Cardiovascular Risk Factors and their Relationship with Endothelial Dysfunction in Rural and Urban South African Children

Edna N. Matjuda
Walter Sisulu University
Godwill Azeh Engwa (engwagodwill@gmail.com)
Walter Sisulu University https://orcid.org/0000-0002-0044-9890
Samuel Nkeh Chungag Anye
Walter Sisulu University
Benedicta N. Nkeh Chungag
Walter Sisulu University
Nandu Goswami
Medizinische Universitat Graz

Research article

Keywords: Endothelial dysfunction, cardiovascular disease, children, obesity, hypertension, oxidative stress, microalbuminuria.

Posted Date: August 13th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-54162/v1

Abstract

Background: Endothelial dysfunction is known to be an initiator to the development and progression of atherosclerotic cardiovascular disease (CVD). However, there is paucity o knowledge on its relationship with cardiovascular risk factors in children. More so, some of these cardiovascular risk factors are known to be influenced by feeding habits and life style changes which often vary between rural and urban settings. This study was aimed to investigate the relationship between cardiovascular risk factors and endothelial function in rural and urban children.

Methods: A cross-sectional study on 6-9 years old children in randomly selected rural and urban schools of the Eastern Cape Province of South Arica was conducted. General anthropometric indices were measured followed by blood pressure (BP) measurements. The pulse wave velocity (PWV) was measured using a Vicorder. Urine sample was collected for the determination of albumin, creatinine, asymmetric dimethylarginine (ADMA), 8-hydroxy-2deoxyguanosine (8-OHdG) and thiobarbituric acid reactive substance (TBARS). Albumin to creatinine ratio (ACR) was calculated.

Results: Children from urban settings (10.8%) had a higher prevalence of overweight/obesity than their rural counterparts (8.5%) while the prevalence of elevated/high blood pressure was higher in rural children (23.2%) than in urban children (19.0%). Diastolic blood pressure (DBP) and mean arterial blood pressure (MAP) significantly (p<0.005) increased with increasing quartiles of PWV. ADMA positively associated with HR in rural girls and showed a weak risk for elevated SBP and MAP. Body mass index (BMI) increased with increasing PWV and predicted endothelial dysfunction. 8-OHdG significantly (p<0.005) increased with increasing quartiles of ADMA and positively correlated with ADMA. Creatinine, albumin and ACR significantly (p<0.005) increased with increasing ADMA and ADMA associated positively with creatinine.

Conclusion: Endothelial dysfunction was associated with obesity, high blood pressure, oxidative stress and microalbuminuria in children, and this relationship varied between rural and urban children.

Background

The endothelium is increasingly been recognized as a vascular barrier which is important in the regulation of blood flow into micro- and macrovascular circulation [1]. Endothelial cells lining the arterial lumen are known to regulate vascular tone and also maintain vascular homeostasis by keeping a delicate balance between vasodilation and vasoconstriction [1]. One key regulator in the endothelium is nitric oxide (NO), which is generated from L-arginine by endothelial nitric oxide synthase (eNOS) in the presence of cofactors such as tetrahydrobiopterin (BH4) [1]. However, alternation in the production of NO or impaired functioning of the endothelium leads to a condition known as endothelial dysfunction [2]. High level of pulse wave velocity (PWV) is indicative of endothelial dysfunction and thus, PWV has served as a marker for endothelial dysfunction [3]. Endothelial dysfunction has been established to be an initiator and a contributory factor to the progression of atherosclerotic cardiovascular disease (CVD) [4].

CVDs are a worldwide problem because they lead to morbidity and mortality, and are of high economic burden to individual households as well as nations [5]. Cardiovascular disease risk factors including smoking, obesity, hypertension, oxidative stress etc. are known to be associated with endothelial dysfunction.

Studies have shown that childhood obesity is associated with endothelial dysfunction. Adiponectin is a molecule that helps to prevent fat storage in adipocytes and improves endothelial function. However, obesity promotes the activation and infiltration of macrophages into adipose tissues which results to low-grade inflammatory response [6]. The adipocytes release low levels of anti-inflammatory adipokines including adiponectin, while macrophages release elevated levels of pro-inflammatory cytokines including tumour necrosis factor alpha (TNF-α) and interleukin-6 (IL-6) [6]. Decreased plasma level of adiponectin promotes the synthesis of arginase [7] which in turns inhibits the production of NO by competing with eNOS for the substrate L-arginine [8]) thereby leading to endothelial dysfunction. Also, eNOS can be inhibited by asymmetric dimethylarginine (ADMA). ADMA inhibits eNOS by competing with its substrate, L-arginine thereby impairing the production of NO [9]. Thus, decrease availability of NO as a result of obesity and increased ADMA leads to endothelial dysfunction [10]. Also, pro-inflammatory cytokines released from adipose tissue may lead to the generation of reactive oxygen species (ROS) [11]. ROS is reported to change the conformation of eNOS making it less effective for NO synthesis with the consequent decreased in NO production [4]. Also, ROS can react with NO converting them to more NO reactive species such as superoxide ($O_2^{\bullet}$), and peroxynitrite peronitrites ($NOO^{\bullet}$) thereby reducing NO levels [4]. Thus, oxidative stress can promote endothelial dysfunction.

Endothelial dysfunction may also result to hypertension. It has been suggested that endothelium dysfunction may cause some structural and functional changes in the microvascular wall with a predominant and deleterious constrictive tone leading to hypertension [12]. More so, release of endothelium-derived relaxing factors (EDRFs) in the endothelium is impaired by endothelial dysfunction resulting in vasoconstriction which may result to hypertension, another major risk factor of CVD [13]. Also, microalbuminuria has being suggested to be an independent risk factor for endothelial dysfunction [14]. Although it remains unclear, it has been suggested that glomerular leaking of albumin is a reflection of vascular damage which denotes atherosclerosis [15].

In South Africa, there is evidence of increase in cardiovascular risk factors in children. A study in Johannesburg showed a 19% of hypertension in children aged 5–8 years [16]. Also, a longitudinal study in North West of South Africa found that there was an increase in obesity over a period of 3 years from 12.5% at baseline to 16.7% among 6–9 years children [17]. More so, endothelial dysfunction was shown to occur in children exposed to cardiovascular risk factors including a family history of hypertension [18]. Though several studies in developed countries and some developing countries have shown high prevalence of cardiovascular risk factors among children [19, 20, 21], there is limited information on their relationship with endothelial function in South African children and especially children in the Eastern Cape Province, a region undergoing rural to urban transition which has influenced life style changes associated with cardiovascular risk factors. Therefore, the present study was aimed to assess the

relationship between cardiovascular risk factors and endothelial function in South African children in urban and rural areas of the Eastern Cape Province.

