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RESEARCH ARTICLE

# Soluble Forms of Intercellular and Vascular Cell Adhesion Molecules Independently Predict Progression to Type 2 Diabetes in Mexican American Families

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## Abstract

### Objective

While the role of type 2 diabetes (T2D) in inducing endothelial dysfunction is fairly well-established the etiological role of endothelial dysfunction in the onset of T2D is still a matter of debate. In the light of conflicting evidence in this regard, we conducted a prospective study to determine the association of circulating levels of soluble intercellular adhesion molecule 1 (sICAM-1) and soluble vessel cell adhesion molecule 1 (sVCAM-1) with incident T2D.

### Methods

Data from this study came from 1,269 Mexican Americans of whom 821 initially T2D-free individuals were longitudinally followed up in the San Antonio Family Heart Study. These individuals were followed for 9752.95 person-years for development of T2D. Prospective association of sICAM-1 and sVCAM-1 with incident T2D was studied using Kaplan-Meier survival plots and mixed effects Cox proportional hazards modeling to account for relatedness among study participants. Incremental value of adhesion molecule biomarkers was studied using integrated discrimination improvement (IDI) and net reclassification improvement (NRI) indexes.

### Results

Decreasing median values for serum concentrations of sICAM-1 and sVCAM-1 were observed in the following groups in this order: individuals with T2D at baseline, individuals who developed T2D during follow-up, individuals with prediabetes at baseline and normal

glucose tolerant (NGT) individuals who remained T2D-free during follow-up. Top quartiles for sICAM-1 and sVCAM-1 were strongly and significantly associated with homeostatic model of assessment—insulin resistance (HOMA-IR). Mixed effects Cox proportional hazards modeling revealed that after correcting for important clinical confounders, high sICAM-1 and sVCAM-1 concentrations were associated with 2.52 and 1.99 times faster progression to T2D as compared to low concentrations, respectively. Individuals with high concentrations for both sICAM-1 and sVCAM-1 progressed to T2D 3.42 times faster than those with low values for both sICAM-1 and sVCAM-1. The results were similar in women in reproductive age group and the remainder of the cohort. Inclusion of sICAM-1 and sVCAM-1 in predictive models significantly improved reclassification and discrimination. The majority of these results were seen even when the analyses were restricted to NGT individuals.

## Conclusion

Serum concentrations of sICAM-1 and sVCAM-1 independently and additively predict future T2D and represent important candidate biomarkers of T2D.

## Introduction

Vascular endothelial dysfunction is a characteristic feature of type 2 diabetes. An increasing number of studies have shown that the serum concentrations of the adhesion molecules involved in leukocyte adhesion to the endothelial surface are elevated in individuals with T2D as compared to healthy counterparts.[1–8] It appears that the relationship between endothelial dysfunction and T2D may be bidirectional, such that T2D can lead endothelial dysfunction [9, 10] and that endothelial dysfunction can lead to T2D.[11, 12] However, evidence for endothelial dysfunction preceding or correlating with the risk of later T2D is inconclusive.

Meigs and coworkers showed in two large epidemiological studies (using data from the Nurses' Health Study (737 incident cases of type 2 diabetes and 785 controls) [13] and from Framingham Offspring Study (2,011 diabetes-free individuals) [14]) that adhesion molecules that signify endothelial dysfunction independently predict the risk of future T2D. Similarly, based on data from the Women's Health Initiative Observational Study (WHIOS; 1,584 incident diabetes cases matched with 2,198 controls), Song et al [15] independently replicated the association of circulating adhesion molecules with risk of incident T2D. Finding from Thorand et al [16], Stranges et al [17] and Rossi et al (using flow mediated dilation to measure endothelial dysfunction) [18] also support a possible etiological role of endothelial dysfunction in T2D. While the molecular mechanisms for this that directly link endothelial dysfunction and T2D are unclear, it has been posited [19–22] that endothelial dysfunction may induce insulin resistance and thereby facilitate progression to T2D. This is partly attributable to the endocrine role of the adipose tissue since reversion of the adiponectin:leptin ratio correlates with both insulin resistance and endothelial dysfunction.[23, 24] It has also been argued that decreased nitric oxide availability observed in a state of insulin resistance has been correlated with vascular cellular adhesion molecule-1 (VCAM-1) levels.[25]

Yet, the relationship between endothelial dysfunction and subsequent T2D has not been universally observed. Using data from the Framingham Heart Study, Dallmeier and others [26] recently showed that while intercellular adhesion molecule 1 (ICAM-1) concentration in serum is increased in individuals who eventually develop T2D, this association does not hold

after accounting for other clinical covariates commonly used to predict T2D (e.g. age, sex, cohort, body mass index, fasting glucose, systolic blood pressure, high-density lipoprotein cholesterol, triglycerides, and smoking). Similarly, using a subset of data from the WHIOS, Chao et al [27] found that addition of ICAM-1 to traditional risk factors of T2D does not improve prediction. Thus, while there is evidence for an etiological role of endothelial dysfunction in T2D, it is unclear if this relationship is primary, is related to the exact adhesion molecule measures or is secondary to clinical comorbidities.

It is possible that ethnic heterogeneity could explain the variability of results and conclusion across epidemiological studies. Currently, there is increasing evidence to demonstrate that endothelial oxidative stress, inflammatory biomarkers and response to L-arginine supplementation can all differ by race.[28–31] We conducted a prospective study of progression to T2D in initially T2D-free individuals of Mexican American origin in San Antonio. Mexican Americans represent a minority ethnic group in the United States that is at a high risk of type 2 diabetes (reference). Data for this study come from the San Antonio Family Heart Study (SAFHS) which enrolled participants from large and extended Mexican American families.[32–34] Using these data, we tested the hypothesis that circulating levels of soluble intercellular adhesion molecule 1 (sICAM-1) and soluble vascular cell adhesion molecule 1 (sVCAM-1) are independently and additively predictive of future T2D.

