Research Report

Journal of INTERNATIONAL MEDICAL RESEARCH

Journal of International Medical Research
2016, Vol. 44(3) 496–507

DOI: 10.1177/0300060515627561
imr.sagepub.com
SAGE

Fasting serum insulin levels and insulin resistance are associated with blood rheology in Japanese young adults without diabetes

Kensuke Yoshida, Takao Kimura, Tomoyuki Aoki, Katsuhiko Tsunekawa, Osamu Araki, Yoshifumi Shoho, Makoto Nara, Hiroyuki Sumino and Masami Murakami

Abstract

Objectives: To evaluate fasting serum insulin levels and insulin resistance, and their association with blood rheology, in Japanese young adults without diabetes.

Methods: Blood samples were analysed and blood rheology was estimated using haematological parameters. Whole blood passage time was measured using a Hitachi MC-FAN© microchannel array flow analyser.

Results: Out of 151 subjects (mean age, 24.1 ± 1.5 years), fasting serum insulin levels and insulin resistance (using homeostasis model assessment-estimated insulin resistance [HOMA-IR]), were positively correlated with longer whole blood passage times and higher values for haematocrit (Hct), haemoglobin (Hb), fibrinogen, body weight, body mass index (BMI), triglycerides, and low-density lipoprotein cholesterol (LDL-C)/high-density lipoprotein cholesterol (HDL-C) ratio, and were negatively correlated with HDL-C. Whole blood passage time correlated with body weight, BMI, LDL-C/HDL-C ratio, Hct, Hb, white blood cell (WBC) count, platelet count, fibrinogen, fasting serum insulin levels, and HOMA-IR. Multiple regression analysis revealed that whole blood passage time was independently associated with Hct, fibrinogen levels, and WBC count.

Conclusions: Fasting serum insulin levels and insulin resistance were associated with blood rheology, and may influence blood rheology by modulating haematological parameters and lipid parameters in young adults without diabetes.

Department of Clinical Laboratory Medicine, Gunma University Graduate School of Medicine, Maebashi, Gunma, Japan

Corresponding author:
Takao Kimura, Department of Clinical Laboratory Medicine, Gunma University Graduate School of Medicine, 3-39-15, Showa-machi, Maebashi, Gunma 371-8511, Japan.
Email: tkimura@gunma-u.ac.jp

Keywords
Insulin, insulin resistance, blood rheology, fibrinogen, haematocrit

Date received: 3 October 2015; accepted: 24 December 2015

Introduction

The prevalence of obesity and metabolic syndrome is a rising problem in children and adolescents worldwide,[1,2] therefore, prevention, early detection, and intervention for children, adolescents, and young adults at risk, may be important for long term health.[1,2] Insulin resistance has been proposed as the key factor leading to abnormalities observed in metabolic syndrome, such as central obesity, impaired glucose tolerance, diabetes mellitus, hypertension and dyslipidaemia, all of which can contribute to cardiovascular diseases.[1–4] Insulin sensitivity and resistance influence blood rheology in subjects with obesity, hypertension and metabolic disorders,[1–4] and impairment of blood rheology is reportedly increased in patients with lifestyle-related diseases, such as hypertension, dyslipidaemia,[5,6] cardiovascular diseases[7,8] and diabetes.[9–12] In healthy young men, insulin sensitivity was reported to be associated with blood rheology,[13] however, whether fasting serum insulin levels and insulin resistance are associated with blood rheology in young adults remains unclear. In previous studies, blood rheology has been assessed using fibrinogen levels, platelet count, haematocrit (Hct) levels, red blood cell (RBC) aggregation, RBC viscosity, plasma viscosity, and whole blood viscosity,[5,7,8,13] all of which are parameters that partially reflect blood rheology.

The aim of the present study was to estimate the association between two insulin parameters, namely fasting serum insulin levels and insulin resistance, and blood rheology in young adults. Thus, whole blood passage time was evaluated using a MC-FAN© microchannel array flow analyser (Hitachi Haramachi Electronics, Ibaraki, Japan), which has been used in previous studies to clinically evaluate blood rheology using microscopic images.[14] A unique feature of MC-FAN© is that it can mimic microthrombus formation in microvessels, via blood flow through the minute watercourses produced on a siliconized chip.[14–18] MC-FAN is superior to other methods of measuring whole blood passage time, in terms of accuracy of channel dimension measurement and high reproducibility, and a number of studies have reported the clinical advantages of MC-FAN in evaluating blood rheology and microcirculation.[7,8,13–19] Cardinal blood rheological parameters were also assessed in the present study, including Hct and haemoglobin (Hb) levels, platelet count, white blood cell (WBC) count, fibrinogen levels, antithrombin-III activity, and plasminogen activity. Various clinical parameters were also examined for clarifying the clinical significance of blood rheology as an identifying factor for metabolic syndrome in young adults.