Methods

Study population and design

This was a cross-sectional study that recruited primary school children aged 6–9 years from rural and urban areas of the Eastern Cape Province of South Africa. The children were recruited from primary schools in Libode, a rural area and from Mthatha and East London which are urban areas.

Ethical Consideration

This study was conducted in accordance to the guidelines of the Helsinki Declaration (2008 reviewed version) as well as the local and national regulations of South Africa. Ethical approval was obtained from Walter Sisulu University Health Sciences Ethics Committee with approval number: 112/2018. After careful explanation of the purpose and aim of the study, written informed consent was obtained from the parents/legal guardians of the children before enrolment into the study. The study adhered to the standards of reporting and was in accordance to the National Data Protection Acts as the identity of the participants was kept confidential. There were no important changes to the methods after study commencement.

Inclusion/exclusion Criteria

Children aged 6–9 years who are free from any cardiovascular and renal diseases were recruited for the study. Pregnant, lactating, ill, physically challenged, individuals having any self-reported comorbidity or cardiovascular diseases were excluded from the study.

Anthropometric Measurements

Anthropometric measurements were performed in according to the International Standards for Anthropometric Assessments [22] on all the participants. Participants' height was measured using a wall-mounted Harpenden stadiometer and recorded to the nearest 0.1 centimeters (cm). The weight was measured using a wireless Tanita weight scale (BC1000, Tanita Corporation, Tokyo, Japan) connected to a computer. Personal details of the children including sex, age and height were entered into the computer and the body mass index (BMI) and body fat percentage for each participant were determined. BMI was calculated from weight and height as weight/height^2 (Kg/m^2) converted to percentiles for age, sex and height as underweight: <5th percentile, normal weight: ≥5th to < 85th percentile, overweight: ≥ 85th to < 95th percentile and obese: ≥95th percentile [23]. The waist circumference (WC), mid-upper arm circumference (MUAC), neck circumference (NC), ankle circumference (AC), calf circumference (CC) and thigh circumference (TC) were measured using an anthropometric tape in cm.

Blood Pressure Measurements

Blood pressure (BP) was measured using a sphygmomanometer (Omron M500, HEM-7321-D, Omron Corporation, Kyoto, Japan). After resting for 5 minutes, a paediatric cuff attached to the sphygmomanometer was fitted to bare left upper arm of children and they sat upright on a chair with their left arm on the table. Three BP readings which included the systolic blood pressure (SBP), diastolic blood pressure (DBP) and heart rate (HR) were taken at 2 minutes intervals; average BP was determined. The average of the second and third BP readings was determined and converted to BP percentiles for sex, age and height and classified according to the American Academy of Paediatrics (AAP) 2017 guideline as normotensive: SBP and DBP < 90th percentile; Elevated BP: SBP and/or DBP > 90th < 95th percentile or High BP: SBP and/or DBP ≥ 95th percentile [24]. Mean arterial pressure (MAP) was calculated from the formula: MAP = (SBP + (2 x DBP))/3

Endothelial Function Assessment

Endothelial function was assessed using a vicorder (SMT medical, Wuerzburg, Germany) which assessed changes in arterial stiffness by measuring pulse wave velocity (PWV). A standard 10 cm pressure cuff was placed on the upper right thigh as high as possible towards the crotch while a 7 cm pressure cuff was wrapped around the wrist of the same arm. The cuff was closed tight enough to assure a good coupling of the cuff to the femoral artery. The right common carotid artery pulse was palpated on the centre between the base of the neck and chin and a neck band with an attached neck pressure cuff was placed snugly around the neck with the cuff-bladder exactly over the palpated carotid artery pulse. Pressure lines were attached to the cuffs and the test conducted and the resulting PWV (m/s) was determined.

Sample Collection And Biochemical Analysis

Urine was collected from all participants in sterile tubes and was used to quantify the following biochemical parameters. Creatinine was quantified using the Roche Cobas 6000 analyser while albumin, asymmetric dimethylarginine (ADMA) and 8-Hydroxy-2’-deoxyguanosine (8-OHdG) were assayed using ELISA kits (Elabscience, USA) according to manufacturer’s protocol. Lipid peroxidation assay was performed based on the quantification of thiobarbituric acid reactive substances (TBARs) as described by Mallick and colleagues [25].

Statistical analysis

Data was analysed using STATA MP version 14.1. Results were presented as mean ± confidence interval (CI). Analysis of variance (ANOVA) test was used to compare the mean differences of study parameters based on location and sex. Spearman correlation was used to evaluate the relationship of cardiovascular risk factors with PWV and ADMA. Linear regression was used to determine the likelihood of cardiovascular risk factors to promote endothelial dysfunction. A p-values ≤ 0.05 was considered significant.

Results

General characteristics of study participants

Three hundred and six (306) children were recruited for the study which included 152 children from rural area and 154 from urban area. Among the 152 children in the rural area, there were 83 girls and 69 boys while among the 154 children in urban area there were 88 girls and 66 boys. Although data is presented for girls and boys, the statistical comparisons are between urban and rural. Age, height and weight were similar between urban and rural children. NC, MUAC, TC and CC were significantly ($p < 0.05$) higher in urban than rural children. The WC, BMI and AC were similar between urban and rural children. Rural children had significantly ($p < 0.001$) higher SBP and PWV than urban children while DBP, MAP and HR were significantly ($p < 0.001$) different between urban and rural children. Albumin, ACR, TBARS and ADMA were significantly ($p < 0.001$) higher in rural children than in urban children while, creatinine and 8-OHdG were significantly ($p < 0.001$) higher in urban children than in rural children (Table 1).