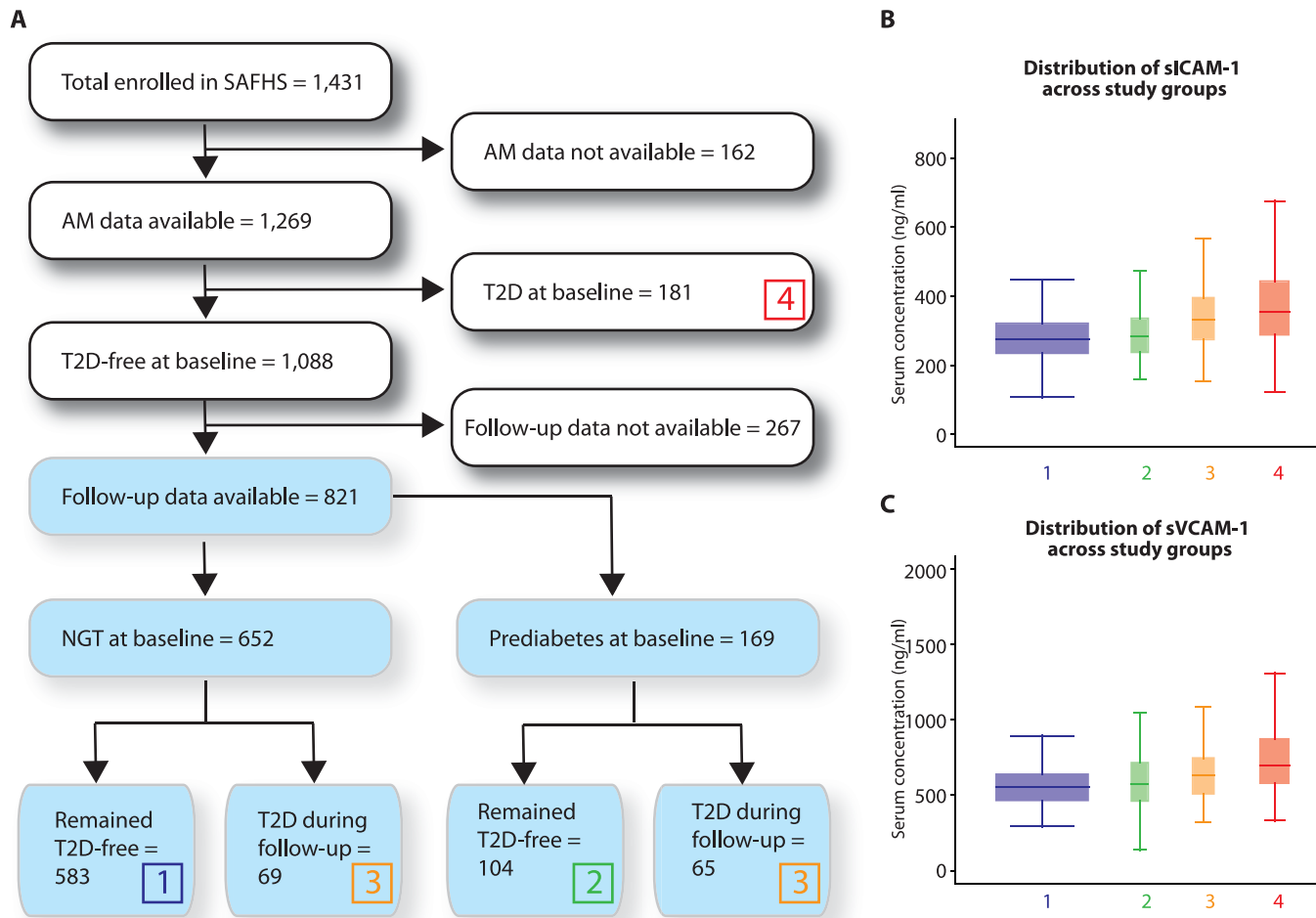
## Materials and Methods

### Study Participants

We used data from the Mexican American families recruited in the SAFHS.[32–34] The Institutional Review Board of the University of Texas Health Science Center at San Antonio approved the study and a written informed consent was obtained from all the study participants. At baseline, this cohort consisted of 1,431 individuals from 42 large and extended pedigrees. We conducted the analyses using the cross-sectional arm of the study (white boxes in Fig 1A) as well as the prospective arm of the study (blue boxes in Fig 1A). The cross-sectional arm of the present study included a total of 1,269 individuals on whom sICAM-1 and sVCAM-1 data was available. We found that 267 (24.5%) of the initially T2D-free individuals did not appear for any follow-up visit later and had to be excluded from longitudinal analyses. The clinical characteristics of those who were lost to follow-up were not statistically significantly different from those who were included (data not shown). For the longitudinal arm of the study, we included a total of 821 individuals without diabetes at baseline who were followed for up to three additional visits spaced approximately 5 years apart (9752.95 person-years of follow-up with a maximum follow-up of 23.53 years). The protocol for selection of the study participants is shown in Fig 1A.

### Phenotypic Data

We included phenotypic data on the following important predictors of type 2 diabetes: age, sex, waist circumference, body mass index, systolic and diastolic blood pressure, fasting and 2-hour plasma glucose, fasting insulin, total serum cholesterol, serum triglycerides, high-density lipoprotein (HDL) cholesterol and use of lipid-lowering and anti-hypertensive drugs. Methods used to measure these variables have been described in details previously. [32–34] Briefly, plasma glucose and insulin levels were measured at fasting and 2 h after administration of 75 g oral glucose, as detailed in [34]. Type 2 diabetes was diagnosed using by the diagnostic criteria of the American Diabetes Association Clinical Practice Recommendations 2004 (fasting plasma glucose level  $\geq 126$  mg/dl [7.0 mmol/l], plasma glucose  $\geq 200$  mg/dl [11.1 mmol/l] at 2 h after oral glucose challenge, or both).[35] Also, individuals were considered to have diabetes if they reported use of antidiabetic medication.[36] For all the participants who reported



**Fig 1. Description of the study cohort and distribution of the serum concentration of adhesion molecules in study participants.** (A) Selection criteria. White colored boxes indicate the cross-sectional component of the study while the blue colored boxes indicate the prospective component of the study. Color-coded numbered boxes represent the four study groups for which distribution of adhesion molecules is given in panels B and C. AM, adhesion molecules (B and C) Box and whisker plots showing the distribution of sICAM-1 (B) and sVCAM-1 (C) concentration in serum. The width of the boxes is proportional to the number of individuals within the group. The boxes are color-coded to match the numbered boxes in panel A.

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T2D during follow-up we had information on the date of T2D diagnosis which was used for censoring. Homeostasis model assessment of insulin resistance (HOMA-IR) values were calculated from fasting glucose and insulin measures according to the formula (fasting glucose [mmol/l] × fasting insulin [μU/ml]/22.5).[37] Prediabetes was defined as presence of either impaired fasting glucose (fasting plasma glucose 100–125 mg/dl [4.56–7 mmol/l]) or impaired glucose tolerance (2 hour post-prandial plasma glucose 140–199 mg/dl [7.78–11.0 mmol/l]) or both. sICAM-1 and sVCAM-1 concentrations were measured in serum samples (diluted 1:20) using an enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN). Measurement of adhesion molecules was based on the same fasting samples used for measurement of glucose, insulin and other biochemical markers.

## Statistical Analysis

**Survival analyses.** Our primary outcome of the study was development of incident T2D. We conducted survival analyses graphically using Kaplan-Meier survival plots. We then

estimated mixed effects Cox proportional hazards (PH) which differs from the classical Cox proportional hazards modeling as mixed effects hazards account for relatedness in family studies. Explicitly, a mixed effects Cox PH model is of the form: [38, 39]

$$\lambda(t) = \lambda_0(t)e^{X\beta+Zb},$$

where  $\lambda$  is the hazard function;  $\lambda_0$  the unspecified baseline hazard;  $X$  and  $Z$  are covariate matrices for fixed and random effects, respectively; and  $\beta$  and  $b$  represent the regression coefficient vectors associated with fixed and random effects, respectively. In this equation the random effects are assumed to be normally distributed as  $N(0, \Sigma(\theta))$  where  $\Sigma$  represents a variance matrix of parameters  $\theta$ . In the case of family studies, the random effects are approximated by the kinship among pairs of individuals. We used this approach to conduct all the multivariable regression modeling in this study. All mixed effects Cox PH models included the following covariates as fixed effects: age, sex, age\*sex interaction, waist circumference, body mass index, systolic and diastolic blood pressures, total serum cholesterol, serum triglycerides, serum HDL-cholesterol, use of anti-lipid medications and use of antihypertensive medication.

**Distributional descriptions.** We used box and whisker plots to describe the distributions of study variables. Continuous variables were also described using the mean and its standard error. To compare median values of a continuous variable across categories of a categorical variable we used Mann-Whitney U (2 categories) or Kruskal-Wallis (3 categories) tests.

