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Associations between serum uric acid and markers of subclinical atherosclerosis in young adults. The cardiovascular risk in Young Finns study

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ABSTRACT

Background and methods: Serum uric acid (SUA) is a suggested biomarker for established coronary artery disease, but the role of SUA in early phases of atherosclerosis is controversial. The relations of SUA with vascular markers of subclinical atherosclerosis, including carotid artery intima-media thickness (cIMT), carotid plaque, carotid distensibility (Cdist) and brachial flow-mediated dilatation (FMD) were examined in 1985 young adults aged 30–45 years. In addition to ordinary regression, we used Mendelian randomization techniques to infer causal associations.

Results: In women, the independent multivariate correlates of SUA included BMI, creatinine, alcohol use, triglycerides, glucose and adiponectin (inverse association) (Model $R^2 = 0.30$). In men, the correlates were BMI, creatinine, triglycerides, C-reactive protein, alcohol use, total cholesterol and adiponectin (inverse) (Model $R^2 = 0.33$). BMI alone explained most of the variation of SUA levels both in women and men (Partial $R^2 \sim 0.2$). When SUA was modeled as an explanatory variable for vascular markers, it directly associated with cIMT and inversely with Cdist in age- and sex-adjusted analysis. After further adjustments for BMI or glomerular filtration rate, these relations were reduced to non-significance. No associations were found between SUA and FMD or the presence of a carotid plaque. Mendelian randomization analyses using known genetic variants for BMI and SUA confirmed that BMI is causally linked to SUA and that BMI is a significant confounder in the association between SUA and cIMT.

Conclusion: SUA is associated with cardiovascular risk markers in young adults, especially BMI, but we found no evidence that SUA would have an independent role in the pathophysiology of early atherosclerosis.

1. Introduction

Higher levels of serum uric acid (SUA) have been associated with coronary artery disease (CAD) [1–3], coronary calcification [4–6], carotid atherosclerotic plaques [7], increased carotid intima-media thickness (cIMT) [8], arterial stiffness [9] and with the metabolic syndrome [10,11]. SUA is produced in humans as an end product of purine metabolism, and impaired urate homeostasis is a causal factor in gout [12]. The role of SUA in the pathophysiology of atherosclerosis is ambiguous and not fully understood. SUA has been suggested be anti-atherogenic and to counterbalance the oxidative stress in the early phases of the atherosclerosis [13,14], and the potential link between SUA and cardiovascular risk may depend on its antioxidative capacity [12–14]. In contrast, association studies of SUA with risk markers of atherosclerosis have suggested that SUA is an independent risk factor in atherosclerosis [1,3–9]. However, in some studies the relations between SUA and

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the markers of atherosclerosis have been confounded by other cardiovascular risk markers such as obesity indices [3,4,15–18].

To clarify the role of SUA in preclinical atherosclerosis, we examined the correlates of SUA concentration and studied the associations between SUA and ultrasonographically measured markers of vascular structure and function, including carotid artery intima-media thickness (cIMT), carotid plaque, carotid distensibility (Cdist) and brachial flow-mediated dilatation (FMD), in a large well-defined cohort of 1985 young adult Finns (age 30–45 years). In addition to conventional regression analysis, we used uric acid- and BMI-associated genetic variants in instrumental variables analyses (Mendelian randomization) [19–21] to study the causal relations between SUA, BMI and cIMT.

2. Methods

2.1. Study design and subjects

The distribution and population determinants of SUA levels were analyzed cross-sectionally in 1985 participants aged between 30 and 45 years drawn from the cardiovascular risk in Young Finns study. We measured SUA levels, markers of vascular structure and function and the following covariates and cardiovascular risk markers: age, sex, BMI, blood pressure, serum lipids, adiponectin, glucose, insulin, C-reactive protein (CRP), creatinine, estimated glomerular filtration rate (GFR and eGFR), cigarette smoking, alcohol use and oral contraceptive use.

The study protocol and population have been described in detail previously [22]. Briefly, The cardiovascular risk in Young Finns study is an ongoing 5-centre follow-up study of atherosclerosis precursors in Finnish children and adolescents. The first cross-sectional survey was conducted in 1980, when 3596 participants, aged 3, 6, 9, 12, 15 and 18 years were randomly chosen from the national population register. A re-examination was performed in 2007 of 2204 of the original participants aged between 30 and 45 years. The study has been carried out in accordance with the Declaration of Helsinki, all participants gave written informed consent and the study was approved by the local ethics committees.

