

Angiopietin-like protein 8/betatrophin correlates with hepatocellular lipid content independent of insulin resistance in non-alcoholic fatty liver disease patients

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Keywords

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ABSTRACT

Aims/Introduction: To explore angiotensin-like protein 8 (ANGPTL-8) levels, and its association with hepatocellular lipid content (HCL) and insulin resistance in patients with different extents of non-alcoholic fatty liver disease (NAFLD).

Materials and Methods: In 48 adults were recruited, of which 12 had no NAFLD (HCL < 5.5%; group 1), 18 had mild NAFLD (5.5% ≤ HCL < 10.0%; group 2) and 18 had moderate-to-severe NAFLD (HCL ≥ 10.0%; group 3). The peripheral insulin sensitivity of all participants was monitored by a hyperinsulinemic-euglycemic clamp (M value), as well as the magnetic resonance image of HCL. Serum ANGPTL-8, blood glucose levels and lipid profiles were also recorded in the study.

Results: Group 3 had a worse metabolic profile, and had the highest ANGPTL-8 level (1,129 ± 351 pg/mL vs 742 ± 252 pg/mL, 765 ± 301 pg/mL, $P = 0.001$) compared with those in group 1 and group 2. In all metabolic profiles, HCL positively correlated the strongest with ANGPTL-8 ($r = 0.436$, $P = 0.042$). Multivariate stepwise linear regression analysis showed ANGPTL-8 and alanine aminotransferase were independent determinants of HCL ($P = 0.002$, $P < 0.001$, respectively), and these two indexes explained 67.4% of the variation of HCL ($P < 0.001$).

Conclusions: ANGPTL-8 was positively correlated with hepatocellular lipid content independent of obesity and insulin resistance, indicating that ANGPTL-8 might be a new and important predictor of the severity of NAFLD.

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) has gained more and more attention in China as a result of a rising prevalence of approximately 15% in large cities¹. NAFLD, ranging from non-alcoholic simple steatosis to non-alcoholic steatohepatitis (NASH), is characterized by hepatic fat accumulation coexistent with inflammation, impaired liver function and subsequent advanced fibrosis that increases the mortality risk in NAFLD patients². In addition to hepatic consequences, NAFLD is also related to systemic metabolic complications, chronic kidney and cardiovascular disease, which contributes to liver-related, and cardiovascular, overall mortality^{3,4}. Despite the severe damage

caused by NAFLD, its underlying pathological mechanism (s) are not yet fully elucidated. The classic “two-hit” hypothesis is that hepatic triglyceride (TG) accumulation sensitizes the liver to the second insults, such as oxidative stress, inflammatory factors and cytokines, all of which will finally result in NASH⁵. However, now this hypothesis is being challenged by some recent emerging evidence^{6–11}. A recent study found that NAFLD is increasingly viewed as a metabolic disorder, as other organs, such as adipose tissue, muscle and the intestine, play important roles in the pathogenesis of NAFLD and its complications¹². Therefore, NAFLD is not merely considered as the hepatic manifestation of metabolic syndrome, but is assumed to precede the development of metabolic syndrome and type 2 diabetes, in which insulin resistance (IR) might play a major role¹³.