**Improvement in predictive performance.** We used the integrated discrimination improvement (IDI) and net reclassification improvement (NRI) indexes to quantify the contribution of adhesion molecules at baseline to prediction of future T2D. [40] These indexes quantify the ability of a marker to better discriminate (as compared to a base model) between cases and non-cases and to better reclassify the cases and non-cases to appropriate groups, respectively.

## Results

### Characteristics of Study Participants

For the longitudinal arm of the study we included a total of 821 initially T2D-free individuals on whom data for sICAM-1, sVCAM-1, follow-up and all phenotypic traits was available. The clinical characteristics of these individuals at baseline are described in [Table 1](#). The participants were young with a majority of females. Both central and general obesity was common (~42% and 35%, respectively) but the prevalence of hypertension was low (9%). Over 20% individuals had prediabetes at baseline. Lipid profile of the study participants did not reveal any major abnormalities. Very few participants were receiving lipid-lowering and antihypertensive drugs (<2% and ~5%, respectively). A total of 134 individuals (16.32%) developed T2D during follow-up.

### Distribution of sICAM-1 and sVCAM-1

As can be seen from [Fig 1B and 1C](#), the distribution of sICAM-1 and sVCAM-1 displayed interesting patterns. For example, highest median values for these adhesion molecules (356.01 ng/ml and 695.26 ng/ml, respectively) were observed for individuals who had T2D at baseline (group 4 in [Fig 1B and 1C](#)) followed by individuals who developed T2D during follow-up (group 3; 332.16 ng/ml and 635.86 ng/ml, respectively). Next, those with prediabetes at baseline but who did not develop T2D during follow-up (group 2) had even lower concentrations of the adhesion molecules (283.19 ng/ml and 575.94 ng/ml, respectively) but the lowest median concentrations were observed for those individuals who had normal glucose tolerance (NGT,

**Table 1. Clinical characteristics of the SAFHS included in the longitudinal arm of the study.**

Characteristic	SAFHS cohort(n = 821)*
Age at enrolment (y)	35.39 (0.51)
Average length of follow-up (y)	11.88 (0.13)
Females	511 (62.24)
Waist (cm)	92.53 (0.61)
Central obesity (Waist circumference $\geq 102$ cm for males and $\geq 88$ cm for females)	343 (41.78)
Body Mass Index (BMI, Kg/m <sup>2</sup> )	28.86 (0.23)
Obesity (BMI $\geq 30$ Kg/m <sup>2</sup> )	288 (35.08)
Systolic blood pressure (SBP, mmHg)	117.0 (0.58)
Diastolic blood pressure (DBP, mmHg)	70.2 (0.34)
Hypertension (SBP $\geq 140$ mmHg and/or DBP $\geq 90$ mmHg)	75 (9.14)
Fasting glucose (mmol/l)	4.86 (0.02)
2-hour post challenge glucose (mmol/l)	5.67 (0.06)
Prediabetes at baseline	169 (20.58)
Impaired fasting glucose only	51 (6.21)
Impaired glucose tolerance only	81 (9.87)
Impaired fasting glucose and impaired glucose tolerance	37 (4.50)
Total serum cholesterol (mg/dl)	187.32 (1.34)
Serum triglycerides (mg/dl)	138.69 (4.57)
HDL cholesterol (mg/dl)	51.12 (0.45)
Participants taking lipid lowering medication	13 (1.58)
Participants taking anti-hypertensive medications	44 (5.36)

\*, numbers indicate mean(SE) for continuous variables and n (%) for categorical variables

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group 1; 274.74 ng/ml and 553.64 ng/ml, respectively). Compared to group 1 the sICAM concentrations in groups 3 and 4 were highly significantly different (Mann-Whitney test  $p = 7.13 \times 10^{-11}$  and  $< 1 \times 10^{-22}$ , respectively) but those in group 2 did not differ significantly from group 1 ( $p = 0.1466$ ). Similarly, the Mann-Whitney test significance values for difference in median concentration of sVCAM in groups 2, 3 and 4 compared to group 1 were 0.2690,  $1.46 \times 10^{-7}$  and  $< 1 \times 10^{-22}$ , respectively.

### Association of sICAM-1 and sVCAM-1 with Insulin Resistance

We investigated if the HOMA-IR values differed significantly based on categories of serum concentration of adhesion molecules. To simplify the use of sICAM-1 and sVCAM-1 concentrations for practical use, we dichotomized the study participants into high and low values based on sICAM-1 and sVCAM-1 concentrations. The high value group represented the top quartile of the distribution while the low value group was a conglomeration of the lower three quartiles. The cutoff points used for high values of sICAM-1 and sVCAM-1 were  $> 336.21$  ng/ml and  $> 670.42$  ng/ml, respectively. To examine if these two adhesion molecules exhibit a potentially independent and additive influence on the risk of future T2D, we created a three-category variable. The categories were: low values for both sICAM-1 and sVCAM-1, high value for either sICAM-1 or sVCAM-1 and high values for both sICAM-1 and sVCAM-1.

The results are shown in [Table 2](#). We observed that with the exception of sVCAM-1 categories in the NGT individuals, the categories of sICAM-1 and sVCAM-1 concentrations were



**Table 2. Median HOMA-IR values based on serum concentrations of sICAM-1 and sVCAM-1.**

Biomarker and categories	Median HOMA-IRAll individuals	Median HOMA-IRNGT individuals
sICAM-1		
High	2.90	2.35
Low	2.04	1.90
Mann-Whitney p	4.08x10 <sup>-9</sup>	0.0012
sVCAM-1		
High	2.67	2.15
Low	2.08	1.94
Mann-Whitney p	3.20x10 <sup>-5</sup>	0.1121
Combined groups (sICAM-1/sVCAM-1)		
High/High	3.28	2.35
High/Low or Low/High	2.53	2.26
Low/Low	1.99	1.87
Kruskal-Wallis p	3.04x10 <sup>-9</sup>	0.0096

