The association of inflammatory and fibrinolytic proteins with 5 year change in insulin clearance: the Insulin Resistance Atherosclerosis Study (IRAS)

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Abstract

Aims/hypothesis Insulin clearance may decline as an early mechanism compensating for deteriorating insulin sensitivity. However, no previous studies have investigated the association between subclinical inflammation or impaired fibrinolysis and insulin clearance. We examined the association between plasminogen activator inhibitor (PAI)-1, C-reactive protein (CRP), TNF- α , leptin and fibrinogen and the progression of metabolic clearance rate of insulin (MCRI) over time.

Methods We studied 784 non-diabetic white, Hispanic and African-American individuals in the Insulin Resistance Atherosclerosis Study (IRAS). Insulin sensitivity, acute insulin response and MCRI were determined from frequently sampled intravenous glucose tolerance tests at baseline and at 5-year follow-up. Inflammatory and fibrinolytic proteins were measured in fasting plasma at baseline.

Results MCRI had declined significantly by 29% at the 5-year follow-up. We observed a significant association between higher plasma PAI-1 levels and the decline in MCRI in

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multivariable-adjusted regression models ($\beta = -0.045$ [95% CI $-0.081, -0.0091$]). Higher plasma CRP and leptin levels were associated with a decline in MCRI in unadjusted models, but these associations were non-significant after adjusting for BMI and waist circumference ($\beta = -0.016$ [95% CI $-0.041, 0.0083$] for CRP; $\beta = -0.044$ [95% CI $-0.10, 0.011$] for leptin). A higher plasma TNF- α concentration was associated with a decline in MCRI in unadjusted ($\beta = -0.071$ [95% CI $-0.14, -0.00087$]) but not in multivariable-adjusted ($\beta = -0.056$ [95% CI $-0.13, 0.017$]) models. Plasma fibrinogen level was not associated with the change in MCRI.

Conclusions/interpretation We identified that higher plasma PAI-1 (but not CRP, TNF- α , leptin or fibrinogen) levels independently predicted the progressive decline of insulin clearance in the multiethnic cohort of the IRAS.

Keywords C-reactive protein · Fibrinogen · Leptin · Metabolic clearance of insulin · Plasminogen activator inhibitor-1 · TNF- α

Abbreviations

AIR	Acute insulin response
ALT	Alanine aminotransferase
CRP	C-reactive protein
FSIGTT	Frequently sampled intravenous glucose tolerance test
IRAS	Insulin Resistance Atherosclerosis Study
MCRI	Metabolic clearance rate of insulin
NHANES	National Health and Nutrition Examination Survey
PAI-1	Plasminogen activator inhibitor-1
S _I	Insulin sensitivity

Introduction

Insulin resistance and beta cell dysfunction have long been recognised as critical pathophysiological disorders underlying type 2 diabetes. Risk factors for these important metabolic traits have also been extensively researched [1, 2]. Insulin clearance is an integrated part of the metabolism of insulin, and reduced insulin clearance serves as an adaptive mechanism in response to insulin resistance to preserve beta cell function in impaired glucose tolerance states [3]. However, the determinants of reduced insulin clearance have not been well studied.

It is well established that type 2 diabetes is associated with low-grade inflammation involving a cytokine-mediated acute-phase response and other inflammatory processes related to obesity [4]. In addition, impaired fibrinolysis has been linked to type 2 diabetes [5]. Previous prospective studies have demonstrated that inflammatory and fibrinolytic proteins predict incident diabetes [6–8]. The secretion, sensitivity and

clearance of insulin are closely related physiological processes. Subclinical inflammation and impaired fibrinolysis may act through insulin resistance [9–11] and possibly beta cell dysfunction to impact on insulin clearance, hence increasing the risk of diabetes.

