Fibrinogen concentration and its role in CVD risk in black South Africans – effect of urbanisation

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Summary

The aim of this study was to investigate correlates of fibrinogen concentration in black South Africans. as well as its association with cardiovascular disease (CVD) risk and whether urbanisation influences this association. A total of 1,006 rural and 1,004 urban black South Africans from the PURE study were cross-sectionally analysed. The association of fibrinogen with CVD risk was determined by investigating the association of fibrinogen with other CVD risk markers as well as with predicted CVD risk using the Reynolds Risk score. The rural group had a significantly higher fibrinogen concentration than the urban group, despite higher levels of risk factors and increased predicted CVD risk in the urban group. Increased levels of CVD risk factors were, however, still associated with increased fibrinogen concentration. Fibrinogen correlated significantly, but weakly, with overall predicted CVD risk. This correlation was stronger in the urban than in the rural group. Multiple regression analysis showed that a smaller percentage of the variance in fibrinogen is explained by the traditional CVD risk factors in the rural than in the urban group. In conclusion, fibrinogen is weakly associated with CVD risk (predicted overall risk as well as with individual risk factors) in black South Africans, and is related to the degree of urbanisation. Increased fibrinogen concentration, in black South Africans, especially in rural areas, is largely unexplained, and likely not strongly correlated with traditional CVD-related lifestyle and pathophysiological processes. This does. however, not exclude the possibility that once increased, the fibrinogen concentration contributes to future development of CVD.

Keywords

African, cardiovascular disease, cardiovascular disease risk factors, fibrinogen

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Introduction

In black South Africans high fibrinogen concentrations are seen despite a historically low prevalence of cardiovascular disease (CVD) (1, 2). In Caucasian populations, fibrinogen is a consistent risk marker for CVD, and in Caucasians, the fibrinogen concentration is associated with many factors. These include known CVD risk factors (3) as well as factors not associated with CVD (4-6). Very little is, however, known regarding the association between fibrinogen and CVD risk in African-Americans and Africans. A different association may be expected since both African-Americans and Africans have consistently been shown to have higher fibrinogen concentrations than Caucasians (1, 4, 7). Only limited data is available on this association and this comes from the ARIC study, where coronary heart disease incidence was found to be positively associated with fibrinogen in a group of almost 4,000 African-Americans (8). An important aspect when considering the relationship between fibrinogen and CVD risk in the black South African population is the fact that black South Africans are undergoing a process of rapid urbanisation. This process is characterised by a demographic and health transition that is marked by circumstances and behaviours leading to a double burden of disease: poverty- and undernutrition-related infectious diseases as well as increased prevalence of CVD risk factors and CVD itself (9). In the rural settings, however, CVD is much less prevalent (9, 10).

Recognised CVD risk factors that have been shown to increase with urbanisation in South Africa include hypertension, obesity, smoking and serum lipids (9), factors which are considered to be associated with increased fibrinogen (3). Factors not associated with CVD in developing countries, such as poverty, lack of education, psychosocial stress, malnutrition and infections are, however, also considered to be associated with increased fibrinogen (4–6) and are abundant in the rural communities in South Africa (11, 12).

The questions that now arise are i) which factors are associated with increased fibrinogen in black South Africans, ii) how fibri-

nogen relates to CVD risk and iii) whether degree of urbanisation influences this relationship given the difference in levels of CVD risk factors as well as the non-CVD related fibrinogen effectors between rural and urban communities. We investigated these questions by comparing the fibrinogen concentration with non-CVD related effectors, other CVD risk factors as well as predicted CVD risk, using the Reynolds Risk Score, in a thousand rural and a thousand urban black South Africans who were part of the Prospective Urban and Rural Epidemiological (PURE) study.

Materials and methods

The PURE study is a large-scale cohort study that tracks changing lifestyles, risk factors and chronic disease using periodic standardised data collection in urban and rural areas of 17 countries in transition. The present study used baseline data from just over 2,000 randomly selected subjects of the South African arm of the PURE study, collected over a twelve week period in 2005. The Ethics Committee of the North-West University, South Africa, approved this study, and subjects signed informed consent before commencement of the study and after the study was explained to them in their home language. All data was treated confidentially and all analyses were performed with coded data.

Black South African men (n= 1,260) and women (n=750) between the ages of 35 to 60 years were recruited from 6,000 randomly selected households. From these household, 1,006 volunteers were recruited from rural (living under tribal law) and 1,004 from urban areas (living in informal and formal settlements surrounding cities) in the North-West province of South Africa. Volunteers were included if they were apparently healthy. Exclusion criteria were use of chronic medication for non-communicable diseases and/or any self-reported acute illness.

