, insulin
259 resistance, HbA1C, diabetes and other factors. The results showed that PRL had
260 contrary associated with NAFLD. We took into consideration that PRL levels are
261 affected by many conditions including various drugs, stress, and exercise. We ruled
262 out the following cases: such as using drugs that affect PRL (metoclopramide,
263 methyl-
264 dopa, opiates, and cimetidine), the levels of thyroid stimulating hormone, cortisol,
265 estradiol and testosterone are higher than the normal range. In terms of medication
266 history, there was no statistical difference between the two groups of male and female
267 patients in the treatment of hypoglycemic programs, which could exclude the
268 influence of hypoglycemic drugs on the study.

269 In the meantime, the secretion of PRL may be affected by whether women were
270 menopausal or not, this paper analyze the menopausal and non-menopausal subtypes
271 of women, we found that postmenopausal women with NAFLD had lower PRL
272 levels. In addition, Zhang Zhuzi et al[27] divided the included women into

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premenopausal group and postmenopausal group, and also found that in both groups, PRL of patients with NAFLD was lower than that of patients without NAFLD, the decrease of PRL levels in postmenopausal women with NAFLD was more significant. It was suggested that the decrease of PRL in NAFLD patients was affected by menopausal factors.

Researchs have shown a correlation between PRL levels and the components of Met S, which could explain the role of PRL in NAFLD. According to the basic researchs, in the obese mouse model induced by high-fat diet, severe metabolic changes would occur in mice with PRL receptor failure. Injection of PRL could improve insulin sensitivity and prevent visceral adipocyte hypertrophy [28]. Clinical studies had found that low serum PRL levels in physiological range was related to poor metabolic outcome of Met S and T2DM[11]. In overweight and obese men, serum PRL levels were lower [28]. Friedrich et al[29] found that PRL levels were negatively correlated with waistline in 1857 healthy women aged 20-79. The endocrine characteristics of Met S and polycystic ovary syndrome (PCOS) have a relatively high coincidence rate [30]. A systematic retrospective analysis of 2052 PCOS patients found that the lower the serum PRL, the higher the BMI. PRL had opposite related with TG, TC and LDL-C [31]. Arterial hypertension is a component of Met S. A prospective study of 874 postmenopausal women found that PRL levels increased by 1 standard deviation during 8 years of follow-up, and the relative risk of hypertension was 1.31[32]. Our study found that in male subjects, the levels of PRL were negatively correlated with hipline, TG and HOMA-IR(CP), and positively associated with HDL, in female subjects, PRL levels were negatively related with BMI, DBP, waistline, hipline and TG. In female subjects, the prevalence rates of Met S in the fourth quartile groups of PRL were significantly lower than those in the first, second and third group. Furthermore, premenopausal and postmenopausal women with NAFLD had higher BMI, TG and the incidence of Met S. As we all know, NAFLD is very common in obese and dyslipidemia patients. Obese individuals produce relatively excessive proinflammatory factors, some of which inhibit the treatment of liver fat and promote the accumulation of lipid in hepatocytes [33]. Dyslipidemia, especially hypertriglyceridemia, may

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303 subsequently increase the transportation of TG and other fats into hepatocytes, result-
304 ing in hepatic steatosis [34].

305 As a retrospective analysis, this study has many limitations. First of all, the diag-
306 nosis of NAFLD is based on ultrasound examination, which cannot distinguish NASH
307 from fibrosis. Secondly, because this is a cross-sectional study, we can't infer the
308 direct cause and influence between PRL and NAFLD, and need further mechanical st-
309 udy to clarify their exact relationship. Thirdly, PRL secretion appears in pulse form,
310 the best time to draw blood for PRL is from 9: 00 to 11: 00 a.m., and patients should
311 avoid emotional excitement. Finally, due to the limited number of samples in this
312 study, the effects of drugs for treating cardiovascular diseases, controlling blood lipid
313 on PRL levels have not been meditated, which requires further layered analysis in the
314 future work. Moreover, the small sample size can not reflect the large scale
315 population based cross-sectional epidemiological study, so it is necessary to increase
316 the samp-
317 le size.

318 **Conclusions**

319 In a word, our research shows that serum PRL levels are related to NAFLD in
320 T2DM population in physiological range, and are also connected to known metabolic
321 risk factors. Our research results may help to predict the risk of developing NAFLD,
322 so as to better understand the disease, importantly, and to formulate effective preventi-
323 on strategies.

324 **Abbreviations**

325 NAFLD: Nonalcoholic fatty liver disease; T2DM: Type 2 diabetes mellitus; PRL: Prolactin;
326 Met S: Metabolic syndrome; NASH: Non-alcoholic steatohepatitis; SBP: Systolic pressure;
327 DBP: Diastolic pressure; BMI: Body mass index; AST: Aspartic acid aminotransferase; ALT:
328 Alanine aminotransferase; GGT: Glutamyltransferase; FBG: Fasting blood glucose; TG:
329 Triglyceride; TC: Total cholesterol; HDL: High density lipoprotein; LDL: Low density lipoprotein;
330 Homa-IR (CP): Modified homeostasis model assessment for insulin resistance (C-peptide);
331 HOMA- β (CP-DM): homeostasis model assessment for beta (C-peptide- diabetes mellitus);
332 HbA1c: Glycosylated hemoglobin; SD: Standard deviation; OR: Odds ratio; CI: Confidence
333 interval

334 **Footnotes**

335 **Contributorship statement**

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YZ conceived the study, collected clinical data, analyzed and interpreted the data and wrote the manuscript. HL made a revised version. All authors read and agreed to the final version of the manuscript.

Competing interests

The authors declare that they have no competing interests.

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Data Sharing Statement

All data generated or analyzed during this study are included in the article. The data that support this study are available from the corresponding author only upon reasonable request, once the study has been published.

Ethics approval and consent to participate

This study was a retrospective research, so it was exempted from informed consent with the approval of the Ethics Committee of The First Affiliated Hospital of Anhui University of Traditional Chinese Medicine (2020MCZQ09).

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1 Cross-sectional correlations of prolactin levels and nonalcoholic fatty liver disease in
2 patients with type 2 diabetes mellitus: a retrospective analysis of patients from a
3 single hospital in China

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11 **Abstract:**

12 **Objective:** This research aims to retrospectively assess the association between
13 prolactin (PRL) and nonalcoholic fatty liver disease (NAFLD), specifically in patients
14 with type 2 diabetes mellitus (T2DM).

15 **Design and setting:** A cross-sectional study was conducted in Anhui, China.

16 **Participants:** A total of 406 patients with T2DM (153 men, 110 women) were
17 selected.

18 **Primary and secondary outcome measures:** P values for the independent T test,
19 Mann - Whitney rank sum test, Spearman correlation analysis, and multiple logistic
20 regression models were used to explore the association between PRL and NAFLD in
21 patients with T2DM.

22 **Results:** The results indicated that in both men and women, the levels of PRL were
23 significantly lower in the T2DM with NAFLD group than that in the T2DM without
24 NAFLD group (men: 9.56 ng/mL vs. 10.36 ng/mL, women: 10.38 ng/mL vs. 12.97
25 ng/mL). In male subjects, the levels of PRL were negatively correlated with hip
26 circumference, homeostasis model assessment for insulin resistance (C-peptide) and
27 triglyceide (TG), and inversely correlated with high density lipoprotein (HDL)
28 ($r=-0.141$, $p=0.032$, $r=-0.141$, $p=0.032$, $r=-0.252$, $p=0.000$, $r=0.147$, $p=0.025$,
29 respectively). In female subjects, PRL levels were negatively related with body mass
30 index, diastolic blood pressure, waist circumference, hip circumference and TG
31 ($r=-0.192$, $p=0.011$, $r=-0.220$, $p=0.003$, $r=-0.152$, $p=0.044$, $r=-0.157$, $p=0.037$,

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32 $r=-0.258$, $p=0.001$, respectively). Logistic regression analysis revealed a negative
33 relationship between PRL and NAFLD (men: OR95% CI: 0.891 (0.803-0.989),
34 $p=0.031$, women: OR95% CI: 0.874 (0.797-0.957), $p=0.004$, respectively).

35 As PRL levels increased, NAFLD prevalence decreased in both sexes (men: $p=0.012$,
36 women: $p=0.013$, respectively).

37 **Conclusion:** Our results supported that a low levels of PRL in the physiological range
38 was a markers of NAFLD in T2DM patients and suggested that prolactin within the
39 biologically high range may play a protective role in the pathogenesis of NAFLD.

40 **Keywords:** Type 2 diabetes mellitus; Nonalcoholic fatty liver disease; Prolactin;

41 **Strengths and limitations of this study**

42 ▶ Abdominal colour ultrasonography is a common and simple method for the clinical
43 diagnosis of nonalcoholic fatty liver disease (NAFLD).

44 ▶ The normal range of serum prolactin (PRL) levels differs by sex, so we conducted a
45 sex stratification analysis of patients with type 2 diabetes mellitus (T2DM) and found
46 that in both men and women, the levels of PRL were significantly lower in the T2DM
47 with NAFLD group than that in without NAFLD group.

48 ▶ The change of PRL levels promotes medical workers to pay attention to the
49 occurrence of NAFLD in patients with T2DM.

50 ▶ P values for independent T tests and multiple logistic regression models were used
51 to assess the association between PRL and NAFLD in patients with T2DM.

52 ▶ This was a cross-sectional study and cannot provide evidence of causal
53 relationships.

54 **Introduction**

55 The liver is an important organ of glycolipid metabolism in the body. When
56 triglyceride deposition in hepatocytes increases and exceeds 5% and other factors
57 causing liver steatosis (such as alcohol consumption and viral hepatitis) are excluded,
58 NAFLD can be diagnosed[1]. In China, with the gradual improvement of living
59 standards, NAFLD has surpassed chronic viral hepatitis to become the primary cause
60 of chronic liver diseases[2]. Currently, the global incidence of NAFLD is 25.2%[3],
61 while the prevalence of NAFLD diagnosed by ultrasound in patients with T2DM is

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73.7%[4]. T2DM is an important factor associated with the progression of NAFLD to NASH and fibrosis[1].

NAFLD is closely related to central obesity, hypertension, hyperlipidaemia, T2DM and metabolic syndrome (MetS)[5]. Among MetS-related diseases, only NAFLD is considered a strong predictor of MetS, and the incidence of MetS in fatty liver patients is more than 4 times that in nonfatty liver patients[6]. Therefore, NAFLD is considered the expression of MetS in the liver.