2.2. Data collection and measurements

The clinical measurements were performed at each study center by trained study nurses according to a standardized study protocol. Height and weight were measured, and BMI was calculated as kg/m^2. Blood pressure was measured with a random zero sphygmomanometer (Hawksley & Sons, Lancin, UK), and an average of three measurements was used in the analysis. For the determination of markers in blood, venous blood samples were drawn after an overnight fast. Separated plasma or serum was frozen in aliquots and stored at −70 °C until thawed for the first time for the analyses. SUA was determined enzymatically (Uric acid reagent, Olympus, Ireland) on an AU400 analyzer (Olympus, Japan) and the inter-assay coefficient of variation was 1.6%. The analyses of serum total cholesterol, triglycerides, adiponectin, glucose and insulin were performed as described previously [23]. Serum CRP was determined turbidimetrically (CRP Latex reagent, Olympus, Ireland) and serum creatinine fotometrically (Creatinine reagent, Olympus, Ireland) on an AU400 analyzer (Olympus, Japan). GFR was estimated using the Cockcroft–Gault formula, and eGFR using the Modification of Diet in Renal Disease (MDRD) formula. The Cockcroft–Gault formula for women was GFR = 0.85 * ((140 − age (years)) * weight (kg))/(72 * creatinine (mg/dL)) and for men GFR = ((140 − age (years)) * weight (kg))/(72 * creatinine (mg/dL)). The MDRD formula for women was eGFR = 32,788 * (age (years)$^{-0.203}$) * (creatinine (mg/dL)$^{-1.154}$) * 0.742 and for men eGFR = 32,788 * (age (years)$^{-0.203}$) * (creatinine (mg/dL)$^{-1.154}$).

2.3. Ultrasound imaging of vascular structure and function

Carotid and brachial artery ultrasound studies were performed to measure the left common carotid artery IMT, to scan for the presence of carotid atherosclerotic plaque (Cplaq) and to assess brachial endothelial function, the brachial artery baseline diameter and FMD using Sequoia 512 ultrasound mainframes (Acuson, Mountain View, CA, USA) with 13.0 MHz linear array transducer, as previously described [22,23]. A minimum of four measurements of the common carotid far wall were taken to derive mean cIMT.

The far and near walls of the left common carotid artery and carotid bulb area were scanned for Cplaq, defined as a distinct area of the vessel wall protruding into the lumen >50% of the adjacent intima-media layer. High-risk IMT was identified as IMT ≥ 90% percentile and/or an existing Cplaq. Cdist measures the ability of the arteries to expand as a response to pulse pressure caused by cardiac contraction and relaxation. Cdist was calculated from the common carotid diameter in end diastole and end systole as ((systolic diameter − diastolic diameter)/diastolic diameter)/(systolic blood pressure − diastolic blood pressure). Left brachial artery diameter was measured at rest and during reactive hyperemia to derive the percentage in arterial diameter relative to resting scan, and the highest of three measurements was chosen as the maximum FMD.

2.4. Statistical analyses

The analysis was based on the cross-sectional data from the 2007 follow-up. Pregnant women ($N = 37$) and participants with a recent infection ($N = 98$) were excluded from the current study, and the final study population consisted of 1985 participants (1062 women and 923 men) with complete data on the vascular measurements and BMI, systolic blood pressure, total cholesterol, alcohol use and smoking status. Values for CRP, insulin and triglycerides were log-transformed prior to analyses due to skewed distributions. There was a significant difference between sexes in SUA concentrations ($p < 0.0001$). Therefore the bivariate associations between SUA and other study variables were explored separately for men and women. By entering SUA as the dependent variable, the independent effects of the cardiovascular risk markers on SUA were estimated with stepwise regression analysis performed separately for men and women. Entering SUA as an independent variable, the effects of SUA on cIMT, Cdist, FMD, Cplaq and high-risk IMT were studied with several linear and logistic regression models adjusted for age and sex, and with fully adjusted models with all cardiovascular risk variables as covariates. Brachial artery baseline diameter was included as a covariate in the models for FMD.

