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RESEARCH ARTICLE

Identifying a urinary peptidomics profile for hypertension in young adults: The African-PREDICT study

Urinary peptidomics and hypertension

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Abstract

Hypertension is one of the most important and complex risk factors for cardiovascular diseases (CVDs). By using urinary peptidomics analyses, we aimed to identify peptides associated with hypertension, building a framework for future research towards improved prediction and prevention of premature development of CVD. We included 78 hypertensive and 79 normotensive participants from the African-PREDICT study (aged 20–30 years), matched for sex (51% male) and ethnicity (49% black and 51% white). Urinary peptidomics data were acquired using capillary-electrophoresis-time-of-flight-mass-spectrometry. Hypertension-associated peptides were identified and combined into a support vector machine-based multidimensional classifier. When comparing the peptide data between the normotensive and hypertensive groups, 129 peptides were nominally differentially abundant (Wilcoxon $p < 0.05$). Nonetheless, only three peptides, all derived from collagen alpha-1(III), remained significantly different after rigorous adjustments for multiple comparisons. The 37 most significant peptides (all $p \leq 0.001$) served as basis for the development of a classifier, with 20 peptides being combined into a unifying score, resulting in an AUC of 0.85 in the ROC analysis ($p < 0.001$), with 83% sensitivity at 80% specificity. Our study suggests potential value of urinary peptides in the classification of hypertension, which could enable earlier diagnosis and better understanding of the pathophysiology of hypertension and premature cardiovascular disease development.

KEYWORDS
hypertension, peptidomics, proteomics, SVM

Abbreviations: ABPM, ambulatory blood pressure; African-PREDICT, The African Prospective study on the Early Detection and Identification of Cardiovascular disease and Hypertension; C, cost parameter; CAD, coronary artery disease; CE-MS, capillary electrophoresis mass spectrometer; CKD, chronic kidney disease; CVD, cardiovascular disease; GWAS, genome-wide association studies; HbA1c, glycated haemoglobin; HF, heart failure; ISAK, International Society for the Advancement of Kinanthropometry; LVM, left ventricular mass; LVMi, left ventricular mass index; *n*, number of participants; NRF, National Research Foundation; SAMRC, South African Medical Research Council; SARChI, South African Research Chairs Initiative; SVM, support vector machine; γ, smoothness parameter .

1 | INTRODUCTION

Hypertension is one of the most important and complex risk factors for cardiovascular diseases (CVDs) including stroke [1], coronary artery disease (CAD) [2] and heart failure [3, 4]. Assessing the presence of cardiovascular risk factors in young adults may provide essential information for the prediction and prevention of premature development of CVD. Countless methods are used to gain better insight into the development of CVD [5, 6], including proteomics.

Proteomics is the large-scale study of proteins [7] in various sample matrices such as serum, plasma and urine, with urine being a stable and easily, non-invasively collectable biofluid containing peptides with clinical significance [8]. Under normal physiological circumstances, urinary protein levels are low, however smaller peptides are filtered from the circulation and actively secreted by the kidneys [9]. These peptides can indicate structural changes within the cardiovascular system, such as extracellular matrix remodelling [10]. Urine-based proteomics approaches have been applied to various complex chronic diseases, including cardiovascular conditions [6] wherein multiple peptides were combined into a single classifier and able to accurately predict and diagnose conditions such as left ventricular dysfunction [11], CAD [12–14], heart failure [11, 15, 16], acute kidney injury [17], chronic kidney disease [18–20] and preeclampsia [21].

Whether a single tool can predict hypertension remains unknown. As such, the identification of urinary peptides-associated with hypertension may provide better insight into the molecular pathways and risk factors underlying CVD and improve the understanding of CVD development. We thus aimed to explore if significant urinary peptides can be observed in young individuals with hypertension. We used urinary peptidomics analyses based on CE-MS to define potential differences between matched normotensive and asymptomatic hypertensive young South African adults.

Significance of statement

Based on the analysis of urinary peptidomics data from 78 hypertensive and 79 normotensive participants (aged 20–30 years old), we were able to identify several urinary peptides potentially associated with hypertension. Our study suggests the value of urinary peptides in hypertension, especially collagen type I and III fragments, and that exploring urinary peptides and risk factors contributing to hypertension and CVD, also in young adults, might be a highly promising approach towards better prediction and prevention of premature development of cardiovascular disease.

2 | MATERIAL AND METHODS

2.1 | Study population and sample size

The African Prospective study on the Early Detection and Identification of Cardiovascular disease and Hypertension (African-PREDICT) is a longitudinal study which collected baseline data from 2013 to 2017 and included 1202 young (aged 20- to 30-years old) and healthy adults [22].

To explore independent associations between 24-h ambulatory blood pressure (ABPM) and urinary peptides, 964 participants with complete urinary proteomics data from the baseline phase of African-PREDICT study were included for analysis. However, $n = 10$ participants with incomplete ABPM data were excluded from further analysis, leaving a final sample size of 954 participants (844 normotensive and 110 hypertensive). In line with the 2018 ESC/ESH guidelines [23], 24-h ABPM was used to distinguish between normotensive and hypertensive participants. Participants with 24-h ABPM (day) of ≥135/85 mmHg or 24-h ABPM (night) of ≥120/70 mmHg or 24-h ABPM (mean) of ≥130/80 mmHg were considered hypertensive. All participants working night shifts were excluded from the study.