Prolactin (PRL) is a type of hormone that is mainly secreted by adenohypophysis. Its main physiological function is to stimulate breast development and milk secretion[7]. Its receptors are widely distributed in various tissues and organs of the body, including fat, liver, pancreas and so on [8]. PRL can increase the proliferation of β cells, stimulate insulin secretion and participate in the regulation of glucose metabolism [9]. PRL can also inhibit lipolysis and activate adipocyte differentiation by activating peroxisome proliferator-activated receptor γ [10]. Studies in China and abroad have found that the decrease in serum PRL at the physiological level is closely related to the occurrence of T2DM. Wang et al[11] discovered that the PRL levels of patients with T2DM and impaired glucose regulation were significantly lower than those of people with normal glucose metabolism. The researchers further pointed out that the decrease in physiological levels of PRL was related to an increased risk of T2DM[9]. Manshaei et al[12] also found that the serum PRL concentration of patients with T2DM was lower than that of healthy people. Because of the high incidence of NAFLD in patients with T2DM, T2DM is also an important factor in MetS. The relationship among PRL, NAFLD and MetS at the physiological level has not been explored. The goal of this research was to explore the relationship among PRL, NAFLD and MetS in patients with T2DM.

Methods

Participants

All participants in this study were recruited from a hospital located Anhui, China. This was a cross-sectional survey. A total of 656 patients with T2DM were investigated in this study, but 15 participants were excluded due to the use of that

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1
2
3
4 92 affect PRL (metoclopramide, methyl dopa, opiates, and imetidine). Thirty participants
5
6 93 were excluded because their levels of thyroid-stimulating hormone, cortisol, oestradiol
7
8 94 and testosterone were higher than the normal range. Four participants had pituitary
9
10 95 diseases, five had hyperglycaemia, 56 exhibited excessive alcohol consumption
11
12 96 (intake of alcohol exceeding 140 g/week for men and 70 g/week for women), 11 had
13
14 97 cancer, 5 were pregnant, 7 had type 1 diabetes, 25 had acute complications of
15
16 98 diabetes, 15 had acute cardiovascular events, 30 had severe hepatic and renal
17
18 99 insufficiency, 8 had viral liver disease, 30 had alcoholic liver disease, 5 had
19
20 100 drug-induced liver disease and 4 had autoimmune liver disease. Finally, 406
21
22 101 participants (230 men and 176 women) were included in this study. This study was a
23
24 102 retrospective study, so it was exempted from the requirement of informed consent and
25
26 103 was approved by the Ethics Committee of The First Affiliated Hospital of Anhui
27
28 104 University of Traditional Chinese Medicine.

105 **Data collection**

106 We collected data on sex, age, menopausal history of women, height, weight,
107 diabetes course, preadmission hypoglycaemic plan (including metformin, insulin and
108 other hypoglycaemic drugs such as sulfonylureas, glinides, thiazolidinediones,
109 α -glucosidase inhibitors, glucagon-like peptide-1 receptor agonists, dipeptidyl
110 peptidase 4 inhibitors, sodium-glucose cotransporter protein 2 inhibitors), history of
111 alcohol consumption, occurrence of cancer, history of other liver diseases, waist
112 circumference, hip circumference, and blood pressure. All patients' morning venous
113 blood samples were collected on the second day after admission, and all blood was
114 extracted with a centrifuge. After separation of serum, fasting blood glucose (FBG),
115 blood fat, liver and kidney function were measured using an automatic biochemical
116 analyser (7600-020; Hitachi). Fasting C-peptide (FCP) was examined using an
117 enzyme-linked immunosorbent assay (A2000 Plus; Autolumo). An automated
118 chemiluminescent immunoassay (Siemens Immulite 2000, UK) was used to measure
119 PRL. The coefficients of intra-assay and interassay variation ranged from 2.49-3.47%
120 and 2.91-3.14%, respectively. PRL levels are affected by many conditions including

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121 the use of various drugs, stress, and exercise, so we took blood samples at 9: 00 am
122 after the patients were admitted to the hospital on the first day and the next morning.
123 We took 2 ml blood samples each time. The patients fasted and rested in a sitting
124 position for 30 minutes, and then the average value of two blood pressure readings
125 was taken. High-performance liquid chromatography was used to check glycosylated
126 haemoglobin (HbA1c) (Variant II; Bio - Rad).

127 **Definitions, counts and groups**

128 The diagnosis of T2DM was based on the diagnostic criteria proposed by the
129 World Health Organization (WHO) Diabetes Expert Committee in 1999. The
130 physiological level of PRL is based on the normal reference range of our hospital,
131 which is 2.78-29.20 ng/mL for premenopausal women, 1.79-20.28 ng/mL for
132 menopausal women and 2.12-17.69 ng/mL for men.

133 NAFLD was diagnosed by ultrasound[13] by a senior technician. The ultrasonic
134 diagnosis of fatty liver is as follows: near-field of liver permeate punctiform
135 hyperecho, composition of the intrahepatic duct not clearly demonstrated by
136 ultrasonography, and weak echo in the distal echo. Diagnosis of NAFLD is based on
137 the following requirements: no history of alcohol consumption, no other types of liver
138 diseases, and unexplained increase in serum alanine aminotransferase (ALT), aspartic
139 acid aminotransferase (AST) or glutamyltransferase (GGT) over 6 months[14].

140 The diagnosis of MetS conformed to the standard put forward in the ninth edition
141 of internal medicine in China [15], and the diagnostic standard included the following
142 three or more items: ① central obesity and/or abdominal obesity: waist
143 circumference is greater than 90 cm for men and 85 cm for women; ②
144 hyperglycaemia: FBG > 6.1 mmol/L or 2-hour blood glucose >7.8 mmol/L and/or
145 confirmation of diabetes diagnosis and treatment with hypoglycaemic therapy; ③
146 Hypertension: blood pressure exceeding 130/85 mmHg and/or diagnosis of
147 hypertension and treatment with antihypertensive therapy; ④ fasting triglyceride
148 (TG) level exceeding 1.7 mmol/l; and ⑤ fasting high-density lipoprotein (HDL)
149 Level below 1.04 mmol/l. Body mass index (BMI) was computed by dividing the
150 body weight (kg) by the square of the height (m²). homeostasis model assessment of

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insulin resistance (C-peptide) (HOMA-IR (CP)) was determined by FCP as a substitute for fasting insulin as follows: $HOMA-IR (CP) = 1.5 + FBG (mmol/L) \times FCP (pmol/L) / 2800$. $HOMA-\beta (CP-DM) = 0.27 \times FCP (pmol/L) / (FBG (mmol/L) - 3.5)$ [16].

In conformity with ultrasonic diagnosis, patients with T2DM who met the inclusion criteria were segmented into the without NAFLD group (77 men, 66 women) and the with NAFLD group (153 men, 110 women).

Patient and public involvement

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

Statistical analysis

SPSS 21.0 statistical software was used for the data analysis, and the Kolmogorov–Smirnov normality test was performed for all data. The measured data with a normal distribution are represented as the mean \pm standard deviation (SD). Comparisons were conducted between two groups, and comparisons were performed using independent T tests. Measurement data with nonnormal distributions are expressed as medians (interquartile intervals). In this situation, two groups were compared by using the Mann - Whitney rank sum test. Categorical variables are shown as the number of cases, and the chi-square test was adopted to demonstrate the differences within two or more groups. Spearman correlation analysis compared the relationship between PRL levels and other variables. The relationships among PRL, NAFLD and MetS were analysed by logistic regression. $P < 0.05$ or $P < 0.01$ represented obvious significant differences.

Results

1. Comparison of general findings and laboratory test targets in each group

The ultrasonic diagnostic rate of NAFLD was 263 cases (153 plus 110 cases) (64.8%) (Table 1). Men with NAFLD had a younger age and higher BMI, waist circumference, hip circumference, diastolic blood pressure (DBP), GGT, FBG, TG, total cholesterol (TC), low-density lipoprotein (LDL), HOMA-IR (CP), HbA1C, and MetS incidence. Women with NAFLD also had higher BMI, ALT, GGT, TG, HOMA-IR (CP), HbA1C, and MetS incidence. HDL and PRL were markedly reduced

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181 in the patients with NAFLD than in those without NAFLD in both sexes (p<0.05 or
 182 p<0.01). In terms of medication history, there was no significant difference between
 183 the two groups of male and female patients in hypoglycaemic programs, which could
 184 exclude the influence of hypoglycaemic drugs on the study.

185 Table 1 Comparison of general characteristics and biochemical indexes of each group

	Men			Women		
	T2DM without NAFLD	T2DM with NAFLD	P value	T2DM without NAFLD	T2DM with NAFLD	P value
N	77	153		66	110	
Age(years)	63(54-63)	54(48-62)	0.000	65(57-71)	61(55-69)	0.077
Metabolic syndrome(%)	64.9	85.6	0.000	59.100	80.000	0.003
Menopause(%)	NA	NA		99.100	83.600	0.117
Diabetes course(years)	10(3-15)	8(3-12)	0.280	10(5-20)	10(4-15)	0.070
BMI(kg/m ²)	24.90±2.97	27.18±2.94	0.000	24.54±3.35	26.33±3.55	0.000
Systolic pressure(mmHg)	130(125-146)	132(121-145)	0.880	130(124-151)	130(123-144)	0.233
Diastolic pressure(mmHg)	81.48±9.59	85.80±9.94	0.002	80.48±8.59	79.07±8.16	0.277
Waist circumference(cm)	90.71±8.02	96.29±8.45	0.000	89.02±9.07	91.38±9.41	0.103
Hip circumference(cm)	96.64±6.77	100.66±6.18	0.000	97.00±6.52	97.73±7.81	0.526
ALT(U/L)	19(13-28)	21(15-32)	0.082	15(12-21)	19(14-33)	0.000
AST(U/L)	18(15-23)	19(15-23)	0.881	17(15-20)	18(15-25)	0.094
GGT (U/L)	24(17-36)	35(23-56)	0.000	19(14-28)	25(19-35)	0.000
FBG (mmol/L)	6.81(5.41-9.49)	7.80(6.21-11.0)	0.002	6.61(5.48-9.34)	7.89(6.05-10.96)	0.050
TG (mmol/L)	1.20(0.79-1.75)	2.01(1.42-3.27)	0.000	1.23(0.93-1.50)	1.81(1.19-2.35)	0.000
TC(mmol/L)	4.32±0.92	4.83±1.10	0.001	4.77±1.24	5.02±1.11	0.158
HDL (mmol/L)	1.04(0.96-1.18)	0.97(0.82-1.11)	0.004	1.23(1.05-1.47)	1.10(0.99-1.28)	0.002
LDL (mmol/L)	2.45±0.78	2.78±0.85	0.004	2.84±1.05	2.98±0.88	0.373
HOMA-IR(CP)	2.90(2.46-3.97)	3.99(3.18-5.20)	0.000	2.97(2.54-3.68)	3.68(2.91-4.41)	0.001
HOMA-β(CP-DM)	46.94(25.29-88.92)	44.33(27.17-83.92)	0.686	38.55(22.52-80.19)	48.27(25.02-73.90)	0.553
HbA1C (%)	7.5(6.7-9.1)	8.3(7.0-9.7)	0.043	7.7(6.7-9.3)	8.5(7.4-9.9)	0.020
PRL(ng/mL)	10.36(9.35-14.72)	9.56(7.81-12.60)	0.001	12.97(10.03-16.58)	10.38(8.43-14.27)	0.001
Hypoglycaemic plan						
Metformin	26(33.8%)	62(40.5%)		17(25.8%)	43(39.1%)	
Other hypoglycaemic drugs	16(20.8%)	44(28.8%)	0.083	23(34.8%)	27(24.5%)	0.150
Insulin	35(45.4%)	47(30.7%)		26(39.4%)	40(36.4%)	

