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ORIGINAL ARTICLE

The link between vascular deterioration and branched chain amino acids in a population with high glycated haemoglobin: the SABPA study

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Abstract Globally the prevalence of non-communicable diseases, such as hypertension and type 2 diabetes, are escalating. Metabolomic studies indicated that circulating branched chain amino acids (BCAAs) are associated with insulin resistance, coronary artery disease and increased risk for cardiovascular events. We aimed to extend the current understanding of the cardiovascular risk associated with BCAAs. We explored whether BCAAs are related to markers of cardiovascular disease in a bi-ethnic population and whether this relationship was influenced by chronic hyperglycaemia. We included 200 African and 209 Caucasian participants, and determined their ambulatory blood pressure and carotid intima-media thickness (cIMT). We analysed blood samples for glycated haemoglobin (HbA1c) and BCAAs. Participants were stratified into two groups according to their HbA1c value using the median cut-off value of 5.6 %. Ambulatory BP, cIMT and BCAAs were significantly higher (all $p < 0.001$) in the high HbA1c group. Single regression analyses indicated significant positive associations of ambulatory blood pressure and cIMT with BCAAs (all $p < 0.05$) in both the groups. These associations between ambulatory systolic blood pressure (SBP) ($r = 0.16$, $p = 0.035$) and cIMT ($r = 0.22$, $p = 0.004$) with BCAAs remained in the high HbA1c group after adjusting for age, gender, ethnicity and body mass index (BMI) and were confirmed in multiple regression analyses (ambulatory SBP: $R^2 = 0.17$, $\beta = 0.21$, $p = 0.005$ and cIMT: $R^2 = 0.30$, $\beta = 0.19$, $p = 0.003$). Our results demonstrate that BCAAs are independently related to ambulatory BP and cIMT in individuals with high HbA1c levels and suggest that potential cardiovascular deterioration accompany the rise in BCAAs in conditions of hyperglycaemia.

Keywords Ambulatory blood pressure · Carotid intima-media thickness · Branched chain amino acids · Glycated haemoglobin

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Introduction

Non-communicable diseases have been listed as one of the primary causes of death worldwide (Kearney et al. 2005; Danaei et al. 2011). Sub-Saharan Africa is no exception, where the incidence of cardiovascular disease, hypertension (Addo et al. 2007), impaired glucose tolerance and diabetes (Mbanya et al. 2010) are escalating at an alarming rate. However, our understanding of all the complex processes involved in the development and progression of non-communicable diseases is still limited. Metabolic changes may occur in disease states. In the case of insulin resistance, metabolic derangements are not only restricted to carbohydrates and fatty acid metabolism (Carvajal and Moreno-Sánchez 2003), but also altered protein and amino acid metabolism, but the extent to which these factors relate to non-communicable diseases has not been sufficiently investigated.

The branched chain amino acids (BCAAs) (leucine, isoleucine and valine) are essential amino acids that cannot be synthesized *de novo*. The metabolic requirements thereof range from the least amount required for normal physiological function to a maximum level (Layman 2003), where excessive intake or inborn errors of metabolism cause accumulation of BCAAs and its metabolites. This may

produce adverse effects ranging from neurological distress to cardiomyopathy (Ogier de Baulny and Saudubray 2002).

Recently, metabolomic studies indicated that BCAAs and related compounds are associated with insulin resistance (Shah et al. 2012). It was also demonstrated that BCAAs and their metabolites are independently associated with coronary artery disease and risk for cardiovascular events (Shah et al. 2010), and that BCAAs and its metabolites can distinguish families with premature coronary artery disease (Shah et al. 2009). However, none of these studies investigated BCAA levels and their associations with vascular deterioration in subjects with impaired glucose metabolism. The aim of this study was therefore to investigate associations of BCAAs with ambulatory blood pressure (BP) and carotid intima-media thickness (cIMT) in a bi-ethnic population stratified according to high and low levels of glycated haemoglobin (HbA1c).

