The relationship of circulating homocysteine with fibrinogen, blood pressure, and other cardiovascular measures in African adolescents

The Relationship of Circulating Homocysteine with Fibrinogen, Blood Pressure, and Other Cardiovascular Measures in African Adolescents

Jacomina P. du Plessis, MS[1,*], Cornelie Nienaber-Rousseau, PhD[1,2,*], Leandi Lammertyn, PhD[2,3], Aletta E. Schutte, PhD[2,3,4], Marlien Pieters, PhD[1,2], and Herculina S. Kruger, PhD[1,2]

Objectives To evaluate the associations between homocysteine (Hcy) and cardiovascular health in South African adolescents.

Study design Circulating Hcy concentrations of 172 South African adolescents (105 girls, ages 13 to <18 years) were measured. Anthropometric and cardiovascular factors were also included and cross-sectionally analyzed through general linear models.

Results Hcy correlated positively with body weight ($P = .03$; after adjusting for multiple testing, it was not regarded as significant) and muscle mass ($P = .01$), but negatively with fibrinogen concentrations ($P = .001$). Across Hcy tertiles, blood pressure produced approximating U-shaped curves, with differences between the middle and upper tertiles (all $P < .02$). Forty percent of the adolescents had elevated blood pressure, of whom 37% fell in the lowest and 38% in the highest Hcy tertiles. Hcy differed between the sexes (with boys having higher Hcy), but not between subgroups based on puberty, weight, stunting, smoking, or alcohol consumption.

Conclusions Both high and low Hcy could be early contributing risk factors to cardiovascular health. The associations between Hcy and blood pressure suggest that dietary and lifestyle manipulation, to achieve the optimal range of Hcy, may be beneficial in preventing Hcy-related hypertension in adulthood. The inverse relationship between Hcy and fibrinogen remains to be clarified. *(J Pediatr 2021;234:158-63)*.

The process leading to the critical stages of cardiovascular disease (CVD) in later life may have already started decades earlier during childhood or young adulthood.[1] The leading cause of CVD worldwide is atherosclerosis elicited by endothelial dysfunction.[2] Several studies show that hyperhomocysteinemia mediates cardiovascular complications by the harmful effects it has on the cardiovascular endothelium and smooth muscle cells, resulting in alterations to arterial structure and function, that is, atherosclerosis.[3,4] Hypohomocysteinemia has been linked to CVD as a result of impaired glutathione metabolism.[5] Moreover, homocysteine (Hcy), a nonproteinogenic, nonessential, sulfhydryl-containing amino acid, has also been associated with coagulation factors, increased blood pressure (BP), and other preclinical CVD markers in adults.[4,6,7] Unfortunately, children are also susceptible to increased hemostatic risk profiles, high BP, and other preexisting CVD risk factors.[1,8,9] Exposure to CVD risk factors in childhood, even during fetal life, may result in early vascular aging.[1]

South African adolescents have increased CVD risk owing to the high prevalence of overweight/obesity and stunting and excessive smoking and alcohol use, as well as ethnicity-related hyperfibrinogenemia, early vascular aging, and hypertension.[10-14] Hcy has been linked to hemostasis, smoking, and alcohol use, as well as being overweight or obese.[6,15-17] Also, stunted growth is known to be associated with lower essential amino acid levels, possibly affecting Hcy as well.[18]

Our aim was to investigate whether a relationship between Hcy and detailed cardiovascular measures in adolescents of African descent exists. Clinical application of Hcy-lowering therapy could potentially decrease Hcy concentrations by one-quarter to one-third if a daily combination of 0.5-5.0 mg folic acid and 0.5 mg vitamin B_{12} is taken.[19]

BF%	Body fat percentage
BMI	Body mass index
BP	Blood pressure
CV	Coefficient of variation
CVD	Cardiovascular disease
HAZ	Height-for-age z-score
Hcy	Homocysteine
NO	Nitric oxide
PLAY	Physical Activity in Youth

From the [1]Center of Excellence for Nutrition, [2]Medical Research Council Unit for Hypertension and Cardiovascular Disease, and [3]Hypertension in Africa Research Team, North-West University, Potchefstroom, South Africa; and the [4]School of Population Health, University of New South Wales; The George Institute for Global Health, Sydney, NSW, Australia

*Contributed equally.

Supported by the National Research Foundation of South Africa. During the writing of the paper, J.d.P. received a scholarship from the South African Medical Research Council through its Division of Research Capacity Development under the National Health Scholarship Programme from funding received from the Public Health Enhancement Fund/South African National Department of Health. Any opinion, findings and conclusions or recommendations expressed in this material are those of the authors and therefore the funding agencies do not accept any liability in regard thereto. The authors declare no conflicts of interest.

https://doi.org/10.1016/j.jpeds.2021.03.034

Methods

Baseline data from the PhysicaL Activity in Youth (PLAY) study were used in a cross-sectional analysis. The PLAY study had a quasi experimental design with longitudinal follow-up in the second and third years. The aim was to assess the effect of increased physical activity on risk factors for noncommunicable disease in adolescents. The sample included ostensibly healthy African adolescents living in low socio-economic conditions in periurban areas while attending 2 separate secondary schools in South Africa. Inclusion and exclusion criteria are listed in **Table I** (available at www.jpeds.com).