186 Note: NAFLD, Nonalcoholic fatty liver disease; T2DM, Type 2 diabetes mellitus; BMI, Body mass index; ALT, Alanine aminotransferase; AST, Aspartic acid
 187 aminotransferase; GGT, Glutamyltransferase; FBG, Fasting blood glucose; TG, Triglyceride; TC, Total cholesterol; HDL, High density lipoprotein; LDL, Low
 188 density lipoprotein; HOMA-IR(CP), homeostasis model assessment for insulin resistance(C-peptide); HOMA-β(CP-DM): homeostasis model assessment for

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189 beta(C-peptide- diabetes mellitus);HbA1c, Glycosylated haemoglobin; PRL, prolactin. The measured data with a normal distribution are represented as the
 190 mean±SD. Measurement data for nonnormal distributions are expressed as medians (interquartile intervals).Normally distributed variables: BMI, diastolic
 191 blood pressure, waist circumference, hip circumference, TC, LDL; Nonnormally distributed variables: Age, diabetes course, systolic blood pressure, ALT, AST,
 192 GGT, FBG, TG, HDL, HOMA-IR (CP), HOMA-β (CP-DM), HbA1C, and PRL.

193 Because women's serum PRL is affected by menopause, we analysed the
 194 metabolic status and PRL levels of female patients with or without NAFLD before
 195 and after menopause (Table 2). Premenopausal women with NAFLD had higher BMI,
 196 FBG, TG, HbA1C and MetS incidence. Postmenopausal women with NAFLD had
 197 higher BMI, ALT, GGT, TG, HOMA-IR (CP) and MetS incidence, while HDL and
 198 PRL were markedly reduced in the patients with NAFLD compared with the levels in
 199 those without NAFLD ($p<0.05$ or $p<0.01$).

200 Table 2 Comparison of clinical data of women with and without NAFLD before and
 201 after menopause

	Premenopause			Postmenopause		
	T2DM without NAFLD	T2DM with NAFLD	P value	T2DM without NAFLD	T2DM with NAFLD	P value
N	6	18		60	92	
Age(years)	44.80±3.76	45.20±4.37	0.848	66.15±8.34	64.61±8.16	0.261
Metabolic syndrome(%)	0	77.8	0.001	65	80.4	0.033
Diabetes course(years)	8.180±6.69	4.78±4.12	0.149	12.86±9.02	10.69±6.88	0.116
BMI(kg/m ²)	22.80±3.87	26.70±3.43	0.029	24.71±3.28	26.26±3.59	0.008
Systolic pressure(mmHg)	121.83±7.08	128.50±8.78	0.107	133(127-152)	131(122-145)	0.167
Diastolic pressure(mmHg)	77.50±7.18	84.06±6.78	0.055	80.78±8.71	78.10±8.08	0.054
Waist circumference(cm)	80.33±12.36	89.06±7.92	0.055	89.88±8.32	91.84±9.65	0.200
Hip circumference(cm)	94.67±6.83	95.61±7.65	0.791	97.23±6.50	98.14±7.81	0.456
ALT(U/L)	13(11-16)	15(13-45)	0.121	15(12-22)	20(15-33)	0.000
AST(U/L)	16(15-18)	16(13-35)	1.000	17(15-20)	19(16-25)	0.073
GGT (U/L)	20.67±14.28	37.67±31.34	0.217	19(14-29)	26(18-35)	0.002
FBG (mmol/L)	6.61±1.59	10.99±3.10	0.003	7.81±2.97	8.08±2.78	0.566
TG (mmol/L)	1.09(0.63-1.30)	2.02(1.37-2.83)	0.003	1.24(0.93-1.56)	1.74(1.17-2.34)	0.000
TC(mmol/L)	4.77±0.84	5.16±1.46	0.534	4.77±1.28	4.99±1.04	0.229
HDL (mmol/L)	1.23±0.17	1.06±0.21	0.086	1.27±0.29	1.14±0.27	0.005
LDL (mmol/L)	2.92±0.83	2.84±0.86	0.838	2.84±1.08	3.00±0.89	0.301
HOMA-IR(CP)	2.30±0.57	4.87±2.98	0.051	3.28±1.00	3.73±1.43	0.036
HOMA-β(CP-DM)	25.07(19.86-28.67)	25.99(13.78-56.47)	0.689	47.00(22.63-85.05)	51.60(28.83-75.27)	0.505
HbA1C (%)	7.62±0.89	9.53±1.66	0.014	8.16±1.82	8.53±1.67	0.196

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PRL(ng/mL) 18.92±8.57 14.54±4.64 0.122 13.16±3.79 10.88±3.77 0.000

202 Note: NAFLD, Nonalcoholic fatty liver disease; T2DM, Type 2 diabetes mellitus; BMI, Body mass index; ALT, Alanine aminotransferase; AST, Aspartic acid
 203 aminotransferase; GGT, Glutamyltransferase; FBG, Fasting blood glucose; TG, Triglyceride; TC, Total cholesterol; HDL, High density lipoprotein; LDL, Low
 204 density lipoprotein; HOMA-IR(CP), homeostasis model assessment for insulin resistance(C-peptide); HOMA-β(CP-DM): homeostasis model assessment for
 205 beta(C-peptide- diabetes mellitus);HbA1c, Glycosylated haemoglobin; PRL, prolactin. The measurement data with a normal distribution are represented as the
 206 mean±SD. Measurement data with nonnormal distributions are expressed as medians (interquartile intervals).¹

207 2. Relationship between PRL levels and MetS-related parameters

208 We further investigated the relationship between PRL levels and MetS-related
 209 parameters (Table 3). We found that in male subjects, the levels of PRL were
 210 negatively correlated with hip circumference, TG and HOMA-IR (CP) and positively
 211 associated with HDL. In female subjects, PRL levels were negatively correlated with
 212 BMI, DBP, waist circumference,

213 Table3 Relationship between PRL levels and MetS-related parameters

	Men		Women	
	r	P value	r	P value
BMI	-0.092	0.166	-0.192	0.011
Systolic pressure	0.046	0.492	-0.045	0.552
Diastolic pressure	-0.125	0.059	-0.220	0.003
Waist circumference	-0.056	0.398	-0.152	0.044
Hip circumference	-0.141	0.032	-0.157	0.037
FBG	-0.109	0.098	-0.034	0.654
TG	-0.252	0.000	-0.258	0.001
TC	-0.096	0.146	-0.061	0.421
HDL	0.147	0.025	0.065	0.390
LDL	-0.042	0.528	-0.110	0.146
HOMA-IR(CP)	-0.141	0.032	-0.049	0.519
HOMA-β(CP-DM)	0.019	0.772	-0.044	0.562
HbA1C	-0.091	0.168	0.057	0.450

214 Note: Met S, metabolic syndrome; BMI, body mass index; FBG, fasting blood glucose; TG, triglyceride; TC, total cholesterol; HDL, high-density
 215 lipoprotein; LDL, low-density lipoprotein; HOMA-IR (CP), homeostasis model assessment for insulin resistance (C-peptide); HOMA-β (CP-DM): homeostasis
 216 model assessment for beta (C-peptide-diabetes mellitus); HbA1c, glycosylated haemoglobin; PRL, prolactin.; PRL, prolactin.

217 3. Multiple-factor logistic regression analysis of serum PRL levels and NAFLD 218 risk

219

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220 The impact index for NAFLD was assessed using multiple logistic regression
 221 analysis, which included age, BMI, menopause, TG, LDL, HOMA-IR (CP), HbA1C
 222 and PRL as variables. We found that PRL levels were independently negatively
 223 associated with NAFLD in both men and women (odds ratio (OR): 0.891, 95%
 224 confidence interval (CI): 0.803-0.989, $p=0.031$, for men; OR: 0.874, 95% (CI):
 225 0.797-0.957, $p=0.004$, for women). Other risk factors included age, BMI, LDL and
 226 HOMA-IR (CP) for men and TG for women (Table 4).

227 Table 4 Multivariate logistic regression analysis of serum PRL levels and NAFLD
 228 risk

	Men			Women		
	β	OR(95% CI)	p value	β	OR(95% CI)	p value
Age	-0.045	0.956(0.924-0.989)	0.010	-0.044	0.957(0.912-1.004)	0.070
BMI	0.255	1.291(1.122-1.484)	0.000	0.090	1.094(0.97-1.224)	0.120
Menopause				0.213	1.237(0.281-5.441)	0.778
TG	0.176	1.193(0.959-1.483)	0.113	0.981	2.666(1.404-5.064)	0.003
LDL	0.493	1.637(1.046-2.561)	0.031	-0.121	0.886(0.596-1.318)	0.550
HOMA-IR(CP)	0.360	1.134(1.062-1.936)	0.019	0.215	1.240(0.859-1.788)	0.250
HbA1C	0.057	1.059(0.872-1.287)	0.564	0.047	1.048(0.840-1.308)	0.676
PRL	-0.115	0.891(0.803-0.989)	0.031	-0.135	0.874(0.797-0.957)	0.004

229 Note: The risk factors for NAFLD were assessed using multiple logistic regression analysis in men and women. The ORs with corresponding 95% CIs were
 230 adjusted for age, BMI, menopause, TG, LDL and HOMA-IR (CP), HbA1C and PRL levels as variables. NAFLD, nonalcoholic fatty liver disease; BMI, body mass
 231 index; FBG, fasting blood glucose; TG, triglyceride; LDL, low-density lipoprotein; HOMA-IR (CP), homeostasis model assessment for insulin resistance
 232 (C-peptide); HbA1c, glycosylated haemoglobin; PRL, prolactin.; PRL, prolactin. OR: Odds ratio; CI: Confidence interval

