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Biomarker Insights

Risk Factors Associated with Serum Levels of the Inflammatory Biomarker Soluble Urokinase Plasminogen Activator Receptor in a General Population

Thomas H. Haupt[1], Thomas Kallemose[1], Steen Ladelund[1], Line J. H. Rasmussen[1], Christian W. Thorball[1], Ove Andersen[1], Charlotta Pisinger[2] and Jesper Eugen-Olsen[1]

[1]Clinical Research Centre, Department 056, Hvidovre Hospital, Hvidovre, Denmark. [2]Research Centre for Prevention and Health, Department 84–85, Glostrup University Hospital, Glostrup, Denmark.

ABSTRACT: The soluble urokinase plasminogen activator receptor (suPAR) is a biomarker of mortality risk in various patient populations. However, little is known about the implications of lifestyle for suPAR levels in the general population. Lifestyle, demographic, and cardiovascular disease (CVD) risk factor data were collected from 5,538 participants in the Danish population-based Inter99 study. Their suPAR levels were measured using a sandwich enzyme-linked immunosorbent assay. In the final adjusted model, smoking and morbid obesity were strongly associated with higher suPAR levels ($P < 0.001$). An unhealthy diet and alcohol abstinence in men were also associated with higher suPAR levels. Physical activity in leisure time had a modest impact on suPAR levels in univariate analysis, but not in the final adjusted model. In conclusion, smoking and morbid obesity were strongly associated with higher serum suPAR levels in this general population. Diet and alcohol consumption also seemed to impact suPAR levels. Lifestyle changes are likely to affect suPAR since ex-smokers had suPAR levels comparable to those of never-smokers.

KEYWORDS: biomarkers, epidemiologic studies, risk factors, urokinase plasminogen activator receptor

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CORRESPONDENCE: thomas.huneck.haupt.01@regionh.dk

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Introduction

Inflammation is thought to be a key player in the development of an increasing number of diseases. Many different markers are used to measure an increased inflammatory state, including pro-inflammatory proteins such as tumor necrosis factor alpha (TNF-α), elevated levels of C-reactive protein (CRP), and increased leukocyte count. CRP is currently the gold standard biomarker for measuring low-grade or chronic inflammation. However, since CRP is an acute phase reactant, measurements of CRP in otherwise healthy individuals may not reflect the basal inflammatory level if acute inflammation is present.

The soluble urokinase plasminogen activator receptor (suPAR) is a recently established biomarker of inflammation and immune activation, and elevated suPAR levels are associated with disease severity and mortality in various patient populations.[1–8] In the general population, suPAR is thought to reflect a state of chronic inflammation, similar to CRP. The Danish part of the MONICA studies showed that elevated suPAR levels are associated with an increased risk for cardiovascular disease (CVD), type 2 diabetes, cancer, and premature mortality in the general population, independently of CRP.[9] Another population-based study in Malmö, Sweden, confirmed that elevated suPAR levels are associated with a higher

risk of CVD.[10] These studies also report some associations of suPAR with baseline subject characteristics like age, sex, and smoking habits; however, these findings were not discussed extensively and only univariate analyses were conducted as the studies were designed to associate suPAR levels with specific long-term outcomes, rather than with baseline data.

As a result, little is known about which factors impact suPAR levels in the general population. We hypothesized that known risk factors for mortality, such as smoking, would also be associated with higher suPAR levels. The primary aim of this study was to investigate the possible link between suPAR levels and lifestyle factors in a general population. We also wanted to investigate the associations between suPAR levels and demographic variables and risk factors for CVD in a general population.

Methods

Study sample. The aim of the Inter99 study was to decrease the incidence of CVD in a general population cohort through systematic computer-aided risk screening and non-pharmacological intervention, such as advising people to adopt a healthy diet, quit smoking, keep alcohol consumption low, and engage in high levels of physical activity. The study design and results are reported in detail elsewhere.[11,12] Briefly, the study population comprised all 61,301 persons born in 1939–40, 1944–45, 1949–50, 1954–55, 1959–60, 1964–65, and 1969–70, who were living in the 11 municipalities in the south-western part of Copenhagen County on December 2, 1998. These people were identified using their unique Danish civil registry identity numbers. From the study population, a random sample of 13,016 persons was invited to participate. A total of 6,784 persons agreed to participate (52.5%). We excluded those who did not have serum samples available for suPAR measurement (n = 1,244, 18.3%) and those in whom the serum suPAR level was out of range of the enzyme-linked immunosorbent assay (ELISA) (n = 2, 0.04%). Thus, a total of 5,538 participants were included in the final analysis. Data from the extensive questionnaire, physical measurements, and blood samples were collected at the baseline examinations, which were conducted from March 1999 until January 2001.

All participants provided written informed consent before taking part in the study. The study was approved by the local ethics committee (KA 98 155), by the Danish Data Protection Agency, and it was conducted in accordance with the Declaration of Helsinki. The study is registered with ClinicalTrials.gov (NCT00289237).

Demographic variables. The participants' age and sex were confirmed using their civil registry identity numbers. Regarding socioeconomic status (SES), participants were asked about the duration of their vocational/higher education, which was categorized as low (<2 years), medium (2 to 4 years), or high (>4 years).

Cardiovascular risk factors. The participants had their CVD risk assessed at baseline.[11] Participants were asked to fast after midnight on the day of the baseline examination, which was defined as no smoking, eating, or drinking except for a little water together with routine medications in the morning. All had their blood pressure measured twice with a mercury sphygmomanometer after resting for 5 minutes in a supine position. If the systolic blood pressure (SBP) was above 140 mmHg, the measurement was repeated twice more, and the lowest measurement was reported. Height was measured without shoes to the nearest centimeter, weight without shoes and overcoat to the nearest kilogram, and the body mass index (BMI) was calculated as weight/height^2. Fasting blood samples were drawn and stored at –18 °C until analysis. Plasma total cholesterol, triglycerides, and high-density lipoprotein (HDL) cholesterol were determined by an enzymatic technique (Boehringer Mannheim, Germany). The personal history of myocardial infarction (MI) and the family history of MI were self-reported in the questionnaire. Participants who reported incident diabetes were classified as such. Those who reported no diabetes underwent a standard 75 g oral glucose tolerance test (OGTT). If fasting plasma glucose (FPG) was ≥7.0 mmol/L or 2-hour plasma glucose was ≥11.1 mmol/L, the subjects were reclassified as having incident diabetes.