Table 1
General characteristics of participating children by sex and location

	Rural (95% CI)		Urban (95% CI)		*p*-value
	Girls	Boys	Girls	Boys	
N	83	69	88	66	
Age (years)	7.91(7.65–8.17)	7.88(7.56–8.19)	8.34(8.08–6.67)	8.11(7.72–8.49)	0.320
HT (cm)	124.81 (122.93–126.70)	126.87(124.41-129.32)	129.81(125.29-134.33)	127.86(125.01-130.11)	0.198
WT(kg)	25.44(24.40–26.40)	27.46(25.11–29.82)	28.61(26.60-30.61)	28.10(26.03–30.17)	0.137
BMI (m^2/kg)	16.4(15.8–16.9)	16.8(15.8–17.9)	17.2(16.4–18.0)	17.1(16.0-18.2)	0.276
NC(cm)	24.9(24.5–25.3)	26.2(25.6–26.7)	26.0(25.4–26.5)	26.6 (25. 9-23.4)	< 0.001
MUAC (cm)	19.3(18.8–19.8)	19.4 (18.5–20.2)	20.5(19.7–21.3)	20.3(19.4–21.2)	< 0.01
WC (cm)	58.8(57.4–60.3)	59.6(56.9–61.2)	58.5(56.5–60.5)	57.9(55.3–60.5)	0.184
TC (cm)	34.2(33.2–35.3)	36.1(34.4–37.7)	39.6(38.1–41.1)	35.9(33.5–38.3)	< 0.001
CC (cm)	24.4 (23.7–25.1)	25.5(24.5–26.5)	26.9(25.9–27.8)	26.6(25.4–27.7)	0.01
AC (cm)	18.3 (17.9–18.7)	19.1(18.4–19.8)	18.6(17.9–19.3)	18.9(18.1–19.9)	0.514
SBP (mmHg)	108.8(106.1-111.6)	107.9(104.0-111.9)	108.4(104.9-111.8)	107.7(104.7–110.0)	< 0.001
DBP (mmHg)	71.1(69.1–73.1)	68.4(65.9–71.0)	69.3(66.9–71.7)	69.5(66.2–72.9)	<0.001
HR (bpm)	89.8(86.3–93.3)	89.4(86.3–92.5)	93.2(90.4–96.0)	87.6(84.4–90.8)	<0.001
MAP (mmHg)	83.7(81.6–85.7)	82.18(79.87–84.50)	82.33(79.82–84.85)	82.54(79.54–85.54)	<0.001
PWV (m/s)	5.6(5.4–5.8)	8.9(2.3–15.6)	5.5(5.2–5.8)	8.8(2.5–15.1)	<0.001
Creatinine (mmo/L)	7.17(6.16–8.11)	8.65(6.52–10.78)	10.79(9.03–12.57)	8.46(6.53–10.37)	< 0.001

	Rural (95% CI)		Urban (95% CI)		*p*-value
ADMA (ng/ml)	72.08(68.8-75.42)	76.41(73.80–79.60)	75.12(72.56–77.68)	73.18(66.32–80.04)	< 0.001
Albumin (mg/L)	47.06(-7.48-101.71)	38.66(-9.75-87.07)	41.78(-9.09-92.65)	5.02(2.65–7.38)	< 0.001
ACR (mg/mmol)	6.16(-0.01-12.33)	3.40(-0.90-7.71)	4.17(-1.51-9.85)	0.58(0.38–0.77)	< 0.001
TBARS (µM)	0.08(0.07–0.08)	0.09(0.05–0.12)	0.08(0.06–0.09)	0.07(0.05–0.08)	< 0.001
8-OHdG (ng/ml)	61.64(57.40-65.88)	66.66(58.34–74.97)	64.92(60.24–69.59)	65.53(59.55–71.51)	< 0.001

Results are expressed as mean (min CI –max CI); CI: Confidence interval; N: Number of children; HT: Height; WT: Weight; BMI: Body Mass Index; NC: Neck circumference; MUAC: Mid upper arm circumference; WC: Waist circumference; TC: Thigh circumference; CC: Calf circumference; AC: Ankle circumference; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; HR: Heart rate; MAP: Mean arterial pressure; PWV: Pulse wave velocity; ADMA: Asymmetric dimethylarginine; ACR: Albumin to creatinine ratio; TBARS: Thiorbarbituric acid reactive substances; 8-OHdG: 8-hydroxyl-deoxy-guanosine.

Prevalence Of Overweight/obesity And Pre-hypertension/hypertension Among Participating Children

Children were separated by location and sex to determine the prevalence of overweight/obesity and pre-hypertension/hypertension as shown in Table 2. The prevalence of overweight /obesity was 19.3% and was higher in children from urban settings (10.8%) compared to those from rural settings (8.5%). Overweight/obesity was more prevalent in girls than in boys. The prevalence of pre-hypertension/hypertension was 42.2% and was high in rural children (23.2%) than in urban children (19.0%). The prevalence of pre-hypertension/hypertension in rural girls (14.7%) almost doubled that of rural boys (8.5%) while the prevalence of pre-hypertension/hypertension in urban settings was the same (9.5%) in both girls and boys.

Table 2
Prevalence of overweight/obesity and pre-hypertension/hypertension

	Cohort (%)		Rural (%)		Urban (%)	
	Rural	Urban	Girls	Boys	Girls	Boys
Overweight/Obesity	26(8.5)	33(10.8)	17(5.55)	9(2.9)	20(6.5)	13(4.2)
EBP/HBP	71(23.2)	58(19.0)	45(14.7)	26(8.5)	29(9.5)	29(9.5)
EBP: Elevated blood pressure; HBP: High blood pressure; %: Percentage indicates prevalence						

Effect of pulse wave velocity on cardiovascular risk factors

Children were classified by four inter-quartile ranges of their PWV in order to determine the effect of PWV on cardiovascular risk factors. SBP, MAP and DBP increased with increasing PWV and the differences were significant ($p < 0.05$) for MAP and DBP. However, HR did not show any specific pattern with the increasing quartiles of PWV. Similarly, BMI, ADMA, 8-OHdG, TBARS, creatinine, albumin and ACR were not significantly ($p > 0.05$) different among the PWV quartiles (Table 3).

Table 3
Effect of pulse wave velocity on cardiovascular risk factors

PWV quartiles	1st quartile	2nd quartile (95% CI)	3rd quartile (95% CI)	4th quartile (95% CI)	*p*-value
N	3	77	79	134	
BMI (m^2/Kg)	22.1	17.1 (16.4–17.3)	16.2 (15.7–16.8)	16.8 (16.2–17.3)	0.60
SBP (mmHg)	105.2	106.9 (104.4-109.4)	108.3 (105.3-111.3)	109.4 (106.4-112.4)	0.51
DBP (mmHg)	68.26	68.5 (66.4–70.6)	71.0 (68.8–73.2)	71.1 (69.8–72.5)	< 0.001
HR (bpm)	90.49	89.7 (85.5–92.9)	90.4 (86.6–94.1)	89.9 (85.6–94.1)	0.89
MAP (mmHg	74.4	82.5 (80.1–85.0)	81.3 (79.4–83.3)	84.3 (82.5–86.0)	0.06
ADMA (ng/ml)	62.3	64.6 (60.3–68.9)	72.7 (69.6–76.8)	70.7 (66.8–74.5)	0.23
8-OHdG (ng/ml)	68.65	62.9 (58.2–67.7)	75.1 (51.2–99.0)	68.71 (59.1–78.5)	0.844
TBARS (μM)	0.02	0.07(0.06–0.09)	0.07 (0.06–0.08)	0.07 (0.06–0.09)	0.461
Creatinine (mmol/L)	7.05	7.9 (6.7–9.2)	8.2 (6.9–9.5)	7.9 (6.9–9.1)	0.915
Albumin (mg/L)	1.5	29.3 (-7.1-65.7)	31.3 (1.2–61.4)	29.2 (-2.8-61.2)	0.32
ACR (mg/mmol)	0.3	3.4 (-0.7-7.5)	4.7 (-0.5-9.9)	2.9 (0.3–5.5)	0.318

Results are expressed as mean (min CI – max CI); CI: Confidence interval; N: Number of children; BMI: Body mass index; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; HR: Heart rate; MAP: Mean arterial pressure; PWV: Pulse wave velocity; ADMA: Asymmetric dimethyl arginine; ACR: Albumin to creatinine ratio; TBARS: Thiorbarbituric acid reactive substances; 8-OHdG: 8-hydroxyl-deoxy-guanosine. PWV quartiles (m/s): 1st quartile:<3.38; 2nd quartile: 4.1and 5.20; 3rd quartile: 5.3 and 5.7; 4th quartile:>6.10.