2.5. Instrumental variables analysis

Two-stage least squares instrumental variables analysis, also known as Mendelian randomization was performed to study the causal relations between SUA, BMI and IMT. The predicting variable is deemed to be in causal relation to the outcome if 1) there is a statistically significant association between predictor and outcome in both the ordinary least squares and in the instrumental variables regression and 2) the assumptions of instrumental variables regression are satisfied. We performed the analyses in multiple directions to reveal the direction of the effects.

The genetic instruments were rs13129697, a genetic variant of the uric acid transporter *SLC2A9* gene, and the weighted genetic

Table 1
Characteristics of the study participants.

Variable	Women	Men
Number of subjects	1062	923
Age (years)	37.9 ± 4.9	37.6 ± 5.0
BMI (body mass index, kg/m^2)	25.3 ± 4.9	26.7 ± 4.3
Waist circumference (cm)	83.4 ± 12.4	94.2 ± 12.0
Waist-hip ratio	0.83 ± 0.07	0.94 ± 0.07
Uric acid (μmol/L)[a]	241.3 ± 52.5	330.7 ± 67.7
Mean carotid IMT (mm)	0.613 ± 0.087	0.643 ± 0.107
Carotid plaque (%)	1.6	3.7
High-risk IMT (IMT > 90th percentile or a carotid plaque) (%)	10.6	21.1
Carotid artery distensibility (Cdist)	2.02 ± 0.72	1.75 ± 0.61
Brachial artery flow-mediated dilatation (FMD) (%)	9.88 ± 4.84	7.64 ± 3.79
Creatinine (μmol/L)	68.8 ± 8.4	82.8 ± 10.2
GFR (Glomerular filtration rate, Cockroft–Gault) (mL/min)	111.5 ± 27.6	135.0 ± 29.5
eGFR (Glomerular filtration rate, MDRD) (mL/min/1.73^2)	91.9 ± 12.7	100.1 ± 13.2
Systolic blood pressure (mmHg)	116 ± 14	126 ± 13
Diastolic blood pressure (mmHg)	73 ± 11	79 ± 11
Total cholesterol (mmol/L)	4.89 ± 0.81	5.21 ± 0.94
Triglycerides (mmol/L)	1.15 ± 0.60	1.64 ± 1.12
Adiponectin (μg/mL)[b]	12.36 ± 5.50	7.72 ± 3.78
Insulin (mU/L)	8.58 ± 8.41	9.39 ± 9.43
Glucose (mmol/L)	5.2 ± 0.8	5.5 ± 1.0
Impaired fasting glucose, ≥5.6 mmol/L (%)	14.7	30.8
C-reactive protein (mg/L)	1.85 ± 3.29	1.52 ± 3.95
Current daily smokers (%)	14.8	22.3
Oral contraceptives (%)	16.3	–
NSAID medication (%)	3.8	2.2
Antihypertensive medication (%)	6.8	7.1
Lipid lowering medication (%)	1.3	3.2
Oral antidiabetic medication (%)	0.4	0.6
Insulin medication (%)	0.6	0.7
Alcohol use (standard drinks per day)	0.6 ± 0.7	1.4 ± 1.9

Probabilities calculated with *T*-test for continuous and Chi-square test for categorical variables. NSAID = Non-steroidal anti-inflammatory drug. All comparisons significant between sexes ($p < 0.05$) except age, antihypertensive medication, oral antidiabetic medication and insulin medication (all $p > 0.05$).

[a] Corresponding to 4.06 ± 0.88 mg/L in women and 5.56 ± 1.14 mg/L in men.

[b] Adiponectin data available in 1939 participants.

risk score (wGRS) of BMI [24–26]. Both genotype and clinical data were available for 1776 participants (948 women and 828 men). Genotyping was done with Illumina 670k custom chip in 2009 at the Wellcome Trust Sanger Institute, Hinxton, Cambridge, UK. Genotype imputation was performed using MACH 1.0 and HapMap II release 22 CEU haplotypes as reference. wGRS of BMI was calculated based on single nucleotide polymorphism (SNP) variants identified by the recently published BMI genome wide association study (GWAS) [25]. Thirty-one SNPs out of total 38 SNPs identified in BMI GWAS were either genotyped or imputed in our data, and the wGRSs were calculated by first weighting SNP risk allele dosages (0–2) with beta estimates reported in the GWAS and then summing up the 31 weighted dosages.

