ARTICLE

Lipid and inflammatory biomarkers and kidney function decline in type 2 diabetes

J. Lin · F. B. Hu · C. Mantzoros · G. C. Curhan

Abstract
Aims/hypothesis Potentially modifiable biomarkers may influence the decline in estimated GFR (eGFR), but few data are currently available in type 2 diabetic adults.
Methods We studied 516 women with type 2 diabetes in the Nurses' Health Study with data on lipid and inflammatory biomarkers from plasma collected in 1989 and plasma creatinine in samples collected in 1989 and 2000. An estimated GFR decline of ≥25% over 11 years was the outcome of interest.
Results Comparing the highest with the lowest quartile, soluble tumour necrosis factor receptor 2 (sTNFR-2) was independently associated with an eGFR decline of ≥25% (multivariate OR 5.81; 95% CI 2.90–11.65); this association was stronger in obese women (OR 16.76; 95% CI 4.69–59.90 for BMI ≥30 kg/m^2; OR 2.78, 95% CI 1.12–6.89 for BMI <30 kg/m^2; *p* for interaction=0.02). No lipids (total cholesterol, LDL-cholesterol, HDL-cholesterol, non-HDL-cholesterol, triacylglycerols, lipoprotein(a), or apolipoprotein B) or other markers of inflammation (C-reactive protein, fibrinogen, E-selectin, intracellular cell adhesion molecule 1, leptin or adiponectin) were significantly associated with eGFR decline after multivariable adjustment.
Conclusions/interpretation Elevated sTNFR-2 levels may be an important and potentially modifiable risk factor for eGFR decline in type 2 diabetes, especially in those with a BMI of ≥30 kg/m^2.

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Abbreviations

ACE-I	Angiotensin-converting enzyme inhibitor
CARE	Cholesterol and Recurrent Events
CRP	C-reactive protein
DSQ	Diabetes supplementary questionnaire
eGFR	Estimated GFR
NHS	Nurses' Health Study
sTNFR-2	Soluble tumour necrosis factor receptor-2

Introduction

Identifying biomarkers predictive of estimated GFR (eGFR) decline could point to pathways that may help identify new therapeutic agents. With the recognition that type 2 diabetes itself may be a chronic inflammatory state, the role of

potentially modifiable lipid and inflammatory biomarkers has been of particular interest.

Notably, higher baseline C-reactive protein (CRP) was associated with eGFR decline in people with hyperlipidaemia and a history of myocardial infarction [1] and in older community-dwelling adults [2]. No relation between CRP and eGFR decline was identified, however, in an analysis of the Modification of Diet in Renal Disease study [3], but mean duration of follow-up was only 2.2 years. Soluble tumour necrosis factor receptor 2 (sTNFR-2) has also been associated with eGFR decline [1]. These investigations included less than 15% diabetic patients, however.

We previously reported that dyslipidaemia, as well as higher fibrinogen, vascular cell adhesion molecule 1 and sTNFR-2, were independently associated with lower eGFR in a cross-sectional study of men with type 2 diabetes [4]. In this study we expanded our investigations by examining the relation between lipid and inflammatory biomarkers with respect to the 11-year eGFR decline in women with type 2 diabetes.

Methods

This study was approved by the Partners' Healthcare Brigham and Women's Hospital Human Research Committee Institutional Review Board.

Participants The Nurses' Health Study (NHS) was initiated in 1976 with the enrolment of 121,700 US nurses aged 30–55 years. Questionnaires are mailed biennially; return of questionnaires constitutes informed consent under the study's institutional review board protocol. Between 1989 and 1990, 32,826 participants provided blood samples that were shipped on ice by overnight delivery and stored at −130°C as previously described [5]. In the year 2000, many of these participants submitted a second blood sample under the same shipment and storage conditions.

We mailed a diabetes supplementary questionnaire (DSQ) to all women reporting diabetes on biennial questionnaires. We used the National Diabetes Data Group criteria [6] to define diabetes self-reported up to the 1996 biennial questionnaire. Self-reported diagnosis using the DSQ was 98% accurate in a validation study with medical records review [7].

We included women who reported being diagnosed with type 2 diabetes up to the year 2000 (because glucose intolerance can precede the diagnosis of type 2 diabetes by ≥10 years [8]) and who had plasma samples from both 1989 and 2000. We excluded women with a serum creatinine concentration ≤44 mmol/l (felt to be implausible) (n=12) and those for whom data on plasma creatinine were missing (n=19). After applying these criteria, 516 women had eGFR change data available for analysis (Fig. 1).

