academicJournals

CONFERENCE PROCEEDINGS ■
Scientific Research and Essays

# Cardiovascular and Exercise Physiology Session

## NRF-1 overexpression increases GLUT4 expression and MEF2A/MEF2D dimerization in C2C12 myotubes

**D. B. GUMEDE AND E. O. OJUKA**

MRC/UCT Research Unit for Exercise Science and Sports medicine, University of Cape Town, South Africa.

20 December 2011

Previous studies have reported that over-expression of nuclear respiratory factor (NRF)-1 increase glucose transporter-4 (GLUT4) and myocyte enhancer factor (MEF)-2A protein contents in skeletal muscle but the mechanisms involved have not been investigated. MEF2A is known to bind to the *glut4* gene as a MEF2A/MEF2D heterodimer for transcriptional regulation. NRF-1 has a binding site on the *mef2a* but not the *glut4* promoter. The aims of this study were to determine whether NRF-1 over-expression (a) enhanced GLUT4 expression indirectly via MEF2A and (b) alters MEF2A/D dimer formation in C2C12 myotubes. The Tet-on gene expression system was used to over-express NRF-1 in C2C12 myotubes (C2C12-Tet-On-NRF-1) after treatment with 2 µg/ml of doxycycline (Dox) for 72 h. Control myotubes were treated with vehicle. To assess if the effects of NRF-1 were mediated via MEF2A, some C2C12-Tet-On-NRF-1 myotubes were transfected with 80 pmol MEF2A siRNA to inhibit MEF2A transcription. NRF-1, GLUT4, MEF2A, MEF2D, δALAS and α-tubulin protein levels were assessed using immunoblotting. Chromatin immunoprecipitation (ChIP) assays were used to measure NRF-1 and MEF2A binding onto *mef2a* and *glut4* genes respectively. Co-IP assays using MEF2A antibody were carried out to assess MEF2A/2D dimer formation following NRF-1 over-expression. Myotubes treated with 2 µg/ml of Dox showed ~1.5 fold increase in NRF-1 expression compared to controls. Dox treated myotubes showed a 50% increase in GLUT4, MEF2A and δALAS but not MEF2D or α-tubulin. However, C2C12-Tet-On-NRF-1 myotubes transfected with MEF2A siRNA and treated with 2 µg/ml Dox resulted in 50% decrease in MEF2A and GLUT4 expression. ChIP assays indicated increased binding of NRF-1 to *mef2a* as well as MEF2A to *glut4* (50%). Co-IP assays indicated increased MEF2A/2D dimerization by 60% in Dox treated myotubes compared to controls. The study shows that NRF-1 over-expression increases GLUT4 content via MEF2A; and it enhances MEF2A/2D dimerization required for GLUT4 transcription in C2C12 myotubes.

**Key words:** Myotubes, myocyte enhancer factor, GLUT 4.

## HDL Cholesterol, and its sphingolipid Sphingosine-1-Phosphate, are cardioprotective via their actions on the mitochondrial permeability transition pore

**D. Hacking[1], M. Frias[1], D. Hausenloy[3], L. Opie[1], R. James[2] and S. Lecour[1]**

[1]Hatter Cardiovascular Research Institute, University of Cape Town, South Africa.
[2]Lipidology Laboratory, University of Geneva, Switzerland.

academicJournals

CONFERENCE PROCEEDINGS ■
Scientific Research and Essays

[3]Hatter Institute, University College London, UK.

20 December 2011

High density lipoprotein cholesterol (HDL) and its component sphingosine-1-phosphate (S1P) protect against myocardial infarction. Recently, the SAFE (survivor activating factor enhancement) pathway, involving the activation of the immune system and the transcription factor STAT3, has been identified as a key signaling pathway in this cardio-protective effect; however the end effector remains to be elucidated. Inhibition of opening of the mitochondrial Permeability Transition Pore (mPTP) is a crucial component of many cardio-protective pathways. Therefore, we hypothesized that HDL/S1P protect via the inhibition of the mPTP opening after activation of the SAFE pathway. Isolated adult mouse cardiomyocytes (wildtype or STAT3 deficient mice (KO)) were exposed to 2 h of Simulated Ischemia(SI) with/without pre-treatment with either HDL (300 mg/ml) or S1P (10 nM). Cell viability was assessed using trypan blue staining, and mPTP opening was measured using TMRM fluorescence and normalised to the normoxic control. Cell viability in the normoxic control group was 84.3±1.5%. Hypoxia reduced viability to 61.0±2.5% ($p< 0.05$ vs control). Cell viability was restored with S1P (80.4±1.4%) or HDL (76.8±1.8%) ($p<0.05$ vs. hypoxia for both). TMRM fluorescence in the hypoxic group was 77±6% arbitrary units (AU) ($p<0.05$ vs. control), and restored to 95±4% AU in the S1P group and 88.4±11% AU in the HDL group ($p<0.05$ vs. hypoxia for both). In the STAT-3 KO mice, no cell viability improvement or restoration of the TMRM fluorescence was observed with either HDL or S1P. Both HDL and S1P protect against SI and our data strongly suggest that this effect is mediated via the activation of STAT3 and the inhibition of mPTP opening.

**Key words:** Mitochondrial permeability transition pore, simulated ischaemia, STAT3.

# Exploring novel therapeutic targets to blunt hexosamine biosynthetic pathway-induced myocardial insulin resistance

**D. Joseph and M. F. Essop**

Cardio-Metabolic Research Group (CMRG), Department of Physiological Sciences, Stellenbosch University, South Africa.