To our knowledge, no studies have investigated the association of subclinical inflammation and impaired fibrinolysis to insulin clearance. Exploring these relationships is crucial in order to gain insight into potential pathophysiological mechanisms for the development of diabetes. We therefore aimed to examine the association between inflammatory and fibrinolytic proteins at baseline, including plasminogen activator inhibitor (PAI)-1, C-reactive protein (CRP), TNF- α , leptin and fibrinogen, and the change in metabolic clearance rate of insulin (MCRI) at 5-year follow-up in the Insulin Resistance Atherosclerosis Study (IRAS).

Methods

Study population The study population comprised participants in the IRAS, a multicentre epidemiological study designed to investigate the relationships between insulin resistance and atherosclerosis, as well as known risk factors for these conditions, among non-Hispanic white, Hispanic and African-American individuals. Between October 1992 and April 1994, IRAS recruited 1,625 participants from four clinical centres located in San Antonio, TX, San Luis Valley, CO, Oakland, CA and Los Angeles, CA, USA. The recruitment strategy aimed to identify sufficient numbers of participants in each ethnic, age, sex and glucose tolerance group to allow for comparison between and within these groups. Although the IRAS investigators purposely oversampled individuals with impaired glucose tolerance, 44% of the study population had normal glucose tolerance at baseline. In addition, the fasting blood glucose levels in the IRAS were comparable to those in the second National Health and Nutrition Examination Survey (NHANES II) in participants with normal or impaired glucose tolerance [12]. The institutional review boards at each site approved the study protocol, and all participants provided written informed consent.

The current analysis included 784 non-diabetic participants who attended both the baseline and follow-up examinations. Participants with prevalent diabetes at baseline ($n = 537$) and missing data on MCRI from either examination ($n = 304$) were excluded from the study. Among the 304 participants without MCRI data, 191 did not attend the follow-up examinations. MCRI was not determined at baseline or follow-up examination in the remaining 113 participants due to technical issues. The characteristics of these 304 participants at baseline were similar to those with data on MCRI in term of age, sex, ethnicity, BMI and glucose tolerance status.

Clinical measurements and procedures The IRAS protocol required two visits, 1 week apart. Both baseline and follow-up examinations were conducted in the morning using identical procedures. Participants were asked before each visit to fast for 12 h, to abstain from alcohol and heavy exercise for 24 h, and to abstain from smoking on the morning of the examination. The duration of fasting was recorded and confirmed by asking ‘What time and date did you last eat and/or drink anything other than water, including candy and chewing gum?’ No participants were excluded from the study because they did not fast properly.

During the first visit, a 75-g OGTT was administered. During the second visit, insulin sensitivity (S_I), acute insulin response (AIR) and MCRI were determined from a frequently sampled intravenous glucose tolerance test (FSIGTT), with two modifications to the original protocol [13]. First, insulin, instead of tolbutamide, was injected to ensure adequate levels of plasma insulin to calculate S_I accurately across a broad range of glucose tolerance [14]. Second, a reduced sampling protocol, using 12 instead of 30 samples, was followed for pragmatic reasons [15]. Insulin resistance, expressed as S_I , was calculated using minimal model analysis. Insulin secretion was measured by AIR, defined as the average increase in plasma insulin at time points 2 and 4 min after infusing glucose [16]. MCRI was calculated as the ratio of the insulin dose to the incremental area under the curve of insulin from 20 min to infinity [17] using the following equation:

$$\text{Clearance (l/min)} = \frac{\text{Dose} \times 1000}{\int_{t=20}^{\infty} (\text{Ins}(t) - \text{Ins}(0))}$$

where *Dose* represents the amount of insulin injected at 20 min, *Ins*(*t*) the plasma insulin concentration at each FSIGTT sampling point, and *Ins*(0) the fasting plasma insulin concentration determined before injecting glucose during the FSIGTT.

Anthropometric measurements (height, weight and waist circumference) and resting blood pressure were taken. BMI was calculated as weight in kilograms divided by the square of the height in metres. All measurements were taken in duplicate following standardised procedures, and the averages of these measurements were used in the analyses. Participants provided information on demographics (age, sex and ethnicity), lifestyle factors (smoking, alcohol consumption and energy expenditure) and family history of diabetes in validated questionnaires by self-report [12].