Subjects and methods

Study population

Japanese young adults (aged 20–29 years), recruited from the local population of Gunma Prefecture, Japan, were sequentially enrolled in this prospective observational study conducted at the Department of Clinical Laboratory Medicine, Gunma University Graduate School of Medicine, Gunma, Japan between October 2011 and September 2014. Participants with fasting plasma glucose ≥ 110 mg/dl or glycosylated haemoglobin (HbA_{1c}) ≥ 6.0 % were excluded. Body mass index (BMI) was calculated as body weight (kg) divided by the square of height (m^2). Insulin resistance was calculated using

homeostasis model assessment-estimated insulin resistance (HOMA-IR), calculated using the following formula:[20]

$$\text{HOMA} - \text{IR} = \text{fasting plasma glucose (mg/dl)} \times \text{fasting serum insulin}(\mu\text{IU/ml})/405$$

Insulin resistance was also calculated using the updated model assessment of insulin resistance (HOMA 2-IR) using the Oxford Diabetes Trials Unit calculator software.[21,22] Insulin sensitivity was calculated using the McAuley insulin resistance index,[23] using the equation:

$$\text{McAuley index} = \exp(2.63 - 0.28\ \ln[\text{basal insulin in mIU/l}] - 0.31\ \ln[\text{basal triglycerides in mmol/l}])$$

This study was approved by the Ethics Committee of Gunma University Graduate School of Medicine (Approval number 10-26), and written informed consent was obtained from all subjects.

Clinical and laboratory examination

In the morning following a 12-h fast, anthropometric measurements were made, and blood samples were collected into three polypropylene tubes for serum and plasma analyses and for whole blood rheology measurements.[14,16–18] Blood samples (2 ml) were obtained by puncture of an antecubital vein using 23-G needles while the subject was in a sitting position. For plasma samples, blood was collected into tubes containing 3.2% sodium citrate anticoagulant, then centrifuged at 1 500 *g* for 15 min at 4°C and analysed immediately following centrifugation. For serum samples, blood was collected and allowed to clot at room temperature for 10 min, then centrifuged at 1 710 *g* for 10 min at 4°C and analysed immediately following centrifugation. Blood samples from all subjects were analysed using the following systems according to the manufacturer's instructions: Hct and Hb levels, WBC count and platelet count were measured using an XE-5000 haematology system (Sysmex, Kobe, Japan); HbA_{1c} levels were determined using an ADAMS™ A1c HA-8180 glycohaemoglobin analyser (ARKRAY, Kyoto, Japan); fasting plasma glucose levels were determined using an ADAMS™ Glucose CA-1170 system (ARKRAY); insulin levels were determined using an AIA-2000 LA automated immunoassay analyser (TOSOH Bioscience, Tokyo, Japan); total cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and triglyceride concentrations were determined using a LABOSPECT 008 automatic analyser (Hitachi, Tokyo, Japan); plasma fibrinogen levels were determined via an automated Clauss assay using Thrombocheck Fib (L) reagent (Sysmex) and Sysmex® CS-5100 system (Sysmex); plasma antithrombin-III and plasminogen activity were determined using a Sysmex® CS-2100i system (Siemens, Erlangen, Germany).[14,16–18]

Blood rheology

For whole blood rheology, blood was collected into tubes containing heparin solution (0.1 ml, 1000 IU/ml) and immediately analysed[14,16–18] by measuring whole blood passage time with a MC-FAN microchannel array flow analyser (Hitachi Haramachi Electronics) as previously reported.[7,8,13–19] Briefly, a 200 μl aliquot of each blood sample (kept between 24–28°C) was introduced into a cylinder connected to the inlet hole of a silicon chip holder using a 1 ml disposable syringe and a thin catheter. The blood sample was allowed to flow through the microchannel array (Bloody 6–7; Hitachi Haramachi Electronics; V-shaped groove width, 7 μm; length, 30 μm; depth, 4.5 μm) by applying a pressure difference of

20 cm of water. The flow rate was determined by recording the times when the meniscus of the sample crossed the graduation marks (10 µl intervals between 0 and 100 µl) on the sample cylinder. Simultaneously, the flow of blood cells through individual microchannels was observed and recorded using an inverted metallographic microscope, video camera and video recorder. The passage time of 100 µl saline was determined before each blood measurement to check the accuracy of the equipment (permissible range, 10–14 s), which was then used to correct the whole blood passage time of 100 µl of whole blood to that expected when the passage time for saline was 12 s. The corrected passage time of whole blood was calculated as (observed passage time of whole blood-$\times$ 12)/observed whole blood passage time of saline. Inter- and intra-assay coefficients of variation for the whole blood passage time were 8% and 5%, respectively.

Statistical analyses

To detect any significant associations using simple linear regression and multiple regression analyses, the present study was determined to require > 150 subjects. Data are presented as mean ± SD. Simple linear regression analysis was used to assess the relationship between whole blood passage time and various factors. Multiple regression analysis was performed to assess the independent predictors of whole blood passage time. All probability values were two-tailed. A P value < 0.05 was considered statistically significant. All statistical analyses were performed using IBM SPSS® software, version 21.0 (IBM Corporation, Armonk, NY, USA).