233 4. Relationship between PRL levels and the prevalence of NAFLD and MetS

234 According to the quartiles of PRL levels, the subjects were divided into four
 235 groups: T1 < 8.29 (n = 57 cases), 8.29 ≤ T2 < 9.93 (n = 58 cases), 9.93 ≤ T3 < 12.68 (n = 57
 236 cases), T4 ≥ 12.68 (n = 58 cases) ng/mL in men (n = 230 cases) and T1 < 8.95 (n = 44
 237 cases), 8.95 ≤ T2 < 11.32 (n = 44 cases), 11.32 ≤ T3 < 14.95 (n = 44 cases), T4 ≥ 14.95 (n =
 238 44 cases) ng/mL in women (n = 176 cases). The chi-square test was used to compare
 239 the prevalence and composition ratio among different groups. The prevalence of
 240 NAFLD exhibited a decreasing trend with the rise of the quartile of PRL in both sexes
 241 (T1: 84.2%, T2: 63.8%, T3: 59.6%, T4: 58.6%, $p=0.012$ in men; T1: 79.5%, T2:
 242 65.9%, T3: 54.5%, T4: 50%, $p=0.013$ in women). However, the prevalence rates of
 243 MetS were T1: 86%, T2: 79.3%, T3: 77.2%, T4: 72.4% ($p=0.354$) in men and T1:

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84.1%, T2: 70.5%, T3: 77.3%, T4: 56.8% (p= 0.031) in women. Therefore, in female subjects, the prevalence rate of Met S in the fourth quartile of PRL was significantly lower than those in the first, second and third quartiles

Discussion

At present, due to the rapid increase in the incidence of obesity and obesity-related diseases, NAFLD has become an important public health problem[17]. NAFLD is considered the manifestation of MetS in the liver, especially in patients with T2DM[18]. In this study, it was found that the incidence of NAFLD diagnosed by abdominal liver colour Doppler ultrasound was 64.8%. Compared with non-NAFLD patients, NAFLD patients had higher BMI, TG, GGT, HOMA-IR (CP), HbA1C, and MetS incidence and lower HDL in both sexes. Zhang et al[19] obtained similar results. BMI, TG and HDL are components of MetS. Therefore, T2DM complicated with NAFLD promotes abnormalities in metabolic indexes.

PRL is a hormone closely related to metabolism[20]. Recent findings have shown that there is a close association between PRL and T2DM. A cross-sectional study included 2377 adults from the community population (excluding those with hyperprolactinemia) and found that individuals with impaired glucose regulation and T2DM had lower PRL levels. Researchers controlled for age, sex, BMI and other confounding factors and still discovered that the risk in the abovementioned people with high serum PRL was significantly reduced [11]. Further follow-up of 3.7 years revealed that female patients had a lower risk of T2DM in the highest quartile of PRL, with a risk ratio of 0.48[9]. Another cross-sectional study also found that the risk of MetS and T2DM in women with lower baseline PRL were increased[21]. A large meta-analysis indicated that higher serum PRL levels in the normal range were related to a low risk of T2DM[22]. Jha et al[23] also found that serum PRL had a significant correlation with liver disease and predicted mortality. In adipose tissue, PRL intervention can reduce the production of malonyl coenzyme A in human primary adipocytes, thus inhibiting triglyceride synthesis[24]. The PRL receptor can also directly inhibit the expression of fatty acid synthetase and fatty acid synthesis in 3T3L1 cells[25]. PRL reduced the accumulation of triglycerides in the liver through

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274 the PRL receptor, thus improving liver steatosis [10]. These results indicate that
275 higher PRL levels had a positive protective effect on glucose and lipid metabolism.

276 Considering that PRL secretion may differ according to sex, we studied male and
277 female subjects separately. We found that compared with non-NAFLD patients, the
278 PRL value of NAFLD patients was lower in both sexes. Age, BMI, TG, LDL,
279 HOMA-IR (CP), and HbA1C were adjusted; additionally, among female subjects,
280 menopausal factors were adjusted, and the study suggested that PRL levels had a
281 negative relationship with the risk of NAFLD. In line with the quartile of PRL, the
282 incidence of NAFLD showed a general decrease with the increase in PRL levels in
283 both sexes. Zhang et al[26] noted that when PRL increased by one standard deviation
284 the risk among male NAFLD patients decreased by 12.3%, and that among female
285 patients decreased by 21.4%. PRL was proven to be a protective factor, that affected
286 the existence and progression of NAFLD. In another study, Zhang et al[19] also found
287 that the PRL levels of NAFLD patients diagnosed by ultrasound were significantly
288 lower than those of non-NAFLD patients, whether male or female. In addition, with
289 the increase in PRL quartile, the incidence of NAFLD decreased. All analyses were
290 corrected for age, sex, BMI, insulin resistance, HbA1C, diabetes and other factors.
291 The results showed that PRL an inverse association with NAFLD. We took into
292 consideration that PRL levels are affected by many conditions including, various
293 drugs, stress, and exercise. We ruled out the following cases: the use of drugs that
294 affect PRL (metoclopramide, methyl-dopa, opiates, and cimetidine) and levels of
295 thyroid-stimulating hormone, cortisol, oestradiol and testosterone that were higher
296 than the normal range. In terms of medication history, there was no significant
297 difference between the two groups of male and female patients in regard to
298 hypoglycaemic programs, which could exclude the influence of hypoglycaemic drugs
299 on the study.

300 In addition, the secretion of PRL may be affected by menopausal status. This paper
301 analysed menopausal and nonmenopausal women and found that postmenopausal
302 women with NAFLD had lower PRL levels. In addition, Zhang Zhuzi et al[27]
303 divided the included women into a premenopausal group and a postmenopausal group

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304 and found that in both groups, the PRL of patients with NAFLD was lower than that
305 of patients without NAFLD, and the decrease in PRL levels in postmenopausal
306 women with NAFLD was more significant. It was suggested that the decrease in the
307 PRL of patients with NAFLD was affected by menopausal factors.

308 Studies have shown a correlation between PRL levels and the components of
309 Met S, which could explain the role of PRL in NAFLD. According to basic studies, in
310 an obese mouse model induced by a high-fat diet, severe metabolic changes would
311 occur in mice with PRL receptor failure. Injection of PRL could improve insulin
312 sensitivity and prevent visceral adipocyte hypertrophy [28]. Clinical studies have
313 found that low serum PRL levels in the physiological range are related to poor
314 metabolic outcomes of MetS and T2DM[11]. In overweight and obese men, serum
315 PRL levels were lower [28]. Friedrich et al[29] found that PRL levels were negatively
316 correlated with waist circumference in 1857 healthy women aged 20-79 years. The
317 endocrine characteristics of MetS and polycystic ovary syndrome (PCOS) have a
318 relatively high similarity rate [30]. A systematic retrospective analysis of 2052 PCOS
319 patients revealed that the lower the serum PRL was, the higher the BMI. PRL had the
320 opposite relationship with TG, TC and LDL-C [31]. Arterial hypertension is a
321 component of MetS. A prospective study of 874 postmenopausal women found that
322 PRL levels increased by 1 standard deviation during 8 years of follow-up, and the
323 relative risk of hypertension was 1.31[32]. Our study found that in male subjects, the
324 levels of PRL were negatively correlated with hip circumference, TG and HOMA-IR
325 (CP) and positively associated with HDL. In female subjects, PRL levels were
326 negatively correlated with BMI, DBP, waist circumference, hip circumference, and
327 TG. In female subjects, the prevalence rates of MetS in the fourth quartile of PRL
328 were significantly lower than those in the first, second and third quartiles.
329 Furthermore, premenopausal and postmenopausal women with NAFLD had higher
330 BMI, TG and MetS incidence. NAFLD is very common in obese and dyslipidaemic
331 patients. Obese individuals produce relatively excessive proinflammatory factors,
332 some of which inhibit the treatment of liver fat and promote the accumulation of
333 lipids in hepatocytes [33]. Dyslipidaemia, especially hypertriglyceridaemia, may

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334 subsequently increase the transportation of TG and other fats into hepatocytes,
335 resulting in hepatic steatosis [34].

336 As a retrospective analysis, this study has many limitations. First, the diagnosis
337 of NAFLD was based on ultrasound examination, which cannot distinguish NASH
338 from fibrosis. Second, because this was a cross-sectional study, we cannot infer the
339 direct cause and effect relationship between PRL and NAFLD and need further
340 mechanical studies to clarify the exact relationship. Third, PRL secretion appears in
341 pulse form, the best time to draw blood for PRL is from 9:00 to 11:00 a.m., and
342 patients should avoid emotional excitement around this time. Finally, due to the
343 limited number of participants in this study, the effects of drugs for treating
344 cardiovascular diseases and controlling blood lipids on PRL levels have not been
345 investigated, which requires further layered analysis in future work. Moreover, the
346 small sample size cannot replace a large-scale population-based cross-sectional
347 epidemiological study, so it is necessary to increase the sample size.

348 **Conclusions**

349 In summary, our research shows that serum PRL levels are related to NAFLD in
350 the T2DM population in the physiological range and are also connected to known
351 metabolic indicators. Our research results may help to predict the risk of developing
352 NAFLD to better understand the disease and to formulate effective prevention
353 strategies.

354 **Abbreviations**

355 NAFLD: nonalcoholic fatty liver disease; T2DM: type 2 diabetes mellitus; PRL: prolactin;
356 Met S: metabolic syndrome; NASH: nonalcoholic steatohepatitis; SBP: systolic pressure;
357 DBP: diastolic pressure; BMI: body mass index; AST: aspartic acid aminotransferase; ALT:
358 alanine aminotransferase; GGT: glutamyltransferase; FBG: fasting blood glucose; TG:
359 triglyceride; TC: total cholesterol; HDL: high-density lipoprotein; LDL: low-density lipoprotein;
360 HOMA-IR (CP): Modified homeostasis model assessment for insulin resistance (C-peptide);
361 HOMA- β (CP-DM): homeostasis model assessment for beta (C-peptide-diabetes mellitus);
362 HbA1c: glycosylated haemoglobin; SD: standard deviation; OR: odds ratio; CI: confidence
363 interval

364 **Footnotes**

365 **Contributorship statement**

366 YZ conceived the study, collected clinical data, analysed and interpreted the data

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3
4 367 and wrote the manuscript. HL made a revised version. All authors read and agreed to
5
6 368 the final version of the manuscript.

7 369 **Competing interests**

8
9 370 The authors declare that they have no competing interests.

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19
20 374 study; the collection, analysis, and interpretation of data; or in writing the manuscript.

21 375 **Data availability statement**

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23
24 376 The data that support this study are available from the corresponding author upon
25
26 377 reasonable request.