Effect Of Asymmetric Dimethylarginine On Cardiovascular Risk Factors

There are currently no cut off values for ADMA in children. In order to study the effect of ADMA on cardiovascular risk factors, children were classified into inter-quartile ranges of ADMA quartiles. Creatinine, albumin, ACR and 8-OHdG significantly ($p < 0.001$) increased with increasing quartiles of

ADMA. Though there was a trend of SBP, DBP, and HR to increase with increasing quartiles of ADMA, the differences were insignificant (p>0.05). Moreover, BMI, MAP and TBARS insignificantly (p > 0.05) did not show any specific pattern of distribution (Table 4).

Table 4
Effect of asymmetric dimethylarginine on risk factors of cardiovascular diseases

ADMA quartiles	1st quartile (95%CI)	2nd quartile (95%CI)	3rd quartile (95%CI)	4th quartile (95%CI)	*p*-value
N	47	48	47	48	
BMI (m^2/Kg)	17.5(16.2–18.8)	16.4(15.9–16.9)	17.1(15.9–18.3)	16.6(16.1–17.2)	0.503
SBP (mmHg)	100.5(100.3-100.8)	107.9(104.8–111.0)	108.0(104.7-111.2)	108.3(105.2-111.4)	0.98
DBP (mmHg)	68.1(65.9–70.2)	68.7(66.4–70.9)	69.8(67.5–72.1)	70.2(68.5–71.9)	0.27
HR (bpm)	89.8(85.3–94.4)	90.5(86.2–94.9)	90.8(86.0-95.5)	91.3(87.5–93.6)	0.97
MAP (mmHg	85.9(82.8–88.9)	82.8(79.7–85.9)	82.2(70.2–84.8)	82.0(80.2–83.9)	0.664
8-OHdG (ng/ml)	64.6(36.4–92.9)	65.2(55.3–75.0)	66.7(63.3–70.1)	77.9(65.6–90.1)	< 0.001
TBARS (µM)	0.07(0.06–0.08)	0.08(0.06–0.11)	0.07(0.06–0.07)	0.07(0.06–0.08)	0.820
Creatinine (mmol/L)	5.6(4.3–6.9)	9.1(7.1–11.1)	9.7(8.2–11.1)	7.3(6.5–8.2)	<0.001
Albumin (mg/L)	6.0(2.7–5.3)	22.0(8.9–35.1)	36.9(-7.1-80.9)	35.4(-0.7-71.5)	0.016
ACR(mg/mmol)	1.2(0.5–1.9)	2.1(1.3–2.9)	3.8(-1.2-8.7)	4.6(0.6–8.6)	0.022

Results are expressed as mean (min CI –max CI); CI: Confidence interval; N: Number of children; BMI: Body mass index; SBP: Systolic blood pressure; DBP = Diastolic blood pressure; HR: Heart rate; MAP: Mean arterial pressure; ADMA: Asymmetric dimethylarginine; ACR: Albumin to creatinine ratio; TBARS: Thiorbarbituric acid reactive substances; 8-OHdG: 8-hydroxyl-deoxy-guanosine. ADMA quartiles (ng/ml): 1st quartile: <68.00; 2nd quartile: 68.4 and 75.1; 3rd quartile: 75.2 and 79.58; 4th quartile: >79.06.

Relationship Between Endothelial Function Markers And Cardiovascular Risk Factors

Study participants were separated by sex and location to assess their relationship between endothelial function markers and cardiovascular risk factors. MAP positively correlated (p < 0.05) with ADMA and PWV in urban and rural girls. A positive relationship (p < 0.05) was observed between DBP and PWV in urban girls while rural girls showed a positive relationship (p < 0.05) between HR and ADMA. BMI

positively correlated ($p < 0.001$) with PWV in rural and urban males. Creatinine positively correlated ($p < 0.05$) with ADMA in urban children and in rural girls. 8-OHdG positively correlated ($p < 0.05$) with ADMA in both urban and rural children while ACR showed a negative relationship with ADMA for rural boys and urban girls (Table 5).

Table 5
Correlation between endothelial function markers and cardiovascular risk factors

Correlation coefficient	**ADMA**				**PWV**			
Location	**Rural**		**Urban**		**Rural**		**Urban**	
Sex	**Girls**	**Boys**	**Girls**	**Boys**	**Girls**	**Boys**	**Girls**	**Boys**
BMI	-0.09	-0.01	-0.05	-0.05	0.10	0.2***	0.01	0.767***
DBP (mmHg)	0.1	0.1	0.2	0.2	0.1	0.1	0.4*	0.2
SBP (mmHg)	0.1	-0.2	0.1	0.2	0.1	-0.02	0. 0	-0.04
HR (bpm)	0.3*	0.02	0.1	-0.3	-0.1	-0.2	0.1	0.1
MAP	0.09	0.05	0.33*	-0.24	0.26*	0.01	0.18	0.08
Creatinine (mmol/L)	0.5*	0.1	0.4*	0.4*	-0.2	0.1	0.1	0.01
Albumin (mg/L)	0.1	-0.23	0.01	0.3	-0.1	0.2	0.1	-0.02
ACR (mg/mmol)	0.03	-0.4*	-0.3*	0.1	-0.03	0.2	-0.01	0.2
8-OHdG (ng/ml)	0.5*	0.5*	0.4*	0.7*	0.1	-0.2	0.08	0.01
TBARS (μM)	0.1	0.04	-0.1	-0.1	-0.2	0.1	-0.1	0.3

BMI: Body mass index; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; HR: Heart rate; MAP: Mean arterial pressure; BMI: Body mass index; PWV: Pulse wave velocity; ADMA: Asymmetric dimethylarginine; ACR: Albumin to creatinine ratio; TBARS: Thiorbarbituric acid reactive substance; 8-OHdG: 8-hydroxyl-deoxy-guanosine. ∗indicates significant relationship ($p < 0.05$).