Lifestyle variables. Regarding *diet*, participants answered a 52-item food frequency questionnaire (reference period: one week). To simplify the data, we used the dietary quality score, which is a three-class quality variable generated for each of four food groups (fish, vegetables, fruits, and fats), and classified the diet as a healthy diet, an average diet, or an unhealthy diet. This score was validated previously.[13] For *smoking*, participants were asked about their smoking habits (daily, occasional, ex- or never-smoker) and grouped accordingly. Occasional smokers were excluded from the analysis (n = 207, 3.7%) because of the group's small size. Daily smokers were asked about their average daily tobacco use. We measured grams of tobacco per day in the following way: one cigarette/gram of pipe tobacco = 1 g; one cheroot = 3 g; one cigar = 5 g. Daily smokers were categorized by daily tobacco use into these categories: <15 g, 15–24.9 g, or ≥25 g per day. For *alcohol*, participants reported their average weekly consumption of beer, strong beer, wine, fortified wine, and liquor. The weekly alcohol consumption was calculated in units of alcohol per week (one unit = 12 g of alcohol). We classified the participants' alcohol use as abstinent (0 units per week), within recommendations (1–21 units per week for men and 1–14 units per week for women according to the Danish Health Authorities' guidelines at the time), or overuse (>21 units per week for men and >14 units per week for women). *Physical activity* was based on self-reported leisure time physical activities and dichotomized as sedentary (mainly sedentary) or active (moderate activity, regular sports/exercise, or athletic training/participation in competitive sports).

suPAR measurement. Serum suPAR concentration (in ng/mL) was measured in frozen serum samples from the Inter99 biobank in 2011 using a Conformité Européene

(CE)-approved sandwich ELISA (suPARnostic®, ViroGates A/S, Birkeroed, Denmark) according to the manufacturer's protocol.

Statistical methods. Univariate associations between suPAR and categorical variables were analyzed by non-parametric one-way analysis (Kruskal–Wallis test[14]) using SAS 9.3 (SAS Institute, Cary, NC, USA). Correlation analysis was performed for continuous variables, and we report the Kendall's tau-b correlation coefficient.[15]

Multiple linear regression was used in the adjusted models. The residuals appeared to be heteroscedastic, with larger values that did not support the normality assumption. Parametric 95% bootstrap confidence intervals (CIs) were calculated based on the results for each variable in all models. The differences in the CIs based on the models and the corresponding bootstrap estimates were all between −0.1 and 0.1, leading us to accept the model results. BMI had a non-linear association with suPAR levels and was included as a cubic spline with knots 18 and 40. All multiple linear regressions were performed using R 3.02 (R Foundation, Vienna, Austria). A *P*-value less than 0.05 was considered statistically significant.

Modeling strategy. We analyzed three adjusted models (CVD risk factor, Lifestyle, and Combined models) in order to assess the effects of multiple mutually adjusted downstream factors. All models included multiple linear regressions with serum suPAR concentration as output, and the Combined model consisted of the statistically significant variables from the CVD and Lifestyle models. The models were tested for pair-wise interactions between sex, smoking, alcohol consumption, and BMI. Sex interactions were present in all models, and estimates are therefore presented separately for men and women. The other interactions showed no clear trend and appear to be the result of residual confounding – they are therefore not reported in the final models.

In order to have a reference point for each explanatory variable, we defined the reference point as someone who was 30 years old at the time of the baseline examination, a never-smoker who ate a healthy diet, was physically active, and who drank alcohol within the recommended limits. The reference point also had a total cholesterol level of 4 mmol/L, HDL cholesterol of 2 mmol/L, a triglyceride level of 1 mmol/L, a SBP of 120 mm Hg, a BMI of 20 kg/m^2, no incident diabetes, and no personal or family history of MI. Estimates from the same models are displayed in two separate tables, for men and women, because of interactions between sex and other variables. The effect of sex thus accounted for the difference in suPAR levels between the male and the female reference points.

To visualize how different risk factor profiles impact on suPAR levels, we defined six illustration persons: a healthy man and woman with the same characteristics as the above-mentioned reference point; a man and woman at high CVD risk who instead ate an unhealthy diet, was physically inactive, and who had an overuse of alcohol. The high CVD risk illustration persons also had a total cholesterol level of 8 mmol/L, HDL cholesterol of 0.8 mmol/L, a triglyceride level of 6 mmol/L, a SBP of 160 mm Hg, a BMI of 40 kg/m^2, incident diabetes, as well as both a personal and family history of MI. Finally, we defined a man and woman with the same high CVD risk factors as above, but who in addition had a daily tobacco use of ≥25 g/day.

Since BMI was included as a cubic spline, direct interpretation of the estimates is difficult and they are therefore not listed in the tables. Instead, the relationship between suPAR level and BMI is displayed graphically, and the tests for BMI were performed on the spline basis variables.

Results

Simple associations between suPAR levels and demographic characteristics. Men had a mean serum suPAR level of 3.51 ng/mL, whereas women had a mean suPAR level of 3.90 ng/mL ($P < 0.0001$). suPAR levels increased with age for both men and women, but the increase per year was greater for men (Table 1). Higher SES was associated with lower suPAR level (Table 2).

Simple associations between suPAR levels and lifestyle factors. An unhealthy diet was associated with higher serum suPAR levels in both men and women (Table 2). Only one out of 10 men reported eating a healthy diet, whereas twice as many women as men ate a healthy diet.