Results

A total of 179 Japanese young adults were enrolled; 28 were subsequently excluded due to fasting plasma glucose ≥ 110 mg/dl or $HbA_{1c} \geq 6.0$ %. Thus, 151 Japanese young adults without diabetes (mean age, 24.1 ± 1.53 years) were included in the final analyses (Table 1).

Table 1. Demographic and clinical characteristics of 151 Japanese young adults without diabetes.

Characteristic	Value
Male/Female	88/63
Age, years	24.10 ± 1.53
Height, cm	166.51 ± 8.66
Weight, kg	59.09 ± 11.29
BMI, kg/m^2	21.16 ± 2.75
Whole blood passage time, s	39.90 ± 7.72
Total cholesterol, mg/dl	185.99 ± 34.46
HDL-C, mg/dl	65.12 ± 12.96
LDL-C, mg/dl	100.46 ± 31.31
LDL/HDL ratio	1.62 ± 0.67
Triglycerides, mg/dl	73.83 ± 40.84
Fibrinogen, mg/dl	233.69 ± 47.18
Antithrombin III activity, %	106.23 ± 10.04
Plasminogen activity, %	103.29 ± 16.97
White blood cell, $\times 10^3$/µl	5.33 ± 1.51
Haemoglobin, g/dl	14.75 ± 1.46
Haematocrit, %	43.86 ± 3.81
Platelet count, $\times 10^3$/µl	238.49 ± 45.24
Fasting plasma glucose, mg/dl	89.87 ± 6.13
Fasting serum insulin, µlU/ml	5.45 ± 2.81
HOMA-IR	1.22 ± 0.63
HOMA 2-IR	0.72 ± 0.35
McAuley index	5.78 ± 1.38
HbA_{1c}, %	5.33 ± 0.22

Data presented as n prevalence or mean ± SD.
BMI, body mass index; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment-insulin resistance; HOMA 2-IR, updated homeostasis model assessment-insulin resistance; HbA_{1c}, glycosylated haemoglobin.

Association between fasting serum insulin levels and other parameters

Simple linear regression analyses of fasting serum insulin levels and physical and biochemical parameters in Japanese young adults without diabetes are shown in Table 2. Fasting serum insulin levels were positively correlated with whole blood passage time

Table 2. Simple linear regression analyses showing the correlation between fasting serum insulin levels and other parameters in 151 Japanese young adults without diabetes.

	Correlation	
Characteristic	*r*	Statistical significance
Age, years	0.081	NS
Height, cm	0.039	NS
Weight, kg	0.344	$P < 0.001$
BMI, kg/m^2	0.455	$P < 0.001$
Whole blood passage time, s	0.249	$P = 0.002$
Total cholesterol, mg/dl	0.039	NS
HDL-C, mg/dl	−0.212	$P = 0.009$
LDL-C, mg/dl	0.103	NS
LDL/HDL	0.226	$P = 0.005$
Triglycerides, mg/dl	0.333	$P < 0.001$
Fibrinogen, mg/dl	0.222	$P = 0.006$
Antithrombin III activity, %	0.158	NS
Plasminogen activity, %	0.120	NS
White blood cell count, $\times 10^3/\mu l$	0.131	NS
Haemoglobin, g/dl	0.202	$P = 0.013$
Haematocrit, %	0.218	$P = 0.007$
Platelet count, x $10^3/\mu l$	0.141	NS
Fasting plasma glucose, mg/dl	0.160	$P = 0.049$
HbA_{1c}, %	−0.036	NS

BMI, body mass index; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; HbA_{1c}, glycosylated haemoglobin.
NS, no statistically significant correlation ($P > 0.05$).

(Figure 1a), body weight, BMI, LDL-C/HDL-C ratio, triglyceride, fasting plasma glucose, Hct, Hb, and fibrinogen levels. Fasting serum insulin levels were negatively correlated with serum HDL-C levels.

Association between insulin resistance and other parameters

Simple linear regression analyses of estimated insulin resistance (calculated via HOMA-IR), and physical and biochemical parameters in Japanese young adults without diabetes, are shown in Table 3. HOMA-IR was positively correlated with whole blood passage time (Figure 1b), body weight, BMI, LDL-C/HDL-C ratio, triglyceride, Hct, Hb, antithrombin-III activity and fibrinogen levels. HOMA-IR was negatively correlated with HDL-C levels.