Interviewer based Quantitative Food Frequency Questionnaires, designed and validated for this population (13), were completed to determine the dietary intakes of volunteers. The *Foodfind e r3®* programme (Medical Research Council, Tygerberg, South Africa), based on the South African food composition tables (14), was used to analyse nutrient intakes. Anthropometrical measurements included hip and waist circumferences as well as weight and height, wearing minimal clothing. A battery of psychological questionnaires, investigating markers of psychosocial wellbeing, which was validated for this population, was also completed. These included: The Mental Health Continuum- Short Form (15), the Sense of Coherence Scale (16) and the General Health Questionnaire (17). Socio-demographic information was obtained during personal interviews by trained fieldworkers who completed questionnaires on health and socio-economic issues in the language of the participants' choice. This questionnaire was used by all countries participating in the PURE study after being adapted and validated to be country specific.

Fasting blood samples, with minimal stasis were collected by qualified nursing sisters from the antecubital vein using a sterile winged infusion set and syringes from 07.00--11.00. Blood was collected in tubes without anticoagulant and serum prepared for the analysis of lipids and C-reactive protein (CRP). Blood was collected in citrated tubes for the analysis of plasma plasminogen activator inhibitor (PAIHact and fibrinogen and kept on ice until centrifugation. Blood was collected into fluoride tubes for the determination of plasma glucose and into EDTA tubes for homocysteine determination. Samples were centrifuged within 30 minutes (min) of collection at 2,000 x g for 15 min at 10"C. Aliquots were then frozen on dry ice, stored in the field at -18"C and after 2--4 days in the laboratory at -82°C until analysis.

CRP and serum lipids were measured by using a Sequential Multiple Analyzer Computer (SMAC), using the Konelab™ auto-analyzer (Thermo Fischer Scientific, Vantaa, Finland) which is a clinical chemistry analyser for colourimetric, immunoturbidimetric and ionselective electrode measurements. Fibrinogen was measured using a modified Clauss method (Multifibrin U-test, Dade Behring, Deerfield, IL, USA) on the Dade Behring BCS coagulation analyzer. PAl-1act was analysed using an indirect enzymatic method (Spectrolyse PAl-1, Trinity Biotech, Bray, Ireland). Total homocysteine was determined using the Abbott automated immunoassay analyzer (AxSYM, Abbott, Abbott Park, IL, USA) based on fluorescence polarisation immunoassay technology. Plasma glucose was measured with a hexokinase method using the Synchron® System(s) (Beckman Coulter Co., Fullerton, CA, USA) and reagents. The coefficient of variance (CV) for the above-mentioned assays was $< 10\%$.

The computer software package Statistica® (Statsoft Inc., Tulsa, OK, USA) was used for the statistical analyses. A p-value ≤ 0.05 was regarded as statistically significant. Normally distributed variables are reported as mean (95% confidence interval [CI]). Not normally distributed data was log transformed to improve normality and reported as geometric mean (95% CI) or median [25th_75th percentile].

T-tests for independent samples for parametric data and the Mann-Whitney U test for non-parametric data were used for comparison between two groups. The Chi-square test was used to determine differences between groups for categorical variables. Analysis of variance (ANOVA) was used for comparison between three or more groups. Analysis of co-variance (ANCOVA) was used when comparison between groups required adjustment for confounders. Pearson correlations were used to determine associations between variables using normally distributed data or log-transformed data for non-parametric variables. Forward Stepwise Multiple Regression was used to determine the main predictors of fibrinogen. Factor analysis using principal component analysis was performed to determine whether fibrinogen cluster differently with CVD risk markers in rural and urban groups. The oblique rotation method indicated low correlation between the different factors (r<0.15) and therefore we used a varimax raw rotation. Only summary factors with an eigenvalue >1 were selected and factor loadings of >0.3 were used for interpretation of results.

Participants were stratified according to their predicted 10-year CVD risk using the Reynolds Risk Score (calculation for women (18); for men: unpublished calculation obtained from authors including coefficient for HbA1C). This risk score was selected be-

cause, apart from the traditional Framingham risk score factors, it also includes CRP, of which increased concentrations are associated with increased poverty as well as with non-white ethnicities, as is the case with the PURE study population (19). The Reynolds Risk Score has furthermore been shown to reclassify African Americans to a different risk category than the Framingham Vascular Disease Risk Score, using CRP testing (20). Risk score categories were: low risk (<5%); low to moderate risk (5-10%); moderate risk (> 10-20%); and high risk (>20%). In order to determine whether there was an interaction between level of urbanisation and the Reynolds Risk Categories regarding fibrinogen concentration, factorial ANOVA (unadjusted model) and factorial ANCOVA (adjusted models) were used.