Predictors Of Endothelial Dysfunctions

Age adjusted linear regression of a fitted model ($F_{females}$=2.204; F_{males}=3.15; $p = 0.05$) for the relationship of ADMA with cardiovascular risk factors showed increased 8-OHdG to predict increased ADMA in rural females ($R^2 = 0.4$, Adj.$R^2 = 0.219$; $p < 0.01$) and rural males ($R^2 = 0.623$, Adj.$R^2 = 0.425$; $p < 0.001$). In urban females, an unfitted model ($F = 2.128$; $p = 0.101$) showed increased 8-OHdG to predict increased ADMA ($R^2 = 0.621$, Adj.$R^2 = 0.329$; $p < 0.05$) while in urban males a fitted model ($F = 3.119$; $p = 0.036$) showed creatinine, albumin, ACR and 8-OHdG to predict increased ADMA ($R^2 = 0.757$, Adj.$R^2 = 0.514$; $p < 0.05$). Age adjusted linear regression of an unfitted model in rural females ($F = 1.201$; $p = 0.316$) for the relationship of PWV with cardiovascular risk factors showed increased creatinine to predict increased PWV (R^2 =

0.207, Adj.R^2 = 0.035; p = 0.018) while a fitted model in rural males (F = 2.603 p = 0.026) showed increased BMI to predict increased PWV (R^2 = 0.555, Adj.R^2 = 0.341; p < 0.001). Also, a fitted model for urban boys (F = 4.326; p = 0.005) showed increased BMI to predict increased PWV (R^2 = 0.76, Adj.R^2 = 0.585; p < 0.001).

Table 5
Regression analysis on the relationship between cardiovascular risk factors and endothelial function markers

Regression coefficient	**ADMA**				**PWV**			
Location	**Rural**		**Urban**		**Rural**		**Urban**	
Sex	**Girls**	**Boys**	**Girls**	**Boys**	**Girls**	**Boys**	**Girls**	**Boys**
BMI	0.02	0.134	-0.66*	-0.02	-0.17	0.75***	0.38	0.99***
DBP (mmHg)	-0.04	0.05	0.15	.037	0.03	0.34	0.25	0.30
SBP (mmHg)	-0.01	-0.30	0.29	-0.27	-0.05	-0.09	-0.27	-0.29
HR (bpm)	0.24	-0.05	-0.04	0.04	0.05	-0.28	0.27	0.14
MAP	-0.19	.022	-0.25	-0.06	0.22	0.22	-0.22	-0.04
Creatinine (mmol/L)	0.13	-0.09	0.78	1.00*	-0.37*	-0.17	0.01	-0.32
Albumin (mg/L)	-0.23	1.41	-0.53	-1.13*	0.32	0.38	-1.71	-0.12
ACR (mg/mmol)	0.42	-1.54	0.71	1.00*	-0.26	-0.42	1.62	0.19
8-OHdG (ng/ml)	0.49**	0.74***	0.69*	0.67*	0.16	-0.09	-0.21	0.17
TBARS (μM)	-0.20	0.01	-0.74	0.16	-0.13	0.29	0.32	-0.22

BMI: Body mass index; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; HR: Heart rate; MAP: Mean arterial pressure; BMI: Body mass index; PWV: Pulse wave velocity; ADMA: Asymmetric dimethylarginine; ACR: Albumin to creatinine ratio; TBARS: Thiorbarbituric acid reactive substance; 8-OHdG: 8-hydroxyl-deoxy-guanosine; ∗indicates p < 0.05; ∗∗indicates p < 0.01; ∗∗∗indicates p < 0.001

Discussion

Endothelial dysfunction has been established to be an initiator as well as an important marker to the progression of atherosclerosis and other CVDs [4]. Endothelial dysfunction has been shown to be associated with known cardiovascular risk factors which include obesity, hypertension, oxidative stress, dyslipidaemia and microalbuminuria [13]. There is evidence of increase prevalence of cardiovascular risk factors, especially obesity and hypertension in children [17, 20, 21]. Childhood obesity is increasing in South Africa. A study conducted among children aged 7–10 years old in Port Elizabeth, South Africa

showed 20.9% and 9.8% prevalence of overweight and obesity respectively [26]. In this present study, the prevalence of overweight/obesity was 19.3%. The prevalence by sex revealed that girls had a higher prevalence of overweight/obesity compared to boys. Possible reasons for the overall higher prevalence of overweight/obesity by sex could be due to lower physical activity in girls as well as biological and socio-cultural differences [27, 28]. More so, children from urban settings had a higher prevalence of overweight/obesity (10.8%) than their rural counterparts (8.5%). In addition, urban children showed higher anthropometric measures compared to rural children. This is in agreement with a study conducted by Monyeki and colleagues [29] who reported that the prevalence of obesity among South African children was higher in urban (6.1%) than in rural (3.7%) children aged 0–18 years old. The high prevalence of obesity in urban children could be as a result of the availability of inexpensive processed and lower nutrient foods in urban areas as it has been reported by McKersie and Baard [26] to be linked with the incidence of childhood obesity. More so, with the advancement of television, most children in urban areas spend a greater part of their time indoors [30].

High blood pressure, a condition associated with obesity is becoming more prevalent in children [31, 32]. Our findings showed 42.3% prevalence of elevated/high blood pressure in children. The elevated blood pressure observed among school-going children in this study is not a new phenomenon in Africa though the prevalence of elevated/high blood pressure in the present study is higher compared to other studies. A prevalence of 9.8% for high blood pressure was shown in a study conducted in Gambia in children aged 5–9 years old with females presenting higher prevalence [32]. Another study conducted in urban and rural settings of Der es Salaam in Tanzania showed a 15.2% prevalence of elevated/high blood pressure [31]. This present study showed high prevalence of elevated/high blood pressure in children from rural (23.2%) areas as compared to their urban counterparts (19.0%). More so, blood pressure measures of children from rural areas were significantly ($p < 0.05$) higher than that of urban areas.