Of the 248 youth who were recruited, varying numbers were present on the baseline measurement days and 184 volunteered to give blood samples. We were able to associate Hcy and cardiovascular measures in 172 adolescents (105 girls). All participants had written informed consent from their parent/legal guardians and assented to take part. Ethical approval was granted in accordance with the Declaration of Helsinki for the PLAY study (ethics number: 04M01) and this affiliated study (ethics number: NWU-00142-18-A1) by the Health Research Ethics Committee of the Faculty of Health Sciences, North-West University. To avoid bias, the study had clear objectives, validated methods, and standardized and blind data collection, as well as data coding by multiple researchers.

The body weight of participants was measured to the nearest 0.1 kg with a portable electronic scale (Precision Health Scale, A&D Company). Height was determined to the nearest 0.1 cm with a stadiometer (IP 1465, Invicta). Height-for-age z-scores (HAZ) were calculated using the World Health Organization AnthroPlus software version 3.2.2 and a HAZ of less than −2 meant stunting. Weight and height were used to compute body mass index (BMI). BMI, sex, and age were used to determine BMI z-scores. Body fat percentage (BF%) and muscle mass were measured by air displacement plethysmography in a BODPOD machine (LifeMeasurement Inc. A Lufkin steel tape (Cooper Tools, Apex) was used to quantify waist and hip circumferences to the nearest 0.1 cm. Waist circumference was measured at the narrowest point between the 10th rib and iliac crest and the hip circumference was recorded at the greatest protrusion of the buttocks. Demographic and health data were obtained from structured validated questionnaires. Information regarding smoking, drinking, and habitual substance use was also recorded. Participants were asked to indicate their level of physical maturation by selecting Tanner photographs that best reflected their current pubertal stage (stage I represented physical immaturity and stage V indicated full physical maturity).[20]

Continuous records of cardiovascular variables under resting conditions were accomplished with a validated Finometer device (FMS, Finapres Measurement Systems), for a period of at least 5 minutes.[21] Variables included systolic BP, diastolic BP, mean arterial pressure, stroke volume, cardiac output, ejection time, total peripheral resistance, and Windkessel arterial compliance. Standardized conditions, as set by the National High Blood Pressure Education Program Working Group on High Blood Pressure in Children and Adolescents (2004) and the Joint National Committee on Prevention, Detection, Evaluation and Treatment of High Blood Pressure, were adhered to.[22]

Blood samples were collected after an overnight fast. Serum samples were allowed to coagulate at room temperature for 30 minutes. Serum for insulin and C-reactive protein analyses was aliquoted and stored at −80°C. Citrated and clotted blood was centrifuged for 15 minutes at 2000 × *g* to yield plasma. Plasma samples, used for the quantification of total Hcy, glucose, fibrinogen, plasminogen activator inhibitor type-1 activity, thrombin-antithrombin complex, and factor VIII coagulant activity were aliquoted and frozen on dry ice, until stored at −80°C.

Hcy concentrations were quantified using the Abbott automated immunoassay analyzer (AxSYM), which is based on fluorescence polarization immunoassay technology (coefficient of variation [CV], 4.5%). Plasma fibrinogen concentrations and factor VIII coagulant activity were measured by the modified method of Clauss and the activated partial thromboplastin time assay, respectively, on an automated coagulation analyzer (ACL-200, Instrumentation Laboratories; CV, 4.5% and 6.2%, respectively). Plasminogen activator inhibitor type-1 activity was assayed by an indirect enzymatic method (Spectrolyse pL, Biopool; CV, 16.7%). The thrombin-antithrombin complex was measured by an enzyme-linked immunosorbent assay (Enzygnost TAT micro, Dade Behring; CV, 20.6%). C-reactive protein was determined by rate turbidimetry with a high-sensitivity kit (CRPH, IMMAGE, Immunochemistry Systems on the Synchron LX System (Beckman Coulter Inc; CV, 8.6%). Fasting insulin was measured with the Microparticle Enzyme Immunoassay method on the AxSYM system (Abbott Diagnostics Division; CV, 5.7%). Fasting glucose was measured by an enzymatic method on a Vitros DT60 II Chemistry Analyzer (Ortho-Clinical Diagnostics; CV, 2.1%). The Homeostasis Model Assessment was used for the calculation of insulin resistance (formula: Homeostasis Model Assessment = [Fasting venous insulin (μIU) × fasting venous glucose (μmol/L)]/22.5).

Data were analyzed using Statistica version 13.3 (TIBCO Software Inc) and R statistical software version 3.5.0 (R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing; 2017). Data were tested for normality using the Shapiro-Wilk W-test and the Kolmogorov-Smirnov test. Excluding those with a C-reactive protein of more than 10 mg/L did not influence correlation or general linear model results; therefore, they were included in the results presented here. Normally distributed data were expressed as mean ± SD and non-normal data as medians (25th-75th quartiles). Relationships among variables were assessed with partial Spearman correlations, controlling for age, sex and, in certain instances, BMI. We applied thresholds for BF% by distinguishing between age and sex and using BF% percentiles (>85 overweight; >95 obese) as cut-off points.[23] Using the updated 2016 European Society of Hypertension guidelines for the

