

Type 2 diabetic patients with non-alcoholic fatty liver disease exhibit significant haemorheological abnormalities

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Abstract Haemorheological abnormalities have been described in diabetes mellitus, as well as in non-alcoholic fatty liver disease (NAFLD). However, the relationship between the changes in liver fat content and haemorheology is unknown. The current study aims to show the correlation between haemorheological parameters and intrahepatic lipid content (IHLC) in patients with type 2 diabetes. The serum biochemical markers, such as fasting plasma glucose (FPG), haemoglobin A1c (HbA1c), liver enzymes, lipid profiles, and haemorheological properties, were examined. IHLC was quantified using proton magnetic resonance spectroscopy (¹H-MRS) scanning of the liver. A significant correlation was observed between IHLC and whole blood viscosity at high, middle, and low shear rates. IHLC also positively correlated with haematocrit, the reduced whole blood viscosity at low and middle shear rates, and the erythrocyte aggregation index. Diabetic patients with NAFLD exhibited significant haemorheological abnormalities compared with patients without NAFLD. In summary, haemorheological disorders are linked to non-alcoholic fatty liver in type 2 diabetes.

Keywords diabetes mellitus, type 2; haemorheology; non-alcoholic fatty liver disease

Introduction

Non-alcoholic fatty liver disease (NAFLD), which is the excessive accumulation of triglycerides within the cytoplasm of hepatocytes, is a common disorder related to obesity and type 2 diabetes [1]. An estimated 70% of type 2 diabetic patients have NAFLD [2]. In recent years, several haemorheological disturbances have been described for diabetes mellitus and NAFLD, such as increased blood viscosity, erythrocyte aggregation index, and haematocrit [3,4]. Haemorheological disorders contribute to decreased organ perfusion, impaired microcirculation, and the occurrence of diabetic complications. Experimental studies on animal models with fatty liver have shown that fatty infiltration reduces hepatic blood flow and parenchymal microcirculation [5,6]. A significant reduction in hepatic microcirculation has been found in liver donors with steatosis compared with that

in normal liver donors [7]. However, the relationship between the changes in intrahepatic lipid content (IHLC) and haemorheology in humans is still unknown. The main reason is the lack of a non-invasive method to quantify IHLC in clinical practice. Recent studies have shown that liver proton magnetic resonance spectroscopy (¹H-MRS) can non-invasively evaluate the degree of NAFLD quantitatively [8,9]. In the present study, IHLC was measured using ¹H-MRS scans of the livers of type 2 diabetic patients. The correlation between haemorheological parameters and IHLC was analyzed, as well as the incidences of significant haemorheological abnormalities in type 2 diabetic patients with NAFLD compared with their counterparts without NAFLD.

Materials and methods

Study subjects

A total of 55 type 2 diabetic patients were recruited from the clinic service of Tongji Hospital, Wuhan, Hubei Province, China, between March 1, 2008 and July 1, 2008 based on the

following inclusion criteria: (1) 25–70 years old; (2) no known acute or chronic history of disease, physical examination, and standard laboratory tests (blood counts, serum creatinine, and electrocardiogram); (3) alcohol consumption of < 20 g/day; and (4) no evidence of hepatitis A, B, or C infection, autoimmune hepatitis, clinical signs or symptoms of inborn errors of metabolism, or a history of use of toxins or drugs that induce hepatitis. Exclusion criteria included thyroid disease, the use of antihypertensive agents that could possibly influence glucose metabolism (β -blockers and thiazides), the use of thiazolidinedione or of insulin, and pregnant or lactating women. Up to 25 males and 30 females with type 2 diabetes mellitus were recruited. Their ages ranged from 39 to 70 (54.6 ± 9.2) years old, with diabetic histories from 3 months to 10 years. Of the 55 subjects, 24 had hypertension and are taking antihypertensive medications (ACE inhibitors or calcium channel blockers). Seven subjects were treated for diabetes with diet alone. The others were treated with metformin, sulphonylureas, α -glucosidase inhibitor, or a combination of these drugs. The study was conducted before the initiation of statin therapy. There were no other adjunctive medications. The nature and potential risks of the study were explained to all subjects before obtaining their written informed consent. The experimental protocol was approved by the ethics committee of Tongji Hospital, affiliated to Tongji Medical College, following the ethical principles outlined in the Declaration of Helsinki.