Results

Table 1 provides details on the CVD risk factors for the total population as well as for the rural and urban groups separately. Gender differences are also indicated for variables with gender specific cut-offs. Most of the CVD risk factors were significantly higher in the urban compared to the rural group, including blood pressure, body mass index (BMI), waist circumference in the women, triglycerides, plasma glucose and PAl-1act However, fibrinogen (3.0 vs. 2.7g/l) and homocysteine were significantly higher in the rural compared to the urban group, in both genders (fibrinogen: men: 2.8 vs. 2.5g/l; women: 3.2 vs. 2.9g/l), and CRP showed a similar, non-significant trend. Women had significantly higher waist circumferences and fibrinogen than men and significantly

Variable	Total population N=2,010	Urban N=1,004	Rural N=1,006	Rural vs. Urban p-value
Age (years)	48 (41-56)	48 (42-57)	47 (41-55)	0.0002
Gender M/F (%)	37.3/62.7	39.9/60.1	34.6/65.4	0.01
HIV+(%)	16.2	15.7	16.8	0.5
Smoking status(%)				
Never	43.8	42.7	44.9	0.32
Past	3.80	3.90	3.8	0.65
Current	51.8	52.6	51.1	0.5
Blood pressure (mmHg)				
Systolic	133.5 ± 24.5	137±25.1	129.7± 23.3	<0.0001
Diastolic	87.7 ± 14.5	89.3 ± 14.5	86.2 ± 14.5	<0.0001
Body mass index (kg/m^1)	22.9 (19.3-28.6)	23.4 (19.5-29.4)	22.4 (19.1-28.1)	0.003
Waist circumference (em)	77.5 (70.2-87.7)	78.5 (70.9-89.0)	76.0 (69.7-86.9)	0.002
Men	74.4 (69.9-81.3) j	74.3 (69.7-81.8) j	74.5 (70.2-80.5) j	0.61
Women	81.0 (70.6-91.3) j	82.8 (73.1-92.8) j	78.8 (69.5-89.5) j	<0.0001
Serum total cholesterol (mM)	5.01 ± 1.38	5.05 ± 1.4	4.96 ± 1.36	0.17
Serum LDL-cholesterol (mM)	2.92 ± 1.17	2.93 ± 1.18	2.92 ± 1.17	0.86
Serum HDL-cholesterol (mM)	1.52 ± 0.63	1.52 ± 0.65	152 ± 0.62	0.91
Men	1.58 ± 0.66 j	1.61 ± 0.66 j	1.55 ± 0.66	0.22
Women	1.48 ± 0.62 j	1.46 ± 0.63 j	1.50 ± 0.61	0.26
Serum triglycerides (mM)	1.07 (0.82-1.55)	1.11 (0.84-1.65)	1.05 (0.80-1.43)	<0.0001
Fasting plasma glucose (mM)	5.02 ± 2.73	5.17 ± 3.7	4.87 ± 1.23	0.02
Serum CRP (mg/1)	3.29 (0.96-9.34)	3.25 (1.12-9.85)	3.33 (0.85-9.02)	0.07
Plasma fibrinogen (g/1)	2.90 (2.30-5.00)	2.70 (2.20-4.30)	3.00 (2.40-5.40)	0.0001 *to
Men	2.60 (2.10-3.70) j	2.50 (2.00-3.30) j	2.80 (2.20-4.30) ▪	0.048 *o
Women	3.10 (2.30-5.50) ▪	2.90 (2.30-5.40) ▪	3.20 (2.50-5.70) ▪	0.003 *to
Plasma PAI-1 activity (U/ml)	4.26 (1.27-7.92)	5.01 (1.76-9.11)	3.58 (0.81-6.85)	<0.0001
Plasma homocysteine ().LM)	9.18 (7.45-12.1)	8.90 (7.23-11.4)	9.48 (7.67-12.6)	<0.0001
Men	10.2 (8.30-13.16) [1]	9.58 (8.01-12.06) ▪	11.3 (8.67-14.4) ▪	<0.0001
Women	8.76 (7.09-11.15) ▪	8.39 (6.90-10.7) j	8.76 (7.09-11.15) ▪	0.004

Table 1: Characteristics of total study population, urban and rural participants.