Biochemical analysis Participants provided a fasting blood draw at each examination. Plasma glucose was measured using the glucose oxidase technique on an autoanalyser (Yellow Springs Equipment, Yellow Springs, Ohio, USA).

Plasma samples to be used for the measurement of inflammatory and fibrinolytic proteins were prepared, frozen and stored at -70°C at the study centres within 90 min of the blood draw. Frozen samples were shipped on a monthly basis to the Laboratory for Clinical Biochemistry Research at the University of Vermont for measurement [6].

PAI-1 was determined in citrated plasma using a two-site immunoassay that was sensitive to free PAI-1, but not to PAI-1 complexed with tissue plasminogen activator, with a CV of 14.0% [18]. Plasma CRP was measured by an in-house ultra-sensitive competitive immunoassay, with an interassay CV of 8.9% [19]. Following the original method of Clauss [20], fibrinogen was measured in citrated plasma with a modified clot-rate assay using the Stago ST4 instrument (Diagnostica, Parsippany, NJ, USA), with a CV of 3.0% [21]. Plasma leptin was measured using radioimmunoassay, with an interassay CV of 7.0% [22]. TNF- α was measured in citrated plasma using a Quantikine HS Human TNF- α immunoassay (R&D Systems, Minneapolis, MN, USA) [23]. Plasma lipids and lipoproteins, including triacylglycerol and HDL-cholesterol, were determined using methods from the Lipid Research Clinics [24]. Alanine aminotransferase (ALT) was determined by enzymatic colorimetric assay [25].

Statistical analysis We summarised the characteristics of participants stratified by thirds of the MCRI distribution at baseline, using median and interquartile range for continuous variables, and percentages for categorical variables. We used ANOVA, Kruskal–Wallis tests and χ^2 tests to determine whether continuous and categorical variables differed across the distribution of MCRI.

Since the distributions of PAI-1, CRP, TNF- α , S_I , AIR and MCRI (at baseline and at follow-up) were skewed, we transformed them using $\log_e$ for normality. Because of the presence of zero values for S_I in the data, we added a constant of 1 to all values before log-transformation. We used unadjusted and multivariable linear regression to explore the association between PAI-1, CRP, TNF- α , leptin and fibrinogen at baseline as continuous exposures, and the change in MCRI between two examinations as continuous outcomes. In regression models, we modelled MCRI at follow-up as the continuous outcome, and adjusted for MCRI at baseline simultaneously to determine the estimates for the change in MCRI between two examinations. We presented the regression coefficients (β) with their 95% CIs.

We included covariates in multivariable models if they were associated with both the exposure and the outcome, or if they were of a priori clinical relevance. Potential confounders included age, sex, ethnicity, smoking, alcohol consumption, energy expenditure, family history of diabetes, BMI, waist circumference, HDL-cholesterol, triacylglycerol, ALT, fasting blood glucose, S_I and AIR. Multivariable-adjusted regression models were based on 784 participants with data

on MCRI at baseline and at follow-up; sample sizes in full multivariate models ranged from 750 to 784 due to occasional missing values on exposures and covariates. We examined the interaction of each of the inflammatory and fibrinolytic proteins with age, sex, ethnicity, BMI, S_I , AIR and glycaemic status at baseline on the difference in MCRI between examinations. We plotted the frequency distribution of the yearly change in MCRI from baseline to 5-year follow-up.

In sensitivity analyses, we additionally adjusted for the use of glucose-lowering medications and incident diabetes to determine whether these variables were confounders. To test the robustness of the association between plasma PAI-1 and the change in MCRI, we also adjusted for plasma CRP in this multivariable-adjusted regression model. Statistical analyses were performed using STATA 12.0 (StataCorp, College Station, TX, USA).

Results

The current study included 784 non-diabetic individuals who had measurements of MCRI at both baseline and follow-up examinations in the IRAS cohort. Participants had an average age of 55 years (interquartile range 47–62 years), and 45% were women. The ethnic distribution values for non-Hispanic white, African-American and Hispanic individuals were 40%, 25% and 34%, respectively. The median MCRI was 5.28 l/min (interquartile range 3.92–7.10 l/min) at baseline.