Materials and methods

Study population

This study is rooted in the Sympathetic activity and Ambulatory Blood Pressure in Africans (SABPA) study, a cross-sectional, target population study. Participants included 200 African and 209 Caucasian educators (25–60 years) from the Kenneth Kaunda Education district in the North-West Province of South Africa. Power calculations based on the largest standard deviation of ambulatory BP have shown that 50 participants per group in this type of study are sufficient to show significant differences in biological profiles (Hojo et al. 1997). Exclusion criteria included elevated ear temperature (>37.5 °C), use or abuse of psychotropic substances, use of α- and β-blockers, blood donors, individuals vaccinated 3 months prior to participation and pregnant or lactating women. In addition, 6 participants with missing HbA1c data were excluded, leaving a total of 403 participants. Participants were fully informed about the objectives and procedures of the study prior to their recruitment and informed consent was obtained from the selected participants prior to commencement of the study. The study was approved by the Ethics Review Board of the North-West University (Potchefstroom campus) (NWU-00036-07-S6) and fulfilled all applicable requirements of the international regulations as in the Helsinki declaration of 1975 (revised in 2008).

Study protocol

On the first day the ambulatory blood pressure monitoring (ABPM) apparatus was attached to the participants' non-dominant arm (07:00) at their workplace to measure ambulatory BP during the working day. At 16:30 the participants were transported to the Metabolic Unit Research Facility of the North-West University, where they completed a general health questionnaire. At dinnertime they received a standardised meal and had their last beverages at 20:30. They were encouraged to go to bed at around 22:00. After the last BP measurement at 06:00 the next morning, the ABPM apparatus was removed and subsequent measurements commenced.

Anthropometric and physical activity measurements

Height and weight of participants wearing minimal clothing were measured to the nearest 1.0 cm or 1.0 kg using calibrated instruments (Precision Health Scale, A & D Company, Tokyo, Japan; Invicta Stadiometer, IP 1465, Invicta, London, UK; Holtain unstretchable flexible 7 mm wide metal tape), and body mass index (BMI) was calculated. Anthropometric measurements were taken in triplicate using standard methods (International Society for the Advancement of Kinanthropometry 2001). The Actical® (Mini Mitter, Bend OR, Montréal, Québec), an omnidirectional accelerometer monitor was worn around the waist and determined physical activity for 24 h taking the metabolic rate into account.

Cardiovascular measurements

The ABPM apparatus (Meditech Cardiotens CE120®, Budapest, Hungary) was programmed to measure BP at 30 min intervals during the day (07:00–22:00) and 1 h intervals during night-time (22:00–06:00). The ambulatory BP data were loaded onto a database using the CardioVisions 1.19 Personal Edition (Meditech, Budapest, Hungary). The successful inflation rate over the 24-h period was 78.8 %. Hypertensive status was classified from the ambulatory BP measurements as SBP and/or diastolic blood pressure (DBP) >125/80 according to the Guidelines of the European Society of Hypertension for a 24-h period (Mancia et al. 2007).

Carotid intima-media thickness (cIMT) was obtained with the SonoSite Micromaxx ultrasound system (Sonosite Inc., Bothell, WA, USA) and a 6–13 MHz linear array transducer. Images from at least two optimal angles of both the left and right common carotid arteries were recorded, analysed and measured with the Artery Measurement Systems (AMS) II v1.139 (Gothenburg, Sweden) software. Far wall measurements of cIMT were used. The carotid cross-sectional wall area (CSWA), as measure of structural rather than functional changes in luminal diameter was calculated as follows: $\mathrm{CSWA} = (d/2 + \mathrm{CIMT})^2 - (d/2)^2$, where d denotes the luminal diameter.