In terms of other markers of insulin resistance, simple linear regression analyses of McAuley index and physical and biochemical parameters revealed that McAuley index was negatively correlated with body weight ($r = -0.309$, $P < 0.001$), BMI ($r = -0.341$, $P < 0.001$), LDL-C ($r = -0.171$, $P = 0.035$), LDL-C/HDL-C ratio ($r = -0.315$, $P < 0.001$), Hct ($r = -0.332$, $P < 0.001$), Hb ($r = -0.313$, $P < 0.001$), fibrinogen levels ($r = -0.235$, $P = 0.004$), and whole blood passage time ($r = -0.245$, $P < 0.001$), and was positively correlated with HDL-C levels ($r = 0.287$, $P < 0.001$). Simple linear regression analyses of HOMA 2-IR and physical and biochemical parameters revealed that HOMA 2-IR was positively correlated with body weight ($r = 0.354$, $P < 0.001$), BMI ($r = 0.467$, $P < 0.001$), LDL-C/HDL-C ratio ($r = 0.243$, $P = 0.004$), triglyceride ($r = 0.342$, $P < 0.001$), Hct ($r = 0.233$, $P = 0.004$), Hb ($r = 0.217$, $P = 0.008$), antithrombin-III activity ($r = 0.165$, $P = 0.043$), fibrinogen levels ($r = 0.206$, $P = 0.011$) and whole blood passage time ($r = 0.243$, $P = 0.003$), and was negatively correlated with HDL-C levels ($r = -0.213$, $P = 0.009$).

A strong linear correlation between HOMA-IR and HOMA 2-IR ($r = 0.988$, $P < 0.001$), and HOMA-IR and McAuley index ($r = -0.748$, $P < 0.001$), was detected in the present study population.

Association between whole blood passage time and other parameters

Simple linear regression analyses of whole blood passage time and physical and biochemical parameters are shown in Table 4.

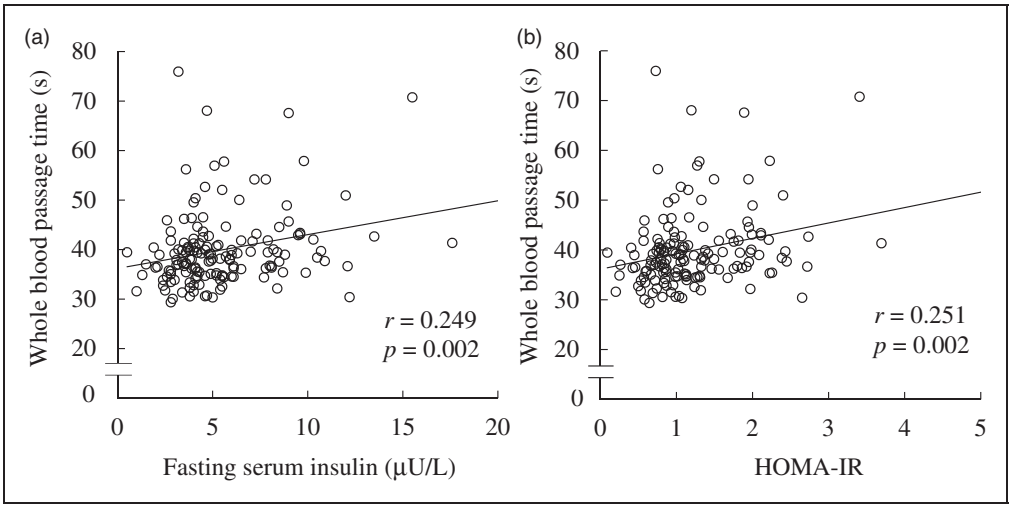

Figure 1. Correlation analyses of blood rheology and insulin parameters in 151 Japanese young adults without diabetes, showing: (a) a positive correlation between fasting serum insulin levels and whole blood passage time ($r = 0.249$; $P = 0.002$); and (b) a positive correlation between insulin resistance estimated by homeostasis model assessment-insulin resistance (HOMA-IR) and whole blood passage time ($r = 0.251$; $P = 0.002$).

Longer whole blood passage times were associated with higher values of height, body weight, BMI, LDL-C/HDL-C ratio, fasting serum insulin, HOMA-IR, HOMA 2-IR, McAuley index, Hct, Hb, plasminogen activity, antithrombin-III activity, fibrinogen levels, platelet count, and WBC count (all $P < 0.05$).

Independent predictors of whole blood passage time

Independent predictors of whole blood passage time were analysed using multiple linear regression (Table 4), and showed that Hct levels, fibrinogen levels and WBC count independently predicted whole blood passage time in Japanese young adults without diabetes.

Discussion

The present study showed that in Japanese young adults without diabetes, fasting serum insulin levels and insulin resistance were correlated with whole blood passage time (a blood rheology parameter). Fasting serum insulin levels and insulin resistance were also found to be positively correlated with fibrinogen levels in this study population, in addition to cardinal parameters of blood rheology, namely Hct, and Hb.

Significant associations have been reported between high Hct values and increased risk of diabetes,[9,24–26] and higher Hct values are shown to decrease blood flow as a result of an increase in whole blood viscosity.[13] Decreased blood flow is thought to lead to insulin resistance by the reduction of glucose delivery to skeletal muscle.[13,25] Blood flow reduction to the pancreatic β-cells, by way of vasoconstriction, has been shown to result in β-cell dysfunction.[24] Increasing evidence supports the finding that insulin levels, insulin resistance, and insulin sensitivity are closely related to haematological parameters such as Hct, Hb and WBC count.[9,10,25,27–30] Higher body weight is also shown to be closely

Table 3. Simple linear regression analyses showing the correlation between insulin resistance, estimated using homeostasis model assessment-insulin resistance (HOMA-IR), and other parameters in 151 Japanese young adults without diabetes.