management of high BP in children and adolescents, we categorized BP values for those less than 16 years of age according to the sex, age, and height tables.[24] Adolescents older than 16 years were classified the same as adults (normal BP, <120/80 mm Hg; prehypertensive, ≥120/80 to ≤139/89; and hypertensive, ≥140/90 mm Hg or those reporting antihypertensive drug use). Additionally, we used the American Academy of Pediatrics 2017 clinical practice guidelines for screening and management of high BP in children and adolescents and categorized BP values for 13 to less than 18 years according to the age, sex, and height tables.[25] Furthermore, the participants were subdivided into tertiles based on Hcy concentrations, that is, less than 5.61 μmol/L (tertile 1), 5.61 μmol/L or greater and less than 6.91 μmol/L (tertile 2), and 6.91 μmol/L or greater (tertile 3). CVD variable differences among the Hcy tertiles were determined after adjusting for age, sex, and BMI by using general linear models followed by post hoc tests. Subgroup data were divided into the following categorical and binary variables: sex (male/female); puberty (prepubertal/pubertal); weight status (normal weight/overweight or obese); nutrition status (nonstunted/stunted); smoking status (yes/no); and consumes alcohol (yes/no). Comparisons among the subgroup categories were performed by using general linear models while adjusting selectively for age, sex, and BMI. To compare the prevalence of normotension and prehypertension/hypertension among the Hcy tertiles, frequency tables with χ^2 tests were used. A P value of .05 or less was regarded as statistically significant with a stricter P value of .02 or less applied to adjust for multiple testing with a 25% Hochberg and Benjamini false discovery rate when 11 CVD markers were regarded as independent ($R^2 < 0.2$; 1/11 [0.25] = 0.022).

Results

Baseline characteristics and correlations between Hcy and detailed CVD markers of the participants are specified in Table II. Only 16 participants (10%) were prepubertal (stages I-II); therefore, 152 (90%) of the adolescents had reached puberty (stages III-V). According to the HAZ, 37 participants (22%) were stunted (HAZ of <−2). Using BF % cut-off points, 21 (27%) boys and 59 (47%) girls were overweight, of whom 10 (13%) boys and 24 (19%) girls were classified as obese.[23] Boys had a higher mean muscle mass (39.6 kg) and Hcy concentrations (6.95 μmol/L) than girls (34.2 kg and 6.05 μmol/L, respectively). Hcy correlated positively with weight and muscle mass, but negatively with fibrinogen concentrations. After accounting for multiple testing, only the positive muscle mass and negative fibrinogen correlation remained.

Hcy differences between CVD risk subgroups were investigated and reported in Table III. A difference in Hcy was detected between sex categories, with boys having higher Hcy concentrations than girls; however, no differences were observed between the other subgroups after adjusting accordingly.

As reported in Table IV, post hoc tests revealed differences across Hcy tertiles for some of the cardiovascular markers after adjusting for age, sex, and BMI. With increasing Hcy tertiles, BP had an approximate U-shaped curve with significant differences between the middle and upper tertiles (Figures 1 and 2; available at www.jpeds.com), whereas fibrinogen concentrations decreased from the lower to the higher Hcy tertiles (Figure 3; available at www.jpeds.com). The difference in systolic BP was no longer significant after adjusting for the false discovery rate.

Using the 2016 European Society of Hypertension guidelines, we determined that 28 adolescents had a prehypertensive status (15%) and 49 already revealed hypertension (25%). Additionally, we used the American Academy of Pediatrics 2017 guidelines and observed that 37 participants (19%) had a prehypertensive status and 66 (34%) had hypertension.

Table II. Demographic and cardiovascular-related characteristics of participants

Characteristics	Median (25th-75th) or mean ± SD	Partial Spearman R with Hcy* R	P
Age (years)	15.2 (14.5-6.2)	.09	.21
Anthropometrical markers			
Body weight (kg)	49.4 ± 8.74	.16	.03†
BMI (kg/m^2)	19.1 (17.5-21.4)	.09	.21
BF%	25.2 ± 8.34	−.09	.33
Muscle mass (kg)	36.3 ± 6.99	.20	.01†,‡
HAZ	−1.08 ± 0.98	.02	.84
Waist (cm)	65.0 ± 5.53	.07	.37
Hip (cm)	82.8 ± 7.70	.11	.15
Waist hip ratio	0.79 ± 0.06	−.06	.46
Biochemical markers			
Hcy (μmol/L)	6.07 (5.23-7.25)		
Thrombin-antithrombin complex (μg/L)	3.50 (2.03-6.54)	−.06	.42
Factor VIII coagulant activity %	153 ± 47.3	.07	.34
C-reactive protein (mg/L)	0.34 (0.17-1.05)	−.08	.30
Fibrinogen (g/L)	2.74 ± 0.51	−.24	<.001†,‡
Plasminogen activator inhibitor type-1 activity (IU/mL)	2.63 (0.00-5.06)	−.07	.36
Insulin (μU/mL)	8.10 (5.30-12.1)	.02	.85
Glucose (μmol/L)	5.10 (4.80-5.30)	.08	.26
Homeostasis Model Assessment-insulin resistance	1.79 (1.18-2.83)	.02	.79
Cardiac markers			
Systolic BP (mm Hg)	117 ± 13.3	.05	.52
Diastolic BP (mm Hg)	77 ± 9.24	.11	.21
Mean arterial pressure (mm Hg)	94.1 ± 9.92	.09	.26
Stroke volume (mL)	44.9 ± 12.2	.09	.25
Cardiac output (Lpm)	3.48 ± 0.91	.10	.22
Ejection time (s)	0.29 ± 0.02	−.05	.54
Total peripheral resistance (MU)	1.61 (1.39-1.93)	−.06	.46
Windkessel arterial compliance (MU)	1.36 ± 0.27	.13	.11

Significant partial Spearman correlations (†$P < .05$), with a false discovery rate adjustment (‡$P \leq .02$).
*Partial Spearman correlations adjusted for age, sex, and BMI except for anthropometrical markers, which were adjusted for age and sex only, and age that was adjusted only for BMI and sex.