We performed the instrumental variables analysis in four settings: 1) IMT as outcome, BMI as predictor and wGRS of BMI as instrument, 2) SUA levels as outcome, BMI as predictor and wGRS of BMI as instrument, 3) IMT as outcome, SUA levels as predictor and rs13129697 as instrument and 4) BMI as outcome, SUA levels as predictor and rs13129697 as instrument, as described in Table 5. In setting 3, BMI was not entered as a covariate because in the ordinary regression analyses the association between SUA levels and IMT was lost when the model was adjusted with BMI. Fully adjusted linear regression models were constructed for all four settings, adjusting for all covariates listed in Table 2 except for GFR (a combination of age, sex, BMI and creatinine levels) and for BMI in setting 3. An interaction term of sex*predictor was added into each model. Final models were chosen by backward selection to be used in the instrumental variables analysis. The interaction terms of sex*BMI in setting 1 and sex*SUA in settings 3 and 4 were statistically significant, and in these settings the analyses were performed for men and women separately and the backward selection of covariates was repeated for sex-stratified data. Backward selection dropped SUA from the model in women in setting 3, and therefore instrumental variables analysis was not performed for women in this setting as the association of SUA with IMT was completely lost. Assumptions of the instrumental variables regression analyses were tested by evaluating the associations of the instruments to the covariates in the full models.

The analyses were performed using the Statistical Analysis System SAS (version 9.2) and the AER package in *R* (version 1.1-9, http://CRAN.R-project.org/package=AER). Statistical significance was inferred at a 2-tailed *p*-value < 0.05. Sufficient strength to ensure the validity of instrumental variables methods was inferred at *F*-values ≥ 10 in the first-stage regression.

3. Results

The mean concentration of SUA was 281.5 ± 75.0 μmol/L and there was a significant difference ($p < 0.0001$, Table 1) in SUA concentrations between men (330.7 ± 67.7 μmol/L) and women (241.3 ± 52.5 μmol/L). There were significant gender-related differences in the early atherosclerosis indices, metabolic variables (e.g. impaired fasting glucose, adiponectin) and lifestyle risk factors (e.g. smoking and NSAID use). Almost 30% of the male population and 14% of the females had impaired fasting glucose and the correlation analyses were repeated for the non-impaired fasting glucose group. Several cardiovascular risk markers were associated with SUA in all participants as well as in the normal fasting glucose group (Table 2). The bivariate correlation between SUA levels and carotid IMT was no longer significant in women with normal fasting glucose.

3.1. Multivariate determinants of SUA

The multivariable correlates of SUA were BMI, creatinine, adiponectin, alcohol use, triglycerides and glucose which together explained 30% (Model $R^2 = 0.30$) of the total variation in SUA in women (Table 3). In men, the multivariable correlates of SUA included BMI, creatinine, triglycerides, CRP, alcohol use, total cholesterol and adiponectin (Model $R^2 = 0.33$, Table 3). BMI had the greatest influence both in women (Partial $R^2 = 0.21$) and in men (Partial $R^2 = 0.19$). The interaction term BMI*sex was significant ($p = 0.0002$) suggesting that the magnitude of the association between SUA and BMI differed between sexes: the Pearson's correlation coefficient was $r = 0.45$ in women and $r = 0.43$ in men.

3.2. SUA as an explanatory variable for vascular markers

SUA levels correlated inversely with Cdist in both men ($r = -0.14$, $p < 0.0001$) and women ($r = -0.14$, $p < 0.0001$) in unadjusted correlation analyses (Table 3). SUA levels correlated directly with IMT in men ($r = 0.15$, $p < 0.0001$). When both sexes were combined in multivariable models, SUA initially associated directly with IMT ($p = 0.02$) in a model adjusted for age and sex. The association remained significant after adjustment for total cholesterol, triglycerides, systolic blood pressure, adiponectin, creatinine, insulin, glucose, CRP and alcohol use. However, the association was attenuated to null after further adjustment for BMI ($p = 0.61$) or GFR ($p = 0.37$).

Table 2
Bivariate correlations between serum uric acid, markers of vascular structure and function and the cardiovascular risk markers in young adults in all participants and in participants with normal fasting glucose. Pearson's correlation coefficients were calculated for continuous variables and Spearman's correlation coefficients for categorical variables.