Biomarker analyses Biomarker assays were performed at Children's Hospital, Boston, MA, USA, or in the laboratory of Dr C. Mantzoros at Beth Israel Deaconess Hospital, Boston, MA, USA, using standard methods and assays (see Electronic supplementary material). On blinded quality control samples that were run simultaneously with participant samples, biomarker measurements had CVs of less than 15% with the exception of E-selectin (20%), adiponectin (27.4%) and sTNFR-2 (16.7%).

Measurements of kidney function decline Plasma creatinine was analysed using a modified kinetic Jaffe reaction (CV 10%). In 2007, repeat creatinine assays of 20 NHS plasma samples (with a range of 53 to 124 mmol/l) initially measured in the year 2000 revealed a mean recalibration coefficient (new value/original value) of 0.97 and confirmed that creatinine was stable under our storage conditions.

Glomerular filtration was estimated by the abbreviated MDRD (Modification of Diet in Renal Disease) equation [9]. An eGFR decline of ≥25% between 1989 and 2000 was the primary outcome. An eGFR decline of ≥25% over 11 years was determined a priori and had been used in previous analyses of renal function decline in NHS participants [10].
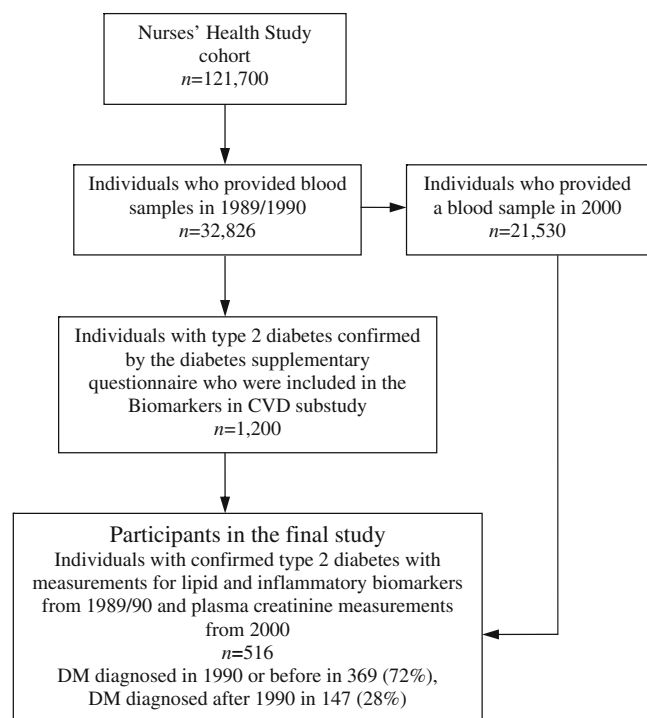
Fig. 1 Biomarkers and type 2 diabetes participant selection in the Nurses' Health Study. DM, diabetes mellitus

Statistical analysis Spearman correlations were calculated for eGFR and individual biomarkers at baseline. Multivariable logistic regression modelling was applied to analyse associations between biomarkers and an eGFR decline of ≥25%. Animal protein intake, alcohol intake and baseline cardiovascular disease were also considered for the adjusted models but were removed as they did not change results. Analyses were performed with SAS software, version 9.1 (SAS Institute, Cary, NC, USA).

Results

Characteristics of the study cohort are summarised in Table 1. In 1989, significant modest cross-sectional inverse correlations were noted between eGFR and leptin ($r=0.16$, $p<0.001$), adiponectin ($r=-0.13$, $p=0.003$) and sTNFR-2 ($r=-0.28$, $p<0.001$). CRP was not associated with eGFR ($r=0.03$, $p=0.57$). The women who experienced an eGFR decline of ≥25% between 1989 and 2000 had higher rates of hypertension, cardiovascular disease, angiotensin-converting enzyme inhibitor (ACE-I)/angiotensin-type 2 receptor blocker medication use, and lower physical activity. In 1989, only 35 women (6.8%) had eGFR <60 ml min^{-1} 1.73 m^{-2}. In 2000, 139 women (27%) had eGFR <60 ml min^{-1} 1.73 m^{-2}.

Those in the highest quartile of sTNFR-2 in 1989 had significantly higher odds for an eGFR decline of ≥25% between 1989 and 2000 (multivariate OR 5.81; 95% CI 2.90–11.65) (Table 2 and Fig. 2). The association between sTNFR-2 and eGFR decline was stronger in women with a BMI of ≥30 (OR 16.76; 95% CI 4.69–59.9) than in women with a BMI of <30 (OR 2.78; 95% CI 1.12–6.89) (*p* for interaction=0.02). No other significant associations between biomarkers and eGFR decline were present after multivariable adjustment. Restricting the analyses to the 369 women diagnosed with diabetes in 1990 or before did not change the results meaningfully.