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Angiopoietin-like protein 8 (ANGPTL-8; also known as betatrophin, hepatocellular carcinoma-associated protein, re-feeding-induced in fat and liver protein, and lipasin)^{14–18} is mainly expressed in the liver in humans, and is highly enriched in the liver, brown adipose tissue and white adipose tissue in mice¹⁶. Mice lacking ANGPTL-8 consistently show lower TG levels owing to enhanced plasma TG clearance by having increased post-heparin lipoprotein lipase activity^{17,19}. Previous studies showed that ANGPTL-8 was increased in NAFLD patients and those with liver cirrhosis, and was also highly expressed in palmitate-treated HepG2, the liver of *db/db* mice and mice consuming a high-fat diet^{19,20}. The relationship between ANGPTL-8 and IR were also reported, most of which showed a positive correlation between them. von Loeffelholz *et al.*²¹ showed that weight loss reduced liver fat by 45% and circulating ANGPTL-8 by 11%. ANGPTL-8 was related to liver steatosis²¹. It is still in question whether increased ANGPTL-8 in NAFLD resulted from hepatic fat accumulation or was only secondary to decreased insulin sensitivity in NAFLD. Given the potential effect of IR, relevant research would be warranted to elucidate the relationship between ANGPTL-8 and NAFLD as possible of eliminating the influence of IR from both peripheral tissue (such as muscle) and the liver. Therefore, the present study aimed to explore ANGPTL-8 and its relationship with hepatocellular lipid content (HLC) in NAFLD patients and peripheral IR, which was measured by a hyperinsulinemic-euglycemic clamp and hepatic IR shown by homeostatic model assessment of insulin resistance (HOMA-IR).

METHODS

Study population

A total of 48 individuals, aged 40–65 years, were recruited from a national cohort study, “Early Recognition and Intervention Technology Study of Metabolic Syndrome,” which was initiated by Shanghai Sixth People’s Hospital, between September 2013 and August 2014 in China. Individuals at high-risk of NAFLD (because of the presence of obesity, metabolic syndrome, type 2 diabetes or abnormal plasma aminotransferases) were recruited in this study. The exclusion criteria were: (i) known history of cardiovascular disease; (ii) current treatment with system corticosteroids; (iii) cirrhosis with or without ascites; (iv) known hyperthyroidism or hypothyroidism; (v) severe disability and psychiatric disturbance; (vi) the presence of cancer; and (vii) pregnancy. Volunteers were also excluded if they had a history of alcohol abuse (>30 g/day for men and >20 g/day for women), type 1 diabetes or any liver disease other than NASH (i.e., hepatitis B or C, autoimmune hepatitis, hemochromatosis, Wilson’s disease or drug-induced hepatitis).

The study was approved by both the Ethics Committee of Shanghai Sixth People’s Hospital and The First Affiliated Hospital of Sun Yat-sen University, and written informed consent was obtained from each patient before their participation.

Measurements

Height and weight were measured, and body mass index (BMI; kg/m²) was calculated by dividing weight (kg) by squared height (m²). The waist circumferences were measured at the midpoint between the lowest rib and the uppermost lateral border of the right iliac crest. The hips circumferences were measured at their widest point, and the waist-to-hip ratio was calculated. Blood pressure was measured using a mercury sphygmomanometer at a resting state.

All blood samples were taken after an overnight fast for 10–12 h. Blood specimens were separated by centrifugation, and serum samples were stored at –80°C until analysis of ANGPTL-8. A 75-g glucose solution was given to every participant orally, and plasma glucose was measured at 30 min and 2 h after glucose administration. In all participants, fasting plasma glucose, 30-min plasma glucose, 2-h plasma glucose, fasting insulin, serum total cholesterol, TG, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, alanine aminotransferase (ALT), aspartate aminotransferase, gamma glutamyl transpeptidase, blood urea nitrogen, serum creatinine and hemoglobin A1c were measured. Serum betatrophin levels were measured using a commercially available human enzyme-linked immunosorbent assay kit (catalog number E11644h; Wuhan Eiaab Science, Wuhan, China) with an intra-assay coefficient of variation of ≤4.8% and an interassay coefficient of variation of ≤7.2%. Human ANGPTL-8 enzyme-linked immunosorbent assay kits were measured twice to ensure the reliability of the experiments according to the manufacturer’s protocol.

Hyperinsulinemic-euglycemic clamp

A hyperinsulinemic-euglycemic clamp was used after a 10-h fast on a different day. In brief, human regular insulin diluted in 0.9% saline was infused intravenously at a constant rate of 80 mU/m² per min for 2 h. Fasting glucose was clamped at 5.6 mmol/L with a coefficient of variation <5% by titrating the infusion rate of 20% dextrose based on the measurement of the arterialized plasma glucose every 5 min^{22,23}. After 2 h of constant insulin infusion, steady-state conditions can typically be achieved for plasma insulin, blood glucose and the glucose infusion rate. Assuming that the hyperinsulinemic state is sufficient to completely suppress hepatic glucose production, and as there is no net change in blood glucose concentrations under steady-state clamp conditions, the glucose infusion rate must be equal to the glucose disposal rate (M value).