Biochemical measurements

Fasting blood samples were obtained and blood spots were made on Guthrie cards (a piece of card with filter paper like texture) (Hannon et al. 2007). Serum and plasma were prepared according to standardised procedures. All samples were stored at –80 °C until analysed. Fasting glucose levels (Konelab™ 20I Sequential Multiple Analyser Computer, Thermo Scientific, Vantaa, Finland and Unicel DXC 800, Beckman and Coulter, Germany) and HbA1c were determined (Integra 400, Roche, Basel, Switzerland) on sodium fluoride plasma and EDTA whole blood, respectively. Total cholesterol, high-density lipoprotein cholesterol, triglycerides, high sensitivity C-reactive protein (CRP) and γ-glutamyl transferase (γ-GT) were analysed in serum (Konelab™ 20I Sequential Multiple Analyser Computer, Thermo Scientific, Vantaa, Finland and Unicel DXC 800, Beckman and Coulter, Germany). Low-density lipoprotein cholesterol levels were calculated with the Friedewald equation (Friedewald et al. 1972). Serum cotinine was determined with a homogeneous immunoassay (Automated Modular, Roche, Basel, Switzerland).

Leucine/isoleucine and valine levels were determined with an electrospray ionisation tandem mass spectrometry method (Chace et al. 1997). Dried blood spots on Guthrie cards were prepared by punching out a 6.35 mm diameter circle into a 1 ml vial with a standard paper punch. This corresponds to approximately 11 µl of whole blood. To the dried blood spots 400 µl of deuterated amino acids (internal standard solution) was added. The internal standard solution contained deuterated amino acids in methanol with the following concentrations: 17.43 µmol/l for [d_{10}]-L-isoleucine, 32.20 µmol/l for [d_8]-L-valine, 15.99 µmol/l for [d_2]-glycine, 3.98 µmol/l for [d_3]-methyl-L-methionine, 5.77 µmol/l for [d_5]-ring-L-phenylalanine, 3.28 µmol/l for [d_5]-L-glutamine, 14.89 µmol/l for [d_5]-indole-L-tryptophan, 14.16 µmol/l for [d_4]-L-lysine:2HCl and 4.21 µmol/l for [d_4]-L-citrulline. The dried blood spots and internal standard solution were allowed to stand for 20 min at room temperature, after which it were centrifuged for 30 min. The samples were transferred to a new tube evaporated to dryness under a gentle stream of nitrogen at 55 °C. To the dried residue, 200 µl 3 N butanolic HCl was added and the samples were incubated at 55 °C for 20 min. The butylated samples were evaporated to dryness again under a stream of nitrogen at 55 °C. The dried residue was reconstituted in water:acetonitrile (50:50) (v/v) containing 0.1 % formic acid. An Agilent 1200 series liquid chromatograph (Santa Clara, CA, USA) with a 96-well plate sampler was used for sample handling as well as mobile phase delivery. Samples (10 µl of each) were injected and a constant flow rate of 0.2 ml/min was maintained throughout the run. The mobile phase consisted of 0.1 % formic acid in water:acetonitrile (50:50) (v/v). The tandem mass spectrometry (MS/MS) analysis was performed on an Agilent 6410 Triple Quadrupole (Santa Clara, CA, USA) in positive ionisation. Amino acids were analysed in MRM mode and quantified by comparison of the signal intensity of amino acids against the signal intensity of the corresponding deuterated analogues. Leucine and isoleucine were not chromatographically separated and are therefore reported as one value. Both the intra- and inter-assay coefficients of variation for all the assays were <10 %.

