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SHORT COMMUNICATION

# Soluble thrombomodulin as a predictor of type 2 diabetes: results from the MONICA/KORA Augsburg case–cohort study, 1984–1998

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**Abstract**
*Aims/hypothesis* Previous studies have shown an inverse association between soluble thrombomodulin (sTM) and incident CHD, but there is a lack of data on the association between sTM and type 2 diabetes. Since CHD and type 2 diabetes share many risk factors, the aim of this study was to assess whether elevated sTM levels are associated with a lower incidence of type 2 diabetes.
*Materials and methods* A case–cohort study was performed in initially healthy middle-aged men and women based on data from the Monitoring of Trends and Determinants in Cardiovascular Disease/Cooperative Health Research in the Region of Augsburg (MONICA/KORA) studies conducted between 1984 and 1998. Levels of sTM were measured with an ELISA in serum samples from 138 men and 86 women who developed type 2 diabetes during follow-up (cases) and 534 men and 446 women who did not develop type 2 diabetes (non-cases).
*Results* An inverse association was found between sTM and type 2 diabetes risk after multivariable adjustment for diabetes risk factors, including several other markers of inflammation and endothelial dysfunction. Markers of inflammation and endothelial dysfunction were particularly strong confounders of the observed association. In the fully adjusted model, a 1 SD increase in sTM was associated with a 27% decrease in the risk of type 2 diabetes (hazard ratio=0.73, 95% CI 0.58–0.91) in the total study population. We did not observe significant risk differences between men and women.
*Conclusions/interpretation* These data suggest that, in initially healthy middle-aged men and women, levels of sTM are inversely associated with the risk of type 2 diabetes.

**Keywords** Case–cohort study · Endothelial dysfunction · Incident type 2 diabetes · Soluble thrombomodulin

**Abbreviations**

| | |
|---|---|
| ARIC | Atherosclerosis Risk in Communities |
| CRP | C-reactive protein |
| HR | hazard ratio |
| KORA | Cooperative Health Research in the Region of Augsburg |
| MONICA | Monitoring of Trends and Determinants in Cardiovascular Disease |
| sE-selectin | soluble E-selectin |
| sICAM-1 | soluble intercellular adhesion molecule-1 |
| sTM | soluble thrombomodulin |

**Electronic supplementary materials** Supplementary material is available in the online version of this article at http://dx.doi.org/10.1007/s00125-006-0568-x and is accessible to authorised users.

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## Introduction

Thrombomodulin is a transmembrane protein that plays a major role in intravascular coagulation. It is an endothelial

cell surface receptor for thrombin and functions as an anticoagulant through formation of a thrombin–thrombomodulin complex. This complex inhibits fibrin formation and platelet activation and accelerates protein C activation. In turn, activated protein C binds to platelet surface protein S, where it degrades factor VIIIa and factor Va, thereby inhibiting further formation of thrombin [1].

Thrombomodulin is not only found bound to endothelial cells, soluble forms of thrombomodulin (sTM) are also present in the circulation. Up to seven molecular subspecies of sTM have been reported, the relative proportions of which appear to differ between diabetes patients and healthy control subjects [2]. Although sTM fragments have anticoagulant activity, their physiological role is unknown. Regulation of sTM concentrations occurs at several levels, including thrombomodulin production by endothelial cells, release of cell-bound thrombomodulin by endothelial injury, cleavage in response to production of proteases and protein clearance. Cellular production and release into the circulation may depend on immune activation and endothelial dysfunction, which have been suggested to represent the underlying causes (or 'common soil') of type 2 diabetes and CHD [3, 4]. Subjects with chronic diseases that are related to inflammation and endothelial dysfunction, such as atherosclerotic arterial diseases and type 2 diabetes, have increased levels of sTM [5, 6]. By contrast, in the prospective Atherosclerosis Risk in Communities (ARIC) study, levels of sTM were inversely associated with the risk of CHD [6]. It has been suggested that a prothrombotic state and endothelial dysfunction could enhance the risk of type 2 diabetes [7]. Thus, as a vasoprotective molecule, thrombomodulin may be involved in the pathophysiology of type 2 diabetes. The aim of the present study was to determine whether elevated concentrations of sTM are associated with a decreased risk of type 2 diabetes.