Hepatocellular lipid content measurement

Participants underwent an upper abdominal coil magnetic resonance imaging (MRI; 3-Tesla whole-body scanner; SIEMENS 3.0T MAGNETOM Verio; Siemens Healthcare Sector, Erlangen, Germany) examination that included an initial set of localizer images and the T1 volumetric interpolated breath-hold examination Dixon sequence to calculate HCL, as previously described^{22,24}. A hepatocellular lipid content of >5.5% was considered a diagnosis of NAFLD, as previously defined²⁵. According

to the severity of NAFLD, volunteers were divided into three groups: group 1 (G1) had no NAFLD (HCL < 5.5%); group 2 (G2) had mild NAFLD (5.5 ≤ HCL < 10.0%); and group 3 (G3) had moderate-to-severe NAFLD (HCL ≥ 10.0%).

Calculations

HOMA-IR values were calculated as fasting plasma glucose (mmol/L) × fasting insulin (μU/mL) / 22.5. HOMA of β-cell function (HOMA-β) values were calculated as 20 × fasting insulin (uU/mL) / (fasting plasma glucose [mmol/L] – 3.5). The estimated glomerular filtration rate values were calculated from creatinine levels using the CKD-EPI formula.

Statistical analysis

Statistical analysis was carried out using SPSS 18.0 software (SPSS, Chicago, IL, USA). The data are presented as mean ± standard deviation (25th and 75th percentiles) for continuous variables. Continuous variables including log-transformed

variables, such as HOMA-IR, TG and HCL, with normal distribution were compared by ANOVA, those that neither followed a normal distribution nor were log transformed were compared using the Kruskal–Wallis *H*-test (paired were compared using the Mann–Whitney *U*-test). Correlation coefficients were analyzed using the Pearson correlation (normally distributed data) or Spearman's correlation (data not normally distributed). Analysis of covariance was used to adjust age, sex and BMI.

RESULTS

Comparison of characteristics and serum ANGPTL-8 levels in participants without or with different extents of NAFLD

The demographic and biochemical characteristics of all participants are summarized in Table 1. The distributions of age, sex, total cholesterol and low-density lipoprotein cholesterol were not statistically different among groups. Compared with G1, G3 had a worse metabolic profile, such as severe obesity, higher prevalence of diabetes mellitus, increased blood pressure and so

Table 1 | Characteristics of participants with different extent of non-alcoholic fatty liver disease