Variable	Correlation with uric acid											
	Women						Men					
	All participants			Normal fasting glucose			All participants			Normal fasting glucose		
	N	Correlation coefficient	*p* value	*N*	Correlation coefficient	*p* value	*N*	Correlation coefficient	*p* value	*N*	Correlation coefficient	*p* value
cIMT	1062	0.09	0.005	905	0.03	0.31	923	0.15	<0.0001	640	0.12	0.002
Carotid plaque	1062	−0.01	0.63	905	−0.04	0.23	923	0.01	0.79	640	0.03	0.47
High-risk IMT	1062	0.03	0.42	905	0.001	0.98	923	0.06	0.09	640	0.01	0.71
Cdist	1062	−0.14	<0.0001	905	−0.10	0.003	923	−0.14	<0.0001	640	−0.11	0.004
FMD	1062	0.01	0.86	905	0.004	0.89	923	0.03	0.29	640	0.07	0.07
Age	1062	0.04	0.21	905	0.02	0.65	923	0.06	0.05	640	0.06	0.14
BMI	1062	0.45	<0.0001	905	0.41	<0.0001	923	0.43	<0.0001	640	0.42	<0.0001
Waist circumference	1062	0.45	<0.0001	905	0.41	<0.0001	923	0.45	<0.0001	640	0.43	<0.0001
Waist-hip ratio	1062	0.35	<0.0001	905	0.32	<0.0001	923	0.37	<0.0001	640	0.35	<0.0001
Creatinine	1062	0.20	<0.0001	905	0.25	<0.0001	923	0.25	<0.0001	640	0.29	<0.0001
Glomerular filtration rate (Cockroft–Gault formula)	1062	0.25	<0.0001	905	0.19	<0.0001	923	0.20	<0.0001	640	0.15	0.0002
Systolic blood pressure	1062	0.16	<0.0001	905	0.08	0.02	923	0.17	<0.0001	640	0.14	0.0005
Diastolic blood pressure	1062	0.20	<0.0001	905	0.12	0.0002	923	0.28	<0.0001	638	0.25	<0.0001
Total cholesterol	1062	0.10	0.0009	905	0.07	0.04	923	0.24	<0.0001	640	0.23	<0.0001
HDL-cholesterol	1061	−0.15	0.01	904	−0.14	<0.0001	914	−0.18	<0.0001	636	−0.20	<0.0001
LDL-cholesterol	1058	0.12	0.0001	902	0.06	0.07	889	0.18	<0.0001	624	0.19	<0.0001
log-Triglycerides	1062	0.30	<0.0001	905	0.23	<0.0001	923	0.40	<0.0001	640	0.38	<0.0001
log-Adiponectin	1036	−0.26	<0.0001	886	−0.22	<0.0001	903	−0.21	<0.0001	624	−0.20	<0.0001
log-Insulin	1060	0.20	<0.0001	905	0.12	0.0002	920	0.24	<0.0001	639	0.21	<0.0001
log-Glucose	1062	0.23	<0.0001	905	0.13	<0.0001	923	0.10	0.06	640	0.06	0.10
log-CRP	1062	0.28	<0.0001	905	0.22	<0.0001	923	0.31	<0.0001	640	0.29	<0.0001
Current smoking	1062	0.04	0.23	905	0.03	0.37	923	−0.04	0.24	640	−0.04	0.29
Alcohol use	1062	0.12	<0.0001	905	0.09	0.006	923	0.14	<0.0001	640	0.11	0.004
Oral contraceptive use	1053	0.01	0.87	898	0.02	0.60	–	–	–	–	–	–

cIMT = mean carotid intima-media thickness; high-risk IMT = IMT > 90th percentile and an existing carotid plaque; Cdist = carotid arterial distensibility; FMD = brachial artery flow-mediated dilatation; BMI = body mass index; HDL = high density lipoprotein; LDL = low-density lipoprotein; CRP = C-reactive protein. Values for triglycerides, adiponectin, insulin, glucose and CRP were log-transformed prior to analyses.

The association of SUA with Cdist in an age- and sex-adjusted model was initially significant ($\beta = -0.0006$, $p = 0.03$), and became borderline significant after further adjustment for GFR ($\beta = -0.0005$, $p = 0.08$). However, in fully adjusted multivariable models performed for men and women separately, SUA was not associated with cIMT, Cdist or brachial FMD or the presence of a Cplaq in either sex (Table 4). The analyses were repeated with eGFR calculated using the MDRD formula with similar results.