Statistical analyses

Statistical analyses were performed with Statistica 11 (Statsoft Inc., Tulsa, OK, USA) and Graphpad Prism 5.03 for Windows (Graphpad Software, San Diego, California, CA). Data were expressed as arrhythmic mean and standard deviation for normal distributed variables. Variables with a non-Gaussian distribution were logarithmically transformed (CRP, leucine/isoleucine, valine, γ-GT) and the central tendency and spread represented by the geometric mean and the 5th and 95th percentile intervals. Interactions of HbA1c levels were tested for the relationship between BP and leucine/isoleucine and valine, and for the relationship between cIMT and leucine/isoleucine and valine, using multiple regression analyses. We stratified the groups into low and high HbA1c groups using the median cut-off of 5.6 %. This stratification level is also in accordance with the American Diabetes Association and close to the HbA1c cut-off for pre-diabetes (5.7–6.4 %) (Standards of Medical Care in Diabetes, Diabetes Care 2010—American Diabetes Association). Means and proportions were compared between the low and high HbA1c groups using independent *t* tests and Chi-square tests. Associations were investigated using single regression analysis, and scatterplots were constructed. Partial regression analyses were performed while adjusting for age, gender, ethnicity and BMI. We investigated independent associations between ambulatory BP and cIMT with BCAAs (leucine/isoleucine and valine, respectively) and included covariates using a forward stepwise procedure. Covariates included ethnicity, gender, age, BMI, total energy expenditure, cotinine, γ-GT, total cholesterol, CRP and leucine/isoleucine or valine. Ambulatory SBP was also included as covariate for the associations with cIMT. Sensitivity analyses included the exclusion of HIV-infected participants ($n = 10$), participants with a HbA1c value >6.5 % or those using diabetes medication ($n = 36$) and participants using anti-hypertensive medication ($n = 62$).

Table 1 Characteristics of the study population

	HbA1c < 5.6 %	HbA1c ≥ 5.6 %	*p* values
N	225	178	
Gender, male, *n* (%)	92 (40.9)	109 (61.2)	<0.001
Ethnicity, African, *n* (%)	57 (33.3)	120 (67.4)	<0.001
Age (years)	43.0 ± 10.1	46.7 ± 8.50	0.016
Anthropometric measurements			
Height (m)	1.70 ± 0.10	1.69 ± 0.10	0.55
Weight (kg)	78.1 ± 18.1	88.7 ± 20.6	<0.001
Body mass index (kg/m^2)	27.1 ± 5.58	31.1 ± 7.15	<0.001
Cardiovascular measurements			
Ambulatory systolic BP (mmHg)	124 ± 12.8	134 ± 15.6	<0.001
Ambulatory diastolic BP (mmHg)	77 ± 9.01	83 ± 10.4	<0.001
Heart rate (beats/min)	74.7 ± 10.1	79.0 ± 11.5	<0.001
Carotid intima-media thickness (mm)	0.64 ± 0.14	0.70 ± 0.14	<0.001
Cross-sectional wall area (mm^2)	12.9 ± 3.76	15.0 ± 3.95	<0.001
Biochemical analyses			
Glucose (mmol/l)	5.30 ± 0.84	6.14 ± 2.05	<0.001
HbA1c (%)	5.33 ± 0.20	6.35 ± 1.15	<0.001
Total cholesterol (mmol/l)	5.08 ± 1.29	5.10 ± 1.32	0.90
HDL cholesterol (mmol/l)	1.22 ± 0.37	1.09 ± 0.35	<0.001
LDL cholesterol (mmol/l)	3.64 ± 1.17	3.69 ± 1.18	0.71
Triglycerides (mmol/l)	1.10 ± 0.68	1.59 ± 1.34	<0.001
C-reactive protein (log mg/l)	2.37 (0.80; 18.0)	3.95 (0.95; 31.0)	<0.001
Leucine/isoleucine (log μmol/l)	105 (53.9; 187)	135 (74.0; 233)	<0.001
Valine (log μmol/)	103 (63.6; 156)	127 (82.7; 187)	<0.001
Lifestyle and medication			
Cotinine (ng/ml)	17.7 ± 67.0	34.5 ± 73.3	0.017
γ-Glutamyl transferase (log U/l)	23.5 (7.00; 116)	40.8 (14.0; 177)	<0.001
Self reported smoking, *n* (%)	28 (12.4)	35 (19.8)	0.045
Self reported alcohol use, *n* (%)	93 (41.3)	59 (33.3)	0.10
Total energy expenditure (kcal)	2,717 ± 750.5	3,149 ± 1,725	<0.001
Obesity, *n* (%)	58 (25.5)	89 (50.0)	<0.001
Hypertensive, *n* (%)	107 (47.6)	130 (73.0)	<0.001
HIV, *n* (%)	9 (4.00)	10 (5.60)	0.45
Statins, *n* (%)	1 (0.44)	10 (5.62)	0.002
Anti-hypertensive medication, *n* (%)	23 (10.2)	39 (21.9)	0.001
Anti-diabetic medication, *n* (%)	3 (1.33)	9 (5.06)	0.029