Table III. Hcy concentrations between CVD risk subgroups

Subcategories (n)	Adjusted means with 95% CI	*P* value
Sex*		
Boys (67)	6.95 (6.57-7.32)	<.001†,‡
Girls (105)	6.05 (5.75-6.34)	
Puberty§		
Prepubertal (16)	6.25 (5.49-7.02)	.75
Pubertal (152)	6.39 (6.12-6.65)	
Weight¶		
Normal (95)	6.47 (6.13-6.82)	.20
Overweight/obese (53)	6.08 (5.64-6.52)	
Nutrition status¶		
Nonstunted (134)	6.46 (6.19-6.72)	.33
Stunted (37)	6.20 (5.70-6.69)	
Smoking status§		
Nonsmoker (92)	6.54 (6.17-6.91)	.39
Smoker (14)	6.95 (6.13-7.77)	
Consume alcohol§		
No (87)	6.54 (6.20-6.87)	.78
Yes (19)	6.43 (5.74-7.12)	

Adjusted for *age, BMI; §age, sex, and BMI; and ¶age and sex. Significant differences in Hcy concentrations between subgroup categories (†$P < .001$), with a false discovery rate adjustment (‡$P \leq .02$).

A frequency table (Table V) with Hcy tertiles and BP categories indicates that those in the highest and lowest Hcy tertiles had a higher risk of being hypertensive than those in the middle tertile ($P = .01$), using the European Society of Hypertension guidelines. It should be noted that BP data were available for 193 adolescents, but only 140 of those participants recorded Hcy data; thus, the hypertensive totals in Table V do not represent the entire BP dataset.

Discussion

The mean Hcy concentration of the PLAY participants fell within the normal range, according to age and ethnicity.[26] We demonstrated U-shaped associations between Hcy and BP, as well as a negative correlation between Hcy and fibrinogen concentrations. Approximately 40%-50% of the adolescents had elevated BP. More than 1 in 3 of the study population presented with prehypertension or hypertension in the highest Hcy tertile, which was also seen in the lowest Hcy tertile. Weight and muscle mass correlated positively with Hcy. Among the subgroups, Hcy differed only between the sexes, with the higher Hcy in boys likely related to their greater muscle mass. After multiple comparisons adjustment, the significance between Hcy, body weight, and systolic BP attenuated; however, the associations between Hcy and muscle mass, diastolic BP, mean arterial pressure, and fibrinogen remained noteworthy.

Skeletal muscle is more than 90% creatine, which is partially replenished by dietary animal protein, but also synthesized when guanidinoacetate is methylated by hepatic S-adenosylmethionine. This process subsequently produces Hcy.[27] During creatine metabolism, Hcy production increases as a consequence of the methyl group transfer. Creatinine is also formed from the intracellular nonenzymatic degradation of creatine. Thus, muscle mass is related to the creatinine-Hcy relationship, possibly explaining part of the sex-related difference in Hcy concentrations. We believe that the muscle mass of our study sample of Black South African adolescents may not be entirely comparable with those of African American populations, and could even be somewhat less than those of European descent based on fat-free soft tissue data and height.[28,29] Height has a strong positive association with appendicular skeletal muscle mass and the high prevalence of stunting in our study population could explain muscle mass differences.[29] There is no universal definition of or assessment method for determining muscle quality in children, but muscle quality vs muscle mass may also be critical here.

Table IV. Cardiovascular markers across Hcy tertiles

	Hcy (adjusted means with 95% CI determined through GML)			
CVD markers	Tertile 1 (n = 57) <5.61 μmol/L	Tertile 2 (n = 58) ≥5.61 and <6.91 μmol/L	Tertile 3 (n = 58) >6.91 μmol/L	ANCOVA *P* value
Thrombin-antithrombin complex (μg/L)	5.48 (3.79-7.18)	6.34 (4.71-7.96)	4.08 (2.39-5.77)	.16
Factor VIII coagulant activity %	153 (140-167)	149 (137-161)	158 (145-170)	.60
C-reactive protein (mg/L)	2.37 (0.40-5.13)	2.72 (0.15-5.28)	1.74 (0.95-4.43)	.87
Fibrinogen (g/L)	2.91 (2.78-3.05)*,†	2.70 (2.58-2.83)*	2.63 (2.50-2.77)†	.01‡,§
Plasminogen activator inhibitor type-1 activity (IU/mL)	3.26 (2.37-4.16)	3.10 (2.27-3.93)	3.12 (2.25-3.99)	.96
Insulin (μU/mL)	9.40 (7.69-11.1)	10.4 (8.82-12.0)	8.24 (6.58-9.90)	.17
Glucose (μmol/L)	5.08 (4.95-5.20)	5.04 (4.92-5.16)	5.15 (5.03-5.27)	.42
Homeostasis Model Assessment -insulin resistance	2.18 (1.74-2.62)	2.37 (1.96-2.77)	1.93 (1.51-2.35)	.33
Systolic BP (mm Hg)	118 (115-122)	113 (110-116)*	119 (115-123)*	.03‡
Diastolic BP (mm Hg)	77.2 (74.6-79.8)	73.6 (71.3-75.9)*	78.2 (75.5-80.9)*	.02‡,§
Mean arterial pressure (mm Hg)	94.8 (91.9-97.6)	90.7 (88.2-93.3)*	96.3 (93.3-99.3)*	.01‡,§
Stroke volume (mL)	43.2 (39.8-46.5)	44.9 (41.9-47.9)	45.2 (41.7-48.7)	.66
Cardiac output (LPM)	3.33 (3.06-3.59)	3.48 (3.25-3.72)	3.44 (3.17-3.72)	.68
Ejection time (s)	0.29 (0.28-0.30)	0.29 (0.28-0.30)	0.29 (0.28-0.29)	.68
Total peripheral resistance (MU)	1.82 (1.66-1.98)	1.69 (1.54-1.84)	1.83 (1.66-2.00)	.36
Windkessel arterial compliance (MU)	1.32 (1.26-1.38)	1.39 (1.34-1.44)	1.33 (1.27-1.40)	.18