3.3. Instrumental variables analysis

The results of ordinary least squares regression analysis and instrumental variables regression analysis are summarized in Table 5. The *F*-statistics from the first-stage regressions of instrumental variables analysis were greater than 10 for all the instruments, except for wGRS of BMI in women in setting 1, indicating sufficient strength of instruments to be valid in these analyses [27]. In every ordinary regression analysis, the predictor was highly significantly associated with the outcome. In instrumental variables regression, the association of BMI with SUA reached statistical significance ($p = 0.02$) in both sexes, and the association of BMI with cIMT only in men ($p = 0.02$). In both analyses, the coefficients in the instrumental variables regression were diluted but their direction was consistent with the coefficients of ordinary regression. The Durbin–Wu–Hausman test did not show statistically significant differences in the coefficients, suggesting causal relation of predictor (BMI) to the outcome (SUA and cIMT) [19]. When testing instrumental variables regression assumptions, the uric acid instrument rs13129697 was not associated with any of the covariates and the BMI instrument wGRS was associated with

Table 3
Multivariable cardiovascular risk correlates of serum uric acid estimated from the stepwise regression analysis performed separately for men (b) and women (a).

	β^c	Partial R^2	*p* value		β	Partial R^2	*p* value
BMI[a]	4.12	0.21	<0.0001	BMI[b]	4.20	0.19	<0.0001
Creatinine	1.48	0.06	<0.0001	Creatinine	1.57	0.06	<0.0001
log-Adiponectin	−14.61	0.02	<0.0001	log-Adiponectin	−9.50	0.003	0.03
Alcohol use	7.56	0.01	<0.0001	Alcohol use	4.17	0.01	<0.0001
log-Glucose	26.46	0.003	0.04	log-Triglycerides	20.89	0.05	<0.0001
log-Triglycerides	7.59	0.004	0.02	log-CRP	8.81	0.02	<0.0001
				Total cholesterol	7.44	0.008	0.0007
Model $R^2 = 0.30$				*Model $R^2 = 0.33$*			

[a]Variables not entered in the stepwise model for women: age, systolic blood pressure, total cholesterol, glomerular filtration rate, CRP, insulin, smoking status and oral contraceptives. β = one mmol/L change in serum uric acid per one unit change in the independent predictor.
[b]Variables not entered in the stepwise model for men: age, systolic blood pressure, glomerular filtration rate, glucose, insulin and smoking status. β = one mmol/L change in serum uric acid per one unit change in the independent predictor.

Table 4
Association of uric acid with carotid artery IMT and distensibility, with the existence of a carotid plaque and a high-risk IMT and with brachial artery FMD in the age- and sex-adjusted and the fully adjusted multivariable models performed separately for men and for women.

	Women		Men	
	Age-adjusted	Fully adjusted[a]	Age-adjusted	Fully adjusted[a]
cIMT	$\beta = 0.0001$ $p = 0.01$	$\beta = -0.00001$ $p = 0.81$	$\beta = 0.0002$ $p < 0.0001$	$\beta = 0.00006$ $p = 0.25$
Cdist	$\beta = -0.0018$ $p < 0.0001$	$\beta = -0.0005$ $p = 0.24$	$\beta = -0.0012$ $p < 0.0001$	$\beta = -0.0005$ $p = 0.15$
Carotid plaque	OR = 1.000 CI = 0.991–1.009 $p = 0.97$	OR = 0.998 CI = 0.986–1.009 $p = 0.71$	OR = 1.000 CI = 0.995–1.005 $p = 0.96$	OR = 0.999 CI = 0.993–1.006 $p = 0.85$
High-risk IMT	OR = 1.001 CI = 0.998–1.005 $p = 0.50$	OR = 1.000 CI = 0.995–1.005 $p = 0.99$	OR = 1.002 CI = 0.999–1.004 $p = 0.15$	OR = 1.000 CI = 0.997–1.003 $p = 0.78$
FMD	$\beta = 0.0069$ $p = 0.01$	$\beta = -0.0047$ $p = 0.13$	$\beta = 0.0036$ $p = 0.04$	$\beta = 0.0011$ $p = 0.60$

[a] Fully adjusted models additionally adjusted for BMI, glomerular filtration rate, systolic blood pressure, total cholesterol, triglycerides, adiponectin, creatinine, insulin, glucose, CRP and alcohol use and for FMD also for the brachial artery baseline diameter; cIMT = mean carotid intima-media thickness; high-risk IMT = IMT > 90th percentile and an existing carotid plaque; Cdist = carotid arterial distensibility; FMD = brachial artery flow-mediated dilatation; β = effect estimate; OR = odds ratio; CI = 95% confidence interval.

glucose ($p = 0.009$), systolic blood pressure ($p = 0.03$) and CRP ($p = 0.18$), which are all known to be mediated through BMI itself. Therefore, the assumptions of instrumental regression analysis were satisfied.