Daily smoking was strongly associated with higher serum suPAR levels. We observed a dose–response-like relationship between tobacco consumption and median suPAR level (Table 2). Interestingly, ex-smokers had suPAR levels comparable to those of never-smokers ($P = 0.06$ for men and $P = 0.8$ for women). The prevalence of daily smokers was similar among men and women, but twice as many men were in the heaviest smoking category for daily tobacco use.

Men who drank alcohol within recommended limits had the lowest suPAR levels. Alcohol overuse as well as alcohol abstinence were associated with higher suPAR levels ($P < 0.0001$). In contrast, women trended toward having lower suPAR levels when they overused alcohol ($P = 0.066$). Similar to the findings for dietary and smoking habits, one out of five men drank more alcohol weekly than recommended, whereas only half as many women exceeded the recommended limits.

Physically active men and women had lower suPAR levels than their sedentary counterparts (both $P < 0.0001$). The prevalence of sedentarism was similar for men and women.

Simple associations between suPAR levels and CVD risk factors. There was a strong positive correlation between BMI and suPAR levels and between triglycerides and suPAR levels ($P < 0.0001$) for both men and women (Table 1). HDL cholesterol showed a strong negative association with suPAR levels ($P < 0.0001$ for both sexes). There was a modest positive association between SBP and suPAR levels in men ($P = 0.035$), but not in women. There was a strong positive association of total cholesterol levels with suPAR levels in women ($P < 0.0001$), but not in men.

Table 1. The association of suPAR levels with continuous variables.

	MEN n = 2,703				WOMEN n = 2,835			
CORRELATION WITH suPAR	KENDALL'S TAU-B	n	%	*P*	KENDALL'S TAU-B	n	%	*P*
Age	0.088	2,703	100	<0.0001	0.047	2,835	100	0.0002
Total cholesterol	0.0020	2,701	99.9	0.87	0.079	2,835	100	<0.0001
HDL cholesterol	−0.099	2,702	99.9	<0.0001	−0.13	2,835	100	<0.0001
Triglyceride	0.074	2,701	99.9	<0.0001	0.13	2,835	100	<0.0001
Systolic BP	0.028	2,703	100	0.035	0.0069	2,834	99.9	0.60
BMI	0.052	2,702	99.9	<0.0001	0.064	2,833	99.9	<0.0001

Abbreviations: BMI, body mass index; BP, blood pressure; HDL, high-density lipoprotein; suPAR, soluble urokinase plasminogen activator receptor.

Table 2. The association of suPAR levels with categorical variables.

	MEN n = 2,703				WOMEN n = 2,835			
	suPAR LEVEL	n	%	*P*	suPAR LEVEL	n	%	*P*
Personal history of MI		2,622	97.0			2,730	96.3	
No	3.16 (2.58–3.96)	2,568	97.9		3.55 (2.93–4.50)	2,700	98.9	
Yes	4.00 (3.20–4.87)	54	2.1	<0.0001	4.55 (3.44–5.44)	30	1.1	0.019
Family history of MI		2,652	98.1			2,819	99.4	
No	3.18 (2.60–4.03)	1,768	66.6		3.50 (2.90–4.45)	1,784	63.3	
Yes	3.20 (2.59–4.00)	884	33.3	0.87	3.63 (2.97–4.65)	1,035	36.7	0.011
Incident diabetes		2,703	100			2,835	100	
No	3.16 (2.58–3.95)	2,507	92.7		3.54 (2.92–4.49)	2,711	95.6	
Yes	3.57 (2.80–4.69)	196	7.3	<0.0001	3.98 (3.35–5.14)	124	4.4	<0.0001
Socioeconomic status		2,464	91.2			2,606	91.9	
Low	3.31 (2.67–4.25)	459	18.6		3.71 (3.05–4.74)	672	25.8	
Medium	3.17 (2.57–3.96)	1,606	65.2		3.53 (2.88–4.48)	1,742	66.8	
High	3.02 (2.51–3.79)	399	16.2	0.0003	3.28 (2.82–4.23)	192	7.4	<0.0001
Diet		2,593	95.9			2,754	97.1	
Healthy	2.95 (2.52–3.59)	251	9.7		3.30 (2.83–4.09)	497	18.0	
Average	3.14 (2.57–3.95)	1,826	70.4		3.57 (2.93–4.57)	1,953	70.9	
Unhealthy	3.44 (2.72–4.41)	516	19.9	<0.0001	3.97 (3.15–5.14)	304	11.0	<0.0001
Smoking		2,570	95.1			2,708	95.5	
Never-smoker	2.88 (2.45–3.48)	894	34.8		3.27 (2.74–3.90)	1,063	39.3	
Ex-smoker	2.96 (2.49–3.60)	709	27.6		3.28 (2.76–3.97)	671	24.8	
Daily smoker		967	37.6			974	36.0	
<15 g tobacco/day	3.29 (2.67–4.09)	251	9.8		3.92 (3.09–4.92)	336	12.4	
15–24.9 g tobacco/day	3.89 (3.15–4.90)	460	17.9		4.68 (3.73–5.94)	520	19.2	
≥25 g tobacco/day	4.50 (3.55–5.67)	256	10.0	<0.0001	4.81 (3.81–5.89)	118	4.4	<0.0001
Alcohol consumption		2,609	96.5			2,672	94.3	
Abstinent	3.33 (2.63–4.74)	175	6.7		3.59 (3.01–4.67)	357	13.4	
Within recommendations	3.11 (2.57–3.88)	1,907	73.1		3.56 (2.90–4.49)	2,006	75.1	
Overuse	3.40 (2.67–4.42)	527	20.2	<0.0001	3.42 (2.92–4.36)	309	11.6	0.066
Physical activity		2,627	97.2			2,780	98.1	
Active	3.14 (2.57–3.89)	2,052	78.1		3.51 (2.90–4.43)	2,168	78.0	
Sedentary	3.38 (2.66–4.36)	575	21.9	<0.0001	3.70 (3.00–4.84)	612	22.0	<0.0001

Notes: Data are reported as median (IQR), the number of participants with available data for each category and the number of participants in each subgroup, the percentage of the total with available data and the percentage in each subgroup, and the *P*-value from the Kruskal-Wallis test for differences in serum suPAR levels between groups.
Abbreviations: MI, myocardial infarction; suPAR, soluble urokinase plasminogen activator receptor.