Characteristics of the study population by thirds of the distribution of MCRI at baseline are shown in Table 1. Demographics, for example age, sex and lifestyle factors such as alcohol consumption and smoking, were similar between thirds of the MCRI distribution. Participants with an MCRI in the bottom third of the baseline distribution, i.e. those with the lowest insulin clearance, were, compared with those in the top third, more likely to be Hispanic, have a family history of diabetes and have a lower energy expenditure. In addition, these participants had higher values of BMI, waist circumference, plasma triacylglycerol, systolic blood pressure, fasting blood glucose and AIR, but lower values of S_I and HDL-cholesterol. At baseline, the median levels of plasma inflammatory and fibrinolytic proteins were 327 pmol/l for PAI-1, 16.2 nmol/l for CRP, 3.4 ng/l for TNF- α , 7.9 μ mol/l for fibrinogen and 12.7 μ g/l for leptin.

The median duration of follow-up was 5.2 years (interquartile range 5.0–5.4 years). MCRI declined by 29% between the baseline and 5-year follow-up examinations ($p<0.001$). The median decline of MCRI per year was 0.28 l/min (interquartile range 0.55–0.066 l/min). More than 50% of the participants had a yearly decline in MCRI in the range 0.01–0.50 l/min (Fig. 1). At follow-up, 124 participants were found to have a diagnosis of type 2 diabetes.

Among these participants, 49 were taking glucose-lowering medications. Values of waist circumference and BMI were significantly higher, whereas values of S_I were significantly lower. In addition, participants reported smoking less and having lower energy expenditure.

Higher PAI-1 values were associated with a decline in MCRI in unadjusted regression models ($\beta=-0.060$ [95% CI $-0.092, -0.028$]; $p<0.001$). The association remained significant after adjusting for age, sex, ethnicity, alcohol consumption, smoking, energy expenditure, family history of diabetes, HDL-cholesterol, triacylglycerol, ALT, BMI, waist circumference, fasting blood glucose, S_I and AIR ($\beta=-0.045$ [95% CI $-0.081, -0.0091$]; $p=0.014$). In unadjusted linear regression analysis, higher plasma CRP levels were associated with a significant decline in MCRI ($\beta=-0.035$ [95% CI $-0.059, -0.011$]; $p=0.004$). The association remained significant after adjusting for demographic factors ($\beta=-0.046$ [95% CI $-0.071, -0.021$]; $p<0.001$). However, additional adjustment for BMI and waist circumference attenuated the association to non-significance ($\beta=-0.016$ [$-0.041, 0.0083$]). Similarly, we observed a significant association between increased plasma leptin and decline in MCRI in an unadjusted linear regression model ($\beta=-0.035$ [95% CI $-0.066, -0.0041$]; $p=0.026$) and after adjusting for demographic characteristics ($\beta=-0.15$ [95% CI $-0.19, -0.099$]; $p<0.001$). The association was no longer statistically significant after adjusting for BMI and waist circumference ($\beta=-0.044$ [95% CI $-0.10, 0.011$]).

Higher plasma TNF- α was associated with a decline of MCRI in unadjusted regression models ($\beta=-0.071$ [95% CI $-0.14, -0.00087$]; $p=0.047$) and in models adjusted for age, sex and ethnicity ($\beta=-0.12$ [95% CI $-0.20, -0.048$]; $p=0.001$). Adjusting for BMI and waist circumference attenuated the association, but it remained statistically significant ($\beta=-0.085$ [95% CI $-0.16, -0.014$]; $p=0.020$). However, the association was not significant after further adjustment for HDL-cholesterol, triacylglycerol and ALT ($\beta=-0.056$ [95% CI $-0.13, 0.016$]). Fibrinogen was not associated with the change in MCRI (Table 2). We did not find significant interactions between any of the inflammatory proteins and age, sex, ethnicity, BMI, S_I , AIR or glycaemic status at baseline in terms of the change in MCRI over time. In sensitivity analysis, including the use of glucose-lowering medications and incident diabetes, or plasma CRP, in the multivariable-adjusted regression models did not materially change the significant association between plasma PAI and the decline in MCRI.