4. Discussion

In this large population-based cohort of young adults, SUA levels correlated with several cardiovascular risk markers, most notably BMI, serum levels of creatinine, triglycerides and adiponectin, as well as alcohol intake. These correlations between SUA and different cardiovascular risk markers in both men and women in this study are supported by previous observations in asymptomatic individuals [16,28,29]. However, we found no evidence that SUA would causally associate with markers of vascular structure and function. Therefore, these results do not support the hypothesis that SUA would have an independent role in the pathophysiology of early atherosclerosis.

The relations between BMI, SUA and cIMT were determined in the instrumental variables analysis (also called Mendelian randomization analysis). Both conventional regression and instrumental variable analyses suggested that there is no direct association between SUA and cIMT. Instrumental variables analysis is based on the idea that an instrumental variable has an effect on a predictor variable, which has an effect on the outcome. Genetic variants may be useful as instrumental variables to control confounding. Another advantage with genotype as the instrument is that a health outcome has no effect on the genes, and therefore associations between genotype and health outcome is not affected by reverse causality [21]. To examine further the role of BMI as a confounder, we applied instrumental variables analysis using BMI-associated wGRS as an instrument and found a causal relation of BMI to SUA in both sexes and to cIMT in men. The lack of causal relationship of BMI to cIMT in women might to be due to the fact that the wGRS instrument appeared to be exceptionally weak (*F*-value = 2.6) in women. Instead, using the uric acid transporter variant rs13129697 as an instrument of SUA, we did not find a causal relation of SUA either to BMI or cIMT, although the instrument was sufficiently strong in all the analyses. The present results suggest that the observed associations between SUA and early atherosclerotic vascular damages are mediated by BMI.

In agreement with the present findings, several cross-sectional and some large epidemiological follow-up studies have concluded that SUA is not independently associated with atherosclerosis or the prevalence or severity of CAD [15,16,30]. Iribarren et al. [16] and Moriarity et al. [30] studied large cohort of middle-aged U.S. population and reported that the association between SUA and cIMT was lost after adjustment with BMI. De Luca et al. [15] showed in 1901 consecutive patients undergoing coronary angiography and carotid ultrasound scans that SUA was not associated with the extent of CAD and cIMT.

However, many studies have suggested SUA to be an independent risk factor for atherosclerosis [6,7,13]. Nieto et al. [13] showed an independent relation between very high SUA levels, i.e. hyperuricemia, and the risk for myocardial infarction in 12,866 men in 6.5 years follow-up time. Hyperuricemia was an independent risk factor for myocardial infarction even after adjustment for alcohol consumption, diabetes, obesity and the presence of metabolic syndrome. In a cross-sectional community-based study of 4866

Table 5
Results of the ordinary least squares regression and the instrumental variables regression analyses. The ordinary least squares beta estimates are presented as standardized *z*-scores (change in outcome in standard deviation units). Note that setting 3 was not repeated for women due to sex*SUA interaction (see text for details).

Setting	Instrument	Predictor	Outcome	*N*	Covariates	*F*-value	Ordinary least squares regression	Instrumental variables regression	DWH test for difference
1	wGRS of BMI	BMI	IMT	824 men	Age, systolic blood pressure	18.9	$\beta = 0.2950$ $p < 0.0001$	$\beta = 0.6180$ $p = 0.023$	$p = 0.49$
				947 women	Age, alcohol use, triglycerides, glucose, oral contraceptives, systolic blood pressure, creatinine	2.6	$\beta = 0.0814$ $p = 0.0038$	$\beta = -0.6930$ $p = 0.34$	$p = 0.56$
2	wGRS of BMI	BMI	Uric acid	1776	Alcohol use, triglycerides, oral contraceptives, creatinine, sex, total cholesterol, CRP, adiponectin	10.9	$\beta = 0.2510$ $p < 0.0001$	$\beta = 0.6350$ $p = 0.024$	$p = 0.39$
3	rs13129697	Uric acid	IMT	829 men	Age, alcohol use, systolic blood pressure, creatinine, insulin, adiponectin	24.8	$\beta = 0.0055$ $p < 0.0067$	$\beta < 0.0001$ $p = 0.999$	$p = 0.90$
4	rs13129697	Uric acid	BMI	828 men	Age, glucose, triglycerides, systolic blood pressure, creatinine, total cholesterol, CRP, insulin	25.9	$\beta = 0.2260$ $p < 0.0001$	$\beta = 0.0420$ $p = 0.82$	$p = 0.60$
				948 women	Age, triglycerides, oral contraceptives, systolic blood pressure, CRP, creatinine, insulin, adiponectin	98.4	$\beta = 0.3600$ $p < 0.0001$	$\beta = 0.0689$ $p = 0.57$	$p = 0.04$