	Correlation	
Characteristic	*r*	Statistical significance
Age, years	0.080	NS
Height, cm	0.059	NS
Weight, kg	0.353	$P < 0.001$
BMI, kg/m^2	0.453	$P < 0.001$
Whole blood passage time, s	0.251	$P = 0.002$
Total cholesterol, mg/dl	0.025	NS
HDL-C, mg/dl	−0.226	$P = 0.005$
LDL-C, mg/dl	0.092	NS
LDL/HDL	0.225	$P = 0.006$
Triglycerides, mg/dl	0.341	$P < 0.001$
Fibrinogen, mg/dl	0.213	$P = 0.009$
Antithrombin III activity, %	0.165	$P = 0.043$
Plasminogen activity, %	0.118	NS
White blood cell count, $\times 10^3/\mu l$	0.147	NS
Haemoglobin, g/dl	0.226	$P = 0.005$
Haematocrit, %	0.237	$P = 0.003$
Platelet count, $\times 10^3/\mu l$	0.133	NS
HbA_{1c}, %	−0.034	NS

BMI, body mass index; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; HbA_{1c}, glycosylated haemoglobin.
NS, no statistically significant correlation ($P > 0.05$).

related to haematological parameters.[9,25] These data suggest that Hct values are influenced by body weight, BMI, insulin levels and insulin resistance, and also suggest that higher Hct values affect the incidence of diabetes by modulating blood rheology in the skeletal muscle and pancreatic β-cells. The present study further investigated the association of blood rheology with insulin parameters, and showed that longer whole blood passage times were associated with higher values of fasting serum insulin and insulin resistance. Consistent with previous reports,[14–19] values of whole blood passage time in the present study were associated with physical parameters (body weight and BMI), lipid parameters, (HDL-C, LDL/HDL ratio, and triglyceride) and haematological parameters (Hct, Hb, platelet count, and WBC count). It has been suggested that insulin and related substances may stimulate erythropoiesis to increase Hct levels, and that higher Hct levels reduce the rate of glucose delivery to the skeletal muscle by way of decreased blood flow.[13] Other reports proposed that insulin resistance-related hyperinsulinaemia may cause vasoconstriction through sympathetic neural activation, which would raise whole blood viscosity due to an increase in Hct levels.[13,31] In the present study, Hct levels were found to be an independent predictor of whole blood passage time, and Hct levels were associated with fasting serum insulin levels and insulin resistance.

Investigations into the association between insulin sensitivity and blood rheology have indicated that low insulin sensitivity increases RBC aggregation, while hyperinsulinaemia increases plasma viscosity.[32–34] Whole blood viscosity is known to be associated with whole blood passage time, and the present authors have previously proposed a simplified model for estimation of whole blood viscosity from whole blood passage time.[35] Whole blood passage time can be estimated by MC-FAN,[15] and this method differs from rotational viscometer, capillary viscometer and filtration methods, as it enables the viewing of blood flow under a microscope connected to a visual display unit while measuring whole blood passage time.[14,15] The present authors have reported previously that whole blood passage time was correlated with RBC deformability.[14] Although the value of whole blood passage time reflects whole blood viscosity and RBC deformability, this method is not able to evaluate RBC specific aggregation or viscosity. To better understand blood rheology, RBC aggregation and viscosity need to be evaluated together with whole blood passage time.

Table 4. Simple linear regression and multiple regression analyses showing the correlation between whole blood passage time and other parameters in 151 Japanese young adults without diabetes.

	Simple linear regression		Multiple regression	
Characteristic	*R*	Statistical significance	β	Statistical significance
Age, years	−0.011	NS	0.065	NS
Height, cm	0.190	$P = 0.019$	0.110	NS
Weight, kg	0.256	$P = 0.002$	0.138	NS
BMI, kg/m^2	0.215	$P = 0.008$	0.099	NS
Total cholesterol, mg/dl	0.069	NS	−0.036	NS
HDL-C, mg/dl	−0.138	NS	−0.018	NS
LDL-C, mg/dl	0.137	NS	−0.016	NS
LDL/HDL	0.206	$P = 0.011$	0.012	NS
Triglycerides, mg/dl	0.129	NS	−0.039	NS
Fibrinogen, mg/dl	0.301	$P < 0.001$	0.250	$P = 0.001$
Antithrombin III activity, %	0.173	$P = 0.033$	−0.002	NS
Plasminogen activity, %	0.207	$P = 0.011$	0.058	NS
White blood cell count, $\times 10^3/\mu$l	0.347	$P < 0.001$	0.232	$P = 0.002$
Haemoglobin, g/dl	0.360	$P < 0.001$	−0.023	NS
Haematocrit, %	0.379	$P < 0.001$	0.347	$P < 0.001$
Platelet count, $\times 10^3/\mu$l	0.174	$P = 0.033$	0.099	NS
Fasting plasma glucose, mg/dl	0.125	NS	0.048	NS
Fasting serum insulin, μlU/ml	0.249	$P = 0.002$	0.109	NS
HOMA-IR	0.251	$P = 0.002$	0.103	NS
HOMA 2-IR	0.243	$P = 0.002$	0.095	NS
McAuley index	−0.245	$P = 0.002$	−0.046	NS
HbA_{1c}, %	0.012	NS	0.001	NS

BMI, body mass index; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment-insulin resistance; HOMA 2-IR, updated homeostasis model assessment-insulin resistance; HbA_{1c}, glycosylated haemoglobin.
NS, no statistically significant correlation ($P > 0.05$).