## Subjects and methods

We conducted a case–cohort study using data from the population-based Monitoring of Trends and Determinants in Cardiovascular Disease/Cooperative Health Research in the Region of Augsburg (MONICA/KORA) cohort study. The study design has been described previously [8]; all subjects provided written informed consent. The source population was stratified by sex and survey and then a subcohort was drawn at random within each stratum. Contrary to previous reports, this subcohort comprised only 1,025 subjects, and the present study included only incident cases that occurred between 1984 and 1998. Cases with self-reported incident diabetes in one of the follow-up questionnaires were validated by a questionnaire mailed to the treating physician or medical chart review. For deceased subjects without a previously reported diagnosis of diabetes, hospital records were searched for and/or the last treating physician was asked whether the patient had a history of diabetes. If the participant had diabetes, the type of diabetes and date of diagnosis were ascertained. The present analysis comprised 1,204 participants (138 men, 86 women with incident type 2 diabetes, 534 men, 446 women without incident type 2 diabetes). The follow-up time (mean ± SD) for the study population was 8.0±4.0 years.

Methods used to assess demographic, lifestyle and clinical characteristics and markers of inflammation have been described elsewhere in detail ([8] and references therein). Non-fasting blood samples were collected from all participants, and all samples were stored at −80°C until analysis. Serum samples were thawed and refrozen twice before analysis of sTM. Levels of sTM were measured in serum using an ELISA (Diaclone, Besançon, France). The intra- and inter-assay CVs were 10.3 and 16.8%, respectively.

Means ± SEM were calculated for sTM using the SAS procedure SURVEYREG, which estimated SEMs appropriate for the sampling scheme. Tests for differences were also based on these procedures. Cox proportional hazards analysis was used to assess the association between sTM and incident type 2 diabetes. Because of the case–cohort design, SEMs were corrected using an SAS macro with a 'sampling weight' approach developed by Barlow et al. ([8] and references therein). Results are presented as hazard ratios (HRs) and 95% CIs per 1 SD increase in sTM in the subcohort, and $p$ values are based on robust variance estimates using the Barlow macro. For all statistical analyses, a $p$ value of less than 0.05 was considered statistically significant. All evaluations were performed with the statistical software package SAS (Version 8.02 for Unix; SAS Institute, Cary, NC, USA).

## Results

Baseline characteristics of cases and non-cases are described in Electronic supplementary material (ESM) Table 1. The mean (±SEM) unadjusted sTM levels were 4.7± 0.12 ng/ml in subjects with incident type 2 diabetes (cases) and 4.6±0.08 ng/ml in non-cases. Levels of sTM were significantly correlated with other markers of subclinical inflammation and endothelial dysfunction and with clinical parameters (Table 1). Associations between sTM and lifestyle factors, drug use and comorbidities have been reported [9] and are shown in ESM Table 2. After adjustment for age, sex, survey, BMI, smoking status, alcohol consumption, physical activity, systolic BP, ratio of total cholesterol:HDL-cholesterol, parental history of diabetes, C-reactive protein (CRP), IL-6, IL-18, soluble

**Table 1** Associations between sTM and markers of endothelial dysfunction and inflammation and other clinical markers using weighted Pearson correlation in the randomly sampled subcohort

| | sTM | | |
|---|---|---|---|
| | Men ($n$=561) | Women ($n$=464) | All ($n$=1,025) |
| sE-selectin | 0.22*** | 0.22*** | 0.24*** |
| sICAM-1 | 0.37*** | 0.16*** | 0.30*** |
| Log CRP | 0.01 | 0.19*** | 0.08* |
| Log IL-6 | 0.11** | 0.22*** | 0.16*** |
| Log IL-18 | 0.11* | 0.15** | 0.12*** |
| BMI | −0.01 | 0.20*** | 0.09** |
| Systolic BP | 0.17*** | 0.26*** | 0.22*** |
| Diastolic BP | 0.03 | 0.17*** | 0.11** |
| Total cholesterol: HDL-cholesterol | 0.05 | 0.27*** | 0.16*** |
| eGFR[a] | −0.33*** | −0.34*** | −0.30*** |

eGFR, estimated GFR

*$p$<0.05, **$p$<0.01, ***$p$<0.001

[a] eGFR; the abbreviated Modification of Diet in Renal Disease Study Group equation [11] was used to calculate eGFR: $\text{eGFR}\left(\text{ml/min per } 1.73\,\text{m}^2\right) = 186.3 \times \left(\text{serum creatinine}^{-1.154}\right) \times \left(\text{age}^{-0.203}\right) \times 0.742(\text{if female}) \times 1.212(\text{if black})$

where serum creatinine is measured in mg/dl, and age in years

intercellular adhesion molecule-1 (sICAM-1) and soluble E-selectin (sE-selectin), mean levels (±SEM) of sTM were significantly lower in cases than in non-cases (4.1±0.18 vs 4.7±0.07, $p$=0.008).