Anthropometric measurements

Waist circumference was measured midway between the superior iliac spine and the lower rib margin; hip circumference was measured at the level of the greater trochanters. Body weight was recorded to the nearest 0.1 kg using a calibrated weighing scale with subjects barefoot and wearing light indoor clothing. Body height was recorded to the nearest 0.5 cm using a ruler attached to the scale.

Blood samples collected and laboratory methods

Blood samples for the measurement of fasting plasma glucose (FPG), haemoglobin A1c (HbA1c), serum total cholesterol (TC), triglyceride (TG), low density lipoprotein cholesterol (LDL-C), and alanine aminotransferase (ALT) were taken after an overnight fast. Haemorrhological parameters were also examined. FPG, TC, TG, and LDL-C concentrations were measured with enzymatic kits (Diasys Diagnostics, Shanghai, China). HbA1c levels were measured via the high performance liquid chromatography (HPLC) method using an automatic glycohaemoglobin analyzer (Tosoh, Japan). Plasma biochemical markers were measured using an Aeroset 2000 automatic biochemical analyzer (Abbott Laboratories, Abbott Park, Illinois, USA). Haemorrhological parameters were measured through the cone and plate stable technology

using an automatic haemorrhological analyzer LBY-N6C (Precil, Beijing, China).

Measurement of intrahepatic lipid content (IHLC) by $^1\text{H-MRS}$

$^1\text{H-MRS}$ scans of the liver were acquired on a 1.5T MRI scanner (GE Healthcare, USA) using a standard body coil and respiratory gating mode. Subjects who fasted overnight lay on their stomachs on the surface coil, which was embedded in a mattress designed to minimize abdominal movement due to breathing. Sagittal, coronal, and axial slices of the liver were recorded. A spectroscopic volume of interest was positioned in the right posterior hepatic lobes, avoiding major blood vessels, intrahepatic bile ducts, and the lateral margin of the liver. Voxel size and position were optimized to prevent contamination of signal from the liver by signal from abdominal adipose fat. The volume of voxel was limited to $2\text{ cm} \times 2\text{ cm} \times 2\text{ cm}$ or $3\text{ cm} \times 3\text{ cm} \times 3\text{ cm}$ proton spectra; a relatively larger voxel was used to collect high quality data in a short time. Saturated zones were placed around the voxel. The single voxel spectra were recorded using a point resolved spectroscopy sequence (PRESS) with an echo time of 35 ms and a repetition time of 1500 ms. The imaging parameters for the PRESS sequence are listed as follows: the length of echo train was 1, the excitation number was 8, and water saturation was not used with the research mode.

Spectroscopic data were processed using the analysis program SAGE (GE Healthcare, USA). Chemical shifts were measured relative to water at 4.77 ppm. The methylene signal, which represents intracellular triglycerides, was measured at 1.40 ppm. The peak area of the water (S_w) and fat resonance (S_f) were measured. Intrahepatic lipid content (IHLC) was calculated as follows:

$$\text{IHCL} = S_f / (S_f + S_w) \times 100$$

This measurement was validated against histologically determined lipid content. All spectra were analyzed by physicists who were unaware of the clinical data.

Statistical analysis

Normally distributed data are shown as the mean \pm SD, whereas non-normally distributed data are shown as the median with the 25th and 75th percentiles in parentheses. An unpaired Student's *t*-test was used to compare mean values between the groups. A nonparametric test was used to compare median values between the groups. Correlation analyses were performed using Spearman's nonparametric rank correlation coefficient. Calculations were performed using GraphPad Prism version 4.03 and SPSS 14.0 software. Statistical significance was defined as $P < 0.05$.

Results

Participant characteristics

The characteristics of the study population, which consisted of 55 diabetic patients, are shown in Table 1. The anthropometric characteristics (body mass index and waist circumference) of healthy persons in China are also shown. The laboratory-defined normal ranges of blood biochemical and haemorheological parameters are listed in Table 1. According to the results of the Dallas Heart Study, liver fat greater than 5.60% (measured by ¹H-MRS) is considered abnormal [10]. Hence, the normal IHLC range was defined as lower than 5.60%.

Relationships between IHLC and haemorheological parameters

As shown in Fig. 1, IHLC (log scale) correlated with whole blood viscosity, including the low, middle, and high shear rates. IHLC also correlated with reduced whole blood viscosity at low shear rates and RBC aggregation index in type 2 diabetic patients.