	Group 1 (HCL < 5.5%)	Group 2 (5.5% ≤ HCL < 10.0%)	Group 3 (HCL ≥ 10.0%)	<i>P</i> -value
<i>n</i>	12	18	18	
HCL (%)	5.1 ± 0.2	7.6 ± 1.3	18.3 ± 4.8 ^{†‡}	<0.0001
Sex (male/female)	6/6	7/11	8/10	0.832
Age (years)	52.2 ± 4.8	56.3 ± 4.9	51.9 ± 7.2	0.059
NC (cm)	34.2 ± 3.7	34.4 ± 2.7	35.8 ± 2.8	0.272
BMI (kg/m ²)	23.6 ± 1.7	23.4 ± 2.1	26.8 ± 2.4 ^{†‡}	<0.0001
WHR	0.86 ± 0.04	0.91 ± 0.05 [†]	0.94 ± 0.07 [†]	0.002
SBP (mmHg)	116 ± 14	120 ± 14	132 ± 19 ^{†‡}	0.024
DBP (mmHg)	71.8 ± 7.3	72.8 ± 9.8	80.4 ± 11.8 ^{†‡}	0.037
FPG (mmol/L)	5.2 ± 0.5	5.8 ± 1.4	7.0 ± 2.4 ^{†‡}	0.017
PG30min (mmol/L)	9.0 ± 1.84	10.6 ± 2.7	11.8 ± 2.5 [†]	0.015
PG2h (mmol/L)	6.5 ± 2.6	9.4 ± 4.0	13.2 ± 4.9 ^{†‡}	<0.0001
HbA1c (%)	5.7 ± 0.5	6.2 ± 0.8	6.8 ± 1.2 [†]	0.006
Diabetes (%)	8.3 (1/12)	27.8 (5/18)	55.6 (10/18) [‡]	<0.0001
CHOL (mmol/L)	5.2 ± 0.8	5.8 ± 1.0	5.6 ± 1.1	0.297
TG (mmol/L)	0.8 (0.7–1.2)	1.4 (1.1–2.0) [†]	1.5 (1.2–2.6) [†]	0.002
HDL-c (mmol/L)	1.6 (1.4–1.7)	1.2 (1.0–1.3) [†]	1.2 (1.0–1.5) [†]	0.001
LDL-c (mmol/L)	3.2 ± 0.76	4.10 ± 1.10	3.88 ± 0.87	0.055
GFR (mL/min/1.73 m ²)	101 ± 23	93 ± 19	100 ± 18	0.458
ALT (U/L)	13 (11–17)	19 (16–23) [†]	28 (18–40) [†]	0.001
AST (U/L)	18 (18–22)	21 (191–23)	22 (17–27)	0.402
GGT (U/L)	18 (14–26)	28 (21–49)	32 (26–61) [†]	0.008
FINS (μU/mL)	5.4 (3.6–5.7)	4.7 (3.0–5.6)	11.0 (8.1–14.4) ^{†‡}	<0.0001
HOMA-IR	1.19 (0.73–1.44)	0.98 (0.79–1.28)	3.16 (2.03–3.40) ^{†‡}	<0.0001
HOMA-β	65.0 (48.4–76.4)	65.5 (23.3–86.7)	76.1 (65.5–134.5)	0.134
M value (mg/kg/min)	11.1 ± 2.4	7.9 ± 2.1 [†]	4.5 ± 1.8 ^{†‡}	<0.0001
ANGPTL-8 (pg/mL)	742 ± 252	765 ± 301	1,129 ± 351 ^{†‡}	0.001

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CHOL, total cholesterol; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; FINS, fasting insulin; FPG, fasting plasma glucose; GGT, gamma-glutamyl transpeptidase; HbA1c, hemoglobin A1c; HCL, hepatocellular lipid content; HDL-c, high-density lipoprotein; HOMA-IR, homeostatic model assessment of insulin resistance; HOMA-β, homeostatic model assessment of β-cell function; LDL-c, low-density lipoprotein; M value, Matsuda value calculated by hyperinsulinemic-euglycemic clamp; PG2h, 2-h plasma glucose during oral glucose tolerance test; PG30min, 30-min plasma glucose during oral glucose tolerance test; SBP, systolic blood pressure; TG, triglyceride; WHR, waist-to-hip ratio. Data are presented as the mean ± standard deviation or median (25th–75th). [†]Group 1 (no non-alcoholic fatty liver disease [NAFLD]) vs group 2 (mild NAFLD) and group 3 (moderate-to-severe NAFLD) *P* < 0.05. [‡]Group 2 vs group 3 *P* < 0.05.