20 December 2011

Hyperglycemia-mediated oxidative stress may increase flux through the hexosamine biosynthetic pathway (HBP) resulting in greater *O*-GlcNAc (HBP end-product) modification of target proteins. Although excess HBP flux is associated with the onset of insulin resistance, underlying mechanisms driving this process in the heart/muscle remain unclear. Here we hypothesized that hyperglycemia-mediated elevation of HBP flux increases *O*-GlcNAcylation of regulators of the insulin signaling pathway (PKB/Akt, AS160), thereby impairing its function and leading to decreased myocardial GLUT4 translocation. We further proposed that HBP inhibition and/or antioxidant treatment would blunt these detrimental effects, thus increasing myocardial insulin sensitivity. Rat cardiac-derived H9c2 myoblasts were cultured in 25 mM glucose (hyperglycemia) for 24 h versus controls (5 mM glucose) ± transfection with a HA-GLUT4-GFP construct (test for GLUT4 translocation) ± 40 µM DON (pharmacologic HBP inhibitor), or 250 µM 4-OHCA (antioxidant). Our data showed decreased insulin-stimulated GLUT4 translocation to the sarcolemma under hyperglycemic conditions. Moreover, immunofluorescence microscopy studies revealed that mitochondrial oxidative stress, overall *O*-GlcNAcylation, and *O*-GlcNAcylation of PKB/Akt and AS160 were significantly increased (versus matched controls) under hyperglycemic conditions. We further found that antioxidant treatment attenuated all these effects, while HBP inhibition resulted in decreased *O*-GlcNAcylation of PKB/Akt and AS160, and improved GLUT4 translocation. Our data show that hyperglycemia-mediated oxidative stress triggers the HBP, thereby increasing myocar-

academicJournals

CONFERENCE PROCEEDINGS ■
Scientific Research and Essays

dial PKB/Akt and AS160 *O*-GlcNAcylation leading to impaired GLUT4 translocation. We suggest that activation of this detrimental signaling cascade may impair insulin-mediated myocardial glucose uptake, while HBP inhibition and/or antioxidant supplementation may be a useful therapeutic strategy in this instance.

**Key words:** Hexoseamine biosynthetic pathway, insulin resistance, therapeutic targets.

# N-terminal prohormone brain natriuretic peptide and cardiovascular function in Africans and Caucasians: The SAfrEIC study

**R. Kruger[1], R. Schutte[1], H. W Huisman[1], P. Hindersson[2], M. H. Olsen[3] and A. E. Schutte[1]**

[1]Hypertension in Africa Research Team (HART), North-West University, Potchefstroom, South Africa.
[2]Department of Biochemistry, Hospital Vendsyssel, Hjørring, Denmark.
[3]Cardiovascular Research Unit, Division of Cardiology, Department of Internal medicine, Glostrup Hospital, University of Copenhagen, Denmark.

20 December 2011

Information about ethnic differences regarding the N-terminal prohormone brain natriuretic peptide (NT-proBNP) and its association with cardiovascular function is limited. This study compared NT-proBNP levels, and explored the associations of NT-proBNP with markers of cardiovascular function between Africans and Caucasians. This cross-sectional study included 264 Africans and 369 Caucasians from the North West province, South Africa. Serum NT-proBNP concentrations were determined using the Elecsys proBNP sandwich immunoassay. We measured blood pressure, pulse wave velocity and arterial compliance, and assessed lifestyle components. NT-proBNP levels were significantly higher ($P$<0.0001) in Africans compared to Caucasians also after adjusting for age, gender, body mass index (BMI) and systolic blood pressure (SBP) ($P$=0.069). However, after full adjustment this significant difference disappeared ($P$=0.59). In single regression, various significant associations were obtained between NT-proBNP and measures of arterial structure and function. After adjusting for age, gender and BMI, as well as after multiple adjustments, only the associations between NT-proBNP, SBP and pulse pressure (PP) remained significant in Africans (SBP: $\beta$=0.240, $P$<0.001; PP: $\beta$=0.231, $P$<0.001), with no significant associations in the Caucasians. The level of Nt-proBNP was higher in Africans independently of age and BMI partly driven by higher SBP in Africans. NT-proBNP was persistently associated with SBP and PP in Africans, but not in Caucasians. These associations suggest early cardiac changes in Africans due to chronically elevated SBP and pulse pressure.

**Key words:** NT-proBNP, blood pressure, ethnicity, cardiovascular function.

# Is an attenuated decline in nocturnal blood pressure associated with target organ changes in people of African ancestry?

**M. J. Maseko, A. J. Woodiwiss and G. R. Norton**

academicJournals

CONFERENCE PROCEEDINGS ■
Scientific Research and Essays

Cardiovascular Pathophysiology and Genomics Research Unit, School of Physiology, Faculty of Health Sciences, University of the Witwatersrand, South Africa.

20 December 2011

Hypertension is a major risk for cardiovascular disease in both developed and developing countries. Blood pressure (BP) normally decreases by 10% or more at night however in some individuals (non-dippers); night-time BP does not decrease substantially. A number of studies have indicated that a reduced nocturnal decline in BP increases the risk for cardiovascular disease. Whether this attenuated decline in nocturnal BP translates into cardiovascular target organ changes in an urban developing community people of African ancestry living in Africa is unknown. We assessed the independent relationship between non-dipping and target organ changes in a randomly selected community sample of nuclear families of African ancestry. Of the 911 participants recruited, 700 had 24 h BP measurements that met pre-specified quality control criteria. Target organ changes were assessed from echocardiographically-determined left ventricular mass indexed to height$^{2.7}$ (LVMI), carotid-femoral pulse wave velocity (PWV) and microalbumin-to-creatinine ratios (ACR-derived from 24-h urine samples). The relationship between non-dipping and target organ changes were assessed in multivariate regression models with adjustments for age, sex, 24 h blood pressure, regular tobacco intake, regular alcohol intake, waist circumference, diabetes mellitus or an HbA1c>6.1%, menopausal status, confirmed with follicle stimulating hormone concentrations and office heart rate (for PWV). Dippers and non-dippers had similar day-time pressures (120±13/76±10 for dippers and 120±16/76±11for non-dippers). There was a difference in night time BP (101±10/59±8 for dippers and 115±18/68±11 for no-dippers). There was no significant difference between the in urine microalbumin concentration between the two groups. However compared to dippers, non-dippers had a significant increase in both LVHI (p=0.0107) and PWV (p<0.0001). We conclude that in a population sample of African ancestry attenuated decline in nocturnal BP results in preclinical vascular and cardiac pathology.

**Key words:** Hypertension, nocturnal blood pressure, dippers.

# Depression, cardiometabolic dysfunction, and left ventricular hypertrophy in black African men and women: The SABPA study

**N. Mashele[1], L. Malan[1], J. M. Van Rooyen[1], J. C. Potgieter[2], B. Harvey[3] and M. Hamer[4]**

[1]School of Physiology, Nutrition and Consumer Sciences, North West University, Potchefstroom, South Africa.
[2]School for Psychosocial Behavioral Sciences, North West University, Potchefstroom, South Africa.
[3]School for Pharmacy, North West University, Potchefstroom, South Africa.
[4]Department of Epidemiology and Public Health, University College London, London, UK.