Difference in biochemical and haemorheological plasma parameters between diabetic patients with and without NAFLD

The subjects were divided into two groups according to their IHLC values. Up to 24 patients with IHLC greater than 5.6% were diagnosed with NAFLD. The remaining 31 patients were without NAFLD. The patients in the two groups were comparable in terms of age, sex, and state of illness. As shown in Table 2, the diabetic patients with NAFLD were abdominally obese compared with those without NAFLD. Plasma ALT, TC, and TG levels in the diabetic patients with NAFLD were higher than those without. However, FPG and HbA1C did not differ between the two groups. Hemorheological disorders were found in diabetic patients with NAFLD, as whole blood viscosity, whole blood reduced viscosity at low and middle shear rates, and RBC aggregation index were all significantly elevated.

Discussion

An alarming trend of type 2 diabetes is developing in China. In 2010, the age-standardized prevalence of total diabetes in China is 9.7%, accounting for 92.4 million adults with diabetes [6]. The main risk factor for the development of diabetes is obesity [11]. An excess of body fat, especially when concentrated within the abdomen, has a range of potentially harmful consequences. NAFLD, for instance, is one of the increasingly recognized conditions associated with

Table 1 Characteristics of the subjects

Variables	Subjects	Normal reference ranges
BMI (kg/m ²)	24.6±3.2	18.5–24
Waist (cm)	Women 83.1±7.7	Women<80
	Men 90.6±7.5	Men<90
ALT (U/L)	25 (18, 33)	0–41
FPG (mmol/L)	7.97±2.10	3.90–6.40
HbA1C (%)	7.62±1.50	4.5–6.3
TC (mmol/L)	4.98±0.99	2.90–5.20
TG (mmol/L)	1.31 (1.04, 2.04)	<1.7
LDL-C (mmol/L)	2.93±0.82	0.00–3.12
Blood viscosity (mPa/s)	Low shear rate	7.38±1.40
	Middle shear rate	4.83±0.60
	High shear rate	4.18±0.52
Plasma viscosity (mPa/s)	1.37±0.07	1.20–1.45
ESR (mm/h)	9 (5, 20)	0–20
Blood reduced viscosity (mPa/s)	Low shear rate	14.80±2.44
	Middle shear rate	8.55±0.96
	High shear rate	6.92±0.81
K-value of ESR	27.90 (17.90, 53.77)	0.00–120.00
RBC rheology	Aggregation index	1.75±0.17
	Rigidity index	5.05±0.58
	Deformation index	0.89±0.05
	Electrophoresis index	4.37±0.50
	IHLC (%)	3.64 (0.82, 10.08)
		1.40–1.90
		3.80–5.80
		0.78–0.92
		3.50–4.60
		<5.60

Normally distributed data are shown as mean ± SD; non-normally distributed data are shown as median with the 25th and 75th percentiles in parentheses. The data in the right column represent the anthropometric characteristics of healthy persons in China and laboratory-defined normal ranges.

diabetes and obesity that may proceed to end-stage liver disease. Given that the liver plays a critical role in glucose homeostasis and lipid metabolism, and that the state of microcirculation reflects its function, the effects of fat accumulation on hepatic microvascular perfusion deficits is important. Experimental studies have demonstrated that systemic and regional haemodynamic changes occur and that the sinusoidal lumen is obstructed by swollen fatty degenerated hepatocytes [4]. Animal models with fatty liver have shown that there is an inverse correlation between the degree of fat infiltration and hepatic blood flow, as well as flow in the microcirculation, as visualized by *in vivo* microscopy [12]. However, no information is available regarding the relationships between IHLC and the systemic haemorheologic changes in humans. The main reason is the lack of a non-invasive quantification method for IHLC in clinical practice.

In the present study, we applied a new method, proton magnetic resonance spectroscopy (¹H-MRS), to detect IHLC.