Discussion

In non-diabetic individuals participating in the IRAS, higher plasma PAI-1 level at baseline was associated with lower MCRI at 5-year follow-up. The significant association

Table 1 Characteristics of non-diabetic participants in the IRAS, stratified by the distribution of MCRI at baseline

Variable	<i>n</i>	Bottom third	Middle third	Top third	<i>p</i> value
Metabolic clearance of insulin (l/min)	784	3.47 (2.79, 3.92)	5.28 (4.79, 5.71)	8.01 (7.11, 9.76)	
Age (years)	784	54 (46, 61)	55 (48, 63)	55 (49, 63)	0.23
Sex (% female)	784	53.8	57.5	53.6	0.61
Ethnicity (%)					
Non-Hispanic white		32.1	38.7	49.8	
African-American	784	25.6	29.5	21.1	<0.001
Hispanic		42.4	31.8	29.1	
Family history of diabetes (%)	784	47.0	42.2	31.4	0.001
Current drinker (%)	784	74.4	72.7	74.0	0.79
Current smoker (%)	784	11.8	13.8	14.6	0.90
Total energy expenditure (kJ kg ⁻¹ day ⁻¹)	780	158 (150, 177)	161 (149, 181)	164 (152, 186)	0.026
BMI (kg/m ²)	783	30.1 (27.7, 35.9)	27.2 (25.2, 29.6)	25.2 (23.2, 27.4)	<0.0001
Waist circumference (cm)	782	97.7 (90.0, 104.5)	89.1 (80.9, 95.3)	84.3 (76.0, 91.0)	<0.0001
Triacylglycerol (mmol/l)	781	1.55 (1.10, 2.08)	1.15 (0.84, 1.76)	1.08 (0.82, 1.59)	<0.0001
HDL-cholesterol (mmol/l)	782	1.01 (0.85, 1.24)	1.17 (0.95, 1.37)	1.27 (1.06, 1.58)	<0.0001
Systolic blood pressure (mmHg)	784	121 (111, 135)	119 (109, 131)	118 (108, 130)	0.015
Fasting blood glucose (mmol/l)	781	5.49 (5.16, 5.99)	5.38 (5.05, 5.77)	5.22 (4.94, 5.72)	<0.0001
AIR (pmol/l)	784	461 (248, 734)	308 (153, 493)	176 (99.3, 317)	<0.0001
S _I × 10 ⁻⁴ min ⁻¹ (pmol/l) ⁻¹	766	0.14 (0.072, 0.20)	0.28 (0.20, 0.39)	0.57 (0.39, 0.80)	<0.0001
CRP (nmol/l)	783	23.1 (13.4, 53.2)	15.8 (6.67, 25.8)	10.2 (5.43, 21.0)	<0.0001
Fibrinogen (μmol/l)	780	8.14 (7.32, 9.32)	7.94 (7.03, 8.99)	7.70 (6.79, 8.58)	<0.0001
Leptin (Cg/l)	750	20.3 (9.0, 33.2)	13.2 (6.2, 23.9)	8.0 (4.2, 16.6)	<0.0001
PAI-1 (pmol/l)	779	462 (288, 692)	327 (173, 500)	250 (154, 365)	<0.0001
TNF-α (ng/l)	783	3.79 (2.90, 4.73)	3.32 (2.50, 4.27)	3.21 (2.55, 4.03)	<0.0001
ALT (μkat/l)	781	1176 (824, 1647)	882 (647, 1176)	834 (529, 1118)	<0.0001
Impaired glucose tolerance (%)	784	43.1	31.4	23.4	<0.0001

