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Research Article

Use of metabolomics to elucidate the metabolic perturbation associated with hypertension in a black South African male cohort: the SABPA study

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Abstract

There is concern about the increasing burden of essential hypertension in urban–dwelling black South Africans, especially males. Several studies have investigated urbanization and hypertension in South Africans, but in–depth metabolomics studies on these urbanized hypertensives are still lacking. We aimed to investigate hypertension via two metabolomics methods in order to explore underlying biological mechanisms, demonstrating the effectiveness of these methods in cardiovascular research. A comprehensive characterization of a group (n = 25) of black male South Africans was performed using urinary gas chromatography–mass spectrometry and liquid chromatography–mass spectrometry metabolic profiling in conjunction with 24–hour ambulatory blood pressure readings and anthropometric, clinical, and biochemical markers. Average 24–hour blood pressure readings served as the grouping variable, and test subjects were divided into quintiles. Statistical analyses were performed on Quintile 1 (normotensive subjects) and Quintile 5 (extreme hypertensive subjects). After feature selection was performed, several metabolites and cardiometabolic risk markers, including abdominal obesity and markers of liver damage, inflammation, and oxidative stress were significantly perturbed in Quintile 5 (hypertensives) compared with Quintile 1 ($P < .05$). Pathway analysis revealed perturbations in several systems involved in ethanol metabolism via shifted global NADH/NAD^+ ratio. Although alcohol abuse has been established as a risk factor for hypertension, this study illustrated a metabolic perturbation associated with alcohol abuse, contributing to the development of hypertension—possibly by altering bioenergetics through a shift in the NADH/NAD^+ ratio. Following this finding, future intervention studies on alcohol moderation, as well as further enhancement of metabolomics methods in cardiovascular research are highly recommended. J Am Soc Hypertens 2015;9(2):104–114.

Keywords: Alcohol abuse; cardiometabolic disease; hypertension; metabolomics.

Introduction

Cardiometabolic disease (including essential hypertension) is an emerging problem among urban–dwelling black Africans in South Africa.[1–3] Peltzer reported that of the estimated 5.5 million people in South Africa with hypertension, three million were black males,[4] which is an alarming statistic. Black adults in an urban setting seem to be most prone to significant increases in cardiovascular disease.[5,6] Walker already predicted, in 1972, that increasing

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Conflict of interest: none.

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urbanization and a rise in socio–economic status in developing populations would increase their proneness to obesity, hypertension, diabetes, and stroke.[7] Unfortunately, with urbanization comes changes in lifestyle, such as poor diet, physical inactivity, and increases in tobacco smoking and alcohol consumption that favor the development of non–communicable diseases. Tracking the influences of these and many other risk factors of hypertension on the body holistically is a challenge, since present clinical markers are measured singularly. However, the field of metabolomics aims to provide a global profile of all small–molecule metabolites in cells and biologic fluids, free of observational biases inherent to more focused studies of metabolism. This may have major advantages over traditional cardiovascular disease studies, as it is not restricted to known patho–physiologic pathways or single biomarker measurements that reflect only a specific aspect of the disease process of interest.[8] Numerous metabolomics studies have contributed to the acquisition of patho–physiologic knowledge of cardiovascular disease, as well as the search for novel biomarkers of this disease.[9–12] Also, the high–throughput nature of metabolomics makes it ideal to perform early screening for diseases or to follow drug efficacy. Metabolomics, in conjunction with known risk factors, can therefore theoretically be used to detect early metabolic signs of hypertension before major systemic and end–organ damage occur, in order to implement successful lifestyle intervention methods. In individuals presenting with hypertension (or other cardiovascular diseases), it is likely that more than one metabolic pathway and process is affected, which in turn will contribute to the overall disease state. Understanding how metabolites relate with each other and with established risk factors will not only be important in assessing their value as potential biomarkers, but will likely shed more light on our understanding of the vast metabolic web of interactions in global pathways and may also lead to novel connections between metabolic pathways.[13]

Knowledge about preventable diseases is important to promote a healthy lifestyle, especially in a recently urbanized and multi–cultural setting like South Africa. The objective of this study was to investigate the systemic perturbations involved in the pathogenesis of essential hypertension in the black South African male in an attempt to explore the main contributing factors or mechanisms. To achieve this goal, a metabolomics approach was used, employing two popular hyphenated methods which cover a wide variety of metabolite classes. Urine was the chosen sample type of this study, as it constitutes the best matrix for systemic investigation of end–products of metabolism.

Methods

Ethics Statement

The study complied with all applicable institutional guidelines and terms of the Declaration of Helsinki of 1975 (as revised in 2004) for investigation of human participants and was approved by the Ethics Review Board of the North–West University, Potchefstroom Campus (00,036–07–S6).[14] The nature, benefits, and risks of the study were explained to the participants in their mother tongue. Written informed consent was obtained from all participants before being included in the study.

Test Subjects

This study was performed on a subset of samples (n = 25) from the Sympathetic activity and Ambulatory Blood Pressure in Africans (SABPA) cross–sectional study conducted between February 2008 and May 2009.[14] To fit the requirements of the SABPA main study, urbanized participants with similar demographic statistics were needed. It was therefore decided during study design to perform the main study on African and Caucasian male and female educators from schools in the same education district, thus minimizing socio–economic variability between participants. Participants for the SABPA study were recruited from the Dr Kenneth Kaunda Education district in the North–West Province, South Africa, and consisted of 409 educators (roughly equally divided into African and Caucasian males and females), aged 25–65 years. Exclusion criteria for the SABPA study included psychotropic substance users, tympanum temperature >37.5°C, and vaccination or blood donation within 3 months prior to participation. For the present sub–study, additional exclusion criteria were used: since hypertension is one of the most common cardiovascular risk factors in the black South African males,[5,15,16] it was decided to only include African men (n = 101) in this metabolomics study. Furthermore, participants that were HIV–positive (n = 13) and/or used anti–hypertensive drugs (n = 17) were excluded from any further analyses. The remaining participants (n = 71) were ranked according to their ambulatory 24–hour systolic blood pressure (SBP) before they were divided into quintiles (Q1 to Q5). Only the participants in Q1 (lowest SBP, n = 13) and the participants in Q5 (highest SBP, n = 12) were further used in this metabolomics study as illustrated in Figure 1. The reason for only using Q1 and Q5 is that metabolomics investigations are most successfully conducted when the two groups are clearly distinguished from one another.[17] According to the 2013 Guidelines of the European Society of Hypertension/European Society of Cardiology for a 24–hour period,[18] all the participants in Q1 can be classified as normotensive while the participants in Q5 are all hypertensive (SBP ≥130 mm Hg and/or DBP ≥80 mm Hg).

Sample Collection and Preparation

Ambulatory blood pressure measurement (ABPM) over a 24–hour period was done, as it reflects a more accurate