Normally distributed data reported as: mean ± SD and non-parametric data as median (25^h·75^h percentile); *Significant difference between rural and urban groups after adjustment for CRP; t Significant difference between rural and urban groups after adjustment for homocysteine; o Significant difference between rural and urban groups after adjustment for HIV status; [1] Significant difference between men and women; M, male; F, female; HIV +, human immunodeficiency virus infected; LDL, low-density lipoprotein; HDL, high-density lipoprotein; CRP, C-reactive protein.

lower high-density lipoprotein cholesterol (HDL-C) and homocysteine in both the rural and urban groups. Fibrinogen remained significantly higher in the rural compared to the urban group after adjustment for CRP or human immunodeficiency virus (HIV) status in both genders as well as after adjustment for homocysteine in women (women: p=0.0001; men: p=0.12).

In order to assess the association of fibrinogen with other individual CVD risk factors, the study population was divided into quartiles of the fibrinogen concentration and the mean± standard deviation (SD) of the risk factors for each quartile reported (Table 2). More HIV+volunteers had fibrinogen concentrations in the lowest quartile. Smoking status does not seem to change significantly over the quartiles. There was furthermore no significant difference in the number of cigarettes smoked per day amongst those individuals that do smoke. Volunteers in the lowest fibrinogen quartile consumed significantly higher volumes of alcohol compared to the other three quartiles. Age, body mass index, waist circumference, total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), triglycerides and CRP increased significantly as the fibrinogen concentration increased. There was, however, no significant difference in blood pressure, HDL-C, homocysteine, PAl-1act and plasma glucose between the fibrinogen quartiles.

Forward stepwise multiple regression was used to determine the main predictors of fibrinogen in the PURE study population (Table 3). A model which included age, gender, CRP, total cholesterol, waist circumference, alcohol consumption, level of urbanisation and HIV status explained 21 % of the variance in fibrinogen concentration. CRP explained 17% of the variance wile alcohol consumption, HIV status, level of urbanisation, age and gender each explained 1% of the variance only. Using the same model in the urban population, this model now explained 24% of the variance in fibrinogen with CRP explaining 20%, HIV status 2.6%, and alcohol consumption and age 1% each. In the rural population, this model explained only 18% of the variance in fibrinogen with CRP explaining 15.6% and gender, age and alcohol consumption, 1% each.

Factor analysis was used to determine with which of the factors that were associated with fibrinogen concentration, it associated most strongly with. In agreement with the multiple regression results, fibrinogen clustered into one factor only with only one additional variable namely CRP. This was the case for the total population as well as for the rural and urban groups separately. The factor loadings for both fibrinogen and CRP in this factor were >0.75 in the total, rural and urban populations.

Table 2: Cardiovascular risk factors and markers of psychosocial wellbeing (mean ± SD) stratified into quartiles of fibrinogen concentration.

Risk factor	Quartile 1 0.6-2.30 g/1 n=517	Quartile 2 2.31-2.90 g/1 n=405	Quartile 3 2.91-5.00 g/1 n=420	Quartile 4 5.01-12.2 g/1 n=440	ANOVA p-value
HIV+(%)	21.7 *	17.6 ▌	16.9 [1]	10.7 *tl	<0.001
Non-smokers(%)	40.4	43.2	44.3	46.4	0.37
Current smokers(%)	56.5	49.9	52.4	50.0	0.27
Cigarettes/day	5.49 ± 4.30	5.85 ± 4.26	5.40 ± 3.70	5.35 ± 3.24	0.66
Alcohol consumption (g/day)	15.7 ± 25.9 *tl	12.7 ± 24.5 *	8.91 ± 20.0 ▌	10.41 ± 24.5 [1]	<0.0001
Age (years)	47.1 ± 9.2 *l	48.3 ± 9.85 ▌	50.0 ± 10.2 *	51.4 ± 11.0 tl	<0.0001
SBP (mmHg)	131.1 ± 23.2	133.9 ± 24.7	133.9 ± 25.7	133.7 ± 24.1	0.30
DBP (mmHg)	86.3 ± 14.6	87.7 ± 14.5	87.9 ± 15.3	87.9 ± 13.9	0.19
BMI(kg/m^2)	23.1±5.9*	24.0 ± 6.04 ▌	24.8 ±7.13*	26.3 ± 7.79 *▌	<0.0001
Waist circumference (em)	77.1 ± 11.1*[1]	78.9 ± 12.2 ▌	80.4 ± 13.4 *	82.2 ± 13.9 tl	<0.0001
Total cholesterol (mM)	4.81 ± 1.32 *l	5.03 ± 1.4	5.13 ± 1.4*	5.10 ± 1.43 ▌	0.001
LDL-cholesterol (mM)	2.71±1.13*[1]	2.88 ± 1.14	3.04 ± 1.19 *	3.08 ± 1.20 ▌	<0.0001
HDL-cholesterol (mM)	1.55 ± 0.62	1.59 ± 0.69	1.51 ± 0.62	1.44 ± 0.62	0.063
Triglycerides (mM)	1.24 ± 0.76	1.27± 0.76	1.35 ± 0.88	1.32 ± 0.76	0.043
CRP (mg/1)	4.15 ± 6.81 *	5.08 ± 7.99 ▌	8.17±11.8 *▌	16.7 ± 16.3 *l	<0.0001
Homocysteine (j.tM)	10.4 ±4.9	10.4± 5.11	10.3 ±4.16	10.3±4.16	0.92
PAI-1 activity (U/ml)	6.25 ± 7.64	5.68 ±7.40	6.37 ± 7.86	5.98 ± 6.26	0.23
Fasting glucose (mM)	4.85 ± 1.23	4.92 ± 1.84	4.92 ± 1.30	4.96 ± 1.66	0.43
Mental Health Continuum (Q-70)	38.5 ± 9.2	38.5 ±10.1	38.9 ± 9.1	38.1 ± 9.8	0.86
General Health Score (Q-28)	8.4 ± 6.3	8.1 ± 6.3	8.6 ± 6.6	9.1 ± 5.9	0.45
Sense of Coherence (29-203)	126 ± 21.1	126 ±23.4	126±23.2	123±22.5	0.37