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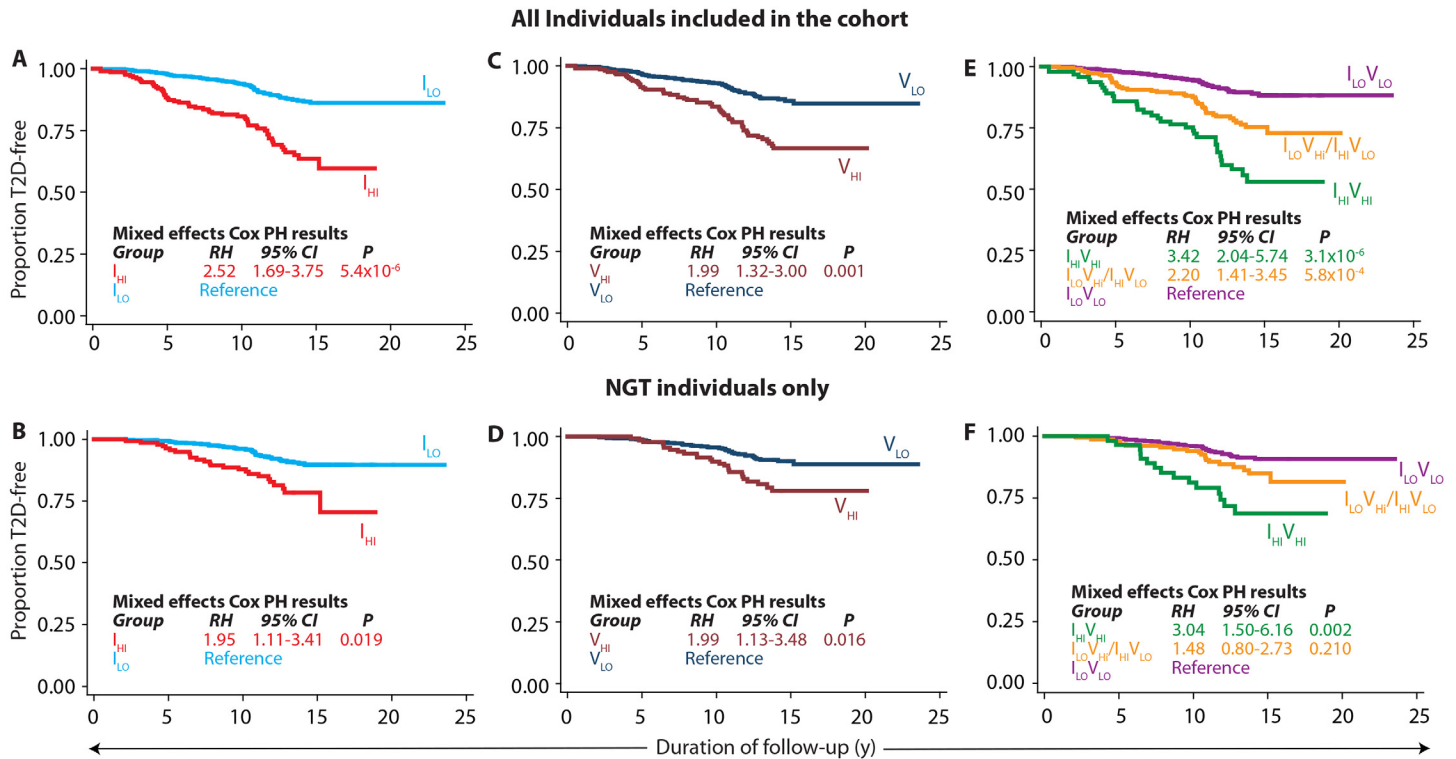
consistently and significantly associated with insulin resistance as measured by HOMA-IR. High values of sICAM-1 and sVCAM-1 concentrations were associated with high values of HOMA-IR and the pattern was even more clearly seen for the variable that combined sICAM-1 and sVCAM-1 concentrations—median HOMA-IR dropped from 3.28 for the High/High group to 1.99 for the Low/Low group for the entire cohort and from 2.35 to 1.87 for NGT individuals.

### Prediction of T2D Based on sICAM-1 and sVCAM-1

We then conducted survival analyses to examine the predictive role of the circulating adhesion molecules in incident T2D. Kaplan-Meier plots for progression to T2D based on independent and combined groups of sICAM-1 and sVCAM-1 concentrations are shown in Fig 2. These results clearly showed that individuals in the top quartile group for both sICAM-1 (Fig 2A) and sVCAM-1 (Fig 2C) progressed to T2D more rapidly than the respective reference groups. Moreover, the influence of these two adhesion molecules was additive as depicted by an even faster progression to T2D when an individual had high values for both sICAM-1 and sVCAM-1 (Fig 2E). These results were seen even in the subset of individuals who had NGT at baseline (Fig 2B, 2D and 2F). The results of multivariable mixed effects Cox PH models for the corresponding analyses demonstrated that indeed, independent of the common clinical predictors of future T2D, high values of sICAM-1 and sVCAM-1 were significantly predictive of incident T2D—singly or in combination. For example, while the individuals with high sICAM-1 and high sVCAM-1 progressed 2.52 and 1.99 times faster than the corresponding reference groups, those who had high values for both sICAM-1 and sVCAM-1 progressed 3.42 times faster to T2D as compared to those with low values for both sICAM-1 and sVCAM-1. With the exception of the group representing a high value for either sICAM-1 or sVCAM-1 concentration, all the results observed the entire cohort were also observed in the NGT subset of the cohort.

### Do Menstruation-Related Variations in Circulating Adhesion Molecules Influence Prediction of T2D?

We investigated the possibility that our results may have been influenced by variations in circulating adhesion molecule concentrations across phases of menstrual cycles. We did not have data on the exact date of last menstrual period. Therefore, we conducted the statistical analyses on two subsets of the original cohort—women in reproductive age group and the remainder of



**Fig 2. Survival analyses for time to onset of type 2 diabetes from the day of enrolment into the study based on the serum concentrations of adhesion molecules.** (A and B) Association of sICAM-1 concentration with incident type 2 diabetes, (C and D) association of sVCAM-1 concentration with incident type 2 diabetes, and (E and F) association of a combination of the serum concentrations of sICAM-1 and sVCAM-1 with incident type 2 diabetes. The group identifiers are as follows: I<sub>LO</sub>, sICAM-1 ≤ 336.21 ng/ml; I<sub>HI</sub>, sICAM-1 > 336.21 ng/ml; V<sub>LO</sub>, sVCAM-1 ≤ 670.42 ng/ml; V<sub>HI</sub>, sVCAM-1 > 670.42 ng/ml. Panels E and F use a combination of these groups as indicated in the Fig. All panels show Kaplan-Meier survival plots for the indicated groups. Inset in each panel are results from mixed effects Cox proportional hazards regression. All regression models used following variables as fixed effects covariates in addition to kinship as random effects: age, sex, age\*sex interaction, waist circumference, body mass index, systolic and diastolic blood pressures, total serum cholesterol, serum triglycerides, serum HDL-cholesterol, use of anti-lipid medications and use of antihypertensive medication. PH, proportional hazards; RH, relative hazards; CI, confidence interval; NGT, normal glucose tolerance.

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the cohort (which included women ≥45 years of age and all males). Notably, only 12.69% of the women in reproductive age group progressed to T2D while 19.54% of the remainder of the cohort progressed to T2D during follow-up. To study the association of circulating adhesion molecules with T2D stratified by the reproductive status of women, we conducted mixed effects Cox regression. Table 3 shows the results of these analyses. We found that in both the

**Table 3. Mixed effects Cox regression for prediction of incident type 2 diabetes in women in reproductive age group (WRAG) and the remainder of the cohort.**

Group	Women in reproductive age group (n = 386)		Remainder of the cohort (n = 435)		P <sub>net</sub> **
	UnadjustedRH (95% CI), p	Adjusted*RH (95% CI), P	UnadjustedRH (95% CI), P	Adjusted*RH (95% CI), p	
I <sub>LO</sub> V <sub>LO</sub>	Ref	Ref	Ref	Ref	--
I <sub>LO</sub> V <sub>HI</sub> /I <sub>HI</sub> V <sub>LO</sub>	3.30 (1.62–6.34), 0.0009	2.46 (1.20–5.00), 0.0130	2.16 (1.26–3.71), 0.0050	1.99 (1.11–3.56), 0.0210	0.944
I <sub>HI</sub> V <sub>HI</sub>	8.61 (3.91–19.0), 9.2x10 <sup>-8</sup>	3.51 (1.59–7.77), 0.0019	3.93 (2.14–7.18), 8.8x10 <sup>-6</sup>	3.39 (1.77–6.52), 0.0003	0.653

\*, All models used following variables as fixed effects covariates in addition to kinship as random effects: age, sex, age\*sex interaction, waist circumference, body mass index, systolic and diastolic blood pressures, total serum cholesterol, serum triglycerides, serum HDL-cholesterol, use of anti-lipid medications and use of antihypertensive medication.

\*\*, test of heterogeneity of the adjusted model results across the two subsets.