Discussion

After multivariable adjustment, sTNFR-2 was a strong predictor of eGFR decline and varied with obesity status. These data support the theory that sTNFR-2, our surrogate marker for the volatile cytokine TNF-α, promotes tubular epithelial apoptosis [11] and progressive renal tubulointerstitial fibrosis [12]. Tubulointerstitial fibrosis is one of the strongest predictors of subsequent kidney disease progression [13], which ultimately manifests as GFR decline. Our findings are consistent with the Cholesterol and Recurrent Events (CARE) trial, in which significantly faster eGFR decline was associated with higher levels of sTNFR-2 [1]. Unlike the CARE study, however, we did not see an association between CRP and eGFR decline. This may be attributable to the difference in populations (type 2 diabetic vs mostly non-diabetic individuals with a history of myocardial infarction).

Table 1 Demographic and clinical characteristics of participants of the Nurses' Health Study with type 2 diabetes in year 2000

Characteristic	eGFR decline ≥25%	
	No (*n*=346)	Yes (*n*=170)
Age (years)	68	70
White (%)	98	97
Hypertension (%)	73	86*
Years since diabetes diagnosis	13.9	16.1
HbA$_{1c}$ in 1989 (%)	6.1	6.5*
Current smoker (%)	6.6	4.1
Ever smoked (%)	51	54
Alcohol intake (g/day)	0.52	0.21
Physical activity level (METs/week)	8.6	4.2*
Cardiovascular disease (%)	19	37*
BMI in 1988 (kg/m^2)	29.0	29.3
BMI in 2000 (kg/m^2)	29.0	30.1
Change in BMI between 1988 and 2000 (kg/m^2)	0.0	0.0
ACE inhibitor or ARB medication use (%)	27	36*
Plasma creatinine in 1989 (mmol/l)	68	63*
Plasma creatinine in 2000 (mmol/l)	70	92*
eGFR in 1989 (ml min^{-1} 1.73 m^{-2})	81	91*
eGFR in 2000 (ml min^{-1} 1.73 m^{-2})	77	56*

Data are percentage or median

*$p<0.05$ vs referent group (no eGFR decline)

ARB, angiotensin-2 receptor blocker; MET, metabolic-equivalent score

Table 2 Logistic regression models for odds ratios for an eGFR decline of ≥25% between 1989 and 2000 for quartile 4 vs quartile 1 of lipid and inflammatory biomarkers

Biomarker	Age-adjusted univariate model (n=516) OR for quartile 4 vs quartile 1 (95% CI)	Full model OR for quartile 4 vs quartile 1 (95% CI)	p value for trend across quartiles for full model
Total cholesterol (mmol/l)	1.48 (0.87, 2.54)	1.26 (0.70, 2.28)	0.41
LDL-cholesterol (mmol/l)	1.74 (1.02, 2.96)	1.36 (0.76, 2.45)	0.24
HDL-cholesterol (mmol/l)	0.89 (0.52, 1.51)	0.94 (0.51, 1.74)	0.66
Non-HDL-cholesterol (mmol/l)	1.78 (1.03, 3.09)	1.39 (0.74, 2.58)	0.32
Triacylglycerols (mmol/l)	1.32 (0.78, 2.23)	1.12 (0.62, 2.03)	0.60
Lipoprotein(a) (μmol/l)	1.19 (0.70, 2.04)	1.11 (0.61, 1.99)	0.49
Apolipoprotein B (g/l)	2.05 (1.20, 3.53)	1.61 (0.87, 2.99)	0.24
CRP (mg/l)	1.43 (0.86, 2.38)	1.34 (0.72, 2.50)	0.45
Fibrinogen (μmol/l)	1.44 (0.85, 2.45)	1.67 (0.90, 3.11)	0.10
E-selectin (ng/ml)	1.49 (0.89, 2.51)	0.96 (0.52, 1.80)	0.84
ICAM-1 (ng/ml)	1.86 (1.09, 3.18)	1.55 (0.84, 2.85)	0.11
Leptin (ng/ml)	1.15 (0.68, 1.94)	1.81 (0.89, 3.68)	0.16
Adiponectin (μg/ml)	0.90 (0.53, 1.52)	1.22 (0.66, 2.25)	0.59
sTNFR-2 (pg/ml)	2.42 (1.39, 4.21)	5.81 (2.90, 11.65)	<0.001