Data are medians (interquartile range) or *n* (%)

remained after adjusting for MCRI at baseline, demographic factors, lifestyle factors and established risk factors for diabetes, including BMI and directly measured S_I. We observed an inverse association of plasma CRP and leptin at baseline with MCRI at follow-up, but these associations

Fig. 1 Frequency distribution of the yearly change in MCRI (l/min) between baseline and 5 year follow-up examination. Categories for the yearly change in MCRI (l/min): 1, minimum to -2.00; 2, 1.99 to -1.50; 3, 1.49 to -1.00; 4, -0.99 to -0.50; 5, -0.49 to 0; 6, 0.01 to 0.50; 7, 0.51 to maximum

were confounded by indexes of obesity and abdominal adiposity, i.e. BMI and waist circumference. We also observed that the inverse association between plasma TNF-α at baseline and the MCRI at follow-up was confounded by HDL-cholesterol, triacylglycerol and ALT values. Plasma fibrinogen was not associated with the change in MCRI. To our knowledge, this is the first study to investigate the association of plasma inflammatory and fibrinolytic proteins with the dynamic change of MCRI over time.

It is well established that higher plasma PAI-1 is a central feature of metabolic syndrome [26], and higher levels prevail through the continuum of insulin resistance, from impaired glucose tolerance to diabetes [27]. Previous observations from the IRAS cohort showed that both plasma PAI-1 at baseline and the progression of plasma PAI-1 over time predicted incident type 2 diabetes, independent of known risk factors for diabetes [6, 28]. Pharmaceutical and lifestyle interventions to treat diabetes, such as dietary modification and weight reduction [29], metformin [30], thiazolidinedione [31] and insulin [32] have been shown to decrease plasma PAI-1. In this study, however, when we

Table 2 Estimated regression coefficients (95% CI) for the association between plasma inflammatory proteins at baseline and at 5-year follow-up in the IRAS

Outcome: follow-up $\log_e$ (MCRI)	β coefficient (95% CI)	<i>p</i> value
$\log_e$(CRP) (<i>n</i>=783)		
Adjusted for baseline $\log_e$ (MCRI)	−0.035 (−0.059, −0.011)	0.004
+age, sex, ethnicity	−0.046 (−0.071, −0.021)	<0.001
+BMI, waist circumference	−0.016 (−0.041, 0.0083)	0.193
+smoking, alcohol consumption, energy expenditure, family history of diabetes	−0.015 (−0.040, 0.0098)	0.234
+HDL, triacylglycerol, ALT	−0.017 (−0.042, 0.0076)	0.174
+Fasting blood glucose, S_I , AIR	−0.013 (−0.038, 0.012)	0.311
$\log_e$(PAI-1) (<i>n</i>=779)		
Adjusted for baseline $\log_e$ (MCRI)	−0.060 (−0.092, −0.028)	<0.001
+age, sex, ethnicity	−0.096 (−0.13, −0.062)	<0.001
+BMI, waist circumference	−0.060 (−0.094, −0.026)	0.001
+smoking, alcohol consumption, energy expenditure, family history of diabetes	−0.057 (−0.092, −0.022)	0.001
+HDL, triacylglycerol, ALT	−0.047 (−0.083, −0.012)	0.009
+Fasting blood glucose, S_I , AIR	−0.045 (−0.081, −0.091)	0.014
Fibrinogen (<i>n</i>=780)		
Adjusted for baseline $\log_e$ (MCRI)	−0.00040 (−0.00088, 0.000075)	0.099
+age, sex, ethnicity	−0.00046 (−0.00095, 0.000028)	0.065
+BMI, waist circumference	0.000040 (−0.00044, 0.00052)	0.870
+smoking, alcohol consumption, energy expenditure, family history of diabetes	0.000077 (−0.00041, 0.00057)	0.758
+HDL, triacylglycerol, ALT	0.000066 (−0.00042, 0.00055)	0.791
+Fasting blood glucose, S_I , AIR	0.000043 (−0.0044, 0.00053)	0.861
$\log_e$(leptin) (<i>n</i>=750)		
Adjusted for baseline $\log_e$ (MCRI)	−0.035 (−0.066, −0.0044)	0.026
+age, sex, ethnicity	−0.15 (−0.19, −0.099)	<0.001
+BMI, waist circumference	−0.044 (−0.10, 0.011)	0.115
+smoking, alcohol consumption, energy expenditure, family history of diabetes	−0.041 (−0.097, 0.014)	0.146
+HDL, triacylglycerol, ALT	−0.059 (−0.11, −0.0037)	0.037
+Fasting blood glucose, S_I , AIR	−0.042 (−0.098, 0.014)	0.140
$\log_e$(TNF-α) (<i>n</i>=779)		
Adjusted for baseline $\log_e$ (MCRI)	−0.071 (−0.14, −0.00087)	0.047
+age, sex, ethnicity	−0.12 (−0.20, −0.048)	0.001
+BMI, waist circumference	−0.085 (−0.16, −0.014)	0.020
+smoking, alcohol consumption, energy expenditure, family history of diabetes	−0.076 (−0.15, −0.0039)	0.039
+HDL, triacylglycerol, ALT	−0.056 (−0.13, 0.016)	0.126
+Fasting blood glucose, S_I , AIR	−0.056 (−0.13, 0.017)	0.129