* [1] *Values with the same symbol differ significantly between quartiles; HIV+, human immunodeficiency virus infected; SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; LDL, low-density lipoprotein; HDL, high-density lipoprotein; CRP, C-reactive protein; PAl-1, plasminogen activator inhibitor 1.

Table 3: Predictors of fibrinogen variance using Multiple Regression.

Variable	Total population R^2 adjusted= 0.21; p<0.0001 $F_{(7, 1658)} = 64.6$				Urban R^2 adjusted = 0.24; p<0.0001 $F_{(7, 799)} = 43.2$				Rural R^2 adjusted = 0.18; p<0.0001 $F_{(7, 853)} = 32.9$			
	β*	Std error of β	p	R2 -change	β*	Std error of β	p	R2 -change	β*	Std error of β	p	R2 -change
CRP	0.41	0.02	<0.0001	0.17	0.42	0.03	<0.001	0.20	0.39	0.03	<0.001	0.16
Alcohol	-0.08	0.02	<0.0001	0.01	-0.06	0.03	0.049	0.009	-0.08	0.03	0.01	0.006
HIV status	0.09	0.02	<0.0001	0.01	0.125	0.03	<0.001	0.026	0.05	0.03	0.1	0.003
Rura1/Urban	-0.08	0.02	<0.0001	0.01								
Age	0.08	0.02	<0.0001	0.01	0.08	0.03	0.02	0.006	0.08	0.03	0.007	0.008
Gender	0.08	0.02	<0.0001	0.01	0.05	0.03	0.1	0.002	0.11	0.03	0.001	0.014
we	-0.02	0.02	0.31	0.0005								
TC					0.07	0.03	0.04	0.005	-0.05	0.03	0.1	0.002

CRP, C-reactive protein; WC, waist circumference; TC, total cholesterol; *Standardised ;-variable did not enter forward stepwise model.

In order to determine the association of fibrinogen with overall CVD risk, instead of with individual CVD risk factors only, predicted CVD risk was calculated for each individual using the Reynolds Risk Score and reported as percentage risk. The mean predicted CVD risk for the PURE study population was relatively low (5.1 ± 9%) with the urban group having a significantly higher mean predicted risk (6.1 ± 9.9%) than the rural group (4.2 ± 8.1%) (p<0.001). Fibrinogen correlated weakly but significantly with predicted CVD risk in the total PURE population (r=0.15, p O.OO1). In order to determine whether this association was different for HIV+ and HIV- individuals, the analysis was also performed stratified for HIV status. HIV status did, however, not significantly influence the association (data not shown). This correlation was borderline significantly stronger (p=0.06) in the urban (r=0.21) than in the rural (r=0.17) group (Table 4). Due to possible differences in prevalence of sub-clinical, low-grade inflammation between rural and urban groups (as illustrated also by higher CRP and homocysteine in the rural group) we adjusted for CRP and homocysteine. Adjustment for CRP, resulted in fibrinogen now no longer associating differently with CVD risk between the two groups (p=0.23). Additionally fibrinogen no longer correlated significantly with predicted CVD risk in the total study population or the rural group. Adjustment for homocysteine did not significantly affect the associations (p=0.043).