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abovementioned subsets of the cohort the association categorized on the basis of sICAM-1 and sVCAM-1 concentrations (as defined in Fig 2E) was statistically significant compared to the reference group. This association could be seen before as well as after adjustment for several other clinically relevant confounders listed in Table 3. Further, the test for heterogeneity of the relative hazards across these two subsets was not significant indicating that women in reproductive age group did not account for the observed associations. Rather, the observations were consistent irrespective of gender and age. These results indirectly affirm the hypothesis that menstruation-related variations in adhesion molecule concentrations were an unlikely driver of the results we observed.

### Improvement in Clinical Prediction of T2D

Considering the independent and additive influence of sICAM-1 and sVCAM-1 concentrations on progression to T2D, we next determined whether addition of these biomarkers to routinely used clinical predictors of T2D leads to a significant improvement in clinical prediction. For this, we estimated the IDI and NRI indexes. Table 4 shows the results of these analyses. Our results indicate that singly or together these two biomarkers significantly improve the net reclassification for the entire cohort as well as for the NGT individuals (p values ranging from 0.0072–2.03x10<sup>-10</sup>). Discrimination between progressors and non-progressors to T2D was statistically significant when examined for the entire cohort but not when restricted to NGT individuals. In the entire cohort, the combined use of sICAM-1 and sVCAM-1 improved discrimination by ~3.5% (p = 0.0002) but in the NGT individuals this improvement was ~1.5% which was marginally non-significant (p = 0.0729).

**Table 4. Improved clinical prediction of incident type 2 diabetes based on sICAM-1 and sVCAM-1 as independent biomarkers\*.**

Biomarker**	IDI (95% CI), p	NRI (95% CI), p
All individuals		
sICAM-1	0.0245 (0.0098–0.0392), 0.0012	0.5482 (0.3595–0.7369), 1.27x10 <sup>-8</sup>
sVCAM-1	0.0195 (0.0062–0.0328), 0.0043	0.4680 (0.2793–0.6567), 1.19x10 <sup>-6</sup>
Combination	0.0346 (0.0166–0.0526), 0.0002	0.6127 (0.4240–0.8014), 2.03x10 <sup>-10</sup>
NGT individuals		
sICAM-1	0.0049 (-0.0049–0.0147), 0.3291	0.3493 (0.0947–0.6039), 0.0072
sVCAM-1	0.0131 (0.0002–0.0260), 0.0485	0.3796 (0.1250–0.6342), 0.0035
Combination	0.0146 (-0.0013–0.0305), 0.0729	0.4005 (0.1459–0.6551), 0.0021

\*, all the results are based on comparison of a model that included the indicated biomarker in addition to the covariates included in the base model. The base model included following covariates: age, sex, age\*sex interaction, waist circumference, body mass index, systolic and diastolic blood pressures, total serum cholesterol, serum triglycerides, serum HDL-cholesterol, fasting glucose, use of anti-lipid medications and use of antihypertensive medication

\*\* , biomarker coding: sICAM-1—serum sICAM-1 concentration >336.21 ng/ml is coded as 1 else 0; sVCAM-1—serum sVCAM-1 concentration >670.42 ng/ml is coded as 1 else 0; Combination—serum sICAM-1 concentration >336.21 ng/ml and serum sVCAM-1 concentration >670.42 ng/ml coded as 2, either serum sICAM-1 concentration >336.21 ng/ml or serum sVCAM-1 concentration >670.42 ng/ml coded as 1, and serum sICAM-1 concentration ≤336.21 ng/ml and serum sVCAM-1 concentration ≤670.42 ng/ml coded as 0

IDI, integrated discrimination improvement; NRI, continuous version of the net reclassification improvement; CI, confidence interval; p, significance value for the null hypothesis of no improvement; NGT, normal glucose tolerance

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## Discussion

Accumulation of soluble adhesion molecules in the serum is a result of proteolytic cleavage of the adhesion molecules bound to the endothelial cell membranes and their consequent release into circulation.[41–43] Therefore serum levels of adhesion molecules are considered to be an indirect measure of endothelial dysfunction. Our findings suggest that soluble versions of ICAM-1 and VCAM-1 are independent and additive biomarkers of incident T2D in Mexican Americans. We observed a clear association of the serum concentrations of these biomarkers with insulin resistance and future T2D. Further, inclusion of these biomarkers into predictive models improved reclassification and discrimination in most instances. The fact that the majority of the results were also observed in the NGT individuals points towards the possibility that endothelial dysfunction may exist years before clinically detectable dysglycemia occurs. Combined with the observation that the highest median values for sICAM-1 and sVCAM-1 were observed in individuals who had T2D at baseline, our study affords an indirect but significant support to the notion that the relationship between T2D and endothelial dysfunction is likely bidirectional. Our results thus agree with the body of literature that supports a contributory role of endothelial dysfunction in type 2 diabetes.

There are two interesting implications of our results in the light of our previous studies. First, we have shown that in healthy adults, smoking is strongly associated with serum concentration of adhesion molecules.[44] On the other hand, smoking—even passive smoking—is a known risk factor for type 2 diabetes.[45, 46] Our results thus provide a potential explanation for the etiological role of smoking in type 2 diabetes. It is conceivable that the smoking increases the risk of T2D by inducing insulin resistance through endothelial dysfunction. This pathway is also supported by the emerging association between smoking and growth differentiation factor-15 [47, 48]—another marker of endothelial dysfunction. Future studies need to test these hypotheses. Second, we have shown previously that there is a significant genetic correlation between sICAM-1 concentration and type 2 diabetes.[49] This finding assumes importance in the light of our observations that the adhesion molecule concentrations in the serum can predict future type 2 diabetes. These observations beckon an examination of the common genetic influences on endothelial dysfunction and type 2 diabetes. Genetic studies such as these can provide novel drug targets for prevention of T2D.

Some limitations of our study need to be considered before generalizing the results. First, our study was based on the high-risk Mexican American individuals and the results should not be readily applied to other populations. Second, even though our study found strong association of the adhesion molecules with insulin resistance, we cannot provide a direct experimental or mechanistic link between these clinical entities. Third, we did not have data on the exact date of last menstrual period which could have helped us directly estimate the putative influence of phases of menstrual cycle on the association of circulating adhesion molecules with incident T2D. This limitation is important in the light of the growing view that concentrations of circulating adhesion molecules vary by phase of menstrual cycle.[50–53] However, we believe that such an influence, even if operational, was unlikely to have confounded our results for the following two reasons: 1. Even after excluding the women in reproductive age groups from analyses (as shown in [Table 3](#)), the remainder of the cohort continued to show the associations with comparable strength and direction of associations. 2. Using the same data on adhesion molecules, we have previously demonstrated a strong genetic control over the circulating levels as well as their significant genetic correlation with insulin resistance.[44, 49, 54] It is conceivable that if the findings were to be confounded by phases of menstruation then the genetic signals observed previously would have obscured.

Notwithstanding these limitations, our study has the strengths of a prospective cohort, single ethnic background, family setting and long follow-up. In conclusion, we show that circulating levels of sICAM-1 and sVCAM-1 are independently and additively predictive of incident T2D. In the continued search for better and more informative biomarkers for T2D, sICAM-1 and sVCAM-1 represent important candidate biomarkers of T2D that need to be investigated in future studies.