DWH = Durbin–Wu–Hausman test for difference between regression estimates, IMT = intima-media thickness, BMI = body mass index, wGRS = weighted genetic risk score, CRP = C-reactive protein.

participants, Neogi et al. [7] showed an independent association between SUA and carotid atherosclerotic plaques measured by ultrasound adjusting for potential confounding factors including BMI. Krishnan et al. [6] studied coronary arterial calcification by computerized tomography in 2498 asymptomatic young adults with a mean age of 40 years. They found that the prevalence of coronary artery calcification increased with SUA concentration even after adjustment for conventional risk factors including waist circumference.

The decrease in SUA levels by various treatment modalities has been independently associated with a reduced risk of vascular events. In the Greek Atorvastatin and Coronary Heart Disease (GREACE) study atorvastatin was associated with an 8.9% reduction in SUA levels, which was independently correlated with a reduced risk of vascular events [31]. In the Losartan Intervention For Endpoint (LIFE) study the reduction in SUA was estimated to contribute to 29% of the treatment effect of losartan [32].

The impact of CRP and adiponectin on SUA has not been fully clarified. Tsioufis et al. [33] addressed the association of SUA with adiponectin and high-sensitivity CRP in 292 essential hypertensive patients and concluded that increased SUA levels are associated with a subclinical inflammatory activation and hypo-adiponectinemia. In the present study, adiponectin was an independent determinant of SUA in both sexes but CRP only in men. Oliveira et al. [34] studied the association between alcohol use and hs-CRP in a population-based sample of 1330 adults, and found that there was a J-shaped association between alcohol intake and CRP in women but a direct linear-shaped association in men. In our study, alcohol use was significantly greater in men that in women and the sex difference in the effect of CRP on SUA may thus partly be due to a sex difference in the effect of alcohol use on CRP. Another putative explanation why CRP levels were only significant in men could have been that NSAID medication use was more prevalent in women as compared to men (3.8 vs. 2.2%).

The strength of this study is the large population-based group of carefully phenotyped participants. A limitation of this study was that there were no cardiovascular events to be used as study endpoints. We used surrogate markers of vascular structure and function such as carotid IMT and FMD. The latter is a surrogate endpoint of vascular damage and not representative of coronary plaques or CHD. The present study was conducted in a homogenous population of healthy young Finnish adults and care must be taken before generalizing to other populations. Importantly, the correlations between the vascular markers and SUA could be different in older adults. Atherosclerosis is a multifactorial disease, and identification of novel biomarkers that could help to predict the silent subclinical stage would be valuable for primary prevention. Multiple epigenetic phenomena may affect the susceptibility to atherogenesis during early life and fetal development, and there may also be several epigenetic mechanisms related to SUA levels and their possible involvement in CVD [35–37]. Furthermore, multiple pathways can be affected by SUA and a more detailed metabolic study would be needed to fully understand the potential role of SUA in the pathogenesis of atherosclerosis. However, our aim was specifically to clarify the ambiguous role of SUA in relation to the subclinical vascular markers of early atherosclerosis. Our study design was cross-sectional and therefore prone to bias when trying to establish causality, and to overcome this shortcoming we used instrumental variable analysis utilizing the recently discovered genetic variants for BMI and SUA to infer causal relations.

5. Conclusions

In a relatively large population-based study of apparently healthy young adults, we found that BMI was causally linked to SUA levels in men and women. However, we found no evidence that SUA would be causally associated with markers of preclinical atherosclerosis. Our observation thus does not support the idea that SUA would have an independent role in the pathogenesis of early atherosclerosis.

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Conflict of interest

No conflict of interest to declare.

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