20 December 2011

There is a recognized association between depression and cardiovascular disease. The intermediate processes that play a role in this relationship are not fully understood. The understanding of these processes is important because of the increasing prevalence of cardiometabolic risk factors in the African population. The objective of this study was to investigate whether cardiometabolic risk markers account for the association between depression and left ventricular hypertrophy (LVH). In a target population study of 200 African men and women (ages 25-60 years), we investigated depressive symptoms (DSM IV criteria, PHQ>10) and psychological distress (GHQ-28≥4) and their association with the metabolic syndrome as defined by the International Diabetes Federation criteria. The main outcome was LVH (measured using the Cornell product >244 mV). Significant interaction between LVH, depression and gender ($p$=0.02) was found, revealing an effect size of $d$= 0.51 in the black African men. LVH in depressed African women is driven pri-

marily by metabolic factor specifically low HDL cholesterol, while in African men this association is mediated by a cardiovascular factor such as increased Systolic blood pressure (SBP). These findings add to the growing evidence suggesting that black African men are at greater risk of developing LVH

**Key words:** Depression, cardiometabolic risk, left ventricular hypertrophy, Africans.

# The most reliable electrocardiographic criteria for left ventricular hypertrophy in a predominantly obese African population

**F. Maunganidze, A. J. Woodiwiss, M. Maseko, C. Libhaber, O.H. I. Majane and G. R. Norton**

Cardiovascular Pathophysiology and Genomics Research Unit, Schools of Physiology and Medicine, Faculty of Health Sciences, University of the Witwatersrand, South Africa.

20 December 2011

Ethnicity, obesity and gender significantly affect the detection of left ventricular hypertrophy (LVH) using electrocardiographic (ECG) criteria. The use of novel ECG criteria (Composite Time-Voltage and Gubner-Ungerleider Product) in subjects of East African ancestry was therefore proposed in preference to classic ECG criteria (Cornell voltage; Sokolow-Lyon voltage; 12-lead voltage; 12-lead QRS sum). However, the validity of these novel ECG criteria have only been assessed in a predominantly lean (mean BMI ~ 25 kg/$m^2$) population. Hence, we aimed to determine the accuracy of both classic and novel ECG criteria in a population of African ancestry with a high prevalence of obesity. We compared LVH determined by ECG to LVH determined by echocardiography (LV mass index>51 g/$m^{2.7}$) using Chi-square analysis. Of the 479 participants assessed, 67% were overweight or obese (24.8% overweight, 42.2% obese), 65.3% were female and 44.0% had hypertension. Using echocardiographic criteria for LVH, 23.8% had LVH (24.7% men, 23.3% women). The Cornell Voltage emerged as the most useful ECG criterion for the detection of LVH [14.8% LVH, sensitivity=29.0%, specificity=89.6%, accuracy=75.16%, negative predictive power (NPP) = 80.15%, area under receiver operating curve (AUC) = 0.646] followed by the Lewis Voltage (10.23% LVH, sensitivity = 18.42%, specificity = 92.33%, accuracy = 74.74%, NPP = 78.37%, AUC = 0.554) and the Gubner-Ungerleider Product (7.72% LVH, sensitivity = 17.54%, specificity = 95.34%, accuracy = 76.43%, NPP = 78.73%, AUC = 0.564). Combining these 3 criteria improved their ability to detect LVH (20.88% LVH, sensitivity = 37.72%, specificity = 84.38%, accuracy = 73.28%, NPP = 81.27%, AUC = 0.611). Sensitivity for the other 7 ECG criteria assessed was generally poor (<10%) in this population although the specificity was high (>90%). In conclusion, in a population of African ancestry with a high prevalence of obesity, the most reliable ECG criterion for LVH was the Cornell Voltage.

**Key words:** Left ventricular hypertrophy, Cornell voltage, ECG criteria.

academicJournals

CONFERENCE PROCEEDINGS ■

Scientific Research and Essays

# Contribution of circulating angiotensinogen concentrations to variations in aldosterone and blood pressure in a group of African ancestry depends on salt intake

**F. S. Michel[1], G. R. Norton[1], O.H.I. Majane[1], M. Badenhorst[1], L. Vengethasamy[1], J. Paiker[2], M. J. Maseko[1], P. Sareli[1] and A. J. Woodiwiss[1]**

[1]Cardiovascular Pathophysiology and Genomics Research Unit, School of Physiology, University of the Witwatersrand, Johannesburg, South Africa.
[2]School of Pathology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa.

20 December 2011

In the presence of high $Na^+$, low $K^+$ diets, which suppress renin release in salt-sensitive groups, the mechanisms that maintain increases in renin-angiotensin-aldosterone system (RAAS) activation downstream from renin and RAAS-induced effects on blood pressure (BP), are uncertain. We aimed to determine whether in the context of dietary $Na^+$ and $K^+$ intake, circulating angiotensinogen (AGT) concentrations contribute to variations in serum aldosterone concentrations and BP in a group of African ancestry. In 579 participants from randomly selected nuclear families, nurse-derived BP, 24 h urine electrolyte excretion rates and circulating concentrations of AGT, renin and aldosterone were measured. The relationships between angiotensinogen and aldosterone concentrations or BP in participants with urinary Na+/K+ ≥ or < median were determined using multivariate regression analysis. An interaction between plasma [AGT] and urinary $Na^+/K^+$ was independently associated with serum [aldosterone] ($p<0.001$) and systolic BP (SBP, $p<0.05$). Independent of confounders, in participants with a urinary $Na^+/K^+$≥median for the sample, plasma [AGT] was positively associated with serum [aldosterone] ($p<0.0001$) and with SBP ($p<0.005$). No independent relationships between plasma [AGT] and either serum [aldosterone] ($p=0.53$) or SBP ($p=0.67$) were noted in participants with a urinary $Na^+/K^+$<median for the sample. The standardised β-coefficients (slopes) of the [AGT]-[aldosterone] and [AGT]-SBP relationships were greater in participants with a urinary $Na^+/K^+$≥median ([AGT] vs. [aldosterone]=0.30±0.06, [AGT] vs. SBP=0.16±0.05) as compared to participants with a urinary $Na^+/K^+$<median ([AGT] vs. [aldosterone]=-0.04±0.06, [AGT] vs. SBP=-0.03±0.05, $p<0.01$-$0.0001$ for comparison of slopes). The [AGT]-SBP relationship in the group with a urinary $Na^+/K^+$≥median for the sample was at least equivalent to the relationship between body mass index and BP. We conclude that, in a group of African ancestry, in the presence of high $Na^+$, low $K^+$ diets, which suppress renin release, RAAS activation and the impact of the RAAS on BP is maintained in-part by circulating AGT concentrations.