included incident diabetes and use of glucose-lowering medications in the regression models, we observed minimal change in the magnitude of the association between plasma PAI-1 and reduced MCRI.

The association between higher plasma PAI-1 and reduced MCRI may be mediated by inflammation. In sensitivity analyses, however, adjusting for plasma CRP level did not alter the significant association between higher plasma PAI-1 values and the decline in MCRI. This observation provides further support for a mechanism specific to PAI-1, which may represent an as yet unmeasured inflammatory mechanism. It has also been suggested that higher PAI-1 values indicate

compromised fibrinolysis more than an inflammatory process [28]. Tissue plasminogen activator antigen, which is less variable than plasma PAI-1, has been shown to predict incident type 2 diabetes [33] and gestational diabetes [34]. Therefore, it is possible that the association between plasma PAI-1 and the decline in MCRI that we observed was linked to the PAI-1 and tissue plasminogen activator antigen complex, through a mechanism involving impaired fibrinolysis.

It is conceivable that the association between higher plasma PAI-1 and reduced insulin clearance over time may reflect hepatic dysfunction, given both the primary role of the liver in insulin clearance [35] and the association of liver injury with

higher plasma PAI-1 levels [36]. Although the significant association remained after adjusting for ALT, transaminases such as ALT are indirect measures of liver function, and we may thus not be able to capture fully the role of liver disease in the present study. As such, we might have observed a stronger magnitude of attenuation had liver function been measured directly, for example by liver biopsy. Although the liver is a crucial site of lipid metabolism, adjusting for HDL-cholesterol and triacylglycerol did not confound the association between plasma PAI-1 and the decline in MCRI. Furthermore, plasma PAI-1 has been significantly associated with degree of liver steatosis [37], and it is possible that liver fat, which was not measured directly in the IRAS, links plasma PAI-1 to reduced insulin clearance.

We observed that the association between plasma CRP and the decline in MCRI over time was significantly confounded by measures of obesity and abdominal adiposity, but not by insulin resistance. Although a meta-analysis of 16 prospective studies demonstrated that plasma CRP was associated with the risk of incident type 2 diabetes, studies that adjusted for abdominal adiposity reported a much weaker association than those that did not make these adjustments, indicating a possibility of residual confounding [7]. To further support the notion that plasma CRP may not be causally related to type 2 diabetes, CRP haplotypes showed null associations with insulin resistance, glycaemia and risk of diabetes in Mendelian randomisation [38]. Since insulin clearance is closely related to insulin resistance [39], it is unlikely that plasma CRP independently predicts the progressive decline of MCRI as shown in our data.