A personal history of MI was associated with higher suPAR levels (Table 2). Women with a family history of MI had slightly higher suPAR levels than those without ($P = 0.011$), but this was not evident in men. Incident diabetes was associated with higher suPAR levels in both men and women.

Adjusted analyses. *Demographic and lifestyle factors.* The sex difference for suPAR levels was evident in all adjusted models, with women having 0.50–0.53 ng/mL higher suPAR levels than men ($P < 0.001$ for every reference point's suPAR level, Tables 3 and 4). A positive association with age was also present in every model ($P < 0.05$ for women in the CVD model, $P < 0.001$ for the others). However, the suPAR level increased more with age in men than in women. Both average ($P < 0.01$) and unhealthy ($P < 0.05$) diets were associated with higher suPAR levels in the Lifestyle model. The effect's size and significance level diminished slightly in the Combined model. As in univariate analysis, ex-smokers had the same suPAR levels as never-smokers. However, all levels of daily tobacco consumption were associated with higher suPAR levels ($P < 0.001$ for all daily smokers). Alcohol showed the same sex-specific associations as in univariate analysis: higher suPAR levels in abstinent men and lower suPAR levels in women who overused alcohol ($P < 0.01$). Physical activity had a very small effect in the Lifestyle model, and it did not reach significance in the Combined model.

There were more missing values for SES than for the other variables (Table 2). In addition, SES had no effect on suPAR levels or on the other parameter estimates (data not shown). Because of this, we did not include SES in the final model.

CVD risk factors. Triglycerides, SBP, and a family history of MI had no effect on suPAR level in the CVD model. Total cholesterol was associated with lower suPAR levels in men and higher suPAR levels in women. However, the associations were weak ($P < 0.05$). In contrast, HDL cholesterol was strongly associated with lower suPAR levels ($P < 0.001$) in both the CVD and Combined models. A personal history of MI was associated with higher suPAR levels in both the CVD ($P < 0.001$) and Combined models, although

Table 3. CVD, Lifestyle, and Combined adjusted models for men.

	MEN		
	CVD MODEL	LIFESTYLE MODEL	COMBINED MODEL
Reference point	2.65***	2.57***	2.49***
Age (per year over 30)	0.021***	0.019***	0.021***
Total cholesterol (per mmol/l over 4)	−0.062*		−0.048*
HDL cholesterol (per mmol/l over 2)	−0.45***		−0.37***
Triglycerides (per mmol/l over 1)	−0.023		
Systolic BP (per mm Hg over 120)	0.0011		
BMI‡	*spline***		*spline***
Personal history of MI	0.55***		0.39*
Family history of MI	0.0053		
Diabetes	0.24**		0.15
Smoking (vs. never)			
Ex-smoker	0.031	0.055	0.035
<15 g tobacco/day	0.42***	0.46***	0.42***
15–24.9 g tobacco/day	1.16***	1.18***	1.12***
≥25 g tobacco/day	1.74***	1.67***	1.63***
Diet (vs. healthy)			
Average		0.15**	0.15*
Unhealthy		0.19*	0.18*
Alcohol consumption (vs. within recommendations)			
Abstinent		0.42***	0.34**
Overuse		−0.044	−0.021
Physical activity (vs. active)			
Sedentary		0.098*	0.0063

Notes: The influence of the listed variables on plasma suPAR levels (ng/ml) in men when mutually adjusted. The Reference point corresponds to the suPAR value of the male reference point as defined in the Methods. *$P < 0.05$. **$P < 0.01$. ***$P < 0.001$. ‡BMI was included as a spline, and the relationship between the suPAR level and BMI is shown in Figure 2.
Abbreviations: BMI, body mass index; BP, blood pressure; CVD, cardiovascular disease; HDL, high-density lipoprotein; MI, myocardial infarction; suPAR, soluble urokinase plasminogen activator receptor.

Table 4. CVD, Lifestyle, and Combined adjusted models for women.

	WOMEN		
	CVD MODEL	LIFESTYLE MODEL	COMBINED MODEL
Reference subject	3.15***	3.10***	3.01***
Age (per year over 30)	0.0073*	0.015***	0.012***
Total cholesterol (per mmol/l over 4)	0.091**		0.071*
HDL cholesterol (per mmol/l over 2)	−0.45***		−0.37***
Triglyceride (per mmol/l over 1)	−0.023		
Systolic BP (per mm Hg over 120)	0.00066		
BMI‡	*spline***		*spline***
Personal history of MI	0.55***		0.39*
Family history of MI	0.0053		
Diabetes	0.23**		0.15
Smoking (vs. never)			
Ex-smoker	−0.025	0.010	−0.0012
<15 g tobacco/day	0.60***	0.68***	0.60***
15–24.9 g tobacco/day	1.44***	1.50***	1.43***
≥25 g tobacco/day	1.49***	1.70***	1.57***
Diet (vs. healthy)			
Average		0.15**	0.15*
Unhealthy		0.19*	0.18*
Alcohol consumption (vs. within recommendations)			
Abstinent		0.18*	0.05
Overuse		−0.37***	−0.28**
Physical activity (vs. active)			
Sedentary		0.098*	0.0063

Notes: The influence of the listed variables on plasma suPAR levels (ng/ml) in women when mutually adjusted. The reference point corresponds to the suPAR value of the female reference point as defined in the Methods. *$P < 0.05$. **$P < 0.01$. ***$P < 0.001$. ‡BMI was included as a spline, and the relationship between suPAR level and BMI is shown in Figure 2.
Abbreviations: BMI, body mass index; BP, blood pressure; CVD, cardiovascular disease; HDL, high-density lipoprotein; MI, myocardial infarction; suPAR, soluble urokinase plasminogen activator receptor.

the association was not as strong in the Combined model ($P < 0.05$). BMI was strongly associated with higher suPAR levels ($P < 0.001$ for both sexes, Fig. 1). Incident diabetes was moderately associated with higher suPAR levels in the CVD model ($P < 0.01$), but this was not statistically significant in the Combined model.