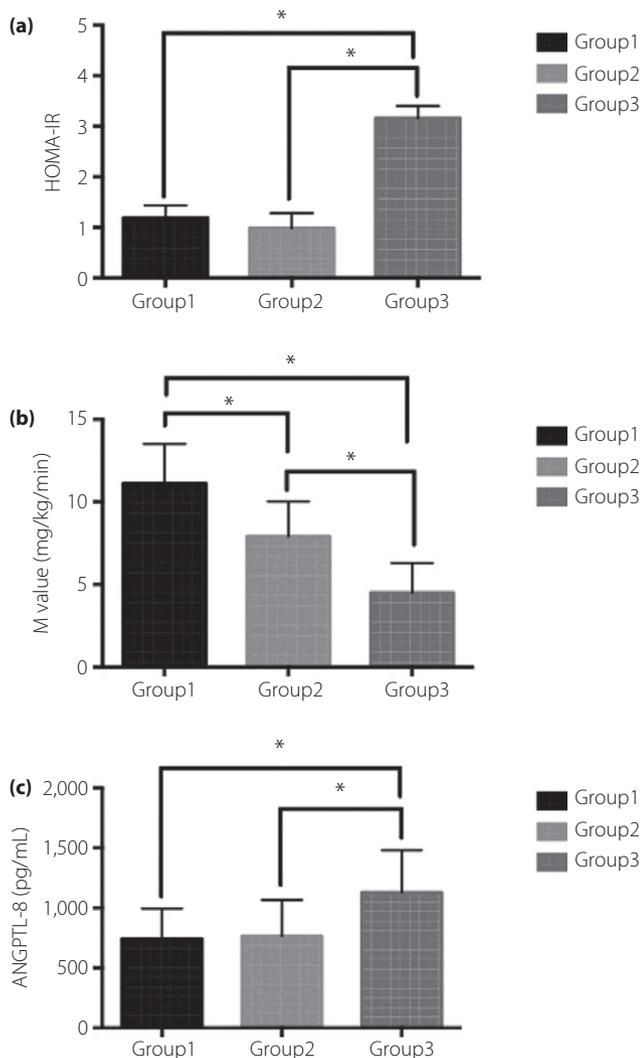


Figure 1 | The (a) homeostatic model assessment of insulin resistance (HOMA-IR), (b) M value and (c) angiotensin-like protein 8 (ANGPTL-8) in different hepatocellular lipid content (HCL) groups (HCL < 5.5% for group 1, 5.5% ≤ HCL < 10.0% for group 2 and HCL ≥ 10.0% for group 3). More severe IR was observed in group 3, shown by both increased HOMA-IR and decreased M value (group 3 vs group 1, group 2; HOMA-IR: 3.16 [2.03–3.40] vs 1.19 [0.73–1.44], 0.98 (0.79–1.28), $P < 0.05$; M value: 4.5 ± 1.8 mg/kg/min vs 11.1 ± 2.4 mg/kg/min, 7.9 ± 2.1 mg/kg/min, $P < 0.05$). The M value of group 2 was significantly lower than group 1 (7.9 ± 2.1 mg/kg/min vs 11.1 ± 2.4 mg/kg/min, $P < 0.05$). Group 3 had a significantly increased ANGPTL-8 compared with group 1 and group 2 ($1,129 \pm 351$ pg/mL vs 742 ± 252 pg/mL, 765 ± 301 pg/mL, $P = 0.001$).

on. In contrast, high-density lipoprotein cholesterol was significantly elevated and the M value was reduced in G3 when compared with those in G2.

More severe IR situations were also observed in G3, shown by both increased HOMA-IR and decreased M value (all $P < 0.05$; Figure 1).

Serum ANGPTL-8 levels ranged from 249 to 1,708 pg/mL, with a mean of 895 ± 354 pg/mL in the 48 participants. G3 had a significantly increased ANGPTL-8 compared with G1 and G2 ($1,129 \pm 351$ vs 742 ± 252 pg/mL, 765 ± 301 pg/mL, $P = 0.001$; Table 1; Figure 1). ANGPTL-8 levels were comparable between G1 and G2.

Correlation of serum ANGPTL-8 level and HCL in participants without or with different extents of NAFLD

HCL was positively associated with ANGPTL-8 ($r = 0.515$, $P < 0.001$; Table 2), and the correlation parameter remained significant after adjusting age, sex and BMI ($r = 0.463$, $P = 0.003$; Table 2; Figure 2). After further adjusting the log HOMA-IR and M value, ANGPTL-8 ($r = 0.436$, $P = 0.042$) was still significantly related with HCL in individuals with NAFLD (G2 and G3).