## Supporting Information

### S1 Data.

(XLSX)

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## Author Contributions

Conceived and designed the experiments: HK MM JEC JB. Performed the experiments: JP JEC. Analyzed the data: HK MM JP MA JB. Wrote the paper: HK MM JB JEC. Reviewed and helped in revising draft: TDD HHG MPJ RD MCM RLO LA DCG SW-B.

## References

1. Nowak M, Wielkoszynski T, Marek B, Kos-Kudla B, Swietochowska E, Sieminska L, et al. Blood serum levels of vascular cell adhesion molecule (sVCAM-1), intercellular adhesion molecule (sICAM-1) and endothelial leucocyte adhesion molecule-1 (ELAM-1) in diabetic retinopathy. *Clinical and experimental medicine*. 2008; 8(3):159–64. Epub 2008/09/16. doi: [10.1007/s10238-008-0173-z](https://doi.org/10.1007/s10238-008-0173-z) PMID: [18791689](https://pubmed.ncbi.nlm.nih.gov/18791689/).
2. Boulbou MS, Koukoulis GN, Makri ED, Petinaki EA, Gourgoulis KI, Germeis AE. Circulating adhesion molecules levels in type 2 diabetes mellitus and hypertension. *International journal of cardiology*. 2005; 98(1):39–44. Epub 2005/01/29. doi: [10.1016/j.ijcard.2003.07.037](https://doi.org/10.1016/j.ijcard.2003.07.037) PMID: [15676164](https://pubmed.ncbi.nlm.nih.gov/15676164/).
3. Matsumoto K, Sera Y, Ueki Y, Inukai G, Niuro E, Miyake S. Comparison of serum concentrations of soluble adhesion molecules in diabetic microangiopathy and macroangiopathy. *Diabetic medicine: a journal of the British Diabetic Association*. 2002; 19(10):822–6. Epub 2002/10/03. PMID: [12358868](https://pubmed.ncbi.nlm.nih.gov/12358868/).
4. Wallen NH, Held C, Rehnqvist N, Hjemdahl P. Elevated serum intercellular adhesion molecule-1 and vascular adhesion molecule-1 among patients with stable angina pectoris who suffer cardiovascular death or non-fatal myocardial infarction. *European heart journal*. 1999; 20(14):1039–43. Epub 1999/06/26. doi: [10.1053/euhj.1999.1451](https://doi.org/10.1053/euhj.1999.1451) PMID: [10381855](https://pubmed.ncbi.nlm.nih.gov/10381855/).
5. Saku K, Zhang B, Ohta T, Shirai K, Tsuchiya Y, Arakawa K. Levels of soluble cell adhesion molecules in patients with angiographically defined coronary atherosclerosis. *Japanese circulation journal*. 1999; 63(1):19–24. Epub 1999/03/20. PMID: [10084383](https://pubmed.ncbi.nlm.nih.gov/10084383/).
6. Albertini JP, Valensi P, Lormeau B, Aourousseau MH, Ferriere F, Attali JR, et al. Elevated concentrations of soluble E-selectin and vascular cell adhesion molecule-1 in NIDDM. Effect of intensive insulin treatment. *Diabetes care*. 1998; 21(6):1008–13. Epub 1998/06/06. PMID: [9614623](https://pubmed.ncbi.nlm.nih.gov/9614623/).
7. Zeitler H, Ko Y, Zimmermann C, Nickenig G, Glanzer K, Walger P, et al. Elevated serum concentrations of soluble adhesion molecules in coronary artery disease and acute myocardial infarction. *European journal of medical research*. 1997; 2(9):389–94. Epub 1997/09/25. PMID: [9300936](https://pubmed.ncbi.nlm.nih.gov/9300936/).
8. Fasching P, Veitl M, Rohac M, Strelci C, Schneider B, Waldhausl W, et al. Elevated concentrations of circulating adhesion molecules and their association with microvascular complications in insulin-dependent diabetes mellitus. *The Journal of clinical endocrinology and metabolism*. 1996; 81(12):4313–7. Epub 1996/12/01. doi: [10.1210/jcem.81.12.8954033](https://doi.org/10.1210/jcem.81.12.8954033) PMID: [8954033](https://pubmed.ncbi.nlm.nih.gov/8954033/).

9. Del Turco S, Gaggini M, Daniele G, Basta G, Folli F, Sicari R, et al. Insulin resistance and endothelial dysfunction: a mutual relationship in cardiometabolic risk. *Current pharmaceutical design*. 2013; 19(13):2420–31. Epub 2012/11/24. PMID: [23173591](#).
10. Matheus AS, Tannus LR, Cobas RA, Palma CC, Negrato CA, Gomes MB. Impact of diabetes on cardiovascular disease: an update. *International journal of hypertension*. 2013; 2013:653789. Epub 2013/03/28. doi: [10.1155/2013/653789](#) PMID: [23533715](#); PubMed Central PMCID: PMC3603160.
11. Polovina MM, Potpara TS. Endothelial dysfunction in metabolic and vascular disorders. *Postgraduate medicine*. 2014; 126(2):38–53. Epub 2014/04/02. doi: [10.3810/pgm.2014.03.2739](#) PMID: [24685967](#).
12. Eringa EC, Serne EH, Meijer RI, Schalkwijk CG, Houben AJ, Stehouwer CD, et al. Endothelial dysfunction in (pre)diabetes: characteristics, causative mechanisms and pathogenic role in type 2 diabetes. *Reviews in endocrine & metabolic disorders*. 2013; 14(1):39–48. Epub 2013/02/19. doi: [10.1007/s11154-013-9239-7](#) PMID: [23417760](#).
13. Meigs JB, Hu FB, Rifai N, Manson JE. Biomarkers of endothelial dysfunction and risk of type 2 diabetes mellitus. *Jama*. 2004; 291(16):1978–86. Epub 2004/04/29. doi: [10.1001/jama.291.16.1978](#) PMID: [15113816](#).
14. Meigs JB, O'Donnell C J, Tofler GH, Benjamin EJ, Fox CS, Lipinska I, et al. Hemostatic markers of endothelial dysfunction and risk of incident type 2 diabetes: the Framingham Offspring Study. *Diabetes*. 2006; 55(2):530–7. Epub 2006/01/31. PMID: [16443791](#).
15. Song Y, Manson JE, Tinker L, Rifai N, Cook NR, Hu FB, et al. Circulating levels of endothelial adhesion molecules and risk of diabetes in an ethnically diverse cohort of women. *Diabetes*. 2007; 56(7):1898–904. Epub 2007/03/29. doi: [10.2337/db07-0250](#) PMID: [17389327](#); PubMed Central PMCID: PMC1952236.
16. Thorand B, Baumert J, Chambless L, Meisinger C, Kolb H, Doring A, et al. Elevated markers of endothelial dysfunction predict type 2 diabetes mellitus in middle-aged men and women from the general population. *Arteriosclerosis, thrombosis, and vascular biology*. 2006; 26(2):398–405. Epub 2005/12/03. doi: [10.1161/01.ATV.0000198392.05307.aa](#) PMID: [16322530](#).
17. Stranges S, Rafalson LB, Dmochowski J, Rejman K, Tracy RP, Trevisan M, et al. Additional contribution of emerging risk factors to the prediction of the risk of type 2 diabetes: evidence from the Western New York Study. *Obesity (Silver Spring)*. 2008; 16(6):1370–6. Epub 2008/03/22. doi: [10.1038/oby.2008.59](#) PMID: [18356828](#).
18. Rossi R, Cioni E, Nuzzo A, Origliani G, Modena MG. Endothelial-dependent vasodilation and incidence of type 2 diabetes in a population of healthy postmenopausal women. *Diabetes care*. 2005; 28(3):702–7. Epub 2005/03/01. PMID: [15735211](#).
19. Chen NG, Holmes M, Reaven GM. Relationship between insulin resistance, soluble adhesion molecules, and mononuclear cell binding in healthy volunteers. *The Journal of clinical endocrinology and metabolism*. 1999; 84(10):3485–9. Epub 1999/10/16. doi: [10.1210/jcem.84.10.6065](#) PMID: [10522984](#).
20. Hak AE, Pols HA, Stehouwer CD, Meijer J, Kiliaan AJ, Hofman A, et al. Markers of inflammation and cellular adhesion molecules in relation to insulin resistance in nondiabetic elderly: the Rotterdam study. *The Journal of clinical endocrinology and metabolism*. 2001; 86(9):4398–405. Epub 2001/09/11. doi: [10.1210/jcem.86.9.7873](#) PMID: [11549682](#).
21. Matsumoto K, Sera Y, Miyake S, Ueki Y. Serum levels of adhesion molecules correlate with insulin resistance. *Atherosclerosis*. 2002; 161(1):243–4. Epub 2002/03/08. PMID: [11882338](#).
22. Weyer C, Yudkin JS, Stehouwer CD, Schalkwijk CG, Pratley RE, Tataranni PA. Humoral markers of inflammation and endothelial dysfunction in relation to adiposity and in vivo insulin action in Pima Indians. *Atherosclerosis*. 2002; 161(1):233–42. Epub 2002/03/08. PMID: [11882337](#).
23. Balsan GA, Vieira JL, Oliveira AM, Portal VL. Relationship between adiponectin, obesity and insulin resistance. *Rev Assoc Med Bras*. 2015; 61(1):72–80. Epub 2015/04/25. doi: [10.1590/1806-9282.61.01.072](#) PMID: [25909213](#).
24. Carbone F, Mach F, Montecucco F. The role of adipocytokines in atherogenesis and atheroprogession. *Current drug targets*. 2015; 16(4):295–320. Epub 2014/11/11. PMID: [25382206](#).
25. Hartge MM, Unger T, Kintscher U. The endothelium and vascular inflammation in diabetes. *Diabetes & vascular disease research*. 2007; 4(2):84–8. Epub 2007/07/27. doi: [10.3132/dvdr.2007.025](#) PMID: [17654441](#).
26. Dallmeier D, Larson MG, Wang N, Fontes JD, Benjamin EJ, Fox CS. Addition of inflammatory biomarkers did not improve diabetes prediction in the community: the framingham heart study. *Journal of the American Heart Association*. 2012; 1(4):e000869. Epub 2012/11/07. doi: [10.1161/JAHA.112.000869](#) PMID: [23130155](#); PubMed Central PMCID: PMC3487343.
27. Chao C, Song Y, Cook N, Tseng CH, Manson JE, Eaton C, et al. The lack of utility of circulating biomarkers of inflammation and endothelial dysfunction for type 2 diabetes risk prediction among postmenopausal women: the Women's Health Initiative Observational Study. *Archives of internal medicine*.