Though there is evidence of increased prevalence of cardiovascular risk factors in children, limited data is available on their relationship with endothelial dysfunction. Endothelial dysfunction is a major early marker of CVDs as it has been shown to be involved in the pathogenesis of atherosclerotic vascular disease [33]. ADMA is a molecule that inhibits eNOS from synthesis NO from L-arginine and has been shown to be associated with endothelial dysfunction in healthy individuals [33]. Reduced availability of L-arginine and increased ADMA contribute to reduced production of NO [34]. Decrease bioavailability of NO leads to endothelial dysfunction, a condition which impairs vasodilation [35]. Impairment of vasodilation results in the constriction of blood vessels and eventually leads to hypertension. It has been reported that endothelial dysfunction is associated with a sustained increase in blood pressure [36]. Uncontrolled hypertension results in subclinical structural changes in the cardiovascular system measured as cardiac wall thickening, carotid intima-media thickness and arterial stiffness characterised by increased PWV [37]. In this study, blood pressure measures increased with increasing quartiles of PWV and ADMA. Also, DBP positively associated with PWV in urban girls. More so, ADMA positively associated with HR in rural girls. This finding concords with a study conducted by Kulsum-Mecci and colleagues [38] which showed that PWV was significantly higher in hypertensive children aged 4–18 years old. It has been reported that high DBP signifies a risk of CVD as pulse wave is reflected during diastole causing the heart to work

harder [39]. Also, a baseline survey which was conducted in 6–15 years old children in 1987 in Hanzhong city who were followed up for 26 years showed that children with high blood pressure had a significant higher incidence of hypertension and brachial-ankle PWV in their adulthood than the normotensive children [40]. These reports suggest that hypertension may result to endothelial dysfunction in children.

Obesity is another factor that has been suggested to be linked with endothelial dysfunction. In obesity, there is adipocytes hypertrophy which leads to excess production of pro-inflammatory markers such as chemokines, adipokines and cytokines [41]. These inflammatory mediators including interleukin 6 (IL-6) and tumour necrosis factor-a (TNF-α) secreted from adipocytes are responsible for decreasing the production and secretion of adiponectin [42], a molecule that increases the production of NO and promotes endothelial function [43]. Therefore, obesity may impair endothelial function. Findings from this study showed a trend of BMI to increase with increasing PWV and BMI was shown to be a predictor of endothelial dysfunction in male children. Previous studies have shown obesity to induce endothelial dysfunction [44].

Oxidative stress is another factor that may be related to endothelial dysfunction. Oxidative stress is as a result of excessive levels of ROS that overwhelms the antioxidant system. These excessive ROS such as superoxide can react with NO to form peroxynitrite ($ONOO^-$) [45], thereby reducing the available NO level for proper endothelial function. Thus, high ROS generation may lead to different abnormalities including endothelial dysfunction [46, 47]. Findings from this study showed 8-OHdG, a marker for oxidative stress to significantly increase with increasing quartiles of ADMA and also positively correlated with ADMA. More so, 8-OHdG was shown to predict endothelial dysfunction in rural and urban children. Although not in children, previous studies have shown oxidative stress to be associated with endothelial dysfunction [48, 49, 50].

Microalbuminuria is known to be an independent risk factor of CVDs [52]. Microalbuminuria which can reliably be defined by elevated ACR [51] has shown to be associated with endothelial dysfunction [14]. Thus, there is possibility of endothelial dysfunction to be related to microalbuminuria in Children. Creatinine, Albumin and ACR significantly increased with increasing ADMA concentration and ADMA positively associated with creatinine. More so, increased ACR as well as creatinine showed risk to predict increased ADMA and PWV. These findings suggest a possible association of microalbuminuria with endothelial dysfunction in children. Urine ACR has been shown to be an early marker for endothelial dysfunction in adolescents independent of glycaemia [52]. Also, microalbuminuria has been shown to positively associate with endothelial dysfunction in HIV-infected patients regardless of known confounders [51]. This study has identified possible cardiovascular risk factors of endothelial dysfunction in children and has provided initial information for further studies. However, these findings may be limiting since it was a cross-sectional study and therefore causal relationship between these risk factors and endothelial dysfunction may not fully be established.

Conclusion

Endothelial dysfunction was associated with obesity, high blood pressure, oxidative stress and microalbuminuria in children and this relationship varied between rural and urban children. Overweight/obesity was more prevalent in urban children while elevated/high blood pressure was more prevalent in rural children. Our findings suggest children in rural and urban areas of the Eastern Cape Province of South African may be at risk of developing CVDs. Thus, there is need for intervention strategies specific for urban and rural primary school children to be instituted to prevent future development of cardiovascular complications.

Abbreviations

CVD, Cardiovascular disease; BP, Blood pressure; PWV, Pulse wave velocity; ADMA, Asymmetric dimethylarginine; 8-OHdG, 8-hydroxy-2deoxyguanosine; TBARS, Thiobarbituric acid reactive substance; ACR, Albumin to creatinine ratio; NO, nitric oxide; eNOS, endothelial nitric oxide synthase; ROS; Reactive oxygen species; TNF-α; Tumour necrosis factor alpha; IL-6, interleukin-6; $O_{2,}^{\bullet}$ Superoxide; $ONOO^{\bullet}$, peroxynitrite; $NOO^{\bullet}$, peronitrite; EDRFs; endothelium-derived relaxing factors; BMI, Body mass index; WC, Waist circumference; MUAC, Mid-upper arm circumference; NC, Neck circumference; AC, Ankle circumference; CC, Calf circumference; TC, Thigh circumference; MAP, Mean arterial pressure; BP, Blood pressure; SBP, Systolic blood pressure; DBP, Diastolic blood pressure; HR, Heart rate; CI, Confidence interval; ANOVA, Analysis of variance; EBP, Elevated blood pressure; HBP: High blood pressure.

Declarations

Data Sharing Statement

We do not wish to share the data included in this manuscript as the participants data are kept confidential in accordance to the South African National Data Protection guidelines of reporting.

Ethical Approval and Informed Consent

Ethical approval was obtained from Walter Sisulu University Health Sciences Ethics Committee with approval number: 112/2018. Written informed consent was obtained from the parents/legal guardians of the children before enrolment into the study.

Acknowledgments

The authors are grateful to the management of the primary schools of the Eastern Cape Province, South Africa for granting permission for the study to be conducted in their schools. We also want to thank the nurses who assisted in the collection of samples from the school children.

Author contributions

BNN and NG designed the study. ENM, GAE and SNCA oversaw data collection. ENM, GAE and BNN analysed the data. BNN, snca and NG interpreted the findings. BNN and GAE wrote the paper. All authors

reviewed the manuscript and approved the final version for submission.

Funding

This study was funded by the South African National Research Foundation NRF-CPRR, Grant No: 106066 to Prof. Benedicta N. Nkeh-Chungag.

Conflict of Interest

The authors declare no conflict of interest.