Data expressed as arrhythmic mean ± standard deviation, geometric mean (5th and 95th percentiles) or % of *n*

n Number of participants, *BP* blood pressure, *HbA1c* glycated haemoglobin, *HDL* high-density lipoprotein, *LDL* low-density lipoprotein, *HIV* human immunodeficiency virus

Results

Characteristics of the study population

The characteristics of the study population are presented in Table 1. Interactions of HbA1c levels were tested for the relationship between BP and leucine/isoleucine ($R^2 = 0.21$, $\beta = 0.23$, $p < 0.001$) and valine ($R^2 = 0.19$, $\beta = 0.23$, $p < 0.001$), and for the relationship between cIMT and leucine/isoleucine ($R^2 = 0.08$, $\beta = 0.17$, $p = 0.016$) and valine ($R^2 = 0.10$, $\beta = 0.14$, $p = 0.035$). We therefore stratified the groups into low and high HbA1c groups using the median cut-off of 5.6 %. The high HbA1c group presented with an unfavourable cardiovascular profile evident by the significantly higher ambulatory SBP and DBP, heart rate and markers of vascular damage (cIMT and CSWA) (all $p < 0.05$). In the high HbA1c group there were significantly more males and Africans, and as expected they were also more obese as seen from the significantly higher weight and BMI (all $p < 0.001$) in comparison to the low HbA1c group. Biochemical analyses revealed significantly higher fasting glucose, BCAAs, triglycerides and CRP levels (all $p < 0.001$) in the high HbA1c group. Subjects in the high HbA1c group reported to use more tobacco