**Key words:** Renin-angiotensin-aldosterone system, salt intake, angiotensinogen.

# Protective effect of female gender against adverse cardiac remodelling in spontaneously hypertensive rats

**F. S. Michel, B. Hodson, L. Booysen and G. R. Norton**

Cardiovascular Pathophysiology and Genomics Research Unit, School of Physiology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa.

academicJournals

CONFERENCE PROCEEDINGS ■
Scientific Research and Essays

20 December 2011

In hypertension, which is the leading cause of heart failure in urban black communities in South Africa, female gender may modify the cardiac phenotype. Indeed, in response to a pressure overload on the heart, women develop more marked concentric hypertrophy and systolic function remains higher than in men. Animal studies have similarly demonstrated that the female gender produce differences in the cardiac response to pressure overload states. However whether these gender-related differences translate into differences in the progression of left ventricular hypertrophy to cardiac dilatation in systemic hypertension has not been established. The aim of the present study was to determine the effect of gender on the progression to cardiac dilatation in Spontaneously Hypertensive Rat (SHR). Premature progression to cardiac dilatation was induced by chronic adrenergic activation. A β-adrenoreceptor agonist, isoproterenol, or the saline vehicle was administrated daily from 9 to 14 months of age in male and female SHR (n = 9 per groups). Left ventricular (LV) chamber dimensions were then determined *in vivo* by echocardiography. LV remodelling was assessed *ex vivo* in a load independent manner with isolated perfused heart preparations. Comparisons between the 4 groups were made with a 2-way ANOVA. LV end diastolic and end systolic diameters, as well as the volume intercept of the LV diastolic pressure-volume relationship were significantly greater in isoproterenol-administrated male compared to the other groups, suggesting more pronounced premature cardiac dilatation in response to chronic adrenergic activation in males. In female rats, similar LV chamber size and remodelling were obtained in the presence or absence of chronic adrenergic activation. In conclusion, in the setting of hypertension, female rats are less susceptible than males to adverse cardiac remodelling produced by long-term β-adrenoreceptor activation.

**Key words:** Gender, cardiac remodelling, spontaneous hypertensive rats.

# The effects of chloroquine on selected markers of cardiovasular disease in male sprague-dawley rats

**P. Murambiwa[1], B. Masola[2], S. Mukaratirwa[3] and C.T. Musabayane[1]**

[1]Discipline of Human Physiology, University of KwaZulu-Natal, South Africa.
[2]Discipline of Biochemistry, University of KwaZulu-Natal, South Africa.
[3]Discipline of Biological Sciences, University of KwaZulu-Natal, South Africa.

20 December 2011

Impairment of cardiovascular function has been ascribed to *Plasmodium falciparum* infection or chloroquine (CHQ) administration in humans and experimental malaria. To distinguish between the pathophysiological effects of malaria and chloroquine on cardiovascular function, we monitored mean arterial pressure and lipid profiles, indicators of the functional status of the cardiovascular system in non-infected (control) and *Plasmodium berghei* infected male Sprague-Dawley rats. *P. berghei* infection is an experimental model of human malaria. Malaria was induced by an i.p. injection of *P berghei* ($10^5$ parasites). Rats treated with deionised water served as controls. Following confirmation of stable malaria status, animals were treated twice daily, eight hours apart with CHQ (60 mg $kg^{-1}$ b.wt, p.o.) for 5 consecutive days. Blood pressure was measured by a tail cuff method at 09 h00 every third day throughout the 21 day experimental period. Animals were euthanized and blood collected on days 7; 14; and 21 post *P. berghei* infection, followed by measurement of plasma Total cholesterol, High Density Lipoprotein-cholesterol (HDL-c) and Low Density Lipoprotein-cholesterol (LDL-c). Mean arterial pressure was not significantly altered by infection or CHQ treatment ($P > 0.05$). However, *P. berghei* infection was associated with a reduction in HDL-c concentration (0.12 ± 0.01 mmol/l,) ($P < 0.05$) in comparison with control animals (0.41 ± 0.06 mmol/l), suggestive of a derangement in the lipid status. On the other hand, *P. berghei* infected animals treated with CHQ were associated with a further reduction in HDL-c to (0.08 ± 0.01 mmol/l) ($P < 0.05$) indicating that the anti-malarial drug alters the lipid status of the animals. Malaria infected animals treated with CHQ

academicJournals

CONFERENCE PROCEEDINGS ■
Scientific Research and Essays

were also associated with elevated total cholesterol (2.86 ± 0.35 mmol/l, n=6) (P <0.05) and LDL-c (2.68 ± 0.40 mmol/l), (P <0.05), in comparison to their respective controls (1.21 ± 0.66 mmol/l) and (0.59 ± 0.14 mmol/l), respectively. Reduced HDL-c, elevated total cholesterol and elevated LDL-c concentration signify dyslipidaemia and progression towards atherosclerosis, a process that precedes development of cardiovascular diseases. We can therefore conclude that *P. berghei* infection may impair cardiovascular function, which is exacerbated by CHQ treatment.

**Key words:** Malaria, cardiovascular function, chloroquine.

# Comparison of vascular function in preeclamptic and normotensive pregnant women in the rural Eastern Cape

**A. Namugowa and A. Meeme**

Physiology department, Faculty of Health Sciences, Walter Sisulu University, South Africa.