Significant CVD variable differences among the Hcy tertiles (‡$P < .05$). Post hoc test revealed significant differences between the categories indicated with symbols (*$P \leq .01$, † <.001), with a false discovery rate adjustment (§$P \leq .02$). Adjusted for age, sex, and BMI.

Table V. Frequency of Hcy tertiles according to hypertension categories

Hcy tertiles	Normotension	Prehypertension/hypertension	Total
Normotension/hypertension, n (%) of category, using European Society of Hypertension guidelines*			
1	27 (31)	19 (37)	46
2	42 (47)	13 (25)	55
3	19 (22)	20 (38)	39
Total	88	52	140
Normotension/hypertension, n (%) of category, using American Academy of Pediatrics guidelines†			
1	22 (31)	24 (34)	46
2	32 (46)	23 (33)	55
3	16 (23)	23 (33)	39
Total	70	70	140

*$\chi 2$, $P = .01$.
†$\chi 2$, $P > .05$.

In our study, almost one-half of the adolescents had an elevated BP. The approximate U-shape relationship between Hcy and BP implies that both high and low Hcy could play a role in BP levels irrespective of age, sex, and BMI. U-shaped curves between Hcy and both peak systolic velocity and end diastolic velocity were recorded among Chinese adults, 30-80 years of age, with no history of cardiovascular-related diseases.[30] Another study on healthy, normotensive, Chinese adults (>40 years of age) reported that Hcy is related to hypertension incidence, with approximating U-shaped curves as result. Men had the highest risk of hypertension in the 2 lowest Hcy quartiles, implying that lower Hcy levels might indicate higher risk of the condition.[31]

Experimental and animal studies have augmented this by reporting an increase in BP as a consequence of hyperhomocysteinemia.[4] There are multifactorial mechanisms that mediate Hcy-induced endothelial dysfunction leading to elevated BP. In particular, Hcy reduces the bioavailability of the potent vasodilator nitric oxide (NO). Reduced NO bioavailability can be caused by the uncoupling of NO synthase activity and NO quenching through oxidative stress.[3] Elevated Hcy concentrations can also cause pathological changes in the structure and function of the arterial wall, which in turn influence arterial stiffness.[32] These processes increase an individual's susceptibility to atherosclerosis, thrombotic processes and lead to elevated BP.[32]

Research on hypohomocysteinemia and related conditions is scarce. Hcy is regulated predominantly through transmethylation or trans-sulfuration. In the Hcy-trans-sulfuration pathway, Hcy acts as an intermediate in the conversion of methionine to cysteine. Low Hcy may be caused by the excessive conversion to cysteine in the trans-sulfuration pathway that produces glutathione, taurine, and sulfate. Cysteine in turn is used for the increased demand of hepatic glutathione synthesis, caused by the rapid response to oxidative stress, which leads to a decline in Hcy concentrations.[33] In contrast, low levels of Hcy could also inhibit the de novo production of glutathione and, thus, increase exposure to oxidative stress, which is directly linked to BP and CVD.[34] Stunting is also associated with reduced serum levels of essential amino acids, possibly caused by decreased intake of protein, which could have lowered Hcy as a result.[18]

Hypohomocysteinemia and hyperhomocysteinemia in adults are classified as Hcy less than 6.0 μmol/L and greater than 15 μmol/L, respectively.[4,35] However, children have lower overall Hcy concentrations that increase with age. Our findings suggest that there may be an optimal or safe Hcy range of 5.0-7.0 μmol/L (above the mean of the first [4.80 μmol/L], but below the mean of the third [8.17 μmol/L] tertile subdivision observed here) for adolescents.

Of the hemostatic markers we measured, only fibrinogen concentrations were associated negatively with Hcy. Little is known about the link between coagulation markers and Hcy concentrations, especially in pediatrics. Similar to what we report here, a study in a group of healthy black South African adults also found an inverse relationship between fibrinogen and Hcy concentrations.[5] Black South Africans manifest higher fibrinogen concentrations than other ethnicities, which could influence the dichotomy in findings observed in other populations.[36-38]

Our study had some limitations. The cross-sectional design prevents us from determining causal relations. In addition, serum lipids and hormones, including thyroid function markers and vitamin data, were not quantified. Other limitations may have included selection bias; we report on only Black South African adolescents, thereby limiting the generalizability of our results. We also had a relatively small sample size.

Although there is evidence of a pathogenic relationship between Hcy and the vasculature in adults, evidence of this relationship in young populations is limited, especially with respect to low Hcy concentrations. The inverse relationship between Hcy and fibrinogen as a marker of cardiovascular health in adolescents of African descent merits further study. ■

Submitted for publication Sep 10, 2020; last revision received Mar 11, 2021; accepted Mar 19, 2021.