Effect sizes and interactions. Effect sizes for the Combined model are illustrated in Figure 2. Given that the reference point was 2.49 ng/mL for men and 3.01 ng/mL for women, most of the investigated factors had a modest influence on suPAR levels (less than 0.5 ng/mL). However, daily heavy smoking and class III/morbid obesity (BMI ≥40 kg/m^2) were associated with a ≥1 ng/mL increase in the suPAR level. Age, total cholesterol, and HDL cholesterol are continuous measures, but the scales are rather restricted, which limits the effect size. The R^2 was 0.22 for the Combined model for both men and women.

To illustrate the combined effect of several risk factors on suPAR, we calculated the suPAR level for six illustration persons based on the Combined model (Fig. 3): two healthy, two with high risk of CVD (but no smoking), and two with high CVD risk including heavy daily smoking. A man with high CVD risk had 1.27 ng/mL higher suPAR, and a woman with high CVD risk had 1.74 ng/mL higher suPAR compared to their healthy counterparts. If heavy smoking was also present, this difference was 2.90 ng/mL and 3.31 ng/mL for men and women, respectively.

When testing for pair-wise interactions between smoking, alcohol consumption, and BMI, we found statistically significant interactions between smoking and alcohol consumption and between BMI and alcohol consumption. However, there was no indication of any synergistic effects. For example, persons who were alcohol-abstinent and smoked 15–24.9 g tobacco/day had 0.5 ng/mL higher suPAR level; abstinent persons who instead smoked ≥25 g tobacco/day had a 0.2 ng/mL lower suPAR level.

Discussion

In this large general population, we found that smoking and morbid obesity were associated with high serum suPAR levels. Diet and alcohol consumption also seemed to impact

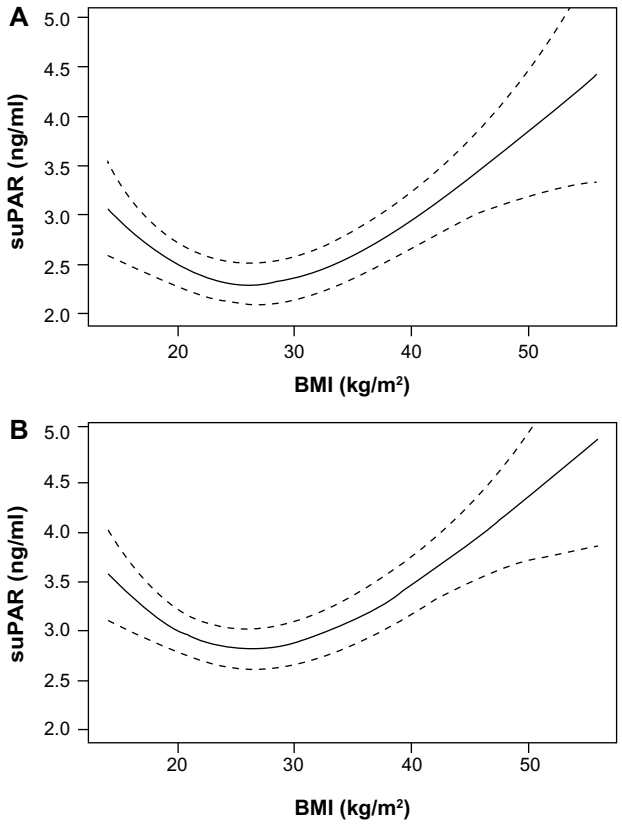

Figure 1. The association between BMI and serum suPAR levels for men (**A**) and women (**B**) in the Combined model with 95% CIs (dotted lines).

suPAR levels. Ex-smokers had suPAR levels comparable to those of never-smokers, most likely indicating that lifestyle changes are subsequently reflected by changes in suPAR levels. Our findings confirmed that suPAR increases with age, that women have higher suPAR levels than men, and that low HDL cholesterol and a history of MI are associated with higher suPAR levels. Surprisingly, incident diabetes had no effect on suPAR levels when adjusted for CVD risk factors and lifestyle factors.

The plasma suPAR level has been shown to be predictive of disease development and premature mortality in the general population. Eugen-Olsen et al stratified baseline data from the Danish MONICA cohort according to suPAR quartiles.[9] In the 3rd and 4th suPAR quartiles (highest suPAR levels), they observed significantly more women and daily smokers, higher age, a higher prevalence of diabetes and CVD, as well as higher total cholesterol, triglyceride, and CRP levels. Higher SBP was also associated with higher suPAR levels, while HDL cholesterol was actually lower in the 1st suPAR quartile (lowest suPAR levels) than in the 2nd and 3rd, although the 4th suPAR quartile had the lowest HDL cholesterol. In contrast, we showed a strong and uniform negative association between HDL cholesterol and suPAR level. Langkilde et al analyzed data from the same cohort, but stratified baseline suPAR according to baseline variables in the same way as in the present study.[16] They found no association between suPAR and BMI in men, and only a modest association in women. However, in the MONICA study, all degrees of obesity were combined into a single category (BMI >30 kg/m^2). This category is likely to contain mostly people with BMIs in the 30–35 kg/m^2 range, and our results indicate that such people have normal or near-normal suPAR levels. The Malmö cohort found the same associations as we did for age, smoking, and CVD, but they did not find an association of suPAR levels with diabetes.[10] However, the Malmö cohort contained a relatively low number of cases with diabetes (n = 72). Also, the incidence of diabetes may have been underestimated in both the Malmö and MONICA cohorts since it was based only on FPG. In the current study, incident diabetes was based on self-reported data as well as on additional per protocol screening with an OGTT.