20 December 2011

Preeclampsia is associated with arterial stiffness and endothelial dysfunction. The aim of the study was to compare vascular function of pregnant women with preeclampsia and normotensives by non invasive techniques. This was a comparative study where participants were recruited from the Mthatha General Hospital complex Antenatal clinics. Fifty four (54) normotensive and 21 preeclamptic women were recruited for the study. Arterial stiffness was assessed using applanation tonometry with SphygmoCor device; central aortic pressures and peripheral and central augmentation index (Alx) and carotid-femoral pulse wave velocity was then calculated. Endothelial function was assessed by an EndoPAT 2000 device. Pneumatic probes were fitted to the index fingers. After baseline recordings a blood pressure cuff was inflated on the non-dominant arm, then released after 5min to induce flow mediated reactive hyperemia. The ratio of the readings before and after occlusion was then used to calculate the score for endothelial function and the reactive hyperaemia index (RHI).RHI was significantly higher; p< 0.001 among preeclamptic women compared to normotensives (1.76±0.5 vs. 1.45± 0.22) indicating good endothelial function. Pulse wave velocity was significantly higher; p<0.001 in preeclamptic than normotensive women (6.7±1.5 vs. 5.1±0.7) indicating arterial stiffness. Alx measured by the EndoPAT 2000 correlated with peripheral Alx (r=0.623, p<0.0001) and central Alx (r=0.60, p< 0.0001) measured by the SphygmoCor. This means that either of these parameters can be used to assess arterial stiffness. In this interim analysis, we have demonstrated that women with preeclampsia have increased pulse wave velocity and peripheral augmentation index suggesting vascular stiffness. Low RHI values indicate endothelial dysfunction in the general population; however our results showed a higher value in preeclampsia than in normal pregnancy. Could there be other factors responsible for RHI in pregnancy?

**Key words:** Pre-eclamsia, vascular function, pulse wave velocity.

academicJournals

CONFERENCE PROCEEDINGS ■
Scientific Research and Essays

# Independent contribution of the forward pressure wave to aortic blood pressure and left ventricular mass in hypertension in young-to-middle aged adults

**G.R. Norton, M. Redelinghuys, M. J. Maseko, O.H.I Majane and A. J. Woodiwiss**

Cardiovascular Pathophysiology and Genomics Research Unit, School of Physiology, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa.

20 December 2011

Although antihypertensive agents modify the reflected wave component (augmentation pressure [AP]) of aortic pulse pressure (PPc), current agents are unable to modify the aortic structural changes that determine the forward pressure wave (P1). Nevertheless, the contribution of P1 to increases in PPc in hypertension (HT) in young-to-middle aged adults is unknown. We determined whether P1 contributes to increases in PPc and left ventricular mass index (LVMI) in young-to-middle aged hypertensive adults. Aortic blood pressure (BP) was determined using applanation tonometry in 1015 randomly recruited participants (age=16-88 years) from a community sample, 35.6% (n=361) of whom had HT with an elevated systolic BP (SBP). LVMI and stroke volume were determined using echocardiography. Independent of mean arterial pressure (MAP) and other confounders, both P1 and AP were independently related to PPc (partial r: P1=0.91, AP=0.73) and central SBP (SBPc; partial r: P1=0.90, AP=0.70) in all participants ($p<0.0001$), but P1 accounted for a greater proportion of the variation in PPc and SBPc than AP ($p<0.0001$ for comparison of r values). Compared to normotensives (NT), HT<43.8 years (median age) had higher multivariate adjusted PPc (mmHg, HT=35±1, NT=28±1); SBPc (mmHg, HT=112±1, NT=107±1) and P1 (mmHg, HT=28±1, NT=21±1) ($p<0.0001$ for all), but not AP values (mmHg, HT=7.2±0.8, NT=6.3±0.2, $p=0.33$); whilst HT>43.8years of age had increases in both P1 (mmHg, HT=31±1, NT=25±1, $p<0.0001$) and AP (mmHg, HT=16.6±0.4, NT=14.0±0.7, $p<0.005$). Neither augmentation index, nor stroke volume accounted for the difference in PPc between HT and NT. P1 was independently associated with LVMI (partial $r=0.14$, $p=0.01$) and adjustments for P1 abolished PPc-LVMI relations ($p=0.47$). In conclusion, independent of steady-state pressures, P1 accounts for a considerable proportion of the increases in PPc and LVMI in hypertension in the young-to-middle aged HT. Thus, therapy targeting the aortic structural changes that contribute toward P1 may be required in young-to-middle aged hypertensive adults.

**Key words:** Aortic blood pressure, hypertension, pulse wave analysis.

# Prevalence and determinants of albuminuria in HIV patients

**K. O. Awotedu[1], C. Ekpebegh[2] and B. Blanco[3]**

[1]Department of Physiology, Walter Sisulu University, Nelson Mandela Drive, South Africa.
[2]Department of Medicine, Walter Sisulu University, Nelson Mandela Drive, South Africa.
[3]Department of Chemical Pathology, Walter Sisulu University, Nelson Mandela Drive South Africa.

20 December 2011

academicJournals

CONFERENCE PROCEEDINGS ■
Scientific Research and Essays

Renal disease which may manifest as albuminuria may be associated with the HIV infection. Our objective was to determine the prevalence and determinants of HIV associated albuminuria. In this cross sectional study we compared albuminuric and non-albuminuric HIV positive- and negative patients. The groups were assessed for differences in demographic, clinical and laboratory parameters. There were 439 subjects comprising 334 females and 105 males. The numbers of HIV positive and negative patients were 323 and 116 respectively. The mean age of all study subjects was 37.2±11.9. Albuminuria was found in 27.6% of HIV positive subjects and 18.1% of HIV negative subjects; P=0.04. Albuminuric in comparison with non-albuminuric HIV patients were younger (35.7±11.3 versus 38.6±11 years; P=0.04), with lower systolic blood pressure (124.4±15.1 versus 132.8±21 mmHg; P=0.004), had lower HDL-Cholesterol (1.1±0.5 versus 1.3±0.7; P=0.01) and CD 4 cells (326.2±247.2 versus 435±302.3; P=0.01).HIV negative patients with albuminuria had higher systolic blood pressure; 140.4±18.4 versus 129.6±21.3; P=0.04. Proteinuria was highly prevalent in our HIV positive patients with low HDL-Cholesterol and low CD 4 cell counts as its determinants.

**Key words:** HIV, albuminuria, renal function, predictors.

# STAT3α interacts with nuclear GSK3ß and cytoplasmic RISK pathway and stabilizes rhythm in the anoxic-reoxygenated embryonic heart.