When dividing the study population according to CVD risk score categories (Table 5), the fibrinogen concentration increased significantly over the risk score categories with participants with a high CVD risk score (>20%) having significantly higher fibrinogen (3.7 vs. 2.8 gil) than those with a low CVD risk score (<5%). In the urban group fibrinogen also increased significantly with increased predicted CVD risk, although no further increase was observed between the moderate and high risk score groups. In the rural group, there was no statistically significant difference in fibrinogen concentration between the four risk score categories, although the mean in the high risk score category was higher than that of the other categories. These statistically significant differences were observed and unchanged in all models tested, with model 1 being an unadjusted model, model 2 – adjusted for age and gender and model 3 adjusted for HIV status and all individual CVD risk factors that showed an association with fibrinogen in Table 2. Factorial ANOVA indicated that the fibrinogen concentration associated differently with the CVD risk score categories in rural and urban groups (p=0.032). Neither adjustment for CRP nor homocysteine influenced this interaction.

From the above results it is clear that other factors, apart from the traditional CVD risk factors investigated above, affect fibrinogen concentration in black South Africans, particularly in the rural setting, resulting in higher fibrinogen levels despite lower CVD risk in this group. Other factors that we investigated in order

Table 4: Pearson correlation of fibrinogen with calculated 10 year CVD risk.

Fibrinogen-CVD risk	Correlation coefficient (r)	P-value	Difference between rural and urban correlation coefficients (p)
Total population-unadjusted	0.146	<0.001	
Adjusted for CRP	0.036	0.14	
Adjusted for Hey	0.159	<0.001	
Urban – unadjusted	0.205	<0.001	Unadjusted: 0.06
Adjusted for CRP	0.080	0.021	Adjusted for CRP: 0.23
Adjusted for Hey	0.227	<0.001	Adjusted for Hey: 0.043
Rura1- unadjusted	0.168	<0.001	
Adjusted for CRP	0.023	0.496	
Adjusted for Hcy	0.131	<0.001	

Table 5: Fibrinogen concentration (median [25th-75th percentile]) of Cardiovascular Risk Categories according to the Reynolds Risk Score.

Fibrinogen (g/1)	low risk <5%	low – moderate risk 5-10%	Moderate risk 10-20%	High risk >20%	Factorial ANOVA p-value
Total group	2.8 [2.2-4.5] n=1287	3.0 [2.4-5.4] n=226	3.2 [2.4-5.8] n=125	3.7 [2.6-6.1] * n=87	
Urban	2.6 [2.1-3.7] n=579	2.8 [2.2-5.4] n=134	3.7 [2.4-5.9] * n=69	3.7 [2.5-6.2] * n=51	0.032 (0.027-adjusted for CRP) (0.019-adjusted for homocysteine)
Rural	3.0 [2.3-5.3] n=708	3.05 [2.5-5.4] n=110	3.0 [2.3-5.5] n=56	3.7 [2.8-5.9] n=36	

*Means differed significantly from low risk score category for all models tested (model1: Unadjusted; model2: adjusted for age and gender; model3: adjusted for age, gender, TC, alcohol consumption, BMI, CRP, HIV, smoking) there were no differences in significance between the tt"ree models.

to determine possible reasons for the increased fibrinogen in the rural group included diet, psychosocial stress and socioeconomic factors.

Possible correlations between fibrinogen concentration and all the macro‑ and micro‑nutrient intake data, expressed as g/day, ob‑tained from the Food Frequency Questionnaires (data not shown) were determined using Pearson correlations. The only dietary variable fibrinogen correlated significantly, but weakly with was alco‑hol consumption (r= -0.13, p<O.OOl).

The association between fibrinogen and three markers of psy‑chosocial wellbeing, determined from questionnaires, with values expressed as continuous variables: a) Mental Health Continuum; b) a General Health Score and c) Sense of Coherence, was also de‑termined. Fibrinogen did not correlate with any of the three psy‑chosocial markers investigated. There was furthermore no differ‑ence in the mean of any of these markers between the fibrinogen quartiles (Table 2). When subjects were divided according to three categories of mental health namely: flourishing individuals, those with moderate mental health and languishing individuals, there was no difference in fibrinogen concentration between the three groups (data not shown).

Lastly, the effect of socio‑economic factors, employment and education, on fibrinogen concentration was investigated. There

was no significant difference in fibrinogen concentration between employed and unemployed individuals (p=0.21) (Table 6). Fi‑brinogen did, however, differ significantly between education cat‑egories, with individuals with no education having the highest fi‑brinogen concentration. Seventy‑one percent of the individuals with no education were in the rural group.