- 2010; 170(17):1557–65. Epub 2010/09/30. doi: [10.1001/archinternmed.2010.312](https://doi.org/10.1001/archinternmed.2010.312) PMID: [20876407](https://pubmed.ncbi.nlm.nih.gov/20876407/); PubMed Central PMCID: [PMC3127580](https://pubmed.ncbi.nlm.nih.gov/pmc/PMC3127580/).
28. Gardner AW, Parker DE, Montgomery PS, Sosnowska D, Casanegra AI, Ungvari Z, et al. Gender and racial differences in endothelial oxidative stress and inflammation in patients with symptomatic peripheral artery disease. *Journal of vascular surgery*. 2015; 61(5):1249–57. Epub 2014/04/08. doi: [10.1016/j.jvs.2014.02.045](https://doi.org/10.1016/j.jvs.2014.02.045) PMID: [24703977](https://pubmed.ncbi.nlm.nih.gov/24703977/); PubMed Central PMCID: [PMC4185015](https://pubmed.ncbi.nlm.nih.gov/pmc/PMC4185015/).
  29. Morris AA, Zhao L, Patel RS, Jones DP, Ahmed Y, Stoyanova N, et al. Differences in systemic oxidative stress based on race and the metabolic syndrome: the Morehouse and Emory Team up to Eliminate Health Disparities (META-Health) study. *Metabolic syndrome and related disorders*. 2012; 10(4):252–9. Epub 2012/03/06. doi: [10.1089/met.2011.0117](https://doi.org/10.1089/met.2011.0117) PMID: [22385338](https://pubmed.ncbi.nlm.nih.gov/22385338/); PubMed Central PMCID: [PMC3449394](https://pubmed.ncbi.nlm.nih.gov/pmc/PMC3449394/).
  30. Gibbons GH. Physiology, genetics, and cardiovascular disease: focus on African Americans. *Journal of clinical hypertension*. 2004; 6(4 Suppl 1):11–8. Epub 2004/04/10. PMID: [15073462](https://pubmed.ncbi.nlm.nih.gov/15073462/).
  31. Houghton JL, Philbin EF, Strogatz DS, Torosoff MT, Fein SA, Kuhner PA, et al. The presence of African American race predicts improvement in coronary endothelial function after supplementary L-arginine. *Journal of the American College of Cardiology*. 2002; 39(8):1314–22. Epub 2002/04/17. PMID: [11955849](https://pubmed.ncbi.nlm.nih.gov/11955849/).
  32. MacCluer JW, Stern MP, Almasy L, Atwood LA, Blangero J, Comuzzie AG, et al. Genetics of atherosclerosis risk factors in Mexican Americans. *Nutrition reviews*. 1999; 57(5 Pt 2):S59–65. Epub 1999/07/03. PMID: [10391028](https://pubmed.ncbi.nlm.nih.gov/10391028/).
  33. Voruganti VS, Lopez-Alvarenga JC, Nath SD, Rainwater DL, Bauer R, Cole SA, et al. Genetics of variation in HOMA-IR and cardiovascular risk factors in Mexican-Americans. *J Mol Med (Berl)*. 2008; 86(3):303–11. Epub 2008/01/22. doi: [10.1007/s00109-007-0273-3](https://doi.org/10.1007/s00109-007-0273-3) PMID: [18204828](https://pubmed.ncbi.nlm.nih.gov/18204828/).
  34. Mitchell BD, Kammerer CM, Blangero J, Mahaney MC, Rainwater DL, Dyke B, et al. Genetic and environmental contributions to cardiovascular risk factors in Mexican Americans. The San Antonio Family Heart Study. *Circulation*. 1996; 94(9):2159–70. Epub 1996/11/01. PMID: [8901667](https://pubmed.ncbi.nlm.nih.gov/8901667/).
  35. Diagnosis and classification of diabetes mellitus. *Diabetes care*. 2004; 27 Suppl 1:S5–S10. Epub 2003/12/25. PMID: [14693921](https://pubmed.ncbi.nlm.nih.gov/14693921/).
  36. Kissebah AH, Sonnenberg GE, Myklebust J, Goldstein M, Broman K, James RG, et al. Quantitative trait loci on chromosomes 3 and 17 influence phenotypes of the metabolic syndrome. *Proceedings of the National Academy of Sciences of the United States of America*. 2000; 97(26):14478–83. Epub 2000/12/20. doi: [10.1073/pnas.97.26.14478](https://doi.org/10.1073/pnas.97.26.14478) PMID: [11121050](https://pubmed.ncbi.nlm.nih.gov/11121050/); PubMed Central PMCID: [PMC18944](https://pubmed.ncbi.nlm.nih.gov/pmc/PMC18944/).
  37. Haffner SM, Miettinen H, Stern MP. The homeostasis model in the San Antonio Heart Study. *Diabetes care*. 1997; 20(7):1087–92. Epub 1997/07/01. PMID: [9203442](https://pubmed.ncbi.nlm.nih.gov/9203442/).
  38. Zhao J. Mixed-effects Cox models of alcohol dependence in extended families. *BMC genetics*. 2005; 6 Suppl 1:S127. Epub 2006/02/03. doi: [10.1186/1471-2156-6-S1-S127](https://doi.org/10.1186/1471-2156-6-S1-S127) PMID: [16451585](https://pubmed.ncbi.nlm.nih.gov/16451585/); PubMed Central PMCID: [PMC1866810](https://pubmed.ncbi.nlm.nih.gov/pmc/PMC1866810/).
  39. Therneau TM. *coxme: Mixed Effects Cox Models*. R package 2.2–3 ed2012.
  40. Pencina MJ, D'Agostino RB Sr., D'Agostino RB Jr., Vasan RS. Evaluating the added predictive ability of a new marker: from area under the ROC curve to reclassification and beyond. *Statistics in medicine*. 2008; 27(2):157–72; discussion 207–12. Epub 2007/06/15. doi: [10.1002/sim.2929](https://doi.org/10.1002/sim.2929) PMID: [17569110](https://pubmed.ncbi.nlm.nih.gov/17569110/).
  41. Price DT, Loscalzo J. Cellular adhesion molecules and atherogenesis. *The American journal of medicine*. 1999; 107(1):85–97. Epub 1999/07/14. PMID: [10403357](https://pubmed.ncbi.nlm.nih.gov/10403357/).
  42. Leeuwenberg JF, Smeets EF, Neefjes JJ, Shaffer MA, Cinek T, Jeunhomme TM, et al. E-selectin and intercellular adhesion molecule-1 are released by activated human endothelial cells in vitro. *Immunology*. 1992; 77(4):543–9. Epub 1992/12/01. PMID: [1283598](https://pubmed.ncbi.nlm.nih.gov/1283598/); PubMed Central PMCID: [PMC1421640](https://pubmed.ncbi.nlm.nih.gov/pmc/PMC1421640/).
  43. Marfella R, Esposito K, Giunta R, Coppola G, De Angelis L, Farzati B, et al. Circulating adhesion molecules in humans: role of hyperglycemia and hyperinsulinemia. *Circulation*. 2000; 101(19):2247–51. Epub 2000/05/16. PMID: [10811590](https://pubmed.ncbi.nlm.nih.gov/10811590/).
  44. Demerath E, Towne B, Blangero J, Siervogel RM. The relationship of soluble ICAM-1, VCAM-1, P-selectin and E-selectin to cardiovascular disease risk factors in healthy men and women. *Annals of human biology*. 2001; 28(6):664–78. Epub 2001/12/01. PMID: [11726042](https://pubmed.ncbi.nlm.nih.gov/11726042/).
  45. Wei X, E M, Yu S. A meta-analysis of passive smoking and risk of developing Type 2 Diabetes Mellitus. *Diabetes research and clinical practice*. 2015; 107(1):9–14. Epub 2014/12/10. doi: [10.1016/j.diabres.2014.09.019](https://doi.org/10.1016/j.diabres.2014.09.019) PMID: [25488377](https://pubmed.ncbi.nlm.nih.gov/25488377/).
  46. Fagard RH, Nilsson PM. Smoking and diabetes—the double health hazard! *Primary care diabetes*. 2009; 3(4):205–9. Epub 2009/10/31. doi: [10.1016/j.pcd.2009.09.003](https://doi.org/10.1016/j.pcd.2009.09.003) PMID: [19875348](https://pubmed.ncbi.nlm.nih.gov/19875348/).