The results from previous studies are hampered by the fact that they are not mutually adjusted, and the studies were not designed to analyze suPAR's associations with baseline data. To our knowledge, we are the first to show that incident diabetes has little effect on suPAR levels when adjusted for CVD risk factors and no effect when also adjusted for lifestyle factors. However, this does not necessarily contradict the conclusion by Eugen-Olsen et al.[9] that high suPAR levels predict the future development of diabetes. Also, in patients with type 1 diabetes, it has recently been shown that elevated suPAR levels are associated with diabetic complications.[17]

We observed that women have higher suPAR levels than men. This is in agreement with previous studies, but the reason for the sex difference is unknown. A recent study indicated that there was a strong negative association between muscle mass and suPAR in a cohort of 1,142 HIV-infected individuals.[18] Since men have a higher muscle mass than women, this may in part explain the sex difference. We were unfortunately unable to adjust for muscle mass. We also show that men had a larger increase in suPAR level per year than did women. Based on our results, the healthy man from this cohort will catch up with the healthy woman when they are both 87 years old. After then, men have higher suPAR levels. However, given the higher prevalence of risk factors in the male population, this suPAR equilibrium between men and women will often occur at an earlier age.

Regarding alcohol, we found that abstinent men had higher suPAR levels. Only 6.7% of males in this cohort did not drink alcohol, whereas twice as many women were abstinent. It is likely that the group of males who are abstinent contains a large proportion of ex-alcoholics and persons taking medications with alcohol interactions, and disease may therefore account for the higher suPAR level in this group. Surprisingly, women with an overuse of alcohol had a lower suPAR level, even in the fully adjusted model. The reason for this is unknown, but in a recent study on 120 alcoholics, alcoholism alone was associated with a moderate increase in suPAR levels, whereas alcoholic liver disease was associated with a large increase in suPAR levels.[19] Further studies are needed to confirm that alcohol overuse in women is associated with lower suPAR levels.

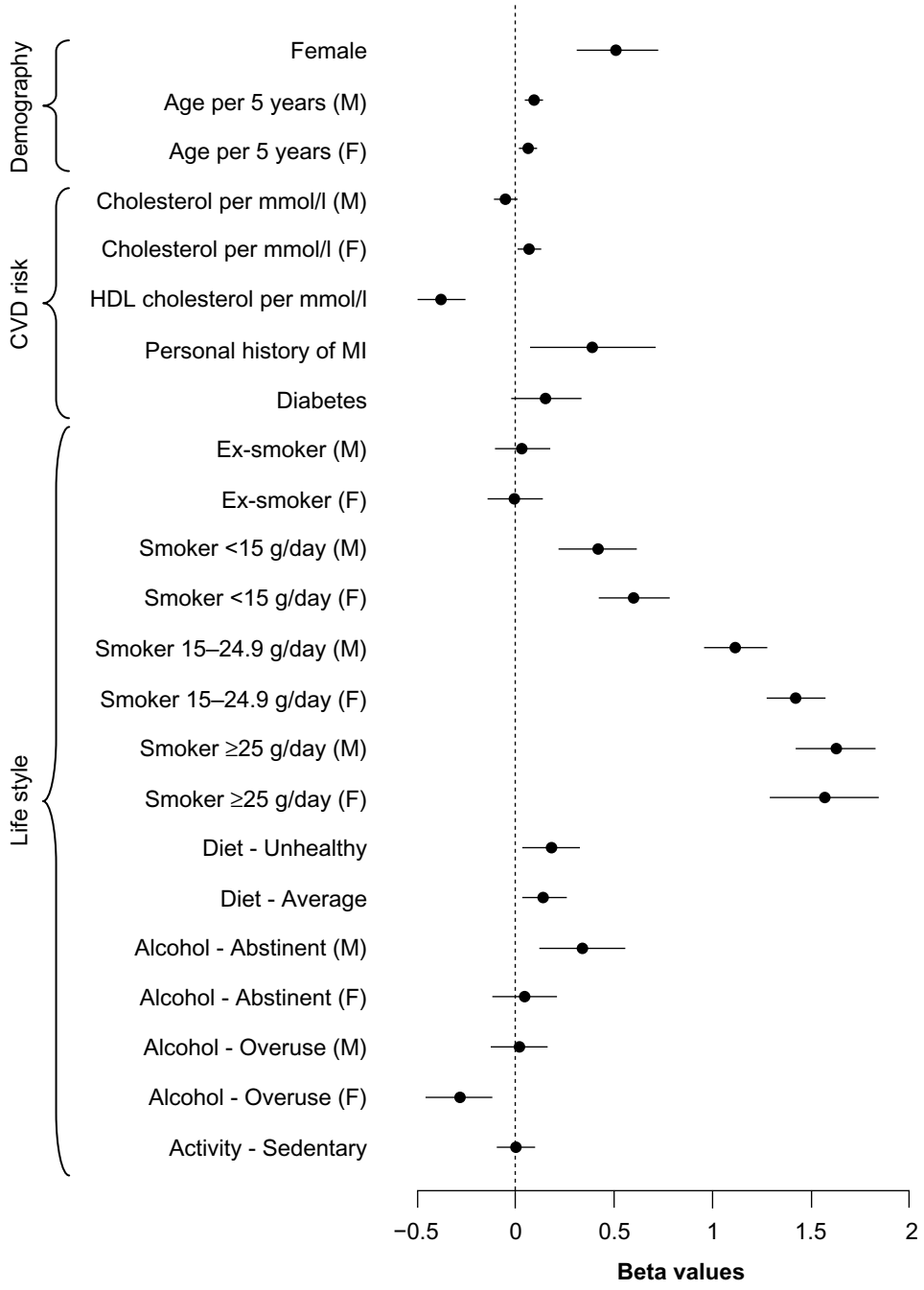

Figure 2. The effects of the indicated factors on serum suPAR levels compared to the reference point of the Combined model. Dots represent the estimate, and lines represent the 95% CIs. If a factor is listed twice, it indicates a sex interaction, and both the female (F) and male (M) estimates are shown.