Discussion

This study investigated factors associated with increased fibri‑nogen in black South Africans and is the first study to examine whether the degree of urbanisation influences the association of fi‑brinogen with CVD risk. From the results it is clear that the rural group had a significantly higher fibrinogen concentration than the urban group, despite higher levels of risk factors and increased pre‑dieted CVD risk in the urban group. Increased levels of CVD risk factors were, however, still associated with increased fibrinogen levels as can be seen from the increase in most of these risk factors over the fibrinogen quartiles. The only risk factor fibrinogen clus‑tered with, in the factor analysis was CRP, in the total population as well as in the rural and urban groups separately. Fibrinogen also

Table 6: Fibrinogen concentration (median [25th-75th percentile]) according to level of education and employment.

Variable	None	Primary school	Secondary school	University / College	ANOVA p-value
Total (g/1)	3.0 [2.3-5.4]	2.9 [2.2-5.0]	2.8 [2.2-4.3]	2.8 [2.2–5.3]	0.048
Rural (g/1) n(%)	3.0 [2.4-5.5] 473 (71%)	3.0 [2.3–5.0] 290 (36%)	2.9 [2.4-5.0] 178 (44%)	2.7 [1.6–4.3] 4 (28%)	0.35
Urban n (gil) (%)	2.8 [2.2-4.3] 197 (29%)	2.7 [2.1-5.0] 516 (64%)	2.6 [2.1-3.9] 227 (56%)	2.8 [2.3-5.3] 10 (72%)	0.31
	Employed		**Unemployed**		**ANOVA p-value**
Total (g/1)	2.7 [2.1-4.1]		2.8 [2.2-5.3]		0.21
Rural (g/1) n(%)	2.7 [2.1-3.9] 28 (14%)		3.0 [2.3–5.9] 341 (46%)		0.07
Urban n (gil) (%)	2.8 [2.1-4.2] 171 (86%)		2.6 [2.1-3.7] 400 (54%)		0.33

correlated significantly with overall predicted CVD risk. Although this association was rather weak, in this black South African population, it was stronger in the urban than in the rural group. Factorial ANOVA also indicated that fibrinogen associated differently with predicted CVD risk in the urban compared to the rural group, with fibrinogen concentration showing an increase already in the lower CVD risk score categories, reaching a plateau from moderate (10--20%) to high (>20%) risk, while in the rural group, fibrinogen tended to increase only in the high risk score group. The multiple regression analysis also showed that a smaller percentage of the variance in fibrinogen is explained by the traditional CVD risk factors in the rural than in the urban group. These results together suggest that while there is an association between fibrinogen and CVD risk in black South Africans, it is not very strong and that something else in the rural group affects/increases fibrinogen concentration, that is either not present or less prevalent in the urban group and which is not related to increased CVD risk.

With fibrinogen being an acute phase protein, the first possibility investigated was the possible difference in inflammatory status between rural and urban volunteers. Higher levels of sub-clinical, low-grade inflammation are suspected in rural communities due to poor access to electricity and running water as well as higher levels of poverty and unemployment (19). This possibility is supported by the borderline significantly higher CRP and significantly higher homocysteine levels in the rural group. Significant differences in fibrinogen concentration between rural and urban groups remained, however, after adjustment for CRP and homocysteine, and neither adjustment significantly altered the fact that fibrinogen associated differently with CVD risk score categories in the rural and urban groups (Table 5). It therefore does not seem as if a difference in inflammatory status is the ca use for the difference in association of fibrinogen with predicted CVD risk between the rural and urban groups. Adjustment for CRP, however, resulted in fibrinogen no longer correlating significantly with predicted CVD risk in the rural population (Table 4) and the correlation between fibrinogen and predicted CVD risk was no longer significantly different between the rural and urban population. These results may be explained by the fact that predicted CVD risk as well as the levels of the other individual CVD risk factors are relatively low in the rural group and that adjustment for CRP in this group, may result in the removal of a major reason why fibrinogen correlated with predicted CVD risk (being an inflammatory marker), especially in the absence of many of the other risk factors that might affect fibrinogen concentration and CVD risk.

Fibrinogen is furthermore known to be influenced by dietary factors such as dietary fats and fibre, albeit moderately (21, 22). It has also been shown in the THUSA study, an epidemiological study performed on a similar black South African Tswana-speaking population of the North-West province of South Africa, about 10 years prior to the PURE study, that lower plasma fibrinogen levels were associated with dietary intakes compatible with prudent dietary guidelines (low intakes of animal protein; trans fatty acids and higher intakes of plant protein, dietary fibre, vitamin E and iron and a high dietary polyunsaturated to saturated fatty acid ratio) (5). It was therefore considered that the differences in fibri

nogen concentration between the rural and the urban groups may be related to differences in dietary intakes between these two groups. Fibrinogen showed, however, no correlation with any nutrient, except for a weak negative correlation with alcohol consumption. What is furthermore interesting is that the lower fibrinogen concentration in the THUSA study was found in the rural group, with the urban group having higher fibrinogen concentration- a situation which seems to have reversed over the last 10 years, despite continued urbanisation and westemisation of the diet.