27 378 **Ethics approval and consent to participate**

28
29 379 This study was a retrospective study, so it was exempted from the requirement of
30
31 380 informed consent and the approval of the Ethics Committee of The First Affiliated
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33 381 Hospital of Anhui University of Traditional Chinese Medicine (2020MCZQ09).

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STROBE Statement—Checklist of items that should be included in reports of *cross-sectional studies*

Section/Topic	Item	Recommendation	Reported on page
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract	1, 2
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3
Objectives	3	State specific objectives, including any prespecified hypotheses	3
Methods			
Study design	4	Present key elements of study design early in the paper	3–4
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4
Participants	6	(a) Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	4–6
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	4–5
Bias	9	Describe any efforts to address potential sources of bias	n/a
Study size	10	Explain how the study size was arrived at	3–4
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	6
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	6
		(b) Describe any methods used to examine subgroups and interactions	6
		(c) Explain how missing data were addressed	n/a
		(d) If applicable, describe analytical methods taking account of sampling strategy	n/a
		(e) Describe any sensitivity analyses	n/a
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	n/a
		(b) Give reasons for non-participation at each stage	n/a
		(c) Consider use of a flow diagram	n/a

Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	n/a
		(b) Indicate number of participants with missing data for each variable of interest	n/a
Outcome data	15*	Report numbers of outcome events or summary measures	6–11
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	6–11
		(b) Report category boundaries when continuous variables were categorized	n/a
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	n/a
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	6–11
Discussion			
Key results	18	Summarise key results with reference to study objectives	11–13
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	14
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	13–14
Generalisability	21	Discuss the generalisability (external validity) of the study results	14
Other information			
Funding	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	15

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at www.strobe-statement.org.

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4 1 **Cross-sectional association between prolactin levels and nonalcoholic fatty liver**
5 2 **disease in patients with type 2 diabetes mellitus: a retrospective analysis of**
6 3 **patients from a single hospital in China**
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11 5 Yuanyuan Zhang^a, Huaizhen Liu^{a*}

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23 12
24 13 **Abstract**

25 14 **Objective:** This study aimed to retrospectively assess the association between
26 15 prolactin (PRL) and nonalcoholic fatty liver disease (NAFLD) in patients with type 2
27 16 diabetes mellitus (T2DM).
28
29

30 17 **Design and setting:** A retrospective, cross-sectional study was conducted at a single
31 18 hospital in Anhui, China.

32 19 **Participants:** A total of 406 patients with T2DM (230 men and 176 women) were
33 20 included.
34

35 21 **Outcome measures:** P values for the independent T test, the Mann-Whitney rank
36 22 sum test, the Spearman correlation analysis, and multiple logistic regression models
37 23 were used to explore the association between PRL and NAFLD in patients with
38 24 T2DM.
39

40 25 **Results:** The results indicated that in both men and women, the levels of PRL were
41 26 significantly lower in the T2DM with NAFLD group than in the T2DM without
42 27 NAFLD group (men: 9.56 ng/mL vs. 10.36 ng/mL, women: 10.38 ng/mL vs. 12.97
43 28 ng/mL). In male patients, the levels of PRL were negatively correlated with hip
44 29 circumference ($r=-0.141$, $p=0.032$), homeostasis model assessment for insulin
45 30 resistance (C-peptide) ($r=-0.141$, $p=0.032$) and triglyceride (TG) ($r=-0.252$, $p=0.000$)
46 31 values and inversely correlated with high-density lipoprotein (HDL) ($r=0.147$,
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4 32 p=0.025) levels. In female patients, PRL levels were negatively related to body mass
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6 33 index ($r=-0.192$, $p=0.011$), diastolic blood pressure ($r=-0.220$, $p=0.003$), waist
7
8 34 circumference ($r=-0.152$, $p=0.044$), hip circumference ($r=-0.157$, $p=0.037$) and TG
9
10 35 ($r=-0.258$, $p=0.001$) values. Logistic regression analysis revealed a negative
11
12 36 relationship between PRL and NAFLD (men: OR 0.891, 95% CI 0.803-0.989,
13
14 37 $p=0.031$; women: OR 0.874, 0.797-0.957, $p=0.004$). As PRL levels increased,
15
16 38 NAFLD prevalence decreased in both sexes (men: $p=0.012$, women: $p=0.013$).

17
18 39 **Conclusion:** Our results suggest that low levels of PRL in the physiological range
19
20 40 were markers of NAFLD in patients with T2DM and that PRL within the biologically
21
22 41 high range may play a protective role in the pathogenesis of NAFLD.

23
24
25 42
26 43 **Keywords:** Type 2 diabetes mellitus; Nonalcoholic fatty liver disease; Prolactin.

27 28 29 44 30 45 **Strengths and limitations of this study**

31 46 ▶ Abdominal colour ultrasonography, as used in the study, is a common and simple
32
33 47 method for the clinical diagnosis of nonalcoholic fatty liver disease (NAFLD).

34
35 48 ▶ The normal range of serum prolactin (PRL) levels differs by sex, so we conducted a
36
37 49 sex-stratified analysis of patients with type 2 diabetes mellitus (T2DM).

38
39 50 ▶ P values for independent T tests and multiple logistic regression models were used
40
41 51 to assess the association between PRL and NAFLD in patients with T2DM.

42
43 52 ▶ This was a cross-sectional study that cannot provide evidence of causal
44
45 53 relationships.

46 47 48 49 54 50 55 **Introduction**

51 56 The liver is an important organ for glycolipid metabolism in the body. When
52
53 57 triglyceride deposition in hepatocytes increases and exceeds 5%, and other factors
54
55 58 causing liver steatosis (such as alcohol consumption and viral hepatitis) are excluded,
56
57 59 NAFLD can be diagnosed[1]. In China, with the gradual improvement of living
58
59 60 standards, NAFLD has surpassed chronic viral hepatitis to become the primary cause
60
61 61 of chronic liver diseases[2]. Currently, the global incidence of NAFLD is 25.2%[3],

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4 62 while the prevalence of NAFLD diagnosed by ultrasound in patients with T2DM is
5
6 63 73.7%[4]. T2DM is an important factor associated with the progression of NAFLD to
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8 64 NASH and fibrosis[1].

9
10 65 NAFLD is closely related to central obesity, hypertension, hyperlipidaemia,
11
12 66 T2DM and metabolic syndrome (MetS)[5]. Among MetS-related diseases, only
13
14 67 NAFLD is considered a strong predictor of MetS, and the incidence of MetS in fatty
15
16 68 liver patients is more than 4 times that in nonfatty liver patients[6]. Therefore,
17
18 69 NAFLD is considered the expression of MetS in the liver.

19
20 70 Prolactin (PRL) is a type of hormone that is mainly secreted by the
21
22 71 adenohypophysis. Its main physiological function is to stimulate breast development
23
24 72 and milk secretion[7]. Its receptors are widely distributed in various tissues and
25
26 73 organs of the body, including in fat, the liver, and the pancreas [8]. PRL can increase
27
28 74 the proliferation of β cells, stimulate insulin secretion and participate in the regulation
29
30 75 of glucose metabolism [9]. PRL can also inhibit lipolysis and activate adipocyte
31
32 76 differentiation by activating peroxisome proliferator-activated receptor γ [10]. Studies
33
34 77 in China and abroad have found that a decrease in serum PRL at the physiological
35
36 78 level is closely related to the occurrence of T2DM. Wang et al.[11] discovered that
37
38 79 the PRL levels of patients with T2DM and impaired glucose regulation were
39
40 80 significantly lower than those of people with normal glucose metabolism. The
41
42 81 researchers further pointed out that a decrease in physiological levels of PRL was
43
44 82 related to an increased risk of T2DM[9]. Manshaei et al.[12] also found that the serum
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46 83 PRL concentration of patients with T2DM was lower than that of healthy people.
47
48 84 Because of the high incidence of NAFLD in patients with T2DM, T2DM is also an
49
50 85 important factor in MetS. The relationship among PRL, NAFLD and MetS at the
51
52 86 physiological level has not been explored. The goal of this research was to explore the
53
54 87 relationship among PRL, NAFLD and MetS in patients with T2DM.

54 88

56 89 **Methods**

58 90 **Participants**

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60 91 All participants in this study were recruited from a hospital located in Anhui, China.

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4 92 This was a cross-sectional survey. A total of 656 patients with T2DM were
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6 93 investigated in this study, but 15 participants were excluded due to the use of
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8 94 medications that affect PRL levels (metoclopramide, methyldopa, opiates, and
9
10 95 cimetidine). Thirty participants were excluded because their levels of
11
12 96 thyroid-stimulating hormone, cortisol, oestradiol and testosterone were higher than
13
14 97 the normal range. Four participants had pituitary diseases, five had hyperglycaemia,
15
16 98 56 exhibited excessive alcohol consumption (intake of alcohol exceeding 140 g/week
17
18 99 for men and 70 g/week for women), 11 had cancer, 5 were pregnant, 7 had type 1
19
20 100 diabetes, 25 had acute complications of diabetes, 15 had acute cardiovascular events,
21
22 101 30 had severe hepatic and renal insufficiency, 8 had viral liver disease, 30 had
23
24 102 alcoholic liver disease, 5 had drug-induced liver disease and 4 had autoimmune liver
25
26 103 disease. Ultimately, 406 participants (230 men and 176 women) were included in this
27
28 104 study. This study was a retrospective study, so it was exempted from the requirement
29
30 105 of informed consent and was approved by the Ethics Committee of The First
31
32 106 Affiliated Hospital of Anhui University of Traditional Chinese Medicine.

33 107 **Data collection**

34
35 108 We collected data on sex, age, menopausal history of women, height, weight, diabetes
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37 109 course, preadmission hypoglycaemic plan (including metformin, insulin and other
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39 110 hypoglycaemic drugs such as sulfonylureas, glinides, thiazolidinediones,
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41 111 α -glucosidase inhibitors, glucagon-like peptide-1 receptor agonists, dipeptidyl
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43 112 peptidase 4 inhibitors, and sodium-glucose cotransporter protein 2 inhibitors), history
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45 113 of alcohol consumption, occurrence of cancer, history of other liver diseases, waist
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47 114 circumference, hip circumference, and blood pressure. Venous blood samples were
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49 115 collected in the morning on the second day after admission, and all blood was
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51 116 extracted with a centrifuge. After the separation of serum, fasting blood glucose
52
53 117 (FBG), blood fat, liver and kidney function were measured using an automatic
54
55 118 biochemical analyser (7600-020; Hitachi). Fasting C-peptide (FCP) levels were
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57 119 examined using an enzyme-linked immunosorbent assay (A2000 Plus; Autolumo). An
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59 120 automated chemiluminescent immunoassay (Siemens Immulite 2000, UK) was used
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4 121 to measure PRL levels. The coefficients of intra-assay and interassay variation ranged
5 122 from 2.49-3.47% and 2.91-3.14%, respectively. PRL levels are affected by many
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7 123 conditions, including the use of various drugs, stress, and exercise, so we took blood
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9 124 samples at 9:00 am on the first day after the patients were admitted to the hospital and
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11 125 the next morning. We took 2 ml blood samples each time. The patients fasted and
12
13 126 rested in a sitting position for 30 minutes, and then the average value of two blood
14
15 127 pressure readings was taken. High-performance liquid chromatography was used to
16
17 128 check glycosylated haemoglobin (HbA1c) (Variant II; Bio-Rad).