For the discovery of a urine-based hypertension peptidomics profile, the 844 normotensive and 110 hypertensive participants were matched for sex and ethnicity. Based on available datasets, a power analysis indicated that 64 subjects in each group should be sufficient to detect a 20% change with an alpha error probability of 0.05 and 80% power. Consequently, a subset of 80 hypertensive and 80 normotensive participants were randomly selected for subsequent statistical analysis.

2.2 | Organisational procedures

Participants were recruited from the North-West province, South Africa. Potential participants were invited to the screening phase to determine their eligibility for the research phase of the African-PREDICT study. The inclusion criteria were self-reported black or white ethnicity, aged 20–30 years of age, men or women with no self-reported chronic illness or use of chronic medication, HIV uninfected and office brachial blood pressure <140/90 mmHg. Participants were required to sign a written informed consent form to participate in the study. The African-PREDICT study was approved by the Health Research Ethics Committee of the North-West University in 2012 (NWU-00001-12-A1). This sub-study also adhered to all applicable requirements of the revised Declaration of Helsinki for investigation on human participants and was approved by the Health Research Ethics Committee of the North-West University (NWU-00495-19-A1).

2.3 | Anthropometric measurements and physical activity monitoring

The International Society for the Advancement of Kinanthropometry (ISAK) [24] guidelines were used by an anthropometrist to measure the following of each participant: height (m) by using the SECA 213 Portable Stadiometer (SECA, Hamburg, Germany), weight (kg) by using

the SECA 813 Electronic Scales with weighing capacity up to 200 kg (SECA, Hamburg, Germany) and also waist circumference (cm) (Lufkin Steel Anthropometric Tape; W606PM; Lufkin, Apex, USA). The participant's body mass index (BMI) was calculated with the use of the following formula: weight (kg)/height (m^2). Waist to height ratio ≥0.55 [25] and/or BMI ≥30 kg/m^2 [26] was used to define obesity. ActiHeart physical activity monitors (CamNtech Ltd., England, UK) were fitted to the chest of the participant over a maximum of 7 days, to record their heart rate variability, and to measure energy expenditure.

2.4 | Questionnaires

Participants completed a general health and demographic questionnaire which provided basic information from the participant, including sex, date of birth, home language, marital status, education, employment, income, self-reported smoking and alcohol consumption, family history of disease and use of medication. Each participant's socio-economic score was calculated using a point system that was adapted from Kuppuswamy's Socioeconomic Status Scale [27] for a South African environment, scoring participants in three categories: skill level, education and household income [22]. The scores were used to categorise the low, middle and high socioeconomic groups.

2.5 | Cardiovascular measurements

2.5.1 | Blood pressure

Twenty-four-hour ABPM was determined with a Card(X)plore device validated by the British Hypertension Society (Meditech, Budapest, Hungary). Also, an appropriately sized cuff was used on the participant's non-dominant arm, which measured blood pressure in 30-min intervals during the day (08:00–22:00), and hourly during nighttime (22:00–06:00). Office brachial blood pressure and heart rate (Dinamap® Procare BP monitor, Wauwatosa, USA) were measured in duplicate on the left and right arm of the participant in a sitting position. The SphygmoCor® XCEL device (AtCor Medical Pty. Ltd., Sydney, Australia) was used to calculate estimate central systolic blood pressure.

2.5.2 | Pulse wave velocity

Carotid-femoral pulse wave velocity was measured non-invasively with the use of the SphygmoCor® XCEL device (AtCor Medical Pty. Ltd., Sydney, Australia). Participants were in a supine position and relaxed before the measurements took place. Pulse wave velocity was performed by placing a brachial cuff on the right upper-arm and measured in duplicate along the descending thoraco-abdominal aorta, using the foot-to-foot velocity method.

2.5.3 | Echocardiography

A standard transthoracic echocardiography procedure was performed by a clinical technologist (registered by the Health Professions Council of South Africa) using a General Electric Vivid E9 device (GE Vingmed Ultrasound A/S, Horten, Norway) along with the 2.5–3.5 MHz transducer and a single ECG-lead. Standard methods were used to ensure high quality recordings, applying the recommendations as stated by the guidelines of the European Association of Echocardiography and the American Society of Echocardiography [28, 29]. A trained ultrasound technician obtained and analyses the data. The updated Devereux and Reichek cube formula was used for determining LVM and subsequently indexed by body height (LVMi) [30, 31].

2.6 | Biochemical analysis

Participants were asked to fast for eight hours prior to biological sampling, which included blood sampling (from the antebrachial vein with the use of a winged infusion set taken by a registered nurse) and an early-morning spot urine sample. We used a fasting early morning spot-urine sample since fasting diminishes the chance for unreliable biochemical parameter values caused by any meals. Biological samples were collected and taken to an on-site laboratory to be prepared, aliquoted into cryovials and stored in bio-freezers (−80°C) for later analyses. Basic biochemical measurements included urinary creatinine and albumin, serum total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides, high sensitivity C-reactive protein, gamma-glutamyl transferase, creatinine and glycated haemoglobin (HbA1c) (Cobas Integra 400 plus, Roche, Basel, Switzerland). Serum cotinine (Immulite, Siemens, Erlangen, Germany) was also measured.