Correlation of HCL or serum ANGPTL-8 with metabolic parameters

HCL was positively correlated with BMI ($r = 0.66$, $P < 0.001$) and waist-to-hip ratio ($r = 0.489$, $P = 0.001$; Table 2). HCL was significantly associated with metabolic profile; that is, blood pressure, blood glucose parameters, lipid profile in the univariate Pearson correlation analysis, and even after adjusting age, sex and BMI (Table 2). HCL was positively related with log HOMA-IR ($r = 0.672$, $P < 0.0001$), and negatively related with M value ($r = -0.785$, $P < 0.001$), although the correlation weakened slightly after adjusting age, sex and BMI (Table 2; Figure 2). No significance of correlation between HOMA- β and HCL was observed.

ANGPTL-8 was positively correlated with waist-to-hip ratio, HOMA-IR and HOMA- β ($P < 0.05$), and was negatively related with M value ($P < 0.05$), which vanished after the adjustment of age, sex and BMI.

Multivariate stepwise linear regression analysis of HCL

To examine the contribution of age, sex, BMI, ALT, ANGPTL-8, log HOMA-IR and M value to HCL in all participants, multivariate stepwise linear regression analysis was used. When age, sex and BMI entered into the equation as covariates, it was ANGPTL-8 (coefficient $\beta = 0.007$, $P = 0.002$) and ALT (coefficient $\beta = 0.215$, $P < 0.001$) that remained independent determinants of HCL, and explained 67.4% of its variation ($P < 0.001$). Similar results were obtained when such regression analysis was carried out only in patients with NAFLD.

DISCUSSION

In the present study, we concluded that individuals with moderate-to-severe NAFLD diagnosed by MRI had increased serum ANGPTL-8 in contrast to age- and sex-matched healthy individuals and those with mild NAFLD. A positive correlation was observed between ANGPTL-8 and HCL, which was independent of obesity and IR. In addition, on the basis of multivariate linear regression analysis, serum ANGPTL-8 and ALT were

Table 2 | Correlation analyses of hepatocellular lipid content or angiopoietin-like protein 8 with metabolic profiles

	Log HCL				ANGPTL-8			
	Unadjusted		Adjusted for age, sex and BMI		Unadjusted		Adjusted for age, sex and BMI	
	<i>r</i>	<i>P</i> -value	<i>r</i>	<i>P</i> -value	<i>r</i>	<i>P</i> -value	<i>r</i>	<i>P</i> -value
BMI	0.660	0.000			0.229	0.118		
WHR	0.489	0.001	0.376	0.018	0.276	0.058	0.233	0.138
SBP	0.352	0.022	0.202	0.219	-0.003	0.985	-0.066	0.677
DBP	0.347	0.024	0.041	0.805	0.187	0.202	0.126	0.425
FPG	0.426	0.005	0.384	0.017	0.104	0.481	0.185	0.242
PG30min	0.390	0.012	0.391	0.015	0.071	0.636	-0.010	0.948
PG2h	0.507	0.001	0.436	0.006	0.260	0.078	0.240	0.126
HbA1c	0.455	0.002	0.359	0.027	0.233	0.111	0.114	0.471
CHOL	0.019	0.907	-0.109	0.525	-0.082	0.581	-0.183	0.246
TG	0.412	0.007	0.337	0.044	0.184	0.211	-0.063	0.692
HDL-c	-0.303	0.051	-0.090	0.600	-0.080	0.590	-0.016	0.919
LDL-c	0.013	0.936	-0.177	0.301	-0.050	0.734	-0.138	0.383
ALT	0.457	0.002	0.449	0.006	0.163	0.270	0.062	0.696
AST	0.256	0.102	0.314	0.062	-0.006	0.967	0.004	0.978
GGT	0.356	0.026	0.344	0.040	0.235	0.121	0.300	0.053
log HOMA-IR	0.672	0.000	0.566	0.002	0.478	0.003	0.306	0.113
log HOMA-β	0.362	0.045	0.304	0.115	0.349	0.034	0.292	0.132
M value	-0.785	0.000	-0.654	0.000	-0.445	0.012	-0.289	0.136
ANGPTL-8	0.515	0.000	0.463	0.003				
HCL					0.457	0.002	0.546	0.000