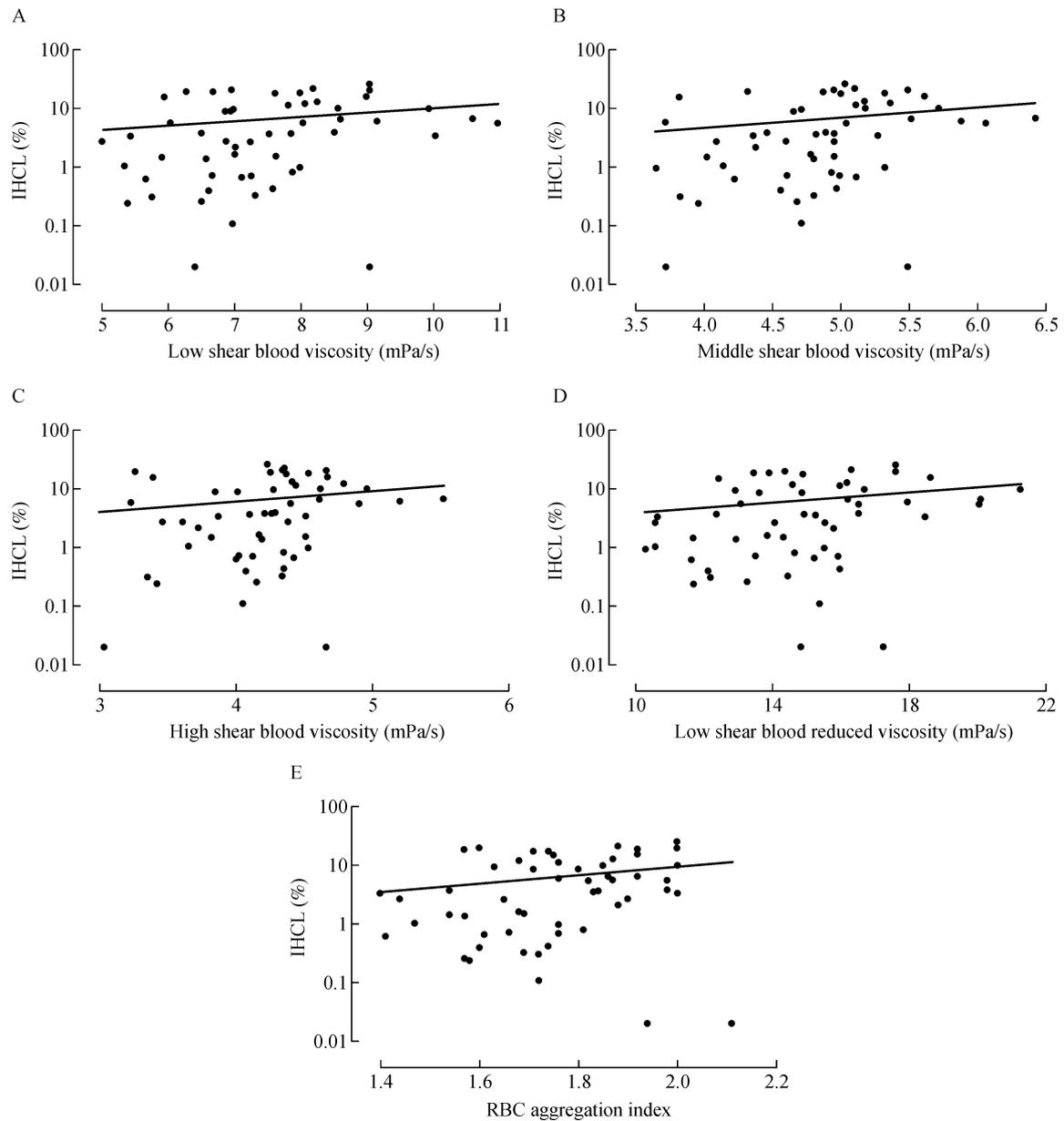


Fig. 1 The relationships between intrahepatic lipid content (log scale) and (A) whole blood viscosity at low shear rate ($r = 0.387$, $P = 0.004$); (B) whole blood viscosity at middle shear rate ($r = 0.390$, $P = 0.003$); (C) whole blood viscosity at high shear rate ($r = 0.336$, $P = 0.012$); (D) whole blood reduced viscosity at low shear rate ($r = 0.334$, $P = 0.013$); (E) RBC aggregation index ($r = 0.317$, $P = 0.018$) in type 2 diabetic patients.

Unlike liver biopsies, localized ^1H -MRS is a reproducible, non-invasive alternative for measuring IHLC without ethical limitations [13]. Although this method gives no information on whether steatosis is microvesicular or macrovesicular, or on inflammation or fibrosis, it allows for relatively larger volumes (routinely 8 to 27 cm^3) of the liver to be sampled, unlike the small sample size of liver biopsy [14,15]. In addition, ^1H -MRS can distinguish intracellular from extracellular lipids based on the different chemical shifts [16]. A previous study has shown that IHLC obtained by

spectroscopy closely coincides with biopsy- or autopsy-derived triglyceride concentrations [10]. Moreover, ^1H -MRS provides a quantitative, rather than qualitative or semiquantitative, assessment of hepatic triglyceride, which is determined by ultrasound and computerized tomography (CT) scanning.