In the present study, WBC count was found to be an independent predictor of whole blood passage time. Blood viscosity depends largely upon RBC, WBC and platelet counts,[36,37] and the present authors have previously reported the significant association between whole blood passage time and Hct levels, RBC count, and WBC count.[17] These previously published results concurred with the present findings that Hct levels and WBC count were positively correlated with whole blood passage time. A significant association has been reported between WBC count and insulin resistance in subjects without diabetes.[9] Filtration parameters and membrane fluidity of polymorphonuclear leukocytes are reported to differ between normal subjects and patients with diabetes,[38] however, in the present study, fasting serum insulin levels and insulin resistance were not found to be associated with WBC count. This discrepancy may be explained by differences in study populations, as subjects in the present study were relatively young, with a mean age of 24.1 years, and the mean age of subjects in

one of the published studies was 49.5 years.[9] This hypothesis is supported by previous reports indicating that aging is associated with a subclinical proinflammatory state in healthy older adults.[39,40]

The present study revealed that fasting serum insulin levels, insulin resistance and whole blood passage time were associated with fibrinogen levels, and indicated that fibrinogen is an independent predictor of whole blood passage time. These results are supported by previous studies in which high fibrinogen levels related to the degree of obesity and insulin resistance.[41,42] Insulin resistance is thought to increase fibrinogen levels by raising plasminogen activator inhibitor-I levels,[43] and plasma insulin levels affect plasminogen activator inhibitor-I release.[44] Plasma viscosity and RBC aggregation are directly influenced by plasma fibrinogen.[45–47] Increased plasma fibrinogen levels have a direct and significant effect on blood rheology.[12] These published reports and the present findings suggest that insulin levels and insulin resistance indirectly influence blood rheology by affecting plasma fibrinogen levels.

In the present 151 Japanese young adults without diabetes, insulin resistance, but not fasting serum insulin levels, weakly related to antithrombin-III activity. Recent studies have suggested a new relationship between insulin and antithrombin-III: Binding of insulin to the plasma protein antithrombin-III via coupling to a pentasaccharide, enhances the half-life of insulin,[48,49] and insulin resistance may influence antithrombin-III, although the relationship between antithrombin-III activity and insulin resistance remains controversial.[50,51]

As HOMA-IR is a surrogate measure of insulin resistance, and has an updated version (HOMA 2-IR), HOMA 2-IR and the McAuley index were also used to assess insulin resistance in the present study. HOMA-IR was strongly correlated with HOMA 2-IR and the McAuley index, and these values of insulin resistance were correlated with whole blood passage time as well as cardinal parameters of blood rheology. Although HOMA-IR is a surrogate measure, HOMA-IR reflects insulin resistance in young adults without diabetes.

The present cross-sectional study included a relatively small number of participants and was performed in a single centre. A prospective multicentre study, including a larger sample size, is necessary to confirm the hypothesis of the present study, and the importance of monitoring physical and haematological parameters as well as insulin levels and insulin resistance to predict the occurrence of metabolic diseases in young adults without diabetes.

In conclusion, the present study showed that fasting serum insulin levels and insulin resistance were significantly associated with haematological parameters and blood rheology in Japanese young adults without diabetes. Insulin levels and insulin resistance affect blood rheology by modulating haematological parameters and fibrinogen levels, in addition to lipid parameters. These results suggest the importance of monitoring physical and haematological parameters to predict the occurrence of metabolic disorders.

Acknowledgements

The authors are grateful to Mayumi Nishiyama for technical assistance.

Declaration of conflicting interests

The authors declare that there is no conflict of interest.

Funding

This work was supported, in part, by Grants-in-Aid 23390146, 26293125 (to M. Murakami) and 26460641 (to T. Kimura) for scientific research

from the Ministry of Education, Culture, Sports, Science, and Technology of Japan.