Reprint requests: Cornelie Nienaber-Rousseau, PhD, Center of Excellence for Nutrition, School Physiology, Nutrition and Consumer Sciences, North-West University, Private Bag X6001, Nutrition, Box 594, Potchefstroom 2520, South Africa. E-mail: cornelie.nienaber@nwu.ac.za

Data Statement

Data sharing statement available at www.jpeds.com.

References

1. Olsen MH, Angell SY, Asma S, Boutouyrie P, Burger D, Chirinos JA, et al. A call to action and a lifecourse strategy to address the global burden of raised blood pressure on current and future generations: the Lancet Commission on hypertension. Lancet 2016;388:2665-712.
2. Barquera S, Pedroza-Tobías A, Medina C, Hernández-Barrera L, Bibbins-Domingo K, Lozano R, et al. Global overview of the epidemiology of atherosclerotic cardiovascular disease. Arch Med Res 2015;46:328-38.
3. Lai WKC, Kan MY. Homocysteine-induced endothelial dysfunction. Ann Nutr Metab 2015;67:1-12.

4. Ganguly P, Alam SF. Role of homocysteine in the development of cardiovascular disease. Nutr J 2015;14:6.
5. Lang CA, Mills BJ, Mastropaolo W, Liu MC. Blood glutathione decreases in chronic diseases. J Lab Clin Med 2000;135:402-5.
6. Nienaber-Rousseau C, de Lange Z, Pieters M. Homocysteine influences blood clot properties alone and in combination with total fibrinogen but not with fibrinogen γ' in Africans. Blood Coagul Fibrinolysis 2015;26:389-95.
7. Bogdanski P, Miller-Kasprzak E, Pupek-Musialik D, Jablecka A, Lacinski M, Jagodzinski PP, et al. Plasma total homocysteine is a determinant of carotid intima-media thickness and circulating endothelial progenitor cells in patients with newly diagnosed hypertension. Clin Chem Lab Med 2012;50:1107-13.
8. Komitopoulou A, Platokouki H, Kapsimali Z, Moschovi M, Kattamis A, Pergantou H, et al. Mutations and polymorphisms in genes affecting haemostasis components in children with thromboembolic events. Pathophysiol Haemos Thromb 2006;35:392-7.
9. Agirbasli M, Dilek HF, Tatlisu MA, Ankarali H. Reliability of normative tables in assessing elevated blood pressure in children. J Hum Hypertens 2020;34:241-7.
10. van Rooyen JM, Kruger HS, Huisman HW, Schutte AE, Malan NT, Schutte R. Early cardiovascular changes in 10-to 15-year-old stunted children: the Transition and Health during Urbanization in South Africa in Children study. Nutr 2005;21:808-14.
11. Symington EA, Gericke GJ, Nel JH, Labadarios D. The relationship between stunting and overweight among children from South Africa: secondary analysis of the National Food Consumption Survey-Fortification Baseline I. S Afr Med J 2016;106:65-9.
12. Peer N, Bradshaw D, Laubscher R, Steyn N, Steyn K. Urban-rural and gender differences in tobacco and alcohol use, diet and physical activity among young black South Africans between 1998 and 2003. Glob Health Action 2013;6:19216.
13. Vorster H. The emergence of cardiovascular disease during urbanisation of Africans. Public Health Nutr 2002;5:239-43.
14. Kruger R, Gafane-Matemane LF, Kagura J. Racial differences of early vascular aging in children and adolescents. Pediatr Nephrol 2021;36:1087-108.
15. Nienaber-Rousseau C, Pisa PT, Venter CS, Ellis SM, Kruger A, Moss SJ, et al. Nutritional genetics: the case of alcohol and the MTHFR C677T polymorphism in relation to homocysteine in a black South African population. J Nutrigenet Nutrigenomics 2013;6:61-72.
16. Kennedy BP, Farag NH, Ziegler MG, Mills PJ. Relationship of systolic blood pressure with plasma homocysteine: importance of smoking status. J Hypertens 2003;21:1307-12.
17. Al-Bayyari N, Hamadneh J, Hamadneh S. Total homocysteine is positively correlated with body mass index, waist-to-hip ratio, and fat mass among overweight reproductive women: a cross-sectional study. Nutr Res 2017;48:9-15.
18. Semba RD, Shardell M, Ashour FAS, Moaddel R, Trehan I, Maleta KM, et al. Child stunting is associated with low circulating essential amino acids. EBioMedicine 2016;6:246-52.
19. Collaboration HLT. Lowering blood homocysteine with folic acid based supplements: meta-analysis of randomised trials. Br Med J 1998;316:894-8.
20. Tanner JM, Whitehouse RH. Clinical longitudinal standards for height, weight, height velocity, weight velocity, and stages of puberty. Arch Dis Child 1976;51:170-9.
21. Heeney ND, Habib F, Brar GK, Krahn G, Campbell DA, Sanatani S, et al. Validation of finger blood pressure monitoring in children. Blood Press Monit 2019;24:137-45.
22. Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, Izzo JL Jr, et al. Seventh report of the joint national committee on prevention, detection, evaluation, and treatment of high blood pressure. Hypertension 2003;42:1206-52.
23. McCarthy H, Cole T, Fry T, Jebb S, Prentice A. Body fat reference curves for children. Int J Obes 2006;30:598-602.
24. Lurbe E, Agabiti-Rosei E, Cruickshank JK, Dominiczak A, Erdine S, Hirth A, et al. 2016 European Society of Hypertension guidelines for the management of high blood pressure in children and adolescents. J Hypertens 2016;34:1887-920.
25. Flynn JT, Kaelber DC, Baker-Smith CM, Blowey D, Carroll AE, Daniels SR, et al. Clinical practice guideline for screening and management of high blood pressure in children and adolescents. Pediatrics 2017;140:e20171904.
26. Mancia G, Fagard R, Narkiewicz K, Redon J, Zanchetti A, Böhm M, et al. 2013 ESH/ESC guidelines for the management of arterial hypertension: the Task Force for the Management of Arterial Hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). Eur Heart J 2013;34:2159-219.
27. Jacques PF, Rosenberg IH, Rogers G, Selhub J, Bowman BA, Gunter EW, et al. Serum total homocysteine concentrations in adolescent and adult Americans: results from the third National Health and Nutrition Examination Survey. Am J Clin Nutr 1999;69:482-9.
28. Silva AM, Shen W, Heo M, Gallagher D, Wang Z, Sardinha LB, et al. Ethnicity-related skeletal muscle differences across the lifespan. Am J Hum Biol 2010;22:76-82.
29. Kruger HS, Micklesfield LK, Wright HH, Havemann-Nel L, Goedecke JH. Ethnic-specific cut-points for sarcopenia: evidence from black South African women. Eur J Clin Nutr 2015;69:843-9.
30. Micklesfield LK, Norris SA, Nelson DA, Lambert EV, Van der Merwe L, Pettifor JM. Comparisons of body size, composition, and whole body bone mass between North American and South African children. J Bone Miner Res 2007;22:1869-77.
31. Wu Z, Wen Y, Liu M, Li H, Zhu Y, Zhang H, et al. A curve model for association of serum homocysteine with carotid artery hemodynamics. Trop J Pharm Res 2017;16:1983-8.
32. Esse R, Barroso M, Tavares de Almeida I, Castro R. The contribution of homocysteine metabolism disruption to endothelial dysfunction: state-of-the-art. Int J Mol Sci 2019;20:867.
33. Vitvitsky V, Mosharov E, Tritt M, Ataullakhanov F, Banerjee R. Redox regulation of homocysteine-dependent glutathione synthesis. Redox Rep 2003;8:57-63.
34. Cervantes Gracia K, Llanas-Cornejo D, Husi H. CVD and oxidative stress. J Clin Med 2017;6:22.
35. Cullen CE, Carter GT, Weiss MD, Grant PA, Saperstein DS. Hypohomocysteinemia: a potentially treatable cause of peripheral neuropathology? Phys Med Reh Clin N Am 2012;23:59-65.
36. Pieters M, Vorster HH. Nutrition and hemostasis: a focus on urbanization in South Africa. Mol Nutr Food Res 2008;52:164-72.
37. Malinowska J, Nowak P, Olas B. Comparison of the effect of homocysteine in the reduced form, its thiolactone and protein homocysteinylation on hemostatic properties of plasma. Thromb Res 2011;127:214-9.
38. Undas A, Brozek J, Jankowski M, Siudak Z, Szczeklik A, Jakubowski H. Plasma homocysteine affects fibrin clot permeability and resistance to lysis in human subjects. Arterioscler Thromb Vas. Biol 2006;26:1397-404.