ANGPTL-8, angiopoietin-like protein 8; ALT, alanine aminotransferase; ANGPTL-8, angiopoietin-like protein 8; AST, aspartate aminotransferase; CHOL, total cholesterol; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; FINS, fasting insulin; FPG, fasting plasma glucose; GGT, gamma-glutamyl transpeptidase; HbA1c, hemoglobin A1c; HCL, hepatocellular lipid content; HDL-c, high-density lipoprotein; HOMA-β, homeostatic model assessment of β-cell function; LDL-c, low-density lipoprotein; M value, Matsuda value calculated by hyperinsulinemic-euglycemic clamp; PG2h, 2-h plasma glucose during oral glucose tolerance test; PG30 min, 30-min plasma glucose during oral glucose tolerance test; SBP, systolic blood pressure; TG, triglyceride; WHR, waist-to-hip ratio.

defined as an independent predictor indicating the existence of significant NAFLD. The innovational point of the present study was that we first showed high serum ANGPTL-8 might result from excessive hepatic lipid accumulation, rather than the provocation of IR in the background of NAFLD. From this assumption, serum ANGPTL-8 was further confirmed to be a non-invasive and independent indicator of the severity of NAFLD.

The results in the present study showed that serum ANGPTL-8 in moderate-to-severe NAFLD patients increased by 52 and 48%, respectively, compared with that in those without or with mild NAFLD. The results were consistent with the study of von Loeffelholz *et al.*²¹, which showed that circulating ANGPTL-8 was numerically increased in NAFLD patients compared with those with non-NAFLD.

Although ANGPTL-8 secretes almost uniquely from the liver in humans and is involved in lipid metabolism, the reciprocal effect of excessive hepatic lipid deposition and the expression of ANGPTL-8 in the liver still need to be fully studied. ANGPTL-8 increased in high-fat diet-fed mice and *db/db* or *ob/ob* mice, and was also highly induced by endoplasmic reticulum stress

that was activated by hyperlipidemia or lipotoxicity²⁰. In contrast, ANGPTL-8 *Gp1hbp1* knockout mice showed decreased serum TG levels and its opposite phenotype (overexpression of ANGPTL-8) severely increased TG levels, which probably resulted from the inhibition of ANGPTL-8 to the lipoprotein lipase activity. These results showed that the expression of ANGPTL-8 could modulate TG metabolism in mice¹⁹. Therefore, it seems that the expression of ANGPTL-8 in the liver and hepatic lipid deposition could promote each other. However, such mechanisms need to be confirmed in humans. What we have learnt until now was that in human liver cells, ANGPTL-8 can activate the autophagic process and mediate the regulation of thyroid hormone 3 in lipid metabolism¹⁹.

Another interesting finding of the present study was the positive relationship between serum ANGPTL-8 and HCL. They were not influenced by the whole body and liver IR. In the multiple linear regression analysis, only ANGPTL-8 and ALT were the independent determinants of HCL. Furthermore, the correlation between ANGPTL-8 and IR indexes including HOMA IR and M value became non-significant after adjusted for age, sex and BMI.