References

1. Deanfield JE, Halcox JP, Rabelink TJ: Endothelial function and dysfunction: testing and clinical relevance. 2007; 115:1285–1295.
2. De Mey JGR and Vanhoutte PM. End o' the line revised: moving on from nitric oxide to CGRP. Life Sciences. 2014; 1: 1–10.
3. Cauwenberghs N, Heyrman Y, Thijs L, Yang W, Wei F, Zhang Z, Staessen JA and Kuznetsova T. Flow-mediated slowing of brachial-radial pulse wave velocity: Methodological aspects and clinical determinants. Artery Research. 2018; 21: 29–37.
4. Park K-H, Park WJ. Endothelial Dysfunction: Clinical Implications in Cardiovascular Disease and Therapeutic Approaches. J Korean Med Sci. 2015; 30: 1213-1225. http://dx.doi.org/10.3346/jkms.
5. World Health Organization, 2013. Global action plan for the prevention and control of non-communicable diseases. World Health Organisation. Geneva, Switzerland.
6. Coelho M , Oliveira T, and Fernandes Biochemistry of adipose tissue: an endocrine organ. Arch Med Sci. 2013; 20: 9(2): 191–200.
7. Durante W, Johnson FK, Johnson RA, Arginase: a critical regulator of nitric oxide synthesis and vascular function. Clin Exp Pharmacol Physiol. 2007; 34(9); 906-911.
8. Ohashi K, Ouchi N, Matsuzawa Y. Adiponectin and hypertension. Am J hypertension. 2011; 24(3): 263-269.
9. Willeit P, Freitag DF, Laukkanen JA, Chowdhury S, Gobin R, Mayr M, Di Angelantonio E, Chowdhury R. Asymmetric dimethylarginine and cardiovascular risk: systemic review and meta-analysis of 22 prospective studies. J Am Heart Assoc. 2015; 4(6): p.e001833.
10. Hwang HM, Lee JH, Min BS, Jeon BH, Hoe KL, Kim YM, Ryoo S. A novel arginase inhibitor derived from scutellavia indica restored endothelial function in ApoE-null mice fed a high-cholesterol diet. J Pharmacol Exp Therapeutics. 2015; 355(1):57-65.
11. Hartigh LJD, Omer M, Goodspeed L, Wang S, Wietecha T, O'Brien KD, Han YC. Adipocyte-Specific Deficiency of NADPH Oxidase 4 Delays the Onset of Insulin Resistance and Attenuates Adipose Tissue Inflammation in Obesity. Arterioscler Thromb Vasc Biol. 2017; 37: 466-475. DOI: 10.1161/ATVBAHA.116.308749.

12. Jacobson JC, Hornbech MS, Holstein-Rathlou NH. Significance of microvascular remodeling for the vascular flow reserve in hypertension. Interface Focus. 2011; 1: 117-131.
13. Barthelmes J, Nägele MP, Ludovici V, Ruschitzka F, Sudano I, Flammer AJ. Endothelial dysfunction in cardiovascular disease and Flammer syndrome—similarities and differences. European Association for Predictive, Preventive and Personalised Medicine (EPMA) Journal. 2017; 8:99–109. DOI 10.1007/s13167-017-0099-1
14. Pirro M, Mannarino MR, Francisci D, Schiaroli E, Bianconi V, Bagaglia F, Sahebkar A, Mannarino E and Baldelli F. Urinary albumin-to-creatinine ratio is associated with endothelial dysfunction in HIV-infected patients receiving antiretroviral therapy. Sci Rep. 2016; 6: 28741; doi: 10.1038/srep28741.
15. Burgert TS, Dziura J, Yeckel C, Taksali SE, Weiss R, Tamborlane W, Caprio S. Microalbuminuria in pediatric obesity: prevalence and relation to other cardiovascular risk factors. Int J Obesity. 2006; 30(2): 273-280.
16. Kagura J, Adair LS, Musa MG, Pettifor JM, Norris SA. Blood pressure tracking in urban black South African children: birth to twenty cohort. BMC Pediatrics. 2015; 15(1): 78. DOI 10.1186/s12887-015-0402-z
17. Pienaar AE, Prevalence of overweight and obesity among primary school children in a developing country: NW-CHILD longitudinal data of 6–9-yr-old children in South Africa. BMC Obesity. 2015. 2(1): 2. DOI 10.1186/s40608-014-0030-4
18. Halcox JPJ. Deanfield JE. Childhood origins of endothelial dysfunction. Heart. 2005; 91(10): 1272-1274
19. Sarganas G, Schaffrath RA, Niessner C, Woll A, Neuhauser HK. Tracking of blood pressure in children and adolescents in germany in the context of risk factors for hypertension. Int J Hypertension. 2018; 2018:1-8. 8429891. https://doi.org/10.1155/2018/8429891
20. Steinthorsdottir SD, Eliasdottir SB, Indridason OS, Agustsdottir IM, Palsson R, Edvardsson VO. Prevalence of hypertension in 9 -to 10 year-old Icelandic school children. J Clin Hypertension. 2011; 13(10): 774-779.
21. Guilherme FR, Molena-Fernandes CA, Guilherme VR, Fávero MTM, Reis EJBD, Rinaldi W. Physical inactivity and anthropometric measures in schoolchildren from Paranavaí, Paraná, Brazil. Revista Paulista de Pediatria. 2015; 33(1): 50-55.
22. Stewart A, Marfell-Jones M, Olds T and Ridder H. International Standards for Anthropometric Assessment. ISAK, Lower Hutt. 2011.
23. Centers for Disease Control and Prevention, A SAS Program for the 2000 CDC Growth Charts (ages 0 to <20 years). Atlanta, GA: Division of Nutrition, Physical Activity, and Obesity, National Center for Chronic Disease Prevention and Health Promotion, Centers for Disease Control and Prevention; 2014.
24. Flynn JT, Kaelber DC, Baker-Smith CM. Clinical Practice Guideline for Screening and Management of High Blood Pressure in Children and Adolescents. Pediatrics. **2017**; 140: e20171904.
25. Mallick C, Mandal S, Barik B, Bhattachary A, Ghosh D. Protection of Testicular Dysfunctions by MTEC, a Formulated Herbal Drug, in Streptozotocin Induced Diabetic Rat. Pharm. Bull. 2007; 30(1) 84

—90.