References

1. Olshansky SJ, Passaro DJ, Hershow RC, et al. A potential decline in life expectancy in the United States in the 21st century. *N Engl J Med* 2005; 352: 1138–1145.
2. Poyrazoglu S, Bas F and Darendeliler F. Metabolic syndrome in young people. *Curr Opin Endocrinol Diabetes Obes* 2014; 21: 56–63.
3. Rask-Madsen C and Kahn CR. Tissue-specific insulin signaling, metabolic syndrome, and cardiovascular disease. *Arterioscler Thromb Vasc Biol* 2012; 32: 2052–2059.
4. Reaven GM. The metabolic syndrome: time to get off the merry-go-round? *J Intern Med* 2011; 269: 127–136.
5. Letcher RL, Chien S, Pickering TG, et al. Elevated blood viscosity in patients with borderline essential hypertension. *Hypertension* 1983; 5: 757–762.
6. Seplowitz AH, Chien S and Smith FR. Effects of lipoproteins on plasma viscosity. *Atherosclerosis* 1981; 38: 89–95.
7. Matsuo K, Ueda Y, Nishio M, et al. Thrombogenic potential of whole blood is higher in patients with acute coronary syndrome than in patients with stable coronary diseases. *Thromb Res* 2011; 128: 268–273.
8. Tzoulaki I, Murray GD, Lee AJ, et al. Relative value of inflammatory, hemostatic, and rheological factors for incident myocardial infarction and stroke: the Edinburgh Artery Study. *Circulation* 2007; 115: 2119–2127.
9. Hanley AJ, Retnakaran R, Qi Y, et al. Association of hematological parameters with insulin resistance and beta-cell dysfunction in nondiabetic subjects. *J Clin Endocrinol Metab* 2009; 94: 3824–3832.
10. Medalie JH, Papier CM, Goldbourt U, et al. Major factors in the development of diabetes mellitus in 10,000 men. *Arch Intern Med* 1975; 135: 811–817.
11. Salazar MR, Carbajal HA, Espeche WG, et al. Relationships among insulin resistance, obesity, diagnosis of the metabolic syndrome and cardio-metabolic risk. *Diab Vasc Dis Res* 2011; 8: 109–116.
12. Simmonds MJ, Meiselman HJ and Baskurt OK. Blood rheology and aging. *J Geriatr Cardiol* 2013; 10: 291–301.
13. Moan A, Nordby G, Os I, et al. Relationship between hemorrheologic factors and insulin sensitivity in healthy young men. *Metabolism* 1994; 43: 423–427.
14. Nara M, Sumino H, Nara M, et al. Impaired blood rheology and elevated remnant-like lipoprotein particle cholesterol in hypercholesterolaemic subjects. *J Int Med Res* 2009; 37: 308–317.
15. Kikuchi Y, Sato K and Mizuguchi Y. Modified cell-flow microchannels in a single-crystal silicon substrate and flow behavior of blood cells. *Microvasc Res* 1994; 47: 126–139.
16. Machida T, Sumino H, Fukushima M, et al. Blood rheology and the low-density lipoprotein cholesterol/high-density lipoprotein cholesterol ratio in dyslipidaemic and normolipidaemic subjects. *J Int Med Res* 2010; 38: 1975–1984.
17. Seki K, Sumino H, Nara M, et al. Relationships between blood rheology and age, body mass index, blood cell count, fibrinogen, and lipids in healthy subjects. *Clin Hemorheol Microcirc* 2006; 34: 401–410.
18. Sumino H, Nara M, Seki K, et al. Effect of antihypertensive therapy on blood rheology in patients with essential hypertension. *J Int Med Res* 2005; 33: 170–177.
19. Kurihara T, Deguchi S, Kato J, et al. Impaired blood rheology by remnant-like lipoprotein particles: studies in patients with fatty liver disease. *Clin Hemorheol Microcirc* 2001; 24: 217–225.
20. Matthews DR, Hosker JP, Rudenski AS, et al. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985; 28: 412–419.
21. Levy JC, Matthews DR and Hermans MP. Correct homeostasis model assessment (HOMA) evaluation uses the computer program. *Diabetes Care* 1998; 21: 2191–2192.
22. Wallace TM, Levy JC and Matthews DR. Use and abuse of HOMA modeling. *Diabetes Care* 2004; 27: 1487–1495.