The detailed reporting of dietary intake in this cohort allowed us to rate the diet of each subject. We found that both average and unhealthy diets were associated with higher suPAR levels, even when adjusted for both CVD risk factors and lifestyle factors. The effect was relatively small (0.18 ng/mL higher suPAR level when eating an unhealthy diet), but the concept of a biomarker that reflects the quality of one's diet is intriguing.

This study has some limitations. suPAR levels were measured in 10-year-old serum samples. While suPAR is a very stable protein over time, it is likely that sublimation of the serum fluids leads to a systematic overestimation of plasma suPAR levels, thus making interpretation of absolute suPAR concentrations difficult. The fact that all samples in this study consisted of serum most likely contributes to this effect. Unfortunately, CRP was not measured in this cohort for comparison. The observation that ex-smokers had suPAR levels comparable to those of never-smokers despite the high levels in daily smokers suggests that suPAR may be used to monitor individual lifestyle changes. Unpublished data from our group show that suPAR decreased after 3 weeks of smoking cessation in 35 out of 36 individuals. However, further

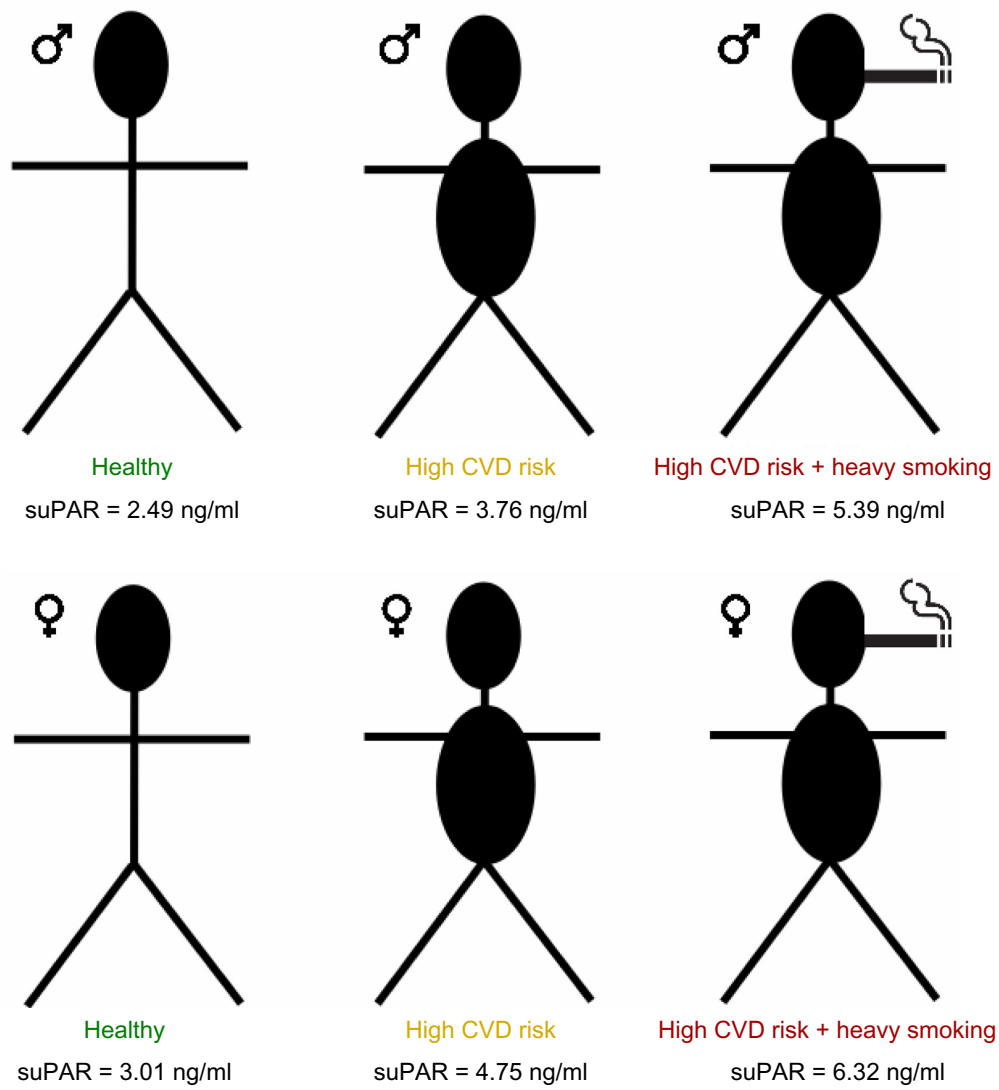

Figure 3. The average suPAR levels in the six illustration persons according to the Combined model. The two healthy persons are equivalent to the reference point in the model (30 years old and healthy). The pair with high CVD risk instead had a total cholesterol level of 8 mmol/L, HDL cholesterol of 0.8 mmol/L, a triglyceride level of 6 mmol/L, a BMI of 40 kg/m^2, incident diabetes, as well as both a personal and family history of MI. The CVD risk + heavy smoking pair also had a daily tobacco use of ≥25 g/day.

studies are needed to test the hypothesis that suPAR actually changes according to a variety of lifestyle changes. Similarly, while there is considerable evidence that elevated suPAR levels reflect a state of low-grade inflammation and increased risk of mortality as well as inflammation-related diseases, we cannot analyze this in a baseline study. It would also be interesting to test the hypothesis that a certain suPAR concentration confers a certain risk, regardless of whether smoking, high BMI, or a third factor is the underlying cause. We plan to do a follow-up analysis of this cohort to address these questions.

In conclusion, here we investigated serum suPAR levels in 5,538 persons aged 30 to 60 years in a Danish general population. In unadjusted analysis, suPAR levels were significantly associated with lifestyle factors, demographic factors, and CVD risk factors. In mutually adjusted analyses, daily smoking, high BMI, unhealthy diet, increasing age, female sex, low HDL, and previous MI were significantly associated with higher suPAR levels. In women, alcohol overuse was associated with lower suPAR levels, whereas alcohol abstinence was associated with higher suPAR levels in men. Physical activity and incident diabetes had no effect on serum suPAR levels for either sex when adjusted for lifestyle and CVD risk factors. Daily smoking and morbid obesity had the greatest effect on serum suPAR levels, followed by female sex and a personal history of MI. We found an R^2 of 0.22 in the final model, indicating that 22% of the suPAR variance can be explained by lifestyle factors. Further analysis is needed to determine whether factors associated with higher suPAR levels also confer an increased mortality risk.