2.7 | Urinary peptidomics

The analytical procedure as well as the performance of the CE-MS platform, including sample preparation and data evaluation, was described in detail in refs. [32] and [33]. Sample preparation for urinary peptidomics analyses included the dilution of 700 µL of urine with 700 µL 2 M urea and 0.1 M ammonium hydroxide containing 0.02% sodium dodecyl sulphate. Ultracentrifugation was performed with a 20 kDa molecular weight cut-off Centrisart centrifugal ultrafiltration unit (Sarorius, Göttingen, Germany) at 3000 *x g* for 1 h at 4°C. The filtrate was then desalted to remove urea, electrolytes and salts with a PD-10 desalting column (Amersham Bioscience, Buckinghamshire, UK) and peptide elution was achieved with 0.01% aqueous ammonium hydroxide. Hereafter samples were lyophilised, stored at 4°C, and re-suspended in high-performance liquid chromatography (HPLC) grade water to a final concentration of 2 µg/µL before analysis [34].

Capillary Electrophoresis Time-of-Flight Mass Spectrometry (CE-TOF-MS) was performed using a P/ACE MDQ capillary electrophoresis

system (Beckman Coulter, Fullerton, USA) coupled with a microTOF mass spectrometer (Bruker Daltonic, Bremen, Germany), as previously described [34]. Samples (250 nL) were injected with 2 psi for 99 s and separation of peptides in the cartridge (maintained at 25°C) was achieved at 25 kV for 30 min followed by a 0.5 psi increase in pressure for another 35 min. The running buffer consisted of 79:20:1 (v/v/v) water, acetonitrile and formic acid, and the sheath liquid consisted of 30% isopropanol, 0.4% formic acid in HPLC grade water. The electrospray ionisation sprayer (Agilent Technologies, California, USA) was grounded, and the ion spray inference potential was set at −4.5 kV. Mass spectra over a mass-to-charge ratio of 350–3000 were collected every 3 s.

The Mosaiques Visu software [35] was used for peak picking, deconvolution and de-isotoping of mass spectral ion peaks. The capillary electrophoresis migration time and ion signal intensity were normalised based on the reference signal from internal peptide standards. Data were calibrated utilising 3151 internal standards as reference data points for mass and migration time by applying global and local linear regression, respectively. Reference signals of 29 abundant peptides were used as internal standards for calibration of signal intensity using linear regression. This procedure is highly reproducible and addresses both analytical and dilution variances in a single calibration step [36]. In 60 independent analytic runs of a single urine sample, the coefficient of variation was 10% [33]. The obtained peak list characterises each polypeptide by its calibrated molecular mass [Da], calibrated CE migration time [min] and normalised signal intensity.

The list of peptides from all the samples which fulfilled the quality control criteria (a minimum of 900 features must be detectable with resolution >10,000 in a minimal migration time interval of 10 min, see also ref. [32], and the sample must be calibrated based on the internal standards) were annotated to a list of clustered peptides as described in ref. [37] and compared in a Microsoft Structured-Query Language database. This relational database is based on Microsoft SQL Server management system as a database server and backend. The primary function is storing and retrieving data as requested by other software applications. As a frontend and user interface applications developed in Microsoft Access, in C++ and in Java are used. The database consists more than 20 tables containing the available clinical, demographic and proteomic data. Data access and manipulation is controlled via SQL Server security. All data were anonymised. The 'masterlist' contains 21,559 features previously defined by mass and migration time. To 5067 of these features, which on average account for 62% of the total signal in a urine sample, sequence could be assigned based on high confidence MS/MS identification. Peptides detected with a frequency of ≥30% in at least one group were considered for further analysis.

2.8 | Statistical analysis

We compared the logarithmic (log_2) transformed signal amplitude of the CE-MS urinary polypeptide profile between the hypertensive and normotensive groups using the Wilcoxon rank sum test, followed by adjustment of multiple testing with Benjamini Hochberg procedure. We tested the null hypothesis that the hypertensive and normotensive groups have the same continuous distribution of signal amplitude of the CE-MS urinary polypeptide profile. Peptides were considered significant according to Wilcoxon rank-sum test ($p < 0.05$). Peptides associated with hypertension were combined into a single summary multidimensional classifier with the use of the support vector machine (SVM)-based MosaCluster software (version 1.6.5). Classification was performed by determining the Euclidian distance (the SVM classification score) of the vector to a maximal margin separating hyperplane. The SVM classifier used the log_2 transformed intensities of the peptides as coordinates in an N-dimensional space. The radial basis function was used as kernel to apply the Kernel trick to transform the original low-dimensional observations into a higher-dimensional space in which the samples can be separable. The cost parameter, C, and smoothness parameter, γ, were optimised through complete take-one-out cross validation. We determined the optimal threshold criterion for the classifier to discriminate between control and hypertensive individuals based on maximised Younden's index determined by Receiver Operating Characteristic (ROC) analysis carried out in MedCalc version 12.7.3.0 (MedCalc Software, Mariakerke, Belgium, https://www.medcalc.org/). The ROC plot was obtained and the area under the ROC curve (AUC) was evaluated.