Similar to plasma CRP, the association between plasma TNF- α and decline in MCRI was attenuated after adjusting for BMI and waist circumference, as well as HDL-cholesterol and triacylglycerol. TNF- α is an inflammatory adipokine found primarily by monocytes and macrophages, and it plays an important role in adipocyte function and obesity-related insulin resistance [40]. There is an overproduction of TNF- α in the adipose tissues of obese persons, and plasma TNF- α is directly related to BMI and hyperinsulinaemia [41]. In addition, TNF- α is a potent regulator of lipid metabolism through mechanisms that increase the synthesis of NEFA, promote lipogenesis, induce lipolysis and inhibit enzymes related to lipid metabolism [42], whereas elevated triacylglycerol and decreased HDL-cholesterol levels are associated with insulin resistance [43]. Cross-sectional studies have shown a positive association between plasma TNF- α and insulin resistance [23, 44]. However, prospective studies on the association between plasma TNF- α and incident diabetes have shown inconsistent results, probably due to confounding by insulin resistance. Although no previous studies have investigated the association between plasma TNF- α and MCRI, the null association that we observed is consistent with the literature on insulin resistance.

Leptin is an adipocytokine secreted almost exclusively by adipose tissues in proportion to total fat mass [45]. Higher plasma leptin was associated with an acute-phase response in individuals with type 2 diabetes, confirming its role as an inflammatory marker [46]. A cross-sectional study involving 30 non-diabetic individuals of different ethnicities (Chinese, Asian Indian and white) reported a significant inverse association between plasma leptin and MCRI, independent of BMI, WHR and percentage of body fat and ethnicity; however, this study did not adjust for S_I [47]. In contrast, we observed that the association between plasma leptin and change in MCRI over time was confounded by BMI and waist circumference. Insulin clearance is a highly heritable trait [48], so ethnic differences between the studies in terms of metabolic characteristics such as plasma leptin concentration, distribution of body fat and degree of S_I and clearance may exist. In addition, the prospective design of our study may also explain the inconsistent findings with respect to previous cross-sectional studies.

Our data showed that plasma fibrinogen at baseline was not associated with the change in MCRI at follow-up. Although previous studies have reported a cross-sectional association between plasma fibrinogen and insulin resistance [27, 49], plasma fibrinogen did not predict incident type 2 diabetes [28, 50]. Therefore, our observation on the null association between plasma fibrinogen and progression of MCRI is plausible.

In addition to its novel findings, the strengths of this study include a well-characterised multiethnic population, a prospective design and detailed measurements of insulin clearance, secretion and sensitivity from FSIGTTs. However, our study may be limited by the relatively short period of follow-up and the limited range of inflammatory markers involved. We also did not have direct measures of insulin clearance. Although the IRAS cohort was enriched by participants with impaired glucose tolerance, it is notable that the fasting blood glucose levels of non-diabetic IRAS participants were similar to those in the NHANES study in the normal glucose tolerance and impaired glucose tolerance categories, and that a considerable proportion of the study population had normal glucose tolerance at baseline. Nevertheless, our findings are likely to be generalisable only to non-diabetic individuals at risk of glucose intolerance in similar ethnic groups.

In conclusion, we observed a significant association between plasma PAI-1, but not plasma levels of CRP, TNF- α , leptin or fibrinogen, and a progressive decline in MCRI over time. The present study has several important implications. First, it extends the scientific literature on the role of inflammatory and fibrinolytic proteins in the aetiology of type 2 diabetes. Second, our findings point to the need for future studies to prove a causal link between higher plasma PAI-1 values and decline in MCRI, and for randomised controlled trials to test the clinical significance of targeting impaired fibrinolysis as a means of decreasing the incidence of type 2 diabetes.

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Contribution statement CCL wrote the manuscript, analysed and interpreted the data, revised it critically for important intellectual content and approved the final version for publication. SMH, LEW and MJR designed the study, collected data, interpreted the data, revised the study critically for important intellectual content and approved the final version for publication. CL, AF, MOG, DS, NCO, JMN and AJH interpreted the data, revised critically for important intellectual content and approved the final version for publication.

Duality of interest The authors declare that there is no duality of interest associated with this manuscript.

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