**S. Pedretti and E. Raddatz**

Department of Physiology, Faculty of Biology and Medicine, University of Lausanne, Switzerland.

20 December 2011

Activation of Janus Kinase 2/Signal Transducer and Activator of Transcription 3 (JAK2/STAT3) pathway is known to play a key role in cardiogenesis and in cardio-protection against ischemia-reperfusion in adult. We previously showed that in ventricle of the anoxic-reoxygenated developing heart, ROS-dependent STAT3α activation leads to nuclear accumulation of STAT3α without DNA-binding. Possible interaction between STAT3α and other signalling pathways [in particular Reperfusion Injury Salvage Kinase (RISK) pathway] and the role of activated STAT3α in functional recovery of the embryonic heart remains unexplored. Hearts isolated from 4-day-old chick embryos were submitted to anoxia (30 min) and reoxygenation (80 min) with or without the JAK2/STAT3 inhibitor AG490 or the PhosphoInositide-3-Kinase (PI3K)/Akt inhibitor LY-294002. Time course of phosphorylation of STAT3α$^{tyr705}$ and RISK proteins [PI3K, Akt, Glycogen Synthase Kinase 3ß (GSK3ß), Glycogen Synthase (GS), Extracellular signal-Regulated Kinase 2 (ERK2)] was determined in homogenate and in enriched nuclear and cytoplasmic fractions of the ventricle. The chrono-, dromo- and inotropic disturbances were also investigated by ECG and mechanical recordings. Phosphorylation of STAT3α$^{tyr705}$ was reduced by AG490 but not affected by LY-294002. STAT3α and GSK3ß were detected both in nuclear and cytoplasmic fractions while PI3K, Akt, GS and ERK2 were restricted to cytoplasm. AG490 decreased the reoxygenation-induced phosphorylation of Akt, GS and ERK2 and phosphorylation/inhibition of GSK3ß in the nucleus, exclusively. Inhibition of JAK2/STAT3 delayed recovery of atrial rate, worsened variability of cardiac cycle length and prolonged arrhythmias compared to control hearts. Thus, besides its nuclear translocation without transcriptional activity, activated STAT3α can rapidly interact with RISK proteins present in nucleus and cytoplasm, without dual interaction, and reduce the anoxia-reoxygenation-induced arrhythmias in the embryonic heart.

**Key words:** JAK2/STAT3 pathway, anoxia-reoxygenation, embryonic heart.

academicJournals

CONFERENCE PROCEEDINGS ■
Scientific Research and Essays

# Metformin prevents isoproterenol-induced cardiac pump dysfunction and dilatation in rats: A therapeutic role for metabolic modulation

**V. R. PETERSON[1], M. MADZIVA[2], G. NORTON[1] AND S. MAKAULA[1]**

[1]Cardiovascular Pathophysiology and Genomics Research Unit, University of the Witwatersrand, South Africa.
[2]Molecular Physiology Laboratory, School of Physiology, Faculty of Health Sciences, University of the Witwatersrand, South Africa.

20 December 2011

Heart failure is characterized by contractile dysfunction and dilatation. Interestingly, chronic administration of isoproterenol, a β-adrenergic agonist, has been shown to induce cardiac dysfunction and dilatation in rats. Also, metabolic dysregulation has been reported in heart failure; however it remains unclear whether isoproterenol exerts its deleterious effect through altered metabolic regulation. Whether metformin, a metabolic modulator, prevents isoproterenol-induced cardiac dysfunction is unknown. Therefore the aim of this study was to determine the role of metformin in the isoproterenol model of cardiac dysfunction. Male Sprague-Dawley rats were assigned into four groups: control (CONT), Isoproterenol (ISO), Metformin (METF) and Isoproterenol + Metformin (ISO+METF). The CONT group received subcutaneous injections of saline; the ISO group received subcutaneous injections of 0.02 mg $kg^{-1}$ $day^{-1}$ of isoproterenol; the METF group received 300 mg $kg^{-1}$ $day^{-1}$ of metformin in drinking water and the ISO+MET group received both drugs, concurrently. All treatments were administered for seven months. Animals were weighed weekly. After seven months, echocardiography was performed to assess cardiac function. Total heart (HM) and left ventricular (LVM) masses were weighed to further investigate cardiac structural changes. After seven months, the ISO group showed significant increases in both left ventricular end diastolic diameter (LVEDD) ($p<0.05$) and end systolic diameter (LVESD) ($p<0.05$) when compared to the CONT group. Also, a significant reduction in both endocardial fractional shortening ($FS_{end}$) ($p<0.05$) and midwall fractional shortening ($FS_{mid}$) ($p<0.05$) was observed in the ISO group compared to CONT group. Administration of metformin alone showed no changes in heart function. However, co-administration of metformin and isoproterenol significantly prevented an increase in both LVEDD ($p<0.05$) and LVESD ($p<0.05$) and a decrease in $FS_{mid}$ ($p<0.05$) and $FS_{end}$ ($p<0.05$) compared to the ISO group. Chronic administration of isoproterenol causes cardiac pump dysfunction and cardiac dilatation. Administration of Metformin prevents isoproterenol-induced progressive pump dysfunction and cardiac dilatation.

**Key words:** cardiac dysfunction, isiproterenol, Metformin.

# The development of hypertension in black South Africans: A 5-year prospective study

**A.E. Schutte, J. M. van Rooyen, R. Schutte, C. M. T. Fourie, H. W. Huisman, L. Malan, N. T. Malan, C. Mels, W. Smith, M. C. P. Glyn and A. Kruger**

Hypertension in Africa Research Team (HART), North-West University, Potchefstroom, South Africa.