19 129 **Definitions, counts and groups**

21 130 The diagnosis of T2DM was based on the diagnostic criteria proposed by the World
22
23 131 Health Organization (WHO) Diabetes Expert Committee in 1999. The physiological
24
25 132 level of PRL was based on the normal reference range of our hospital, which is
26
27 133 2.78-29.20 ng/mL for premenopausal women, 1.79-20.28 ng/mL for menopausal
28
29 134 women and 2.12-17.69 ng/mL for men.

31 135 NAFLD was diagnosed by ultrasound[13] by a senior technician. The ultrasonic
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33 136 diagnosis of fatty liver is as follows: the near-field of the liver permeates a punctiform
34
35 137 hyperecho, the composition of the intrahepatic duct is not clearly demonstrated by
36
37 138 ultrasonography, and a weak echo is present in the distal echo. The diagnosis of
38
39 139 NAFLD is based on the following requirements: no history of alcohol consumption,
40
41 140 no other types of liver diseases, and an unexplained increase in serum alanine
42
43 141 aminotransferase (ALT), aspartic acid aminotransferase (AST) or glutamyltransferase
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45 142 (GGT) levels over 6 months[14].

46
47 143 The diagnosis of MetS conformed to the standard put forward in the ninth edition
48
49 144 of internal medicine in China [15], and the diagnostic standard included three or more
50
51 145 of the following items: ① central obesity and/or abdominal obesity: a waist
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53 146 circumference greater than 90 cm for men and 85 cm for women; ② hyperglycaemia:
54
55 147 an FBG level > 6.1 mmol/L or a 2-hour blood glucose level >7.8 mmol/L and/or the
56
57 148 confirmation of a diabetes diagnosis and treatment with hypoglycaemic therapy; ③
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59 149 hypertension: a blood pressure exceeding 130/85 mmHg and/or a diagnosis of
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150 hypertension and treatment with antihypertensive therapy; ④ a fasting triglyceride

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4 151 (TG) level exceeding 1.7 mmol/l; and ⑤ a fasting high-density lipoprotein (HDL)
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6 152 level below 1.04 mmol/l. Body mass index (BMI) was computed by dividing the body
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8 153 weight (kg) by the square of the height (m²). The homeostasis model assessment of
9
10 154 insulin resistance (C-peptide) (HOMA-IR (CP)) value was determined by the FCP
11
12 155 level as a substitute for the fasting insulin level as follows: HOMA-IR (CP)
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14 156 =1.5+FBG (mmol/L)xFCP (pmol/L)/2800. HOMA-β (CP-DM) =0.27x FCP (pmol/L)
15
16 157 (FBG (mmol/L) -3.5)[16].

17
18 158 In conformity with ultrasonic diagnosis, patients with T2DM who met the
19
20 159 inclusion criteria were divided into the without NAFLD group (77 men, 66 women)
21
22 160 and the with NAFLD group (153 men, 110 women).

23 161 **Statistical analysis**

24
25 162 SPSS 21.0 statistical software was used for the data analysis, and the Kolmogorov–
26
27 163 Smirnov normality test was performed for all data. The measured data with a normal
28
29 164 distribution are represented as the mean and standard deviation (SD). Comparisons
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31 165 were conducted between two groups, and comparisons were performed using
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33 166 independent T tests. Measurement data with nonnormal distributions are expressed as
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35 167 medians (interquartile intervals). In this situation, two groups were compared by using
36
37 168 the Mann–Whitney rank sum test. Categorical variables are shown as the number of
38
39 169 cases, and the chi-square test was adopted to demonstrate the differences within two
40
41 170 or more groups. Spearman correlation analysis compared the relationship between
42
43 171 PRL levels and the other variables. The relationships among PRL, NAFLD and MetS
44
45 172 were analysed by logistic regression. P<0.05 or P<0.01 represented obvious
46
47 173 significant differences.

48 174 **Patient and public involvement**

49
50 175 Neither the patients nor the public were not involved in the design, conduction,
51
52 176 reporting, or dissemination plans of our research.

53 177 54 55 178 **Results**

56 179 **Comparison of general findings and laboratory test targets in each group**

57
58
59 180 The ultrasonic diagnostic rate of NAFLD was 263 patients (153 plus 110 patients)
60

181 (64.8%) (Table 1). Men with NAFLD were younger, had higher BMI, waist
 182 circumference, hip circumference, diastolic blood pressure (DBP), GGT, FBG, TG,
 183 total cholesterol (TC), low-density lipoprotein (LDL), HOMA-IR (CP), and HbA1C
 184 values, and had a higher MetS incidence. Women with NAFLD also had higher BMI,
 185 ALT, GGT, TG, HOMA-IR (CP), and HbA1C values and a higher MetS incidence.
 186 HDL and PRL levels were markedly reduced in the patients with NAFLD compared
 187 with those without NAFLD in both sexes ($p < 0.05$ or $p < 0.01$). In terms of medication
 188 history, there was no significant difference between the two groups of male and
 189 female patients in hypoglycaemic programmes, which could exclude the influence of
 190 hypoglycaemic drugs on the study.

191
 192 **Table 1. Comparison of the general characteristics and biochemical indices of**
 193 **each group**

	Men			Women		
	T2DM without NAFLD	T2DM with NAFLD	P value	T2DM without NAFLD	T2DM with NAFLD	P value
N	77	153		66	110	
Age (years)	63(54-63)	54(48-62)	0.000	65(57-71)	61(55-69)	0.077
Metabolic syndrome (%)	64.9	85.6	0.000	59.100	80.000	0.003
Menopause (%)	NA	NA		99.100	83.600	0.117
Diabetes course(years)	10(3-15)	8(3-12)	0.280	10(5-20)	10(4-15)	0.070
BMI (kg/m ²)	24.90±2.97	27.18±2.94	0.000	24.54±3.35	26.33±3.55	0.000
Systolic pressure (mmHg)	130(125-146)	132(121-145)	0.880	130(124-151)	130(123-144)	0.233
Diastolic pressure (mmHg)	81.48±9.59	85.80±9.94	0.002	80.48±8.59	79.07±8.16	0.277
Waist circumference (cm)	90.71±8.02	96.29±8.45	0.000	89.02±9.07	91.38±9.41	0.103
Hip circumference (cm)	96.64±6.77	100.66±6.18	0.000	97.00±6.52	97.73±7.81	0.526
ALT (U/L)	19(13-28)	21(15-32)	0.082	15(12-21)	19(14-33)	0.000
AST (U/L)	18(15-23)	19(15-23)	0.881	17(15-20)	18(15-25)	0.094
GGT (U/L)	24(17-36)	35(23-56)	0.000	19(14-28)	25(19-35)	0.000
FBG (mmol/L)	6.81(5.41-9.49)	7.80(6.21-11.0)	0.002	6.61(5.48-9.34)	7.89(6.05-10.96)	0.050
TG (mmol/L)	1.20(0.79-1.75)	2.01(1.42-3.27)	0.000	1.23(0.93-1.50)	1.81(1.19-2.35)	0.000
TC (mmol/L)	4.32±0.92	4.83±1.10	0.001	4.77±1.24	5.02±1.11	0.158
HDL (mmol/L)	1.04(0.96-1.18)	0.97(0.82-1.11)	0.004	1.23(1.05-1.47)	1.10(0.99-1.28)	0.002
LDL (mmol/L)	2.45±0.78	2.78±0.85	0.004	2.84±1.05	2.98±0.88	0.373
HOMA-IR (CP)	2.90(2.46-3.97)	3.99(3.18-5.20)	0.000	2.97(2.54-3.68)	3.68(2.91-4.41)	0.001
HOMA-β (CP-DM)	46.94(25.29-88.92)	44.33(27.17-83.92)	0.686	38.55(22.52-80.19)	48.27(25.02-73.90)	0.553
HbA1C (%)	7.5(6.7-9.1)	8.3(7.0-9.7)	0.043	7.7(6.7-9.3)	8.5(7.4-9.9)	0.020

PRL (ng/mL)	10.36(9.35-14.72)	9.56(7.81-12.60)	0.001	12.97(10.03-16.58)	10.38(8.43-14.27)	0.001
Hypoglycaemic plan						
Metformin	26(33.8%)	62(40.5%)		17(25.8%)	43(39.1%)	
Other hypoglycaemic drugs	16(20.8%)	44(28.8%)	0.083	23(34.8%)	27(24.5%)	0.150
Insulin	35(45.4%)	47(30.7%)		26(39.4%)	40(36.4%)	

194 Note: NAFLD, nonalcoholic fatty liver disease; T2DM, type 2 diabetes mellitus; BMI, body mass index; ALT, alanine aminotransferase; AST, aspartic acid
 195 aminotransferase; GGT, glutamyltransferase; FBG, fasting blood glucose; TG, triglyceride; TC, total cholesterol; HDL, high-density lipoprotein; LDL,
 196 low-density lipoprotein; HOMA-IR (CP), homeostasis model assessment for insulin resistance (C-peptide); HOMA-β (CP-DM): homeostasis model assessment
 197 for beta (C-peptide-diabetes mellitus); HbA1c, glycosylated haemoglobin; PRL, prolactin. The measured data with a normal distribution are represented as the
 198 mean±SD. Measurement data for nonnormal distributions are expressed as medians (interquartile intervals). Normally distributed variables: BMI, diastolic
 199 blood pressure, waist circumference, hip circumference, TC, LDL; Nonnormally distributed variables: Age, diabetes course, systolic blood pressure, ALT, AST,
 200 GGT, FBG, TG, HDL, HOMA-IR (CP), HOMA-β (CP-DM), HbA1C, and PRL

201

202 Because women's serum PRL levels are affected by menopause, we analysed the
 203 metabolic status and PRL levels of female patients with or without NAFLD before
 204 and after menopause (Table 2). Premenopausal women with NAFLD had higher BMI,
 205 FBG, TG, and HbA1C values and a higher MetS incidence. Postmenopausal women
 206 with NAFLD had higher BMI, ALT, GGT, TG, and HOMA-IR (CP) values and a
 207 higher MetS incidence, while HDL and PRL values were markedly reduced in the
 208 patients with NAFLD compared with those in patients without NAFLD (p<0.05 or
 209 p<0.01).