23. McAuley KA, Williams SM, Mann JI, et al. Diagnosing insulin resistance in the general population. *Diabetes Care* 2001; 24: 460–464.
24. Annerén C, Welsh M and Jansson L. Glucose intolerance and reduced islet blood flow in transgenic mice expressing the FRK tyrosine kinase under the control of the rat insulin promoter. *Am J Physiol Endocrinol Metab* 2007; 292: E1183–E1190.
25. Barazzoni R, Gortan Cappellari G, Semolic A, et al. The association between hematological parameters and insulin resistance is modified by body mass index - results from the North-East Italy MoMa population study. *PLoS One* 2014; 9: e101590.
26. Facchini FS, Carantoni M, Jeppesen J, et al. Hematocrit and hemoglobin are independently related to insulin resistance and compensatory hyperinsulinemia in healthy, non-obese men and women. *Metabolism* 1998; 47: 831–835.
27. Nakanishi N, Suzuki K and Tatara K. Haematocrit and risk of development of type 2 diabetes mellitus in middle-aged Japanese men. *Diabet Med* 2004; 21: 476–482.
28. Tulloch-Reid MK, Hanson RL, Saremi A, et al. Hematocrit and the incidence of type 2 diabetes in the pima Indians. *Diabetes Care* 2004; 27: 2245–2246.
29. Wannamethee SG, Perry IJ and Shaper AG. Hematocrit and risk of NIDDM. *Diabetes* 1996; 45: 576–579.
30. Wilson PW, McGee DL and Kannel WB. Obesity, very low density lipoproteins, and glucose intolerance over fourteen years: the Framingham study. *Am J Epidemiol* 1981; 114: 697–704.
31. Catalano C, Muscelli E, Natali A, et al. Reciprocal association between insulin sensitivity and the haematocrit in man. *Eur J Clin Invest* 1997; 27: 634–637.
32. Aloulou I, Varlet-Marie E, Mercier J, et al. The hemorheological aspects of the metabolic syndrome are a combination of separate effects of insulin resistance, hyperinsulinemia and adiposity. *Clin Hemorheol Microcirc* 2006; 35: 113–119.
33. Brun JF, Varlet-Marie E, Raynaud de Mauverger E, et al. Minimal model-derived insulin sensitivity, insulin secretion and glucose tolerance: relationships with blood rheology. *Clin Hemorheol Microcirc* 2012; 51: 21–27.
34. Brun JF, Varlet-Marie E and Raynaud de Mauverger E. Relationships between insulin sensitivity measured with the oral minimal model and blood rheology. *Clin Hemorheol Microcirc* 2012; 51: 29–34.
35. Maki Y, Endo Y, Fukushima M, et al. Estimation of viscosity from passage time of liquids flowing through a microchannel array. *J Biorheol* 2013; 26: 69–73.
36. Koenig W and Ernst E. The possible role of hemorheology in atherothrombogenesis. *Atherosclerosis* 1992; 94: 93–107.
37. Turitto VT and Weiss HJ. Platelet and red cell involvement in mural thrombogenesis. *Ann N Y Acad Sci* 1983; 416: 363–376.
38. LoPresti R, Montana M, Canino B, et al. Diabetes mellitus: polymorphonuclear leukocyte (PMN) filtration parameters and PMN membrane fluidity after chemotactic activation. *Metabolism* 1999; 48: 30–33.
39. Anuurad E, Enkhmaa B, Gungor Z, et al. Age as a modulator of inflammatory cardiovascular risk factors. *Arterioscler Thromb Vasc Biol* 2011; 31: 2151–2156.
40. Ferrucci L, Corsi A, Lauretani F, et al. The origins of age-related proinflammatory state. *Blood* 2005; 105: 2294–2299.
41. Caballero AE, Bousquet-Santos K, Robles-Osorio L, et al. Overweight Latino children and adolescents have marked endothelial dysfunction and subclinical vascular inflammation in association with excess body fat and insulin resistance. *Diabetes Care* 2008; 31: 576–582.
42. Giordano P, Del Vecchio GC, Cecinati V, et al. Metabolic, inflammatory, endothelial and haemostatic markers in a group of Italian obese children and adolescents. *Eur J Pediatr* 2011; 170: 845–850.
43. Juhan-Vague I, Thompson SG and Jespersen J. Involvement of the hemostatic system in the insulin resistance syndrome. A study of 1500 patients with angina pectoris. The ECAT angina pectoris study group. *Arterioscler Thromb* 1993; 13: 1865–1873.
44. Festa A, D'Agostino R Jr., Mykkänen L, et al. Relative contribution of insulin and its precursors to fibrinogen and PAI-1 in a large population with different states of glucose

tolerance. The Insulin Resistance Atherosclerosis Study (IRAS). *Arterioscler Thromb Vasc Biol* 1999; 19: 562–568.
45. Avellone G, Garbo D, Panno AV, et al. Haemorheological components in the pre-geriatric and geriatric age range in a randomly selected western Sicily population sample (Casteldaccia study). *Clin Hemorheol Microcirc* 1993; 13: 83–92.
46. Christy RM, Baskurt OK, Gass GC, et al. Erythrocyte aggregation and neutrophil function in an aging population. *Gerontology* 2010; 56: 175–180.
47. Feher G, Koltai K, Kesmarky G, et al. Hemorheological parameters and aging. *Clin Hemorheol Microcirc* 2006; 35: 89–98.
48. Miltenburg AM, Prohn M, van Kuijk JH, et al. Half-life prolongation of therapeutic proteins by conjugation to ATIII-binding pentasaccharides: a first-in-human study of CarboCarrier® insulin. *Br J Clin Pharmacol* 2013; 75: 1221–1230.
49. de Kort M, Gianotten B, Wisse JA, et al. Conjugation of ATIII-binding pentasaccharides to extend the half-life of proteins: long-acting insulin. *Chem Med Chem* 2008; 3: 1189–1193.
50. Ozkul A, Turgut ET, Akyol A, et al. The relationship between insulin resistance and hypercoagulability in acute ischemic stroke. *Eur Neurol* 2010; 64: 201–206.
51. Ragab A, Abousamra NK, Higazy A, et al. Relationship between insulin resistance and some coagulation and fibrinolytic parameters in patients with metabolic syndrome. *Lab Hematol* 2008; 14: 1–6.