20 December 2011

academicJournals

CONFERENCE PROCEEDINGS ■
Scientific Research and Essays

Hypertension prevalence in African populations, including black South Africans, is a well-known concern. Although sufficient evidence is available reporting the stroke and hypertensive heart disease prevalence among South Africans; to date no evidence of longitudinal data have been published. Our aim was therefore to determine which characteristics of Africans with optimal blood pressure (BP)(<120/80 mmHg) relate to the development of hypertension within a 5-year period. The South African arm of the multi-national PURE study (Prospective Urban Rural Epidemiology) included 1994 Africans at baseline in 2005 (aged > 30 years). At baseline and in 2010 we collected the brachial BP data, as well as pulse wave velocity. In 2010 we additionally measured central systolic BP. A total of 47.7% of participants were hypertensive at baseline (>140 and/or 90 mmHg). We identified 258 participants with optimal BP in 2005, with 61 becoming hypertensive at follow-up, and 197 remaining normotensive. Comparing the baseline characteristics of these two groups indicated similar ages, gender distribution, levels of employment, and also similar dietary intake, cholesterol profiles, and glycated hemoglobin (p>0.05). At baseline the group that became hypertensive had higher waist circumferences (p=0.007), body mass index (p=0.03), smoked more (p=0.02) and had elevated gamma glutamyl transferase (p<0.001). Baseline BP of the groups differed slightly: 108±7.20 / 71.9±5.85 vs. 106±8.60 / 70.2 ± 6.40 mmHg; p=0.056 and p=0.066, respectively. Five years later the groups differed significantly: brachial SBP (115±12.6 vs. 145±16.1 mmHg; p<0.001) and central systolic BP (126±13.2 vs. 151±22.4 mmHg; p<0.001). Odds ratios (adjusted for age, BMI, gender and rural/urban location) indicated antihypertensive medication (4.63; p = 0.02), elevated arterial stiffness (2.12; p=0.04), smoking habit (2.70; p= 0.003), and alcohol intake (3.10; p = 0.001) increased the odds for the development of hypertension. Our first longitudinal data of black South Africans demonstrate that the main culprits for the development of hypertension are mainly modifiable risk factors, which therefore clearly deserve attention especially regarding prevention programmes amongst the young population.

**Key words:** Hypertension, Africans, lifestyle.

# The cardiovascular effects of chronic PPARα agonist (K-111) treatment in obese insulin resistant rats

**W. SMITH[1, 2], S. GENADE[1], D. BLACKHURST[3], A. LOCHNER[1] AND E. F. DU TOIT[4]**

[1]DEPARTMENT OF BIOMEDICAL SCIENCES, STELLENBOSCH UNIVERSITY, SOUTH AFRICA.
[2]Hypertension in Africa Research Team (HART), North-West University (Potchefstroom Campus), South Africa.
[3]DIVISION OF LIPIDOLOGY, DEPARTMENT OF INTERNAL MEDICINE, UNIVERSITY OF CAPE TOWN, SOUTH AFRICA.
[4]School of Medical Science, Griffith University (Gold Coast), Queensland, Australia.

20 December 2011

Cardiac specific over-expression of PPARα results in a phenotype resembling the diabetic cardiomyopathy which is also characterized by worse post-ischaemic outcomes. This is concerning as many obese patients at risk for developing myocardial infarction are likely to receive metabolic modulators such as PPARα agonists. The aim of our study was to determine the impact of chronic PPARα agonist (K-111) treatment on systemic metabolic markers, left ventricular (LV) mechanical function, LV substrate metabolism, LV mitochondrial function and susceptibility to ischaemia/reperfusion injury in a rodent model of diet induced obesity. After 8 weeks of male Wistar rats being fed a standard rat chow or high caloric diet, the groups were randomly divided into groups receiving 10 mg/kg/day K-111 or vehicle together with their respective diet for the next 10 weeks. Fasting metabolic markers were determined. Isolated hearts were perfused with a Krebs-Henseleit buffer containing glucose, insulin and fatty acid. Normoxic and post-ischaemic myocardial carbohydrate and lipid metabolism was determined, while myocardial infarct size was determined in a separate series of animals, after 40 min of regional ischaemia and 1 h reperfusion. Isolated mitochondrial respiration was measured in the presence of glutamate or palmitoyl-L-carnitine. K-111 normalized the systemic metabolic abnormalities associated with obesity.

academicJournals

CONFERENCE PROCEEDINGS ■
Scientific Research and Essays

Despite this, neither chronic K-111 treatment nor obesity influenced myocardial mechanical function, mitochondrial respiration rates or infarct size (Control: 37.17±2.63%; Control+K-111: 45.01±2.60% Obese: 44.89±3.2% Obese+K-111: 37.58±3.78%). K-111 however reduced myocardial palmitate oxidation rates in the obese treated groups (obese: 28.39±2.34 vs. obese+K-111: 13.90±1.02nmol palmitic acid/g dry weight/min). K-111 is effective in treating the systemic metabolic abnormalities associated with obesity. From the parameters measured, K-111 does not appear to have any detrimental myocardial effects and could be considered safe for treating obese patients at risk for developing acute myocardial infarction.

**Key words:** insulin resistance, systemic metabolic markers, PPARα-agonist.

# Ambulatory blood pressure, 3-methoxy-4-hydroxyphenylglycol and carotid intima-media thickness in dipper and non-dipper men: The SABPA study

**L. van Lill[1], L. Malan[1], J. van Rooyen[2], F. Steyn[3], T. Ziemssen and M. Reimann[3]**

[1]School for Physiology, Nutrition and Consumer Sciences, North-West University, Potchefstroom Campus, South Africa.
[2]Statistical Consultation Service, North-West University, Potchefstroom Campus, South Africa.
[3]Department of Neurology, Medical Faculty Carl Gustav Carus, Dresden University of Technology, Germany.