2.9 | Results

From the 160 samples selected, 157 gave acceptable results based on QC standards [32]. A table listing the peptides identified in the samples (in at least 30% of hypertensive and/or normotensive patients) is given in Table S1. These data were used to define urinary peptides associated with hypertension (Figure 1). In Table 1, the general characteristics of the hypertensive and normotensive groups are compared. The distribution ethnicity and sex were similar due to matching. All blood pressure measures, as well as body mass index and waist circumference (with 41% obese against 4% in the hypertensive compared to the normotensive group) were higher in the hypertensive compared to the normotensive group (all $p < 0.001$). There were no differences in self-reported smoking or alcohol use, albumin:creatinine ratio and estimated glomerular filtration rate (all $p > 0.96$), but even in these young participants we found higher (although still within normal ranges) left ventricular mass index, pulse wave velocity, C-reactive protein and gamma-glutamyl transferase in the hypertensive compared to the normotensive group (all $p < 0.002$). The hypertensive group had a less favourable lipid profile, with higher triglyceride- and lower high-density lipoprotein cholesterol levels compared to the normotensive group (all $p < 0.019$).

2.9.1 | Peptidomics data

When applying a frequency threshold of 30%, 1478 urine peptides with a known sequence could be detected in at least one investigated group (normotensive and hypertensive, data on the abundance of the 1478

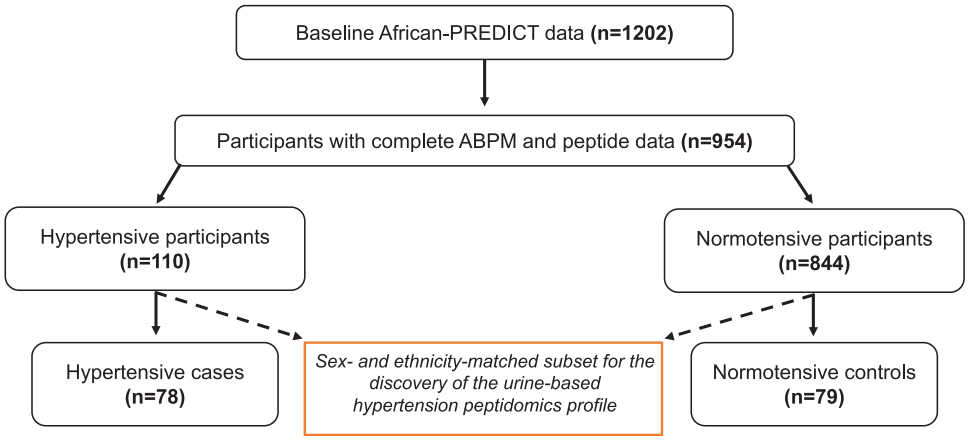


FIGURE 1 Experimental design indicating the selection of the hypertensive ($n = 78$) and normotensive groups ($n = 79$).

peptides in each sample are given in the Table S1). When comparing the peptide data between the normotensive and hypertensive individuals, 129 peptides were nominally differentially abundant (Wilcoxon $p < 0.05$). However, after rigorous adjustment for multiple comparisons (Benjamini Hochberg), only three peptides, all derived from collagen alpha-1(III), remained significantly altered and were higher in the hypertensive compared to the normotensive group.

We combined the 37 nominally most significant peptides (Wilcoxon $p \leq 0.001$) (Table 2) into a classifier and optimised the approach applying a 'take-one-out' strategy to determine if a combination of multiple signals can discriminate between the analysed groups. Applying this approach, a classifier based on 20 peptides (marked in Table 2) was established ($C = 5120$, $\gamma = 0.000102$). When applying the scoring from the complete take-one-out cross-validation, the average score from the classifier differed significantly between the groups ($p < 0.001$). ROC analysis revealed an AUC of 0.85, a sensitivity of 83% and specificity of 80% (Figure 2).

2.9.2 | Individual peptides

Of the 129 urinary peptide fragments that were nominally differentially abundant between the hypertensive and normotensive group (Wilcoxon $p < 0.05$, all listed in Table S2), 98 were derived from different collagens, with collagen alpha-1(I) ($n = 28$) and collagen alpha-1(III) ($n = 19$) being the most prominent represented members of the collagen family. From the 31 non-collagen peptides, at least two peptides were derived from CD99 antigen, polymeric immunoglobulin receptor, keratin type 1 and alpha-1-antitrypsin. Individual peptides were found among others from matrix Gla protein, ProSAAS (Proprotein convertase 1 inhibitor), mucin-16 and apolipoprotein CII.

3 | DISCUSSION

In this study, we explored the peptidomic profiles of young normotensive and hypertensive adults to identify urinary peptides associated with hypertension. Hypertension is an important risk factor for CVD since the early development of high blood pressure (even in childhood) usually tracks into adult life [38]. Hypertension is also a multifactorial disorder, which makes it a complex condition to detect and prevent [4, 39, 40]. To study hypertension in isolation is extremely challenging and nearly impossible since it is linked to numerous modifiable and non-modifiable risk factors such as diet [41] and age [42], as well as other comorbidities including obesity, diabetes, kidney disease and heart disease [43–46]. Genome-wide association studies (GWAS) have shown that there are hundreds of causative genes that contribute to hypertension but with moderate effects [47–51], hence genomics studies require large sample sizes due to the complexity of the genetic mechanisms underlying hypertension. Genomic approaches have shown that many of the genes relating to hypertension also relates to shared mechanisms such as inflammation, diabetes, obesity, CAD and CKD [49, 52–55]. By following a peptidomics approach, such wider pathways may be displayed.