210

211 **Table 2. Comparison of the clinical data of women with and without NAFLD**
 212 **before and after menopause**

	Premenopause			Postmenopause		
	T2DM without NAFLD	T2DM with NAFLD	P value	T2DM without NAFLD	T2DM with NAFLD	P value
N	6	18		60	92	
Age (years)	44.80±3.76	45.20±4.37	0.848	66.15±8.34	64.61±8.16	0.261
Metabolic syndrome (%)	0	77.8	0.001	65	80.4	0.033
Diabetes course (years)	8.180±6.69	4.78±4.12	0.149	12.86±9.02	10.69±6.88	0.116
BMI (kg/m ²)	22.80±3.87	26.70±3.43	0.029	24.71±3.28	26.26±3.59	0.008
Systolic pressure (mmHg)	121.83±7.08	128.50±8.78	0.107	133(127-152)	131(122-145)	0.167
Diastolic pressure (mmHg)	77.50±7.18	84.06±6.78	0.055	80.78±8.71	78.10±8.08	0.054

3	Waist circumference (cm)	80.33±12.36	89.06±7.92	0.055	89.88±8.32	91.84±9.65	0.200
4	Hip circumference (cm)	94.67±6.83	95.61±7.65	0.791	97.23±6.50	98.14±7.81	0.456
5	ALT (U/L)	13(11-16)	15(13-45)	0.121	15(12-22)	20(15-33)	0.000
6	AST (U/L)	16(15-18)	16(13-35)	1.000	17(15-20)	19(16-25)	0.073
7	GGT (U/L)	20.67±14.28	37.67±31.34	0.217	19(14-29)	26(18-35)	0.002
8	FBG (mmol/L)	6.61±1.59	10.99±3.10	0.003	7.81±2.97	8.08±2.78	0.566
9	TG (mmol/L)	1.09(0.63-1.30)	2.02(1.37-2.83)	0.003	1.24(0.93-1.56)	1.74(1.17-2.34)	0.000
10	TC (mmol/L)	4.77±0.84	5.16±1.46	0.534	4.77±1.28	4.99±1.04	0.229
11	HDL (mmol/L)	1.23±0.17	1.06±0.21	0.086	1.27±0.29	1.14±0.27	0.005
12	LDL (mmol/L)	2.92±0.83	2.84±0.86	0.838	2.84±1.08	3.00±0.89	0.301
13	HOMA-IR (CP)	2.30±0.57	4.87±2.98	0.051	3.28±1.00	3.73±1.43	0.036
14	HOMA-β (CP-DM)	25.07(19.86-28.67)	25.99(13.78-56.47)	0.689	47.00(22.63-85.05)	51.60(28.83-75.27)	0.505
15	HbA1C (%)	7.62±0.89	9.53±1.66	0.014	8.16±1.82	8.53±1.67	0.196
16	PRL (ng/mL)	18.92±8.57	14.54±4.64	0.122	13.16±3.79	10.88±3.77	0.000

213 Note: NAFLD, nonalcoholic fatty liver disease; T2DM, type 2 diabetes mellitus; BMI, body mass index; ALT, alanine aminotransferase; AST, aspartic acid
 214 aminotransferase; GGT, glutamyltransferase; FBG, fasting blood glucose; TG, triglyceride; TC, total cholesterol; HDL, high-density lipoprotein; LDL,
 215 low-density lipoprotein; HOMA-IR (CP), homeostasis model assessment for insulin resistance (C-peptide); HOMA-β (CP-DM): homeostasis model assessment
 216 for beta (C-peptide-diabetes mellitus); HbA1c, glycosylated haemoglobin; PRL, prolactin. The measurement data with a normal distribution are represented as
 217 the mean±SD. Measurement data with nonnormal distributions are expressed as medians (interquartile intervals).¹

219 Relationship between PRL levels and MetS-related parameters

220 We further investigated the relationship between PRL levels and MetS-related
 221 parameters (Table 3). We found that in male subjects, the levels of PRL were
 222 negatively correlated with hip circumference, TG and HOMA-IR (CP) values and
 223 positively associated with HDL levels. In female subjects, PRL levels were negatively
 224 correlated with BMI, DBP, and waist circumference values.

226 **Table 3. Relationship between PRL levels and MetS-related parameters**

	Men		Women	
	r	P value	r	P value
BMI	-0.092	0.166	-0.192	0.011
Systolic pressure	0.046	0.492	-0.045	0.552
Diastolic pressure	-0.125	0.059	-0.220	0.003
Waist circumference	-0.056	0.398	-0.152	0.044
Hip circumference	-0.141	0.032	-0.157	0.037
FBG	-0.109	0.098	-0.034	0.654

TG	-0.252	0.000	-0.258	0.001
TC	-0.096	0.146	-0.061	0.421
HDL	0.147	0.025	0.065	0.390
LDL	-0.042	0.528	-0.110	0.146
HOMA-IR(CP)	-0.141	0.032	-0.049	0.519
HOMA- β (CP-DM)	0.019	0.772	-0.044	0.562
HbA1C	-0.091	0.168	0.057	0.450

Note: Met S, metabolic syndrome; BMI, body mass index; FBG, fasting blood glucose; TG, triglyceride; TC, total cholesterol; HDL, high-density lipoprotein; LDL, low-density lipoprotein; HOMA-IR (CP), homeostasis model assessment for insulin resistance (C-peptide); HOMA- β (CP-DM): homeostasis model assessment for beta (C-peptide-diabetes mellitus); HbA1c, glycosylated haemoglobin; PRL, prolactin..

Multiple-factor logistic regression analysis of serum PRL levels and NAFLD risk

The impact index for NAFLD was assessed using multiple logistic regression analysis, which included age, BMI, menopause, TG, LDL, HOMA-IR (CP), HbA1C and PRL as variables. We found that PRL levels were independently negatively associated with NAFLD in both men and women (odds ratio (OR): 0.891, 95% confidence interval (CI): 0.803-0.989, $p=0.031$, for men; OR: 0.874, 95% (CI): 0.797-0.957, $p=0.004$, for women). Other risk factors included age, BMI, LDL and HOMA-IR (CP) for men and TG for women (Table 4).

Table 4 Multivariate logistic regression analysis of serum PRL levels and NAFLD risk

	Men			Women		
	β	OR(95% CI)	p value	β	OR(95% CI)	p value
Age	-0.045	0.956(0.924-0.989)	0.010	-0.044	0.957(0.912-1.004)	0.070
BMI	0.255	1.291(1.122-1.484)	0.000	0.090	1.094(0.97-1.224)	0.120
Menopause				0.213	1.237(0.281-5.441)	0.778
TG	0.176	1.193(0.959-1.483)	0.113	0.981	2.666(1.404-5.064)	0.003
LDL	0.493	1.637(1.046-2.561)	0.031	-0.121	0.886(0.596-1.318)	0.550
HOMA-IR(CP)	0.360	1.134(1.062-1.936)	0.019	0.215	1.240(0.859-1.788)	0.250
HbA1C	0.057	1.059(0.872-1.287)	0.564	0.047	1.048(0.840-1.308)	0.676
PRL	-0.115	0.891(0.803-0.989)	0.031	-0.135	0.874(0.797-0.957)	0.004

Note: The risk factors for NAFLD were assessed using multiple logistic regression analysis in men and women. The ORs with corresponding 95% CIs were adjusted for age, BMI, menopause, TG, LDL and HOMA-IR (CP), HbA1C and PRL levels as variables. NAFLD, nonalcoholic fatty liver disease; BMI, body mass index; FBG, fasting blood glucose; TG, triglyceride; LDL, low-density lipoprotein; HOMA-IR (CP), homeostasis model assessment for insulin resistance (C-peptide); HbA1c, glycosylated haemoglobin; PRL, prolactin.; PRL, prolactin. OR: Odds ratio; CI: Confidence interval

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247 **Relationship between PRL levels and the prevalence of NAFLD and MetS**

248 According to the quartiles of PRL levels, the subjects were divided into four groups:
249 T1<8.29 (n= 57 patients), 8.29≤T2<9.93 (n= 58 patients), 9.93≤T3<12.68 (n= 57
250 patients), and T4≥12.68 (n= 58 patients) ng/mL for men (n= 230 patients) and
251 T1<8.95 (n= 44 patients), 8.95≤T2<11.32 (n= 44 patients), 11.32≤T3<14.95 (n= 44
252 patients), and T4≥14.95 (n= 44 patients) ng/mL for women (n= 176 patients). The
253 chi-square test was used to compare the prevalence and composition ratio among
254 different groups. The prevalence of NAFLD exhibited a decreasing trend with the rise
255 in the PRL quartile in both sexes (T1: 84.2%, T2: 63.8%, T3: 59.6%, T4: 58.6%,
256 p=0.012 in men; T1: 79.5%, T2: 65.9%, T3: 54.5%, T4: 50%, p= 0.013 in women).
257 However, the prevalence rates of MetS were T1: 86%, T2: 79.3%, T3: 77.2%, and T4:
258 72.4% (p= 0.354) in men and T1: 84.1%, T2: 70.5%, T3: 77.3%, and T4: 56.8% (p=
259 0.031) in women. Therefore, in female subjects, the prevalence rate of MetS in the
260 fourth quartile of PRL levels was significantly lower than those in the first, second
261 and third quartiles.

262

263 **Discussion**

264 At present, due to the rapid increase in the incidence of obesity and obesity-
265 related diseases, NAFLD has become an important public health problem[17].
266 NAFLD is considered the manifestation of MetS in the liver, especially in patients
267 with T2DM[18]. In this study, it was found that the incidence of NAFLD diagnosed
268 by abdominal liver colour Doppler ultrasound was 64.8%. Compared with non
269 NAFLD patients, NAFLD patients had higher BMI, TG, GGT, HOMA-IR (CP), and
270 HbA1C values, a higher MetS incidence and lower HDL levels in both sexes. Zhang
271 et al.[19] obtained similar results. BMI, TG and HDL are components of MetS.
272 Therefore, T2DM complicated with NAFLD promotes abnormalities in metabolic
273 indices.