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Author Contributions

Conceived and designed the experiments: TH, TK, SL, LR, CP, CT, OA, JE-O. Collected data: CP. Analyzed the data: TH, SL, TK. Wrote the first draft of the manuscript: TH, TK, LR, CP, JE-O. Contributed to the writing of the manuscript: TH, TK, SL, LR, CP, CT, OA, JE-O. Agree with manuscript results and conclusions: TH, TK, SL, LR, CP, CT, OA, JE-O. Jointly developed the structure and arguments for the paper: TH, TK, SL, LR, CP, CT, OA, JE-O. Made critical revisions and approved final version: TH, TK, SL, LR, CP, CT, OA, JE-O. All authors reviewed and approved of the final manuscript.

REFERENCES

1. Sidenius N, Sier CF, Ullum H, et al. Serum level of soluble urokinase-type plasminogen activator receptor is a strong and independent predictor of survival in human immunodeficiency virus infection. *Blood*. 2000;96(13):4091–5.
2. Fernebro E, Madsen RR, Fernö M, et al. Prognostic importance of the soluble plasminogen activator receptor, suPAR, in plasma from rectal cancer patients. *Eur J Cancer*. 2001;37(4):486–91.
3. Ostergaard C, Benfield T, Lundgren JD, Eugen-Olsen J. Soluble urokinase receptor is elevated in cerebrospinal fluid from patients with purulent meningitis and is associated with fatal outcome. *Scand J Infect Dis*. 2004;36(1):14–9.
4. Wittenhagen P, Kronborg G, Weis N, et al. The plasma level of soluble urokinase receptor is elevated in patients with streptococcus pneumoniae bacteraemia and predicts mortality. *Clin Microbiol Infect*. 2004;10(5):409–15.
5. Haupt TH, Petersen J, Ellekilde G, et al. Plasma suPAR levels are associated with mortality, admission time, and Charlson Comorbidity Index in the acutely admitted medical patient: a prospective observational study. *Crit Care*. 2012;16(4):R130.
6. Tzanakaki G, Paparoupa M, Kyprianou M, Barbouni A, Eugen-Olsen J, Kourea-Kremastinou J. Elevated soluble urokinase receptor values in CSF, age and bacterial meningitis infection are independent and additive risk factors of fatal outcome. *Eur J Clin Microbiol Infect Dis*. 2012;31(6):1157–62.
7. Zimmermann HW, Reuken PA, Koch A, et al. Soluble urokinase plasminogen activator receptor is compartmentally regulated in decompensated cirrhosis and indicates immune activation and short-term mortality. *J Intern Med*. 2013;274(1):86–100.
8. Huttunen R, Syrjänen J, Vuento R, et al. Plasma level of soluble urokinase-type plasminogen activator receptor as a predictor of disease severity and case fatality in patients with bacteraemia: a prospective cohort study. *J Intern Med*. 2011;270(1):32–40.
9. Eugen-Olsen J, Andersen O, Linneberg A, et al. Circulating soluble urokinase plasminogen activator receptor predicts cancer, cardiovascular disease, diabetes and mortality in the general population. *J Intern Med*. 2010;268(3):296–308.
10. Persson M, Engström G, Björkbacka H, Hedblad B. Soluble urokinase plasminogen activator receptor in plasma is associated with incidence of CVD. Results from the Malmö diet and cancer study. *Atherosclerosis*. 2012;220(2):502–5.
11. Jørgensen T, Borch-Johnsen K, Thomsen TF, Ibsen H, Glümer C, Pisinger C. A randomized non-pharmacological intervention study for prevention of ischaemic heart disease: baseline results Inter99. *Eur J Cardiovasc Prev Rehabil*. 2003;10(5):377–86.
12. Jørgensen T, Jacobsen RK, Toft U, Aadahl M, Glümer C, Pisinger C. Effect of screening and lifestyle counselling on incidence of ischaemic heart disease in general population: Inter99 randomised trial. *BMJ*. 2014;348:g3617.
13. Toft U, Kristoffersen LH, Lau C, Borch-Johnsen K, Jørgensen T. The Dietary Quality Score: validation and association with cardiovascular risk factors: the Inter99 study. *Eur J Clin Nutr*. 2007;61(2):270–8.
14. Kruskal WH, Wallis WA. Use of ranks in one-criterion variance analysis. *J Am Stat Assoc*. 1952;47(260):583–621.
15. Kendall MG. A new measure of rank correlation. *Biometrika*. 1938;30(1–2):81–93.
16. Langkilde A, Hansen TW, Ladelund S, et al. Increased plasma soluble uPAR level is a risk marker of respiratory cancer in initially cancer-free individuals. *Cancer Epidemiol Biomarkers Prev*. 2011;20(4):609–18.
17. Theilade S, Lyngbaek S, Hansen TW, et al. Soluble urokinase plasminogen activator receptor levels are elevated and associated with complications in patients with type 1 diabetes. *J Intern Med*. 2014:doi: 10.1111/joim.12269.
18. Langkilde A, Petersen J, Klausen HH, Henriksen JH, Eugen-Olsen J, Andersen O. Inflammation in HIV-infected patients: impact of HIV, lifestyle, body composition, and demography - a cross sectional cohort study. *PLoS One*. 2012; 7(12):e51698.
19. Tuomi H, Kultti J, Danielsson J, Kangastupa P, Akerman K, Niemelä O. Serum soluble urokinase plasminogen activator receptor in alcoholics: relation to liver disease severity, fibrogenesis and alcohol use. *J. Gastroenterol Hepatol*. 2014:doi: 10.1111/jgh.12639.