We explored the peptidomics profiles of a group at early stages of hypertension without overt organ damage by applying statistical methods combined with SVM. We identified several peptides that showed a nominal different distribution between the hypertensive and normotensive groups. When combining these peptides into an SVM-driven classifier, separation of the two groups with an AUC of 0.85 was possible, indicating that the peptides do contain complementary information. However, to assess the degree of association of the peptides or the classifier with high confidence, evaluation in an independent dataset is required. We also do not propose the use of peptides or the classifier as biomarkers for blood pressure, as blood pressure can be easily measured using the devices currently available. The aim of the study was (1) to investigate if urinary peptides are significantly associated with blood pressure, and (2) if this is the case, to initiate an in depth investigation of this association, aiming towards a deeper understanding of the molecular pathophysiology.

After the data were normalised using internal standards [36], the majority of the identified peptides ($n = 129$) were higher in the hypertensive compared to the normotensive group, which may in part be as a result of the increased blood pressure in the hypertensive group, which

TABLE 1 Characteristics of hypertensive and normotensive groups.

	Normotensive (n= 79)	Hypertensive (n= 78)	p-value
Age (years)	24.6 ± 2.98	25.0 ± 3.25	0.49
Ethnicity (black), *n* (%)	39 (49)	38 (49)	0.94
Sex (male), *n* (%)	40 (51)	40 (51)	0.94
Body mass index (kg/m^2)	21.6 ± 3.49	28.8 ± 7.96	<0.001
Waist circumference (cm)	73.3 ± 10.0	88.2 ± 15.7	<0.001
Body height (m)	1.68 ± 0.09	1.70 ± 0.10	0.17
Cardiovascular measurements			
24 h SBP (mmHg)	104 ± 5.34	132 ± 7.29	<0.001
24 h DBP (mmHg)	62 ± 4.10	77 ± 5.49	<0.001
Daytime SBP (mmHg)	109 ± 6.41	137 ± 8.5	<0.001
Daytime DBP (mmHg)	67 ± 5.15	82 ± 6.61	<0.001
Nighttime SBP (mmHg)	95 ± 6.21	123 ± 8.99	<0.001
Nighttime DBP (mmHg)	53 ± 4.85	67 ± 6.98	<0.001
Pulse wave velocity (m/s)[a]	6.26 (5.20; 7.90)	6.69 (4.83; 8.16)	0.002
LVMi (g/m^2)	28.3 ± 6.64	35.7 ± 8.33	<0.001
Biochemical analysis			
Total cholesterol (mmol/L)	3.50 (1.98; 5.97)	3.63 (1.99; 5.82)	0.47
LDL cholesterol (mmol/L)	2.20 (1.16; 4.51)	2.27 (0.98; 4.32)	0.57
HDL cholesterol (mmol/L)	1.14 (0.60; 1.72)	0.98 (0.46; 1.84)	0.019
Triglycerides (mmol/L)	0.65 (0.29; 2.02)	0.81 (0.36; 2.71)	0.018
HbA1c (mmol/L)	5.22 (4.69; 5.82)	5.29 (4.66; 5.89)	0.18
C-reactive protein (mg/L)	0.56 (0.05; 5.80)	1.14 (0.12; 9.53)	0.001
Cotinine (ng/mL)	4.23 (1.00; 382)	4.46 (1.00; 360)	0.89
γ-glutamyl transferase (U/L)	13.3 (4.90; 33.1)	22.0 (7.69; 97.0)	<0.001
eGFR (mL/min/1.73 m^2)	130 (85.7; 170)	128 (86.3; 173)	0.63
ACR (mmol/L)	0.49 (0.20; 2.13)	0.47 (0.13; 2.17)	0.81
Lifestyle			
Socio-economic score	20.1 ± 6.40	20.6 ± 6.69	0.65
Self-reported smoking, *n* (%)	24 (30)	24 (30)	0.96
Self-reported alcohol use, *n* (%)	38 (48.0)	49 (63.0)	0.051

Note: Values are arithmetic mean and standard deviation, geometric mean (5th and 95th percentile).
Abbreviations: ACR, albumin-creatinine ratio; CAD, coronary artery disease; CKD, chronic kidney disease; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; HbA1c, glycated haemoglobin; HDL, high density lipoprotein; LDL, low-density lipoprotein; LVMi, left ventricular mass index; SBP, systolic blood pressure.
[a]Adjusted for mean arterial pressure.

consequently would lead to an increase in glomerular filtration rate. Furthermore, most of the peptides for which a significant difference between the hypertensive and the normotensive group was observed, were fragments of different collagens, especially collagen type I and III. This is to some extent expected since (1) collagen peptides are among the most abundant peptides in urine [33] and (2) hypertension is expected to have an impact on the structure of the extracellular matrix (ECM) [56], hence on collagen. Moreover, collagen type I and III are the major collagens found in the vascular ECM and provide mechanical and tensile strength in blood vessels [57]. With increased blood pressure, the mechanical strain exerted on the vascular wall increases, which may lead to the up-regulation of collagen synthesis and deposition to maintain vascular integrity [58]. Over time, this may lead to stiffer arteries [59] and consequently further increase blood pressure [60]. As such, many of the differences observed in peptides may be due to hypertension.