274 PRL is a hormone that is closely related to metabolism[20]. Recent findings have
275 shown that there is a close association between PRL levels and T2DM. A

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4 276 cross-sectional study included 2377 adults from the community population (excluding
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6 277 those with hyperprolactinemia) and found that individuals with impaired glucose
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8 278 regulation and T2DM had lower PRL levels. Researchers controlled for age, sex, BMI
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10 279 and other confounding factors and still discovered that the risk in the abovementioned
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12 280 people with high serum PRL levels was significantly reduced [11]. A further
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14 281 follow-up of 3.7 years revealed that female patients in the highest quartile of PRL
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16 282 levels had a lower risk of T2DM, with a risk ratio of 0.48[9]. Another cross-sectional
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18 283 study also found that the risk of MetS and T2DM in women with lower baseline PRL
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20 284 levels was increased[21]. A large meta-analysis indicated that higher serum PRL
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22 285 levels in the normal range were related to a low risk of T2DM[22]. Jha et al.[23] also
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24 286 found that serum PRL levels had a significant correlation with liver disease and
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26 287 predicted mortality. In adipose tissue, PRL intervention can reduce the production of
27
28 288 malonyl coenzyme A in human primary adipocytes, thus inhibiting triglyceride
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30 289 synthesis[24]. The PRL receptor can also directly inhibit the expression of fatty acid
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32 290 synthetase and fatty acid synthesis in 3T3L1 cells[25]. PRL reduces the accumulation
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34 291 of triglycerides in the liver through the PRL receptor, thus improving liver steatosis
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36 292 [10]. These results indicate that higher PRL levels have a positive protective effect on
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38 293 glucose and lipid metabolism.

39 294 Considering that PRL secretion may differ according to sex, we studied male and
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41 295 female subjects separately. We found that compared with that of non-NAFLD
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43 296 patients, the PRL value of NAFLD patients was lower in both sexes. Age, BMI, TG,
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45 297 LDL, HOMA-IR (CP), and HbA1C were adjusted; additionally, menopausal factors
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47 298 were adjusted for among female subjects, and the study suggested that PRL levels had
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49 299 a negative relationship with the risk of NAFLD. In line with the quartile of PRL, the
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51 300 incidence of NAFLD showed a general decrease with the increase in PRL levels in
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53 301 both sexes. Zhang et al.[26] noted that when PRL increased by one standard
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55 302 deviation, the risk among male NAFLD patients decreased by 12.3% and that among
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57 303 female patients decreased by 21.4%. PRL was proven to be a protective factor that
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59 304 affected the existence and progression of NAFLD. In another study, Zhang et al.[19]
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305 also found that the PRL levels of NAFLD patients diagnosed by ultrasound were

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4 306 significantly lower than those of non-NAFLD patients, whether they were male or
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6 307 female. In addition, with the increase in PRL quartile, the incidence of NAFLD
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8 308 decreased. All analyses were corrected for age, sex, BMI, insulin resistance, HbA1C,
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10 309 diabetes and other factors. The results showed that PRL levels had an inverse
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12 310 association with NAFLD. We considered that PRL levels are affected by many
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14 311 conditions, including various drugs, stress, and exercise. We excluded the following
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16 312 patients: patients with the use of drugs that affect PRL levels (metoclopramide,
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18 313 methyl-dopa, opiates, and cimetidine) and those with levels of thyroid-stimulating
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20 314 hormone, cortisol, oestradiol and testosterone that were higher than the normal range.
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22 315 In terms of medication history, there was no significant difference between the two
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24 316 groups of male and female patients in regard to hypoglycaemic programmes, which
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26 317 could exclude the influence of hypoglycaemic drugs on the study.

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28 318 In addition, the secretion of PRL may be affected by menopausal status. This paper
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30 319 analysed menopausal and non-menopausal women and found that postmenopausal
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32 320 women with NAFLD had lower PRL levels. In addition, Zhang Zhuzi et al.[27]
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34 321 divided the included women into a premenopausal group and a postmenopausal group
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36 322 and found that in both groups, the PRL levels of patients with NAFLD were lower
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38 323 than those of patients without NAFLD, and the decrease in PRL levels in
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40 324 postmenopausal women with NAFLD was more significant. It was suggested that the
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42 325 decrease in the PRL levels of patients with NAFLD was affected by menopausal
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44 326 factors.

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46 327 Studies have shown a correlation between PRL levels and the components of
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48 328 MetS, which could explain the role of PRL in NAFLD. According to basic studies, in
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50 329 a mouse model with obesity induced by a high-fat diet, severe metabolic changes
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52 330 would occur in mice with PRL receptor failure. The injection of PRL could improve
53
54 331 insulin sensitivity and prevent visceral adipocyte hypertrophy[28]. Clinical studies
55
56 332 have found that low serum PRL levels in the physiological range are related to poor
57
58 333 metabolic outcomes in MetS and T2DM patients[11]. In overweight and obese men,
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60 334 serum PRL levels were lower [28]. Friedrich et al.[29] found that PRL levels were
335 335 negatively correlated with waist circumference in 1857 healthy women aged 20-79

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4 336 years. The endocrine characteristics of MetS and polycystic ovary syndrome (PCOS)
5 337 have a relatively high similarity rate [30]. A systematic retrospective analysis of 2052
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7 338 PCOS patients revealed that the lower the serum PRL level was, the higher the BMI.
8
9 339 PRL levels had the opposite relationship with TG, TC and LDL-C levels [31]. Arterial
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11 340 hypertension is a component of MetS. A prospective study of 874 postmenopausal
12
13 341 women found that PRL levels increased by 1 standard deviation during 8 years of
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15 342 follow-up, and the relative risk of hypertension was 1.31[32]. Our study found that in
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17 343 male subjects, the levels of PRL were negatively correlated with hip circumference,
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19 344 TG and HOMA-IR (CP) values and positively associated with HDL levels. In female
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21 345 subjects, PRL levels were negatively correlated with BMI, DBP, waist circumference,
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23 346 hip circumference, and TG values. In female subjects, the prevalence rates of MetS in
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25 347 the fourth quartile of PRL levels were significantly lower than those in the first,
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27 348 second and third quartiles. Furthermore, premenopausal and postmenopausal women
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29 349 with NAFLD had higher BMI and TG levels and a higher MetS incidence. NAFLD is
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31 350 very common in obese and dyslipidaemic patients. Obese individuals produce
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33 351 relatively excessive proinflammatory factors, some of which inhibit the treatment of
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35 352 liver fat and promote the accumulation of lipids in hepatocytes [33]. Dyslipidaemia,
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37 353 especially hypertriglyceridaemia, may subsequently increase the transportation of
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39 354 TGs and other fats into hepatocytes, resulting in hepatic steatosis [34].

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41 355 As a retrospective analysis, this study has many limitations. First, the diagnosis
42
43 356 of NAFLD was based on ultrasound examination, which cannot distinguish NASH
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45 357 from fibrosis. Second, because this was a cross-sectional study, we cannot infer the
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47 358 direct cause and effect relationship between PRL levels and NAFLD and further
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49 359 mechanical studies are needed to clarify the exact relationship. Third, PRL secretion
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51 360 appears in pulse form, the best time to draw blood for PRL measurement is from 9:00
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53 361 to 11:00 a.m., and patients should avoid emotional excitement around this time.
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55 362 Finally, due to the limited number of participants in this study, the effects of drugs for
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57 363 treating cardiovascular diseases and controlling blood lipids on PRL levels have not
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59 364 been investigated, which requires further layered analysis in future work. Moreover,
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365 the small sample size cannot replace a large-scale population-based cross-sectional

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4 366 epidemiological study, so it is necessary for future studies to increase the sample size.

5
6 367 **Conclusions**

7 368 In summary, our research shows that serum PRL levels in the physiological range are
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9 369 related to NAFLD in the T2DM population and are also connected to known
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11 370 metabolic indicators. Our research results may help to predict the risk of developing
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13 371 NAFLD to better understand the disease and to formulate effective prevention
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15 372 strategies.
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19 374 **Abbreviations**

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21 375 NAFLD: nonalcoholic fatty liver disease; T2DM: type 2 diabetes mellitus; PRL: prolactin;
22 376 MetS: metabolic syndrome; NASH: nonalcoholic steatohepatitis; SBP: systolic blood pressure;
23 377 DBP: diastolic blood pressure; BMI: body mass index; AST: aspartic acid aminotransferase; ALT:
24 378 alanine aminotransferase; GGT: glutamyltransferase; FBG: fasting blood glucose; TG:
25 379 triglyceride; TC: total cholesterol; HDL: high-density lipoprotein; LDL: low-density lipoprotein;
26 380 HOMA-IR (CP): modified homeostasis model assessment for insulin resistance (C-peptide);
27 381 HOMA- β (CP-DM): homeostasis model assessment for beta (C-peptide-diabetes mellitus);
28 382 HbA1c: glycosylated haemoglobin; SD: standard deviation; OR: odds ratio; CI: confidence
29 383 interval.
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35 385 **Contributors**

36 386 YZ conceived the study, collected the clinical data, analysed and interpreted the data
37
38 387 and wrote the manuscript. HL revised the manuscript. All authors read and agreed to
39
40 388 the final version of the manuscript.
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45 390 **Competing interests**

46 391 The authors declare that they have no competing interests.
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49
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52
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56 395 the study; the collection, analysis, and interpretation of the data; or in writing the
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58 396 manuscript.
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397 **Data availability statement**

398 The data that support this study are available from the corresponding author upon
399 reasonable request.

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401 **Ethics approval and consent to participate**

402 This study was a retrospective study, so it was exempted from the requirement of
403 informed consent with the approval of the Ethics Committee of The First Affiliated
404 Hospital of Anhui University of Traditional Chinese Medicine (2020MCZQ09).

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STROBE Statement—Checklist of items that should be included in reports of *cross-sectional studies*

Section/Topic	Item	Recommendation	Reported on page
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract	1, 2
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3
Objectives	3	State specific objectives, including any prespecified hypotheses	3
Methods			
Study design	4	Present key elements of study design early in the paper	3–4
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4
Participants	6	(a) Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	4–6
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	4–5
Bias	9	Describe any efforts to address potential sources of bias	n/a
Study size	10	Explain how the study size was arrived at	3–4
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	6
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	6
		(b) Describe any methods used to examine subgroups and interactions	6
		(c) Explain how missing data were addressed	n/a
		(d) If applicable, describe analytical methods taking account of sampling strategy	n/a
		(e) Describe any sensitivity analyses	n/a
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	n/a
		(b) Give reasons for non-participation at each stage	n/a
		(c) Consider use of a flow diagram	n/a

Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	n/a
		(b) Indicate number of participants with missing data for each variable of interest	n/a
Outcome data	15*	Report numbers of outcome events or summary measures	6–11
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	6–11
		(b) Report category boundaries when continuous variables were categorized	n/a
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	n/a
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	6–11
Discussion			
Key results	18	Summarise key results with reference to study objectives	11–13
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	14
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	13–14
Generalisability	21	Discuss the generalisability (external validity) of the study results	14
Other information			
Funding	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	15

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at www.strobe-statement.org.