47. Berezin AE. Diabetes mellitus related biomarker: The predictive role of growth-differentiation factor-15. *Diabetes & metabolic syndrome*. 2015. Epub 2015/10/21. doi: [10.1016/j.dsx.2015.09.016](https://doi.org/10.1016/j.dsx.2015.09.016) PMID: [26482961](https://pubmed.ncbi.nlm.nih.gov/26482961/).
48. Adela R, Banerjee SK. GDF-15 as a Target and Biomarker for Diabetes and Cardiovascular Diseases: A Translational Prospective. *Journal of diabetes research*. 2015; 2015:490842. Epub 2015/08/15. doi: [10.1155/2015/490842](https://doi.org/10.1155/2015/490842) PMID: [26273671](https://pubmed.ncbi.nlm.nih.gov/26273671/); PubMed Central PMCID: PMC4530250.
49. Kent JW Jr., Comuzzie AG, Mahaney MC, Almasy L, Rainwater DL, VandeBerg JL, et al. Intercellular adhesion molecule-1 concentration is genetically correlated with insulin resistance, obesity, and HDL concentration in Mexican Americans. *Diabetes*. 2004; 53(10):2691–5. Epub 2004/09/28. PMID: [15448102](https://pubmed.ncbi.nlm.nih.gov/15448102/).
50. Bonello N, Norman RJ. Soluble adhesion molecules in serum throughout the menstrual cycle. *Human reproduction*. 2002; 17(9):2272–8. Epub 2002/08/31. PMID: [12202413](https://pubmed.ncbi.nlm.nih.gov/12202413/).
51. Begic I, Cavka A, Mihalj M, Bacun T, Drenjancevic I. Influence of short-term changes in sex hormones on serum concentrations of cellular adhesion molecules in young healthy women. *Medicinski glasnik: official publication of the Medical Association of Zenica-Doboj Canton, Bosnia and Herzegovina*. 2012; 9(1):32–6. Epub 2012/05/29. PMID: [22634905](https://pubmed.ncbi.nlm.nih.gov/22634905/).
52. Somigliana E, Vigano P, Gaffuri B, Guarneri D, Busacca M, Vignali M. Human endometrial stromal cells as a source of soluble intercellular adhesion molecule (ICAM)-1 molecules. *Human reproduction*. 1996; 11(6):1190–4. Epub 1996/06/01. PMID: [8671421](https://pubmed.ncbi.nlm.nih.gov/8671421/).
53. Vigano P, Somigliana E, Gaffuri B, Santorsola R, Busacca M, Vignali M. Endometrial release of soluble intercellular adhesion molecule 1 and endometriosis: relationship to the extent of the disease. *Obstetrics and gynecology*. 2000; 95(1):115–8. Epub 2000/01/15. PMID: [10636513](https://pubmed.ncbi.nlm.nih.gov/10636513/).
54. Kent JW Jr., Mahaney MC, Comuzzie AG, Goring HH, Almasy L, Dyer TD, et al. Quantitative trait locus on Chromosome 19 for circulating levels of intercellular adhesion molecule-1 in Mexican Americans. *Atherosclerosis*. 2007; 195(2):367–73. Epub 2006/11/23. doi: [10.1016/j.atherosclerosis.2006.10.005](https://doi.org/10.1016/j.atherosclerosis.2006.10.005) PMID: [17112530](https://pubmed.ncbi.nlm.nih.gov/17112530/); PubMed Central PMCID: PMC2719976.