In previous studies, we and others have identified multiple molecules associated with blood pressure or the prediction thereof, including among others reactive oxygen species [61, 62], glutathione reductase [63], C-reactive protein [64, 65], nitric oxide-related markers [66, 67], 8-oxo-7,8-dihydro-2′-deoxyguanosine [68], L-carnitine, long-chain acylcarnitine [69], uric acid [70, 71] and amino acids, such

TABLE 2 Thirty seven most significant peptides differing between the normotensive and hypertensive group (all Wilcoxon $p \leq 0.001$).

ID	Mass (Da)	CE time (min)	Sequence	Start AA	Stop AA	Symbol	Protein name	Unadjusted Wilcoxon *p*-value	Fold change
9002	1885.77	20.72	GGEGKGGSDGGGSHRKEGEE	101	120	CD99	CD99 antigen	1.93E-03	2.37
7823	1752.78	19.61	PpGKNGDDGEAGKpGRpG	225	242	COL1A1	Collagen alpha-1(I) chain	8.92E-03	1.77
12661[a]	2377.11	20.84	GKNGDDGEAGKPGRpGERGPpGpQ	227	250	COL1A1	Collagen alpha-1(I) chain	3.75E-03	0.76
2517[a]	1178.54	27.34	QDGRpGpPGPpG	561	572	COL1A1	Collagen alpha-1(I) chain	9.87E-03	1.96
17360[a]	3282.47	30.06	GPSGASGERGPPGPMGPpGLAGpPGESGREGAPGAEG	986	1022	COL1A1	Collagen alpha-1(I) chain	3.43E-03	1.59
18765	3680.70	31.83	GLpGKDGETGAAGpPGPAGPAGERGEQGAPGPSGFQGLPGP	630	670	COL2A1	Collagen alpha-1(II) chain	6.49E-03	2.78
11065[a]	2168.97	32.92	ppGADGQpGAKGEQGEAGQKGDA	838	860	COL2A1	Collagen alpha-1(II) chain	3.22E-03	1.45
7054[a]	1675.70	29.31	GpPGPpGTSGHpGSpGSpG	180	198	COL3A1	Collagen alpha-1(III) chain	3.37E-03	1.72
3389	1272.53	27.73	EpGRDGVpGGPGm	523	535	COL3A1	Collagen alpha-1(III) chain	2.99E-03	1.14
6617	1627.70	29.59	MpGSpGGpGSDGKpGpPG	538	555	COL3A1	Collagen alpha-1(III) chain	4.41E-03	1.27
6961[a]	1664.74	29.98	GLpGTGGPpGENGKPGEp	642	659	COL3A1	Collagen alpha-1(III) chain	8.73E-05	1.32
8526	1834.82	31.17	GLpGTGGPpGENGKpGEpGP	642	661	COL3A1	Collagen alpha-1(III) chain	7.50E-03	1.48
6841[a]	1649.73	22.75	GPpGpAGQpGDKGEGGAPG	768	786	COL3A1	Collagen alpha-1(III) chain	6.13E-05	1.53
10110[a]	2025.86	32.27	SEGSpGHpGQpGpPGPPGApGp	1174	1195	COL3A1	Collagen alpha-1(III) chain	2.07E-05	1.47
9891	1993.88	32.19	SEGSPGHpGQPGpPGpPGApGP	1174	1195	COL3A1	Collagen alpha-1(III) chain	5.27E-03	1.46
7935[a]	1764.78	29.71	QGLpGApGDQGQRGpPGE	495	512	COL9A1	Collagen alpha-1(IX) chain	2.75E-03	1.94
9719	1971.94	24.77	RGDPGPQGppGLALGERGpP	2008	2027	COL7A1	Collagen alpha-1(VII) chain	6.40E-03	1.30
5288	1473.63	22.33	FPGQTGPRGEMGQp	2405	2418	COL7A1	Collagen alpha-1(VII) chain	9.25E-04	1.42
10814[a]	2125.94	32.70	GPpGDDGMRGEDGEIGpRGLp	625	645	COL11A1	Collagen alpha-1(XI) chain	3.54E-03	1.73
4847[a]	1425.59	22.34	GGDkGEDGDPGQPGp	1332	1346	COL11A1	Collagen alpha-1(XI) chain	5.76E-03	1.12
1818[a]	1103.48	26.10	SGGGGSGGGGGVGGAGG	430	446	COL17A1	Collagen alpha-1(XVII) chain	6.95E-03	2.45
2793[a]	1209.49	29.08	GSSGSPGpQGpPGP	668	681	COL17A1	Collagen alpha-1(XVII) chain	3.43E-03	0.52
9697	1969.85	24.84	PGpPGPHGPPGpmGPHGLpGP	531	551	COL25A1	Collagen alpha-1(XXV) chain	2.95E-03	1.53
9548[a]	1950.85	35.51	GTDGpMGpHGpAGPKGERGE	553	572	COL25A1	Collagen alpha-1(XXV) chain	9.19E-03	1.42
4373[a]	1373.60	38.77	pPGPAGNPGPSpNSP	257	271	COL26A1	Collagen alpha-1(XXVI) chain	4.57E-03	2.02
8187	1794.74	30.33	GpRGQLGPEGDEGPmGpP	937	954	COL27A1	Collagen alpha-1(XXVII) chain	3.35E-03	1.51
5227[a]	1466.64	29.87	GpSGPpGPDGNKGEpG	610	625	COL1A2	Collagen alpha-2(I) chain	4.01E-03	4.02
7334	1702.70	29.63	QGDSGPQGPpGSEGFTGp	141	158	COL4A2	Collagen alpha-2(IV) chain	3.30E-03	1.78