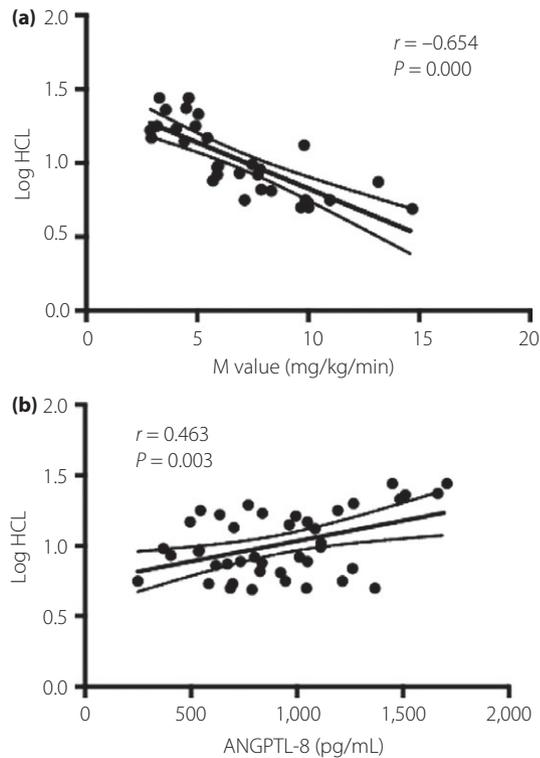


Figure 2 | The relationship between, hepatocellular lipid content (HCL) with (a) M value and (b) angiopoietin-like protein 8 (ANGPTL-8). Log HCL significantly correlates with M value ($r = -0.654$, $P = 0.000$) and ANGPTL-8 ($r = 0.463$, $P = 0.003$).

The positive correlation between ANGPTL-8 and HCL also could be obtained from a population-based study by Hu *et al.*²⁶ However, the differences between the present study and their study might be the interpretation of the results: different methods (MRI vs ultrasound) were used to measure HCL, and a lack of adjustment of IR when assessing the correlation existed in that study. Additionally, increased ANGPTL-8 was found in individuals with cirrhosis and was positively correlated with the severity of cirrhosis, which was speculated to be related to the existence of IR^{21,27}. However, such speculations cannot be approved by the results in the present study.

Contrary to the above speculations, we inferred that ANGPTL-8 could be involved in the pathogenic process of NAFLD independent of the coexistence of IR. In a rodent study, the expression of ANGPTL-8 was not associated with either mild age-related IR in Wistar rats or with severe genetically-based IR in Goto-Kakizaki rats²⁸. In the study by von Loeffelholz *et al.*²¹, after adjustment for age, circulating ANGPTL-8 correlated with the insulin area under the curve, but this correlation disappeared after 45% liver lipid reduction with concomitant ANGPTL-8 decrease²¹, which indirectly inflected the correlation between serum ANGPTL-8 and HCL. However, further confirmation in humans and

studies focused on related mechanism(s) are still required in future.

Increased serum ANGPTL-8 can be observed in NAFLD patients in most studies excluding the one by Cengiz *et al.*²⁹ In that study, serum ANGPTL-8 decreased in NAFLD and NASH patients who had more severe liver steatosis and even fibrosis. In such settings, a large number of inflammatory cytokines were released, which could lead to a low expression of ANGPTL-8.

The regulations of ANGPTL-8 on lipid metabolism have been confirmed in rodent model studies; however, a consensus about the relationship between ANGPTL-8 and serum lipid profile in humans has not yet been reached. In the present study, no significant correlation was observed after the adjustment, which was consistent with previous studies by Espes *et al.*³⁰ and Hu *et al.*³¹ Some studies reported that serum ANGPTL-8 decreased in patients with dyslipidemia and correlated with high-density lipoprotein cholesterol or very low-density lipoprotein levels^{15,32}. The reasons for the discrepancy of these clinical findings need to be elucidated further. The possible reasons might be the different population and methodology applied in these studies.

One of the main limitations of the present study was the cross-sectional design, in which the causal relationship between ANGPTL-8 and HCL in NAFLD cannot be concluded from this study. Another limitation was the relatively small sample size, which might reduce the statistical power of the results.

In summary, the present study showed that serum ANGPTL-8 increased in individuals with moderate-to-severe NAFLD diagnosed by MRI and was positively correlated with hepatocellular lipid content, which was independent of obesity and IR. We speculate that ANGPTL-8 could contribute to the pathogenesis of NAFLD independent of IR in the liver, and could be a potential therapy target of NAFLD. Further investigation is required to investigate the mechanism(s) of ANGPTL-8 in the pathogenesis of NAFLD.

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DISCLOSURE

The authors declare no conflict of interest.

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