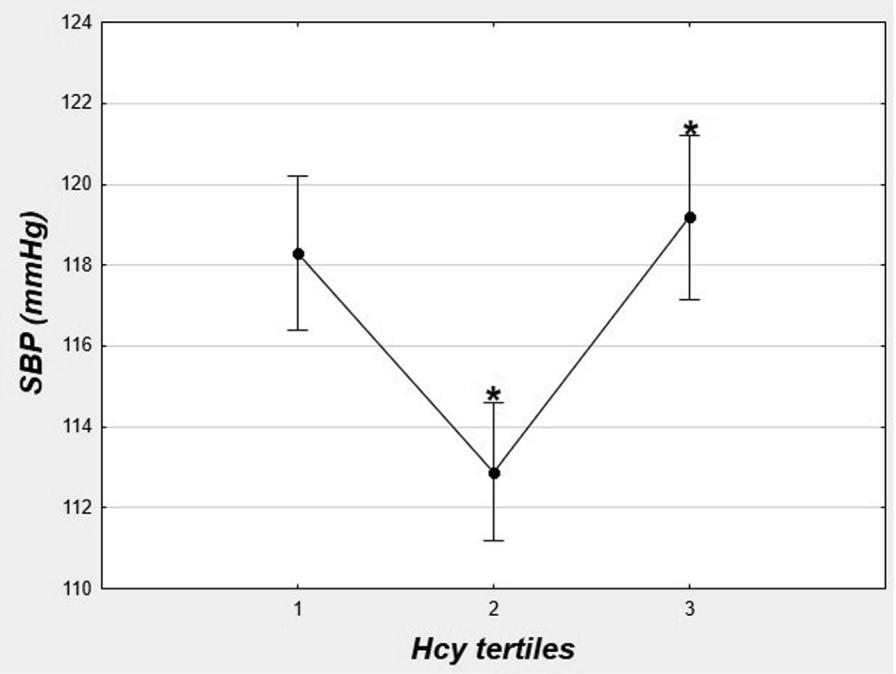

Figure 1. Systolic BP over Hcy tertiles. Results presented as adjusted means (*dots*) and standard error (*whiskers*). Symbols indicate statistically significant differences between groups after post hoc correction. *$P < .01$.