20 December 2011

Research has indicated that non-dipping is often accompanied by an autonomic imbalance. An association has been shown between sympathetic hyperactivity and increased carotid intima-media thickness (CIMT), especially in non-dippers. The objective of this study was to determine the possible association between changes in ambulatory blood pressure, 3-methoxy-4-hydroxyphenylglycol (MHPG) (norepinephrine metabolite) and CIMT (marker of subclinical atherosclerosis) in non-dipper men. The Sympathetic Activity and Ambulatory Blood Pressure in Africans (SABPA) study included 82 African (dippers N=45; non-dippers N=37) and 100 Caucasian male teachers (dippers N=72; non-dippers N=28) aged between 20 and 65 years, recruited in the North-West Province. Ambulatory blood pressure was obtained for a 22 to 23 h period and thereafter, sonar images were obtained to determine CIMT. Fasting saliva and blood samples were collected for biochemical analyses. The African non-dipper men had higher nighttime blood pressure ($p<0.001$) compared to the Caucasian male non-dippers. In the low MHPG tertile night systolic blood pressure (SBP) ($p=0.003$) and diastolic blood pressure (DBP) ($p=0.007$) of the African non-dipper men were higher. The African non-dipper men in the medium MHPG tertile had higher night heart rate ($p=0.051$) and CIMT ($p=0.043$). The night DBP of the African non-dipper men in the high MHPG tertile was higher ($p=0.015$) compared to their Caucasian counterparts. CIMT was significantly predicted by night DBP ($\beta=0.36$; $p=0.025$) and SPB ($\beta=0.397$; $p=0.015$) in the African non-dipper low and medium MHPG groups respectively. Whilst in the Caucasian non-dipper men, CIMT was predicted in the medium and high MHPG groups by night SBP ($\beta=0.67$; $p<0.001$) and heart rate ($\beta=-0.24$; $p=0.049$). In the African and Caucasian non-dipper men, the development or progression of subclinical atherosclerosis is associated with high nighttime blood pressure. Downregulation of vesicular storage of norepinephrine may be present due to possible increased sympathetic activity, specifically in the male African non-dippers.

**Key words:** 3-methoxy-4-hydroxyphenylglycol, carotid intima-media thickness, non-dipping, ambulatory blood pressure.

academicJournals

CONFERENCE PROCEEDINGS ■
Scientific Research and Essays

# Protein phosphatase 2a in cardiac ischaemia/reperfusion: A negative regulator of survival

**D. VAN VUUREN, C. WESTCOTT AND A. LOCHNER**

Division of Medical Physiology, Department of Biomedical Sciences, University of Stellenbosch, South Africa.

20 December 2011

The majority of research regarding the signaling cascades associated with myocardial ischaemia/reperfusion injury (IRI) has focused on the role of protein phosphorylation events (kinase mediated), while the importance of the protein phosphatases in determining the characteristics of the signal remain largely neglected. The aim of our study was to investigate the contribution of a major serine/threonine protein phosphatase, Protein phosphatase 2A (PP2A), to the signalling events associated with cardiac IRI. Isolated rat hearts were exposed to 20 min sustained global ischaemia (GI), followed by 30 min reperfusion. Samples were collected at several time points, fractionated and analyzed using standard Western blotting techniques. We found that sustained ischaemia was associated with an elevation of the measured levels of the catalytic subunit PP2A-C (20 minutes GI: 1.78±0.10 vs. control: 1.03±0.11 arbitrary units (AU)). At 10 min ischaemia there was an accumulation of PP2A-C in the nucleus (GI: 4.97±0.42 vs. control: 1.00±0.07 AU; $p<0.05$), gradually dispersing to the membrane fraction as ischaemia progressed. Administration of the PP2A inhibitor, okadaic acid (OA; 10 nM), immediately before 35 min regional ischaemia reduced infarct size (PreOA: 26.26±5.10% vs. control: 41.53±2.81%; $p<0.05$). This infarct sparing effect was associated with a significant elevation in the phosphorylation of ERK p42/p44 at 5 min reperfusion (PreOA: 5.90±0.77 vs. reperfusion: 1.90±0.51 AU, $p<0.05$). Confirming this, co-immunoprecipitation experiments indicated that OA administration reduced the interaction between PP2A-C and ERK p42/p44 (PreOA: 0.13±0.01 vs. reperfusion: 0.27±0.03 AU). Our results reveal PP2A as an active component of the myocyte's response to IRI. Not only do PP2A levels and its cellular distribution change as ischaemia progresses, but the inhibition of PP2A during ischaemia and initial reperfusion also confers cardioprotection. This latter effect is, at least in part, probably due to PP2A's role as a negative regulator of ERK p42/p44.

**Key words:** Protein phosphatases, cardiac ischaemia/reperfusion, survival signaling.

# Intra-familial aggregation and heritability of aortic pulse pressure and the wave components in a community with a high prevalence of uncontrolled hypertension

**A. J. Woodiwiss, M. Redelinghuys, M. J. Maseko, O.H.I. Majane and G. R. Norton**

Cardiovascular Pathophysiology and Genomics Research Unit, School of Physiology, Faculty of Health Sciences, University of the Witwatersrand, South Africa.

20 December 2011

Central (aortic) pulse pressure (PPc) and the augmented pressure (AP) wave component are more closely associated with cardiovascular outcomes than peripheral PP. Although peripheral PP is inherited, reports on the heritability of PPc are conflicting. Previous studies failed to adjust for steady-state pressures (mean arterial pressure, MAP) or heart rate, which are strong determinants of PPc and AP. We determined the intra-familial aggregation and heritability of PPc, AP and the forward wave component (P1) independent of MAP and other confounders in a community sample with a high

prevalence of uncontrolled hypertension. PPc, central systolic blood pressure (SBPc), P1 and AP were determined by applanation tonometry (SphygmoCor software) in 946 participants from 258 families (23 families included three generations) recruited from an urban developing community of black African descent. Heritability estimates were determined from SAGE software. Echocardiography was evaluated in 480 participants in order to determine stroke volume. 58.4% of parents and 19.8% of offspring had uncontrolled hypertension. Age and MAP were independently associated with PPc, SBPc, P1 and AP ($p<0.0001$); heart rate was independently associated with AP, PPc and SBPc ($p<0.0001$); and height was independently associated with AP ($p=0.0001$). However, stroke volume was not independently associated with PPc or P1 ($p>0.05$). With adjustments for potential confounders including MAP, heart rate and height; significant parent-child ($p<0.05$-$p<0.01$) and sibling-sibling ($p<0.0005$) correlations were noted for PPc, SBPc, P1 and AP; but no correlations between fathers and mothers were observed. Independent of MAP and additional confounders, significant heritability was identified for PPc ($h^2=0.25\pm0.07$, $p<0.0005$), SBPc ($h^2=0.25\pm0.07$, $p<0.0005$), P1 ($h^2=0.28\pm0.07$, $p<0.0001$), and AP ($h^2=0.19\pm0.07$, $p<0.005$). In conclusion, in a community sample with a high prevalence of uncontrolled hypertension, independent of MAP and other confounders, aortic SBP and PPc as well as the component forward and augmented pressure waves of PPc are significantly inherited.

**Key words:** Central pulse pressure, inheritance, intra-familial correlation.