Full multivariable models adjusted for age (continuous, years), hypertension (yes/no), BMI (continuous), ever smoked, physical activity (METS/week), duration of type 2 diabetes (continuous, years), ACE-I/angiotensin-type 2 receptor blocker medication use (yes/no), baseline HbA$_{1c}$ (%) and eGFR in 1989. Cumulative averaged animal protein intake, alcohol intake and baseline cardiovascular disease were also considered for inclusion in the multivariable model but did not change estimates between biomarker and eGFR ≥25% decline and were therefore removed

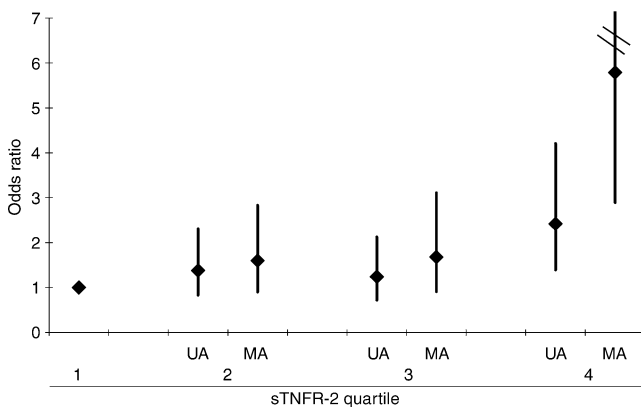ICAM-1, intracellular adhesion molecule

MET, metabolic-equivalent score

Obesity is an independent predictor of chronic kidney disease development [14]; we therefore stratified by obesity status and found that obesity modified the associations between sTNFR-2 and eGFR decline. TNF-α is elevated in obese compared with non-obese women and weight loss decreases these cytokine levels [15]. Because adipocytes

Fig. 2 Odds ratios with 95% CIs for univariate age-adjusted (UA) and multivariable-adjusted (MA) associations of quartiles of sTNFR-2 with an eGFR decline of ≥25% between 1989 and 2000. Quartile 1 is the referent, 939–2,058 pg/ml; quartile 2, 2,062–2,446 pg/ml; quartile 3, 2,450–2,975 pg/ml; quartile 4, 2,979–5,758 pg/ml

are now recognised as important regulators of metabolism and inflammation through their secretion of cytokines, chemokines and hormone-like proteins, we hypothesise that the larger mass of adipose tissue in obese humans may intensify the detrimental effects of elevated sTNFR-2 on kidney function.

We confirmed some of our previous findings from a cross-sectional analysis of 732 men with type 2 diabetes in the Health Professionals Follow-Up Study [4]. The strongest correlation with eGFR was seen with sTNFR-2 (r=−0.39, p<0.001 for men, r=−0.28, p<0.001 for women), whereas CRP and eGFR were weakly or not correlated (r=−0.09, p=0.02 for men and r=0.03, p=0.57 for women). No significant relations were noted between dyslipidaemia and eGFR in NHS diabetic women, whereas in the diabetic men eGFR was significantly and inversely associated with total cholesterol, triacylglycerols, non-HDL, lipoprotein(a) and apolipoprotein B. Therefore, it appears that associations between lipids and eGFR may vary by sex.

In contrast to our findings, an analysis of over 4,000 participants with type 2 diabetes in the UK Prospective Diabetes Study reported that higher baseline triacylglycerols and LDL-cholesterol were independently associated with incident renal impairment (creatinine clearance

<60 ml/min or doubling of plasma creatinine) [16]. The larger number of male and female participants in the UK Prospective Diabetes Study analysis allowed the examination of incident renal impairment, and the modelling approach used lipids as continuous variables rather than in quantile categories; thus, the results may not be directly comparable to the current study.

Limitations of this study include the relatively small number of women with an eGFR decline of ≥25% (thus limiting power for this study) and the high CVs for some of the biomarkers, which resulted in wide confidence intervals; however, the high CVs would be likely to bias the results towards the null, whereas an association with eGFR decline was observed. The participants were mostly older white women so results may not necessarily be generalisable to other populations. Although it is possible that some women with type 2 diabetes who were lost to follow-up would not have been included in these analyses, this would probably bias the results towards the null. Despite these limitations, this study provides important new additional information on biomarkers and kidney function in diabetes, which has not been studied extensively in humans.

We conclude that a higher level of sTNFR-2 is associated with subsequent eGFR decline in women with type 2 diabetes; the relation of sTNFR-2 with eGFR decline appeared stronger in those with a BMI of ≥30. Progressive kidney disease in type 2 diabetes currently represents a major public health problem worldwide and further investigation into its pathogenesis, especially inflammatory pathways, may open new avenues for potential therapeutics. Therefore, future research may include studying whether TNF-α blockade is beneficial in slowing eGFR decline in type 2 diabetes.

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