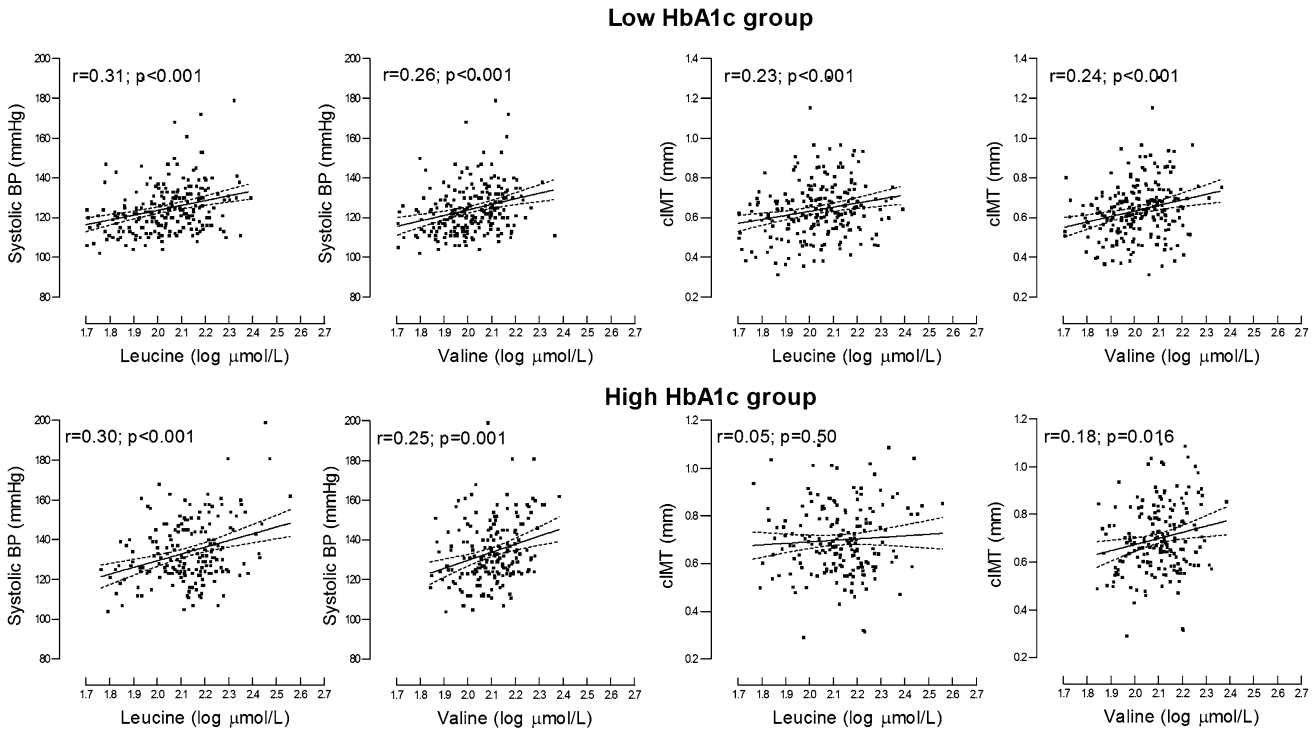

Fig. 1 Unadjusted associations of ambulatory BP and cIMT with BCAAs in the low and high HbA1c groups

Table 2 Partial regression analyses of BCAAs with ambulatory BP, cIMT and CSWA

	HbA1c < 5.6 % ($n = 225$)		HbA1c ≥ 5.6 % ($n = 178$)	
	Leucine/ isoleucine	Valine	Leucine/ isoleucine	Valine
Systolic BP	$r = 0.04$, $p = 0.60$	$r = 0.03$, $p = 0.64$	$r = 0.16$, $p = 0.035$	$r = 0.13$, $p = 0.088$
Diastolic BP	$r = -0.004$, $p = 0.96$	$r = -0.04$, $p = 0.59$	$r = 0.07$, $p = 0.33$	$r = 0.08$, $p = 0.31$
cIMT	$r = -0.007$, $p = 0.92$	$r = 0.02$, $p = 0.82$	$r = 0.02$, $p = 0.76$	$r = 0.22$, $p = 0.004$
CSWA	$r = 0.10$; $p = 0.14$	$r = 0.05$, $p = 0.43$	$r = 0.06$, $p = 0.43$	$r = 0.20$, $p = 0.010$

Relationships adjusted for age, ethnicity, gender and body mass index

n Number of participants, *HbA1c* glycated haemoglobin, *BP* blood pressure, *cIMT* carotid intima-media thickness, *CSWA* cross-sectional wall area

products than their counterparts in the low HbA1c group ($p = 0.045$). In addition, both the serum concentrations of cotinine ($p = 0.017$) and γ-GT ($p < 0.001$) were significantly higher in the high HbA1c group.

Unadjusted analyses

A positive association between the BCAAs and ambulatory SBP ($r = 0.31$, $p < 0.001$; $r = 0.26$, $p < 0.001$) (Fig. 1) and DBP ($r = 0.30$, $p < 0.001$; $r = 0.23$, $p = 0.001$) were indicated in the low HbA1c group. In the same group there were also positive correlations between BCAAs and cIMT ($r = 0.23$, $p < 0.001$; $r = 0.24$, $p < 0.001$) (Fig. 1) and CSWA ($r = 0.29$, $p < 0.001$; $r = 0.33$, $p < 0.001$).