26. McKersie J. Baard ML. Obesity in 7-10-year-old children in urban primary schools in Port Elizabeth. South African J Sports Med. 2014; 26(2): 55-58.
27. Ortlieb S, Schneider G, Koletzko S, Berdel D, von Berg A, Bauer CP, Schaaf B, Herbarth O, Lehmann I, Hoffmann B. Heinrich J. Physical activity and its correlates in children: a cross-sectional study (the GINIplus & LISAplus studies). BMC Public Health. 2013; 13(1): 349. http://www.biomedcentral.com/1471-2458/13/349
28. Sweeting HN. Gendered dimensions of obesity in childhood and adolescence. Nutrition J. 2008; 7(1):1.
29. Monyeki MA, Awotidebe A, Strydom GL, De Ridder JH, Mamabolo RL. Kemper HC. The challenges of underweight and overweight in South African children: are we winning or losing the battle? A systematic review. Int J Env Res Public Health. 2015; 12(2): 1156-1173.
30. Rosiek A, Maciejewska NF, Leksowski K, Rosiek-Kryszewska A. Leksowski Ł. Effect of television on obesity and excess of weight and consequences of health. Int J Env Res Public Health. 2015; 12(8): 9408-9426.
31. Muhihi AJ, Njelekela MA, Mpembeni RN, Muhihi BG, Anaeli A, Chillo O, Kubhoja S, Lujani B, Maghembe M. Ngarashi D, Elevated blood pressure among primary school children in Dar es salaam, Tanzania: prevalence and risk factors. BMC Pediatrics. 2018; 18(1): https://doi.org/10.1186/s12887-018-1052-8
32. Jobe M, Agbla SC, Prentice AM. Hennig BJ. High blood pressure and associated risk factors as indicator of preclinical hypertension in rural West Africa: A focus on children and adolescents in The Gambia. Medicine 2017; 96(13).
33. Antoniades C, Demosthenous M, Tousoulis D, Antonopoulos AS, Vlachopoulos C, Toutouza, M, Marinou K, Bakogiannis C, Mavragani K, Lazaros G. Koumallos N. Role of asymmetrical dimethylarginine in inflammation-induced endothelial dysfunction in human atherosclerosis. Hypertension. 2011; 58(1): 93-98.
34. Groner JA, Joshi M. Bauer JA. Pediatric precursors of adult cardiovascular disease: noninvasive assessment of early vascular changes in children and adolescents. Pediatrics. 2006; 118(4): 1683-1691.
35. Weil BR, Stauffer BL, Greiner JJ. DeSouza CA.. Prehypertension is associated with impaired nitric oxide-mediated endothelium dependent vasodilation in sedentary adults. Am J Hypertension. 2011; 24(9): 976-981.
36. Lobato NS, Filgueira FP, Akamine EH, Tostes RC, Carvalho MHCD. Fortes ZB. Mechanisms of endothelial dysfunction in obesity-associated hypertension. Brazilian J Med Biol Res. 2012: 45(5); 392-400.
37. Vasilevska-Ristovska J, Hudes SZ, Naguleswaran K, Langlois V, Matsuda-Abedini M. Parekh RS. Pediatric Hypertension: Impact on the Heart, Brain, Kidney, and Retina. Curr Cardiovascular Risk Rep.2018; 12(5): 14. https://doi.org/10.1007/s12170-018-0577-6

38. Kulsum-Mecci N, Goss C, Kozel BA, Garbutt JM, Schechtman KB. Dharnidharka VR. Effects of obesity and hypertension on pulse wave velocity in Children. J Clin Hypertension. 2017; 19(3): 221-226.
39. Craig A, Mels CMC, Kruger R. Thiobarbituric acid reactive substances relate to arterial stiffness and blood pressure in 6 to 8- year-old boys stratified by maternal risk. Free Radical Research; 2018; 52 (2): 180-187. DOI: 10.1080/10715762.2017.1421314
40. Mu J, Chu C. Wang M. PP. 38.13: The Association between Childhood Risk Factors and Arterial Stiffness in Young Adults. A 26-Year Follow-Up Study. J Hypertension. 2015; 33: e481. doi: 10.1097/01.hjh.0000468901.34347.8e
41. Hosogai N, Fukuhara A, Oshima K, Miyata Y, Tanaka S, Segawa K, Furukawa S, Tochino Y, Komuro R, Matsuda M. and Shimomura I, Adipose tissue hypoxia in obesity and its impact on adipocytokine Diabetes. 2007; 56(4): 901-911.
42. Matsuzawa Y. The metabolic syndrome and adipocytokines. FEBS Letters. 2006; 580(12): 2917-2921.
43. Szmitko PE, Teoh H, Stewart DJ. Verma S. Adiponectin and cardiovascular disease: state of the art? Am J Physiology-Heart Circulatory Physiol. 2007; 292(4): H1655-H1663.
44. Iantorno M, Campia U, Di Daniele N, Nisticò S, Forleo GB, Cardillo C and Tesauro M. Obesity, Inflammation and Endothelial Dysfunction. J Biol Regulators & Homeostatic Agents. 2014; 28 (2): 169-176.
45. Bae YS, Lee JH, Choi SH, Kim S, Almazan F, Witztum J.L. Miller YI. Macrophages generate reactive oxygen species in response to minimally oxidized low-density lipoprotein: toll-like receptor 4–and spleen tyrosine kinase– dependent activation of NADPH oxidase 2. Circulation Res. 2009; 104(2): 210-218.
46. Tan HY, Wang N, Li S, Hong M, Wang X. Feng Y. The reactive oxygen species in macrophage polarization: reflecting its dual role in progression and treatment of human diseases. Oxidative Medicine and Cellular Longevity, 2016; 2016: 1-16. 2795090. http://dx.doi.org/10.1155/2016/2795090
47. Leucker TM. Jones SP. Endothelial dysfunction as a nexus for endothelial cell-cardiomyocyte miscommunication. Frontiers 2014; 5: 328.
48. Jurado-Gamez B, Fernandez-Marin MC, Go´mez-Chaparro JL, Munoz-Cabrera L, Lopez-Barea J, Perez-Jimenez F and Lopez-Miranda J. Relationship of oxidative stress and endothelial dysfunction in sleep apnoea. Eur Respir J. 2011; 37: 873–879. DOI: 10.1183/09031936.00027910
49. Wallace JP, Johnson B, Padilla J, Mather K. Postprandial lipaemia, oxidative stress and endothelial function: a review. Int J Clin Pract. 2010; 64 (3): 389–403 doi: 10.1111/j.1742-1241.2009.02146.x
50. Neri S, Signorelli S, Pulvirenti D, Mauceri B, Cilio D, Bordonaro F, Abate G, Interlandi D, Misseri M, Ignaccolo L, Savastano M, Azzolina R, Grillo C, Messina A, Serra A, Tsami A. Oxidative stress, nitric oxide, endothelial dysfunction and tinnitus, Free Radical Res. 2006; 40 (6): 615-618, DOI: 1080/10715760600623825

51. Jotwani V, Katz R, Ix JH, Gutiérrez OM, Bennett M, Parikh CR, Cummings SR, Sarnak MJ, Shlipak Urinary Biomarkers of Kidney Tubular Damage and Risk of Cardiovascular Disease and Mortality in Elders. Am J Kidney Dis. 2018; 72 (2): 205-213.
52. Bartz K S, Caldas MC, Tomsa A, Krishnamurthy R, Bacha F. Urine Albumin-to-Creatinine Ratio: A Marker of Early Endothelial Dysfunction in Youth. J Clin Endocrinol Metab. 2015; 100: 3393–3399.