In Cox proportional hazards models (Table 2), sTM was inversely associated with incident type 2 diabetes after adjustment for classical risk factors for diabetes (model 2) or for other markers of inflammation and endothelial dysfunction (model 3). Addition of sE-selectin or sICAM-1 to the model containing age, sex and survey as covariables had the greatest effect on the observed HRs. The strongest inverse association between sTM and type 2 diabetes was observed in the fully adjusted model, which included classical risk factors for diabetes and other markers of inflammation and endothelial dysfunction (model 4). In this model, a 1 SD increment in sTM was associated with a 27% decrease in the risk of type 2 diabetes. HRs for the association between sTM and type 2 diabetes were only slightly lower in men than in women, but results were only significant in men, possibly as a result of the smaller number of incident cases in women ($p$=0.79 for sex interaction). Further adjustment for use of lipid-lowering medication did not affect the results, while additional adjustment for use of antihypertensive medication produced somewhat lower HRs in women (data not shown). Subgroup analyses in subjects with available data on WHR and waist circumference (130 cases, 542 non-cases) revealed that adjustment for WHR instead of BMI led to similar results. Adjustment for waist circumference instead of BMI led to slightly higher HRs; however, associations between sTM and type 2 diabetes remained significant in models 2 and 4 (data not shown). Exclusion of subjects with prevalent myocardial infarction, angina pectoris or stroke or incident myocardial infarction during follow-up ($n$=138) produced slightly lower HRs in men and slightly higher HRs in women (data not shown).

## Discussion

In this population-based sample of middle-aged German men and women we have shown for the first time that elevated levels of sTM are associated with a decreased risk of type 2 diabetes after multivariable adjustment for classical diabetes risk factors and/or markers of inflammation and endothelial dysfunction. Type 2 diabetes was not significantly associated

**Table 2** Hazard ratios for incident type 2 diabetes per 1 SD increment (2.23 ng/ml) in soluble thrombomodulin in middle-aged men and women from the MONICA/KORA Augsburg case–cohort study, 1984–1998

| | HR (95% CI) $p$ value | | | | $p$ value for interaction |
|---|---|---|---|---|---|
| Subjects | Model 1 | Model 2 | Model 3 | Model 4 | |
| All subjects | 0.92 (0.78–1.09) 0.336 | 0.79 (0.62–1.00) 0.047 | 0.75 (0.61–0.94) 0.011 | 0.73 (0.58–0.91) 0.006 | |
| Men | 0.82 (0.65–1.04) 0.098 | 0.74 (0.55–1.00) 0.048 | 0.64 (0.48–0.87) 0.004 | 0.65 (0.48–0.89) 0.007 | 0.790[a] |
| Women | 1.10 (0.84–1.45) 0.483 | 0.84 (0.55–1.27) 0.400 | 0.83 (0.59–1.18) 0.307 | 0.64 (0.39–1.05) 0.080 | |

Model 1, adjusted for age, sex and survey

Model 2, adjusted for variables in model 1 + BMI, smoking status (never smoker, former smoker, current smoker), alcohol consumption (0, 0.1–39.9, ≥40 g/day for men; 0, 0.1–19.9, ≥20 g/day for women), physical activity (inactive, active), systolic BP, Total cholesterol:HDL-cholesterol, parental history of diabetes mellitus (negative, positive, unknown)

Model 3, adjusted for variables in model 1 + CRP, IL-6, IL-18, sICAM-1, sE-selectin

Model 4, adjusted for variables in model 2 + CRP, IL-6, IL-18, sICAM-1, sE-selectin

[a] Interaction between sex and sTM for model 4

with sTM in the unadjusted model, but, consistent with previous results [9], sTM levels were positively associated with diabetes risk factors and other markers of inflammation and endothelial dysfunction, most of which were elevated in subjects with increased diabetes risk [8]. Adjustment for these confounders could be expected to lower the HR for the association between sTM and type 2 diabetes risk and thus revealed an inverse association.