A possible explanation for the above phenomenon may be related to psychosocial wellbeing. Both acute and long-term exposure to psychosocial stress has been shown to activate the haemostatic system, including increasing fibrinogen concentration (23-25). Unpublished data comparing psychosocial wellbeing between rural and urban groups of the THUSA and PURE study, have indicated that while the urban groups consistently had higher psychosocial wellbeing than the rural groups, the trend over time was that satisfaction with life declined in both rural and urban areas and that the gap between the rural and urban groups had enlarged with the rural group now having even lower psychosocial wellbeing and higher stress. Results from three questionnaires used to determine psychosocial wellbeing: the Mental Health Continuum, the Sense of Coherence Scale and the General Health Questionnaire, showed, however, no relation with fibrinogen concentration. It could therefore, not be confirmed that increased psychosocial stress in the rural group contributed to the increased fibrinogen concentration observed in this group, through the use of these three questionnaires.

Lastly the effect of socio-economic factors such as education and employment was investigated as possible reasons for the increased fibrinogen in the rural group. Low levels of these socio-economic factors contribute to increased psychosocial stress and have been shown to increase fibrinogen concentration (4, 25). No difference in fibrinogen concentration was observed between employed and unemployed individuals, but participants with no education had higher fibrinogen concentration than those who had some form of education. Seventy-one percent of the participants who had no education were living in rural areas. Lack of education may therefore contribute to the increased fibrinogen concentrations observed in the rural group. The mechanism underlying this association, however, remains to be elucidated.

A limitation of this study was that individuals already presenting with CVD were excluded from the study and that follow-up data providing information on CVD events is not yet available. The Reynolds Risk Score was therefore employed to stratify the population into risk score categories to enable us to relate fibrinogen to overall CVD risk and not to individual factors only. It should be noted that the risk score was not used to calculate absolute risk but purely to stratify the population into CVD risk score categories. The prospective design of the PURE study will allow determination of actual CVD event rates in future, which will provide additional and much needed insight into the relationship between fibrinogen and CVD in black South Africans.

In conclusion, fibrinogen is associated with CVD risk (individual risk factors as well as with predicted overall risk) in black South

Africans. Although this association is rather weak it is related to degree of urbanisation. It is weaker in the rural group where higher fibrinogen concentrations were observed in the presence of lower predicted CVD risk. A smaller percentage of the variance in fibrinogen is explained by traditional CVD risk factors in the rural group, although it is not entirely clear what the cause for the increased fibrinogen concentrations is. Increased low grade inflammation, psychosocial stress and lack of education may contribute, although this could not be proven. It is not entirely surprising that factors other than CVD-related factors significantly affected fibrinogen concentration. It has already been reported that up to 70% of the explained variance in fibrinogen levels in the Fibrinogen Studies Collaboration, was attributable to non-modifiable factors such as cohort, age, gender, ethnic group and season, whereas only 30% was explained by modifiable factors (4). None of these non-modifiable factors were, however, different between the rural and urban groups in the PURE study. It seems therefore that increased fibrinogen concentration, in black South Africans, while still largely unexplained, is likely not strongly correlated with traditional CVD-related lifestyle and pathophysiological processes. This does, however, not exclude the possibility that, once increased, it may contribute to future development of CVD, especially in urbanised areas where levels of other CVD risk markers are on the rise.

What is known about this topic?

- Factors associated with increased fibrinogen concentration have been investigated in depth in Caucasian populations but much less is known about this in Blacks.
- Fibrinogen is considered to be a cardiovascular disease (CVD) risk factor in Caucasians. Much less is, however, known regarding the association of fibrinogen with CVD risk in Blacks. Some data is available for African Americans (from the ARIC study) but much less is known about this association in Africans. What makes this association particularly interesting in black South Africans, is the fact that high fibrinogen concentrations are seen despite a historically low prevalence of CVD.
- No published data is available on the effect of urbanisation on fibrinogen and its association with CVD.

What does this paper add?

- This study sheds some light on which factors are associated with increased fibrinogen in Blacks and whether it is different from what is found in Caucasians.
- This study, provides data on the association of fibrinogen with other CVD risk factors and predicted CVD risk in black South Africans.
- It demonstrates, for the first time, that urbanisation affects the association of fibrinogen with CVD risk.
- It also demonstrates that especially in rural areas, increased fibrinogen concentration in black South Africans is likely not related to traditional CVD-related lifestyle and pahtophysiological processes.

Conflict of interest

None declared.

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