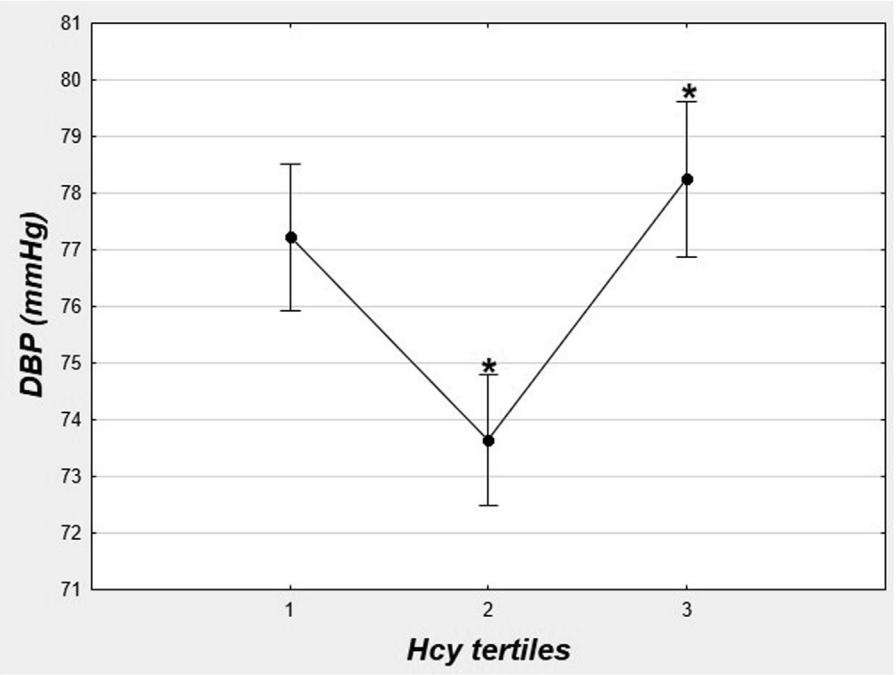

Figure 2. Diastolic BP over Hcy tertiles. Results presented as adjusted means (*dots*) and standard error (*whiskers*). Symbols indicate statistically significant differences between groups after post hoc correction. *$P < .01$.

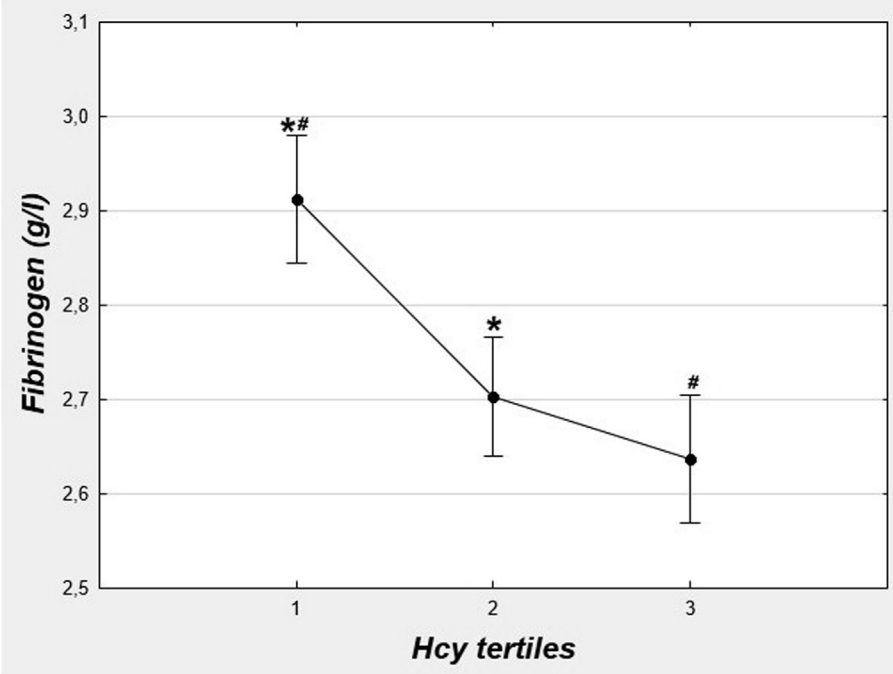

Figure 3. Fibrinogen concentrations over Hcy tertiles. Results presented as adjusted means (*dots*) and standard error (*whiskers*). Symbols indicate statistically significant differences between groups after post hoc correction. $^{*}P < .001$, $^{\#}P < .01$.

Table I. Inclusion and exclusion criteria of the PLAY study

Criteria	Justification
Inclusion criteria	
1 Apparently healthy	1 The intervention included physical exercise and chronic illness that could influence participation/compliance and risk factors that might confound the outcome(s).
2 Grade nine pupil of intervention or reference school	2 Grade nine learners were chosen because higher grade pupils may have already transitioned from adolescence to adulthood. Furthermore, grade nine is not as scholastically demanding as the higher grades and we did not want to interfere with the learner's education. The schools were chosen based on their catchment areas that attract children of low socio-economic status.
3 Age between 13 and 18 years	3 Adolescent age range; the study did not include adults or young children.
4 Voluntary written consent given by parents and assent given by learner	4 No child could participate without parental/guardian's consent and they should also have assented themselves to volunteer.
Exclusion criteria	
2 Learners in other grades or not a learner at the 2 schools	
3 Age <13 and >18 years	
4 Learners that did not get voluntary consent from parents or guardians and/or who did not assent themselves	