(Continues)

TABLE 2 (Continued)

ID	Mass (Da)	CE time (min)	Sequence	Start AA	Stop AA	Symbol	Protein name	Unadjusted Wilcoxon *p*-value	Fold change
7368[a]	1706.78	22.75	pGLKGDRGSpGMDGFQG	916	932	COL4A2	Collagen alpha-2(IV) chain	4.43E-03	1.38
3023[a]	1235.53	19.74	pGApGPPGHPSHP	170	182	COL5A2	Collagen alpha-2(V) chain	3.65E-03	3.20
6852	1651.70	29.58	GPpGFQGEpGpQGEPGP	182	198	COL8A2	Collagen alpha-2(VIII) chain	5.46E-03	1.40
9311	1922.78	40.03	VPGSpGITGPpGPPGPpGPpGA	511	532	COL8A2	Collagen alpha-2(VIII) chain	8.28E-03	2.66
11161	2180.98	33.32	DQGpQGPQGVPGTSKDGQDGApG	617	639	COL9A3	Collagen alpha-3(IX) chain	3.67E-03	1.62
7665	1736.77	32.31	TQLLNNMRSQYEQL	315	328	KRT10	Keratin, type I cytoskeletal 10	5.12E-03	0.33
16880[a]	3173.56	22.89	GRPEAQPPPLSSEHKEPVAGDAVPGPKDGSAP	26	57	VGF	Neurosecretory protein VGF	5.52E-03	0.26
5166[a]	1457.63	28.63	EGASRETAGGGPSEGP	156	171	SERPINE3	Serpin E3	5.17E-03	1.33
9606	1958.88	32.22	LDTYPNDETTERVFPY	161	176	LMAN2	Vesicular integral-membrane protein VIP36	1.77E-03	2.39

Given are the ID in the SQL database, the theoretical mass and the normalised migration time, the sequence ('p' –hydroxyproline, 'm – oxidized methionine'), the start and stop amino acid positions, the parental protein, the unadjusted *p*-value for a significant difference between the groups, and the fold change calculated based on the average peptide abundance in the hypertensive compared to the normotensive group.
[a]Peptides retained in the urinary classifier.

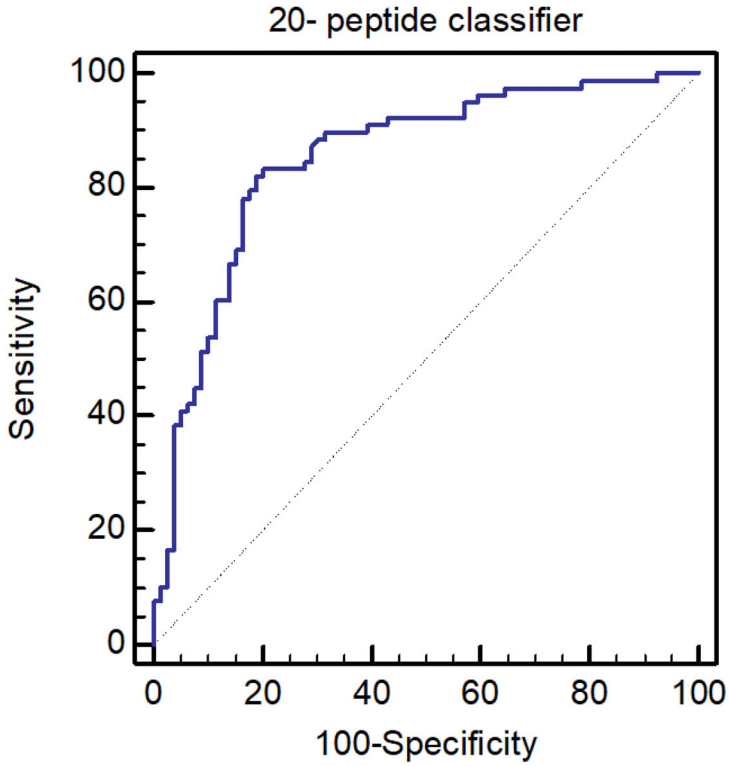
as serine, glycine, methionine, arginine and alanine [44, 72]. Knowing the complexity of hypertension, it might be beneficial to approach these blood pressure associated molecules in a combined way, rather than individually, to better display (and also predict) the complex, multifactorial condition like hypertension and related organ damage.