Our study shows, for the first time, a positive correlation between IHLC and systemic haemorheological parameters, including whole blood viscosity, whole blood reduced viscosity, and erythrocyte aggregation index. However, the “ r ” value, ranging from 0.317 to 0.390, indicates a low degree

Table 2 Comparison of characteristics between diabetic patients with and without NAFLD

Variables	Without NAFLD	With NAFLD
N	31	24
Age (y)	55.2±8.1	53.8±10.6
BMI (Kg/m ²)	23.6±2.8	25.8±3.2*
Waist (cm)	82.4±8.7	88.4±6.3**
ALT (U/L)	21 (16, 28)	30 (25, 38)**
FPG (mmol/L)	7.94±2.31	8.02±1.86
HbA1C (%)	7.54±1.56	7.72±1.46
TC (mmol/L)	4.72±0.95	5.33±0.96*
TG (mmol/L)	1.15 (0.91, 1.43)	2.17 (1.38, 3.32)**
LDL-C (mmol/L)	2.81±0.75	3.10±0.89
Blood viscosity (mPa/s)		
Low shear rate	6.86±1.22	8.05±1.34**
Middle shear rate	4.69±0.48	5.11±0.63**
High shear rate	4.02±0.44	4.39±0.55**
Plasma viscosity (mPa/s)	1.36±0.07	1.39±0.07
HCT (%)	39.29±4.11	41.54±3.27*
ESR (mm/h)	12 (4, 20)	9 (5, 17)
Blood reduced viscosity (mPa/s)		
Low shear rate	13.90±2.11	15.97±2.39**
Middle shear rate	8.2±0.79	8.90±1.06*
High shear rate	6.75±0.65	7.15±0.96
K-value of ESR	41.26 (15.78, 56.47)	26.17 (18.61, 47.43)
RBC rheology		
Aggregation index	1.69±0.19	1.82±0.13**
Rigidity index	4.97±0.53	5.15±0.65
Deformation index	0.89±0.05	0.88±0.05
Electrophoresis index	4.35±0.56	4.39±0.42
IHLC (%)	0.99 (0.40, 2.72)	11.90 (7.24, 18.91)**

Normally distributed data are shown as the mean ± SD; non-normally distributed data are shown as the median with the 25th and 75th percentiles in parenthesis. * $P < 0.05$, ** $P < 0.01$ compared with the patients without NAFLD.

of correlation, suggesting that IHLC and the haemorheological parameters are neither closely related to one another nor have a cause-and-effect relationship. Our previous study has shown that IHLC is more closely related to waist circumference and plasma triglyceride level [17]. Visceral adipose tissue abdominally quantified by ¹H-MRS is also positively related to very low density lipoprotein (VLDL) particle number, low density lipoprotein (LDL) particle number, and VLDL concentration in the peripheral blood [18]. Considering that triglycerides and cholesterol both travel in the blood in lipoprotein packages, elevated triglyceride levels increase large particles, such as VLDL and chylomicrons, in the blood. The same is true for LDL particles, which contain cholesterol. Large particles in the vessels increase the friction and fluid resistance of blood flow, which could increase blood viscosity. Increased blood viscosity and erythrocyte aggregation may lead to impairment of hepatic

sinusoidal perfusion, which account for the injurious effects of hepatocytes. Meanwhile, fat-laden hepatocytes that are swollen and ballooning cause sinusoidal distortion, thereby reducing intrasinusoidal volume and microvascular blood flow [19]. This indirect correlation between IHLC and blood viscosity may partially explain the low correlation coefficient in the present study.

The differences in characteristics between diabetic patients with and without NAFLD have also been compared. Diabetic patients with NAFLD were abdominally obese, with elevated liver enzymes and dyslipidemia, unlike the patients without NAFLD. Significant haemorheological disorders have been found in diabetic patients with NAFLD, suggesting that diabetic patients with fatty liver have more metabolic disturbances than those without fatty livers.

In conclusion, although the correlation coefficient is low, impaired blood rheology is related to liver fat content in diabetic patients. In previous studies, blood rheology was regarded as an important potential contributory factor to diabetic angiopathy and cardiovascular complications of diabetes [20,21]. The present study was the first to document the correlation between blood rheology and liver fat content. Further work is needed to fully understand the relationships between fatty liver disease and hepatic microcirculation.

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