In the high HbA1c group BCAAs also linked positively with ambulatory SBP ($r = 0.30$, $p < 0.001$; $r = 0.25$, $p < 0.001$) (Fig. 1) and DBP ($r = 0.29$, $p < 0.001$; $r = 0.26$, $p = 0.001$), while there were associations only between valine and cIMT ($r = 0.18$, $p = 0.016$) and valine and CSWA ($r = 0.21$, $p = 0.005$) (Fig. 1).

Adjusted analyses

In Table 2, the positive association between leucine/isoleucine and ambulatory SBP ($r = 0.16$, $p = 0.035$) and between valine, cIMT ($r = 0.22$, $p = 0.004$) and CSWA ($r = 0.20$, $p = 0.010$) remained consistent after adjusting for ethnicity, gender, age and BMI, in the high HbA1c group. All the associations previously observed in the low HbA1c group lost significance.

In subsequent multivariate analyses in the high HbA1c group, our findings were confirmed with ambulatory SBP ($R^2 = 0.17$, $\beta = 0.21$, $p = 0.005$) correlating positively with leucine/isoleucine (Table 3) and cIMT ($R^2 = 0.30$, $\beta = 0.20$, $p = 0.003$) with valine (Table 4). The associations between CSWA and valine in the high HbA1c group lost significance. In the low HbA1c group the positive

Table 3 Independent associations between ambulatory SBP and BCAAs in the high HbA1c group

	Systolic blood pressure (mmHg)			
	Leucine/isoleucine (log μmol/l), $R^2 = 0.17$		Valine (log μmol/l), $R^2 = 0.15$	
	β (95 % CI)	p value	β (95 % CI)	p value
BCAA (log μmol/l)	0.21 (0.07; 0.35)	0.005	0.11 (−0.05; 0.27)	0.15
Ethnicity, African/Caucasian	–	–	−0.14 (−0.02; −0.30)	0.089
Gender, male/female	−0.17 (−0.01; −0.33)	0.028	−0.21 (−0.03; −0.39)	0.018
Age (years)	0.13 (−0.01; 0.27)	0.064	0.14 (0.00; 0.28)	0.054
BMI (kg/m^2)	0.23 (0.09; 0.37)	0.002	0.24 (0.10; 0.38)	0.001
Cotinine (ng/ml)	–	–	–	–
GGT (log U/l)	0.17 (0.01; 0.33)	0.026	0.13 (−0.05; 0.31)	0.14
Total cholesterol (mmol/l)	–	–	–	–
CRP (log mg/l)	–	–	–	–
TEE (kcal)	–	–	–	–

Variables included in the model were BCAAs (leucine/isoleucine or valine), ethnicity, gender, age, BMI, total energy expenditure, cotinine, GGT, total cholesterol and CRP

BCAA branched chain amino acids, *BMI* body mass index, *GGT* γ-glutamyl transferase, *CRP* C-reactive protein, *TEE* total energy expenditure

Table 4 Independent associations of cIMT with BCAAs in the high HbA1c group ($N = 178$)

	Carotid intima-media thickness (mm)			
	Leucine/isoleucine (log μmol/l), $R^2 = 0.27$		Valine (log μmol/l), $R^2 = 0.30$	
	β (95 % CI)	p value	β (95 % CI)	p value
BCAA (log μmol/l)	–	–	0.20 (0.06; 0.34)	0.004
Ethnicity, African/Caucasian	–	–	–	–
Gender (male/female)	−0.08 (−0.06; −0.22)	0.31	–	–
Age (years)	0.39 (0.25; 0.53)	<0.001	0.42 (0.28; 0.56)	<0.001
BMI (kg/m^2)	–	–	–	–
Cotinine (ng/ml)	0.16 (0.02; 0.30)	0.014	0.17 (0.05; 0.29)	0.009
GGT (log U/l)	–	–	–	–
Total cholesterol (mmol/l)	0.09 (−0.05; 0.23)	0.16	0.08 (−0.06; 0.22)	0.22
CRP (log mg/l)	0.17 (0.03; 0.31)	0.02	0.14 (0.02; 0.26)	0.034
TEE (kcal)	–	–	–	–
Systolic BP (mmHg)	0.26 (0.12; 0.40)	<0.001	0.23 (0.09; 0.37)	<0.001