Towards this direction, proteomics studies enable a more comprehensive assessment of molecular changes associated with diseases and have been already successfully applied in different disease populations. Examples of such studies include a urinary proteomics study on CAD which identified the urinary polypeptide CAD238 score [73] and successfully validated this score in independent cohorts as a potential cardiovascular biomarker for CAD [12, 13]. A proteomics study done on CKD patients, identified and validated 273 urinary peptides (CKD273) as a proteomic-based classifier of CKD, with a significantly higher CKD273 score in CKD patients compared to healthy controls [20, 74]. A proteomics study on hypertensive patients with left ventricular (LV) dysfunction identified collagen alpha-1(V), WW domain-binding protein 11 (WBP11) and isoform 1 of collagen alpha-1(XXVI) to be significantly downregulated compared to controls [75]. However, studies on proteomics in hypertension are sparse.

A recent review on proteomics in hypertension indicated that proteomic studies do have the potential to deliver biomarkers, identify disease associated pathways and drug targets [76]. However, there are a few challenges that remain to be overcome, such as the multifactorial complexity of hypertension, expensive nature of proteomics, along with the translatability of the pre-clinical research [76]. For the latter, experimental models are mainstay at the moment for proteomics in hypertension, since it appears to be more suitable to study a single primary cause for hypertension, for example angiotensin II infused rats [77]. As such, performing future proteomics studies in patients with a known primary cause for hypertension, such as low-renin hypertension phenotypes [78], might be a promising approach. On the other hand, some clinical studies have been successful in identifying proteomic features that are able to differentiate between hypertensive and normotensive groups [75, 79]. However, such studies are either difficult to compare with each other, small or unstandardised. This indicates a need for setting up a standardised protocol to improve on data comparability. Also, the majority of proteomics research focus on the consequence of hypertension, such as heart failure, rather than the molecular pathways underlying hypertension, indicating a room for future investigation.

Given these facts, and also previous focus of proteomics studies in older subjects with hypertension (between 30 and 50 years old) or in groups with organ damage as a result of hypertension, such as left ventricular hypertrophy [75], the results of our study cannot be easily compared with previous findings.

However, even in this young population with seemingly uncomplicated hypertension, it is difficult to isolate hypertension as we already found higher body mass index, left ventricular mass and arterial stiffness (all p-value <0.002) in the hypertensive compared to the normotensive group. The latter may complicate the ability for a peptidomics approach to identify peptides associated solely with hypertension, as the peptidomic profiles may be affected by these factors.



AUC	0.85
95% CI	0.79 - 0.90
p-value	<0.0001
Sensitivity (95% CI) / Specificity (95% CI) at the cut-off level of -0.043	83.33 (73.2 - 90.8) / 79.75 (69.2 - 88.0)

FIGURE 2 Receiving operating characteristics curve for the 20 peptide model.

Moreover, hypertension, especially at earlier stages, is a functional phenotype where subtle changes in one pathway can be compensated by another. The reason for hypertension in this young cohort may therefore be more functional and not associated with extensive vascular remodelling and organ damage.

The latter is further supported when comparing the urine peptides associated with hypertension defined in this study, with the urine peptides associated with CKD [80, 81] or heart failure (HF) [82]. As evident from Table S3, when comparing the regulation of the 37 most significant peptides in this study with the changes observed in CKD or HF, no convincing concordance can be detected. This may indicate that the molecular mechanisms for onset and progression of CKD and HF differ from the molecular changes relevant in hypertension. Furthermore, the datasets were also matched for blood pressure, hence while hypertension is certainly a risk factor for CKD and HF, the distribution of this risk factor was similar in the comparisons CKD or HF versus controls.

Our study has some limitations. The number of subjects for which peptidomics analysis was conducted was rather small, and as such, was not adequate to perform the adjustment for multiple testing, along with the adjustment for potential confounding factors (e.g., BMI). Along these lines, validation of the performance of the classifier in the independent set of samples was not possible. Given the pre-selected age range of the individuals, it needs to be investigated if the findings can be generalised in other age ranges. Due to the cross-sectional design, the prognostic value of the classifier for detection of future hypertension, or other endpoints (CVD), could not be evaluated. Collectively, the findings of our study require further validation in the larger, statistically well-powered study, and in addition, it would be interesting to assess the prognostic value of the identified peptides/classifier in the longitudinal study. Nonetheless, our results are novel and may help pave the way for future hypertension research in a proteomics setting.

4 | CONCLUSION

Multiple urinary peptides appear to be altered in hypertension, especially collagen type I and III fragments, as seen in these young hypertensive adults. Given that hypertension is a multifactorial health

problem (in young adults and in general), the use of single biomarker (e.g., single peptides) might not be sufficient to comprehensively reflect the complex condition. This could be addressed by combining multiple biomarkers into a panel, or even one step further, by combining different – omics data traits and other risk factors, to better characterise molecular pathophysiology underling hypertension, and improve on prediction and prevention of premature development of cardiovascular disease.

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CONFLICT OF INTEREST STATEMENT

Harald Mischak is the co-founder and co-owner of Mosaiques Diagnostics. Agnieszka Latosinska is employed by Mosaiques Diagnostics. All other authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data sharing is available from the principal investigator of the African-PREDICT study on reasonable request. A data sharing agreement will be set up and submitted to the Health Research Ethics Committee (HREC) of the North-West University, where data will be shared if approved by the HREC.

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SUPPORTING INFORMATION

Additional supporting information may be found online https://doi.org/10.1002/pmic.202200444 in the Supporting Information section at the end of the article.

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