Our data may appear counterintuitive as previous cross-sectional studies reported elevated sTM concentrations in patients with type 2 diabetes [5, 6]. However, similar results have been obtained in studies on cardiovascular and cerebrovascular diseases. Patients with CHD may have higher sTM levels, but in the prospective ARIC study, it was lower instead of higher sTM concentrations that predicted CHD [6 and references therein]. Another study showed that elevated sTM levels predicted mortality in patients with previous stroke, whereas elevated levels of sTM were associated with lower stroke prevalence when only individuals without a history of vascular disease were analysed [10].

As endothelial thrombomodulin production, release of cell-bound thrombomodulin by endothelial injury or proteases and protein clearance probably all help to regulate sTM concentrations in the circulation, these epidemiological findings are difficult to interpret. It is conceivable that high circulating sTM in healthy individuals mainly reflects thrombomodulin production on endothelial cells and thus indicates an intact anticoagulant and vasoprotective state, so that decreased sTM concentrations in otherwise healthy individuals would indicate endothelial disturbances and an increased risk for the development of type 2 diabetes, CHD or stroke. In subjects with type 2 diabetes or CHD, subclinical inflammation and endothelial dysfunction could lead to a considerable increase in the pool of sTM fragments by cell injury, and a proinflammatory milieu including increased protease activities, which could obscure decreases in cellular thrombomodulin production. It can be argued that subjects who will later develop type 2 diabetes or CHD already suffer from subclinical inflammation many years before disease manifestation, but this level of immune activation is certainly less pronounced and may not be sufficient to impair the antithrombotic and anticoagulant activity of thrombomodulin.

Taken together, our results demonstrate that, in an initially healthy population-based cohort, sTM concentrations in the circulation and type 2 diabetes risk are inversely associated when adjustment is made for multiple confounders. The precise mechanisms responsible for this finding are not entirely clear. Further research is required to elucidate the mechanisms involved in regulating sTM concentrations, the impact of diseases on the relative proportions of sTM fragment types in the circulation, and the antithrombotic activity of these sTM forms.

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**Duality of interest** The authors declare that there is no duality of interest.

## References

1. Sadler JE (1997) Thrombomodulin structure and function. Thromb Haemost 78:392–395
2. Uehara S, Gotoh K, Handa H (2001) Separation and characterization of the molecular species of thrombomodulin in the plasma of diabetic patients. Thromb Res 104:325–332
3. Pickup JC, Crook MA (1998) Is type II diabetes mellitus a disease of the innate immune system? Diabetologia 41:1241–1248
4. Pinkney JH, Stehouwer CDA, Coppack SW, Yudkin JS (1997) Endothelial dysfunction: cause of the insulin resistance syndrome. Diabetes 46(Suppl. 2):S9–S13
5. Aso Y, Inukai T, Takemura Y (1998) Mechanisms of elevation of serum and urinary concentrations of soluble thrombomodulin in diabetic patients: possible application as a marker for vascular endothelial injury. Metabolism 47:362–365
6. Salomaa V, Matei C, Aleksic N et al (1999) Soluble thrombomodulin as a predictor of incident coronary heart disease and symptomless carotid artery atherosclerosis in the Atherosclerosis Risk in Communities (ARIC) study: a case–cohort study. Lancet 353:1729–1734
7. Tooke JE, Goh KL (1998) Endotheliopathy precedes type 2 diabetes. Diabetes Care 21:2047–2049
8. Thorand B, Baumert J, Chambless L, for the MONICA/KORA Study Group et al (2006) Elevated markers of endothelial dysfunction predict type 2 diabetes mellitus in middle-aged men and women from the general population. Arterioscler Thromb Vasc Biol 26:398–405
9. Thorand B, Baumert J, Döring A et al (2006) Association of cardiovascular risk factors with markers of endothelial dysfunction in middle-aged men and women. Results from the MONICA/KORA Augsburg Study. Thromb Haemost 95:134–141
10. Olivot JM, Labreuche J, Aiach M, Amarenco P (2004) Soluble thrombomodulin and brain infarction: case–control and prospective study. Stroke 35:1946–1951
11. Levey AS, Coresh J, Balk E, National Kidney Foundation et al (2003) National Kidney Foundation practice guidelines for chronic kidney disease: evaluation, classification, and stratification. Ann Intern Med 139:137–147