Variables included in the model were BCAAs (leucine/isoleucine or valine), ethnicity, gender, age, BMI, total energy expenditure, cotinine, GGT, total cholesterol, CRP and systolic BP

BCAA branched chain amino acids, *BMI* body mass index, *GGT* γ-glutamyl transferase, *CRP* C-reactive protein, *TEE* total energy expenditure, *BP* blood pressure

correlations between cIMT and leucine demonstrated in the unadjusted analyses was also significant in multivariate analysis ($R^2 = 0.43$, $\beta = 0.14$, $p = 0.046$), while all the other associations in the low HbA1c group remained non-significant.

All the multiple regression analyses were repeated after excluding HIV-infected participants ($n = 10$), participants with a HbA1c value >6.5 % or those using diabetes medication ($n = 36$), and participants using anti-hypertensive medication ($n = 62$). By doing so, the associations between ambulatory SBP and leucine in the high HbA1c group lost significance after the exclusion of diabetics and participants on anti-hypertensive medication, while the associations between cIMT and valine did not change materially. As in the low HbA1c group the positive association between cIMT and leucine lost significance after the exclusion of HIV-infected participants and diabetics, while it remained after exclusion of participants on anti-hypertensive medication.

Discussion

Our study showed that individuals with elevated levels of glycated haemoglobin have higher BCAAs, which is significantly associated with ambulatory BP and cIMT. It therefore not only confirms the interplay between BCAAs and chronic high glucose levels, but also proposes that cardiovascular deterioration is part of the process. Metabolite profiling in the Framingham Offspring Study indicated that the concentrations of circulating amino acids (BCAAs and aromatic amino acids) are elevated before any alterations in insulin sensitivity can be detected by standard biochemical measures (Wang et al. 2011), suggesting a significant early predictive value of BCAAs. Our study also adds to the current knowledge by indicating a link between BCAAs and vascular deterioration. We also indicated that these associations are independent of both gender and ethnicity, and after excluding HIV-infected participants, diabetics and those using anti-hypertensive medication.

Although the BCAA levels obtained in our study are lower than those previously reported (Tai et al. 2010), the significantly higher concentrations of BCAAs in a group of participants with high HbA1c levels confirm the previous results from metabolomic studies (Shah et al. 2012; Newgard et al. 2009). Newgard et al. (2009) demonstrated that by comparing obese/insulin resistant and lean/insulin-sensitive subjects, that levels of BCAAs, the aromatic amino acids, C3 and C5 acyl-carnitines as well as glutamic acid, glutamine and alanine differed the most. In addition, this component demonstrated the strongest association with a decrease in insulin sensitivity and accordingly it was hypothesised that the reduced insulin sensitivity noted in the obese subjects may be due to an overload of BCAA catabolism (Newgard et al. 2009). An inverse association between BCAAs and related metabolites with insulin sensitivity was also demonstrated in sedentary subjects with the metabolic syndrome (Huffman et al. 2009).

Our results indicated a significantly higher cIMT in the high HbA1c group, although these values are still within the reference range of <0.9 mm (Mancia et al. 2007). Previous studies also demonstrated that in addition to known correlations between higher glycaemic status and clinical cardiovascular disease in diabetic (Selvin et al. 2005) and non-diabetic populations (Sasso et al. 2004), HbA1c levels are associated with subclinical cardiovascular disease in the absence of clinically evident diabetes (McNeely et al. 2009). The independent association of cIMT with valine indicated in this study also contributes to previous results obtained by Shah et al. (2010), which indicated that BCAAs and related analytes are independently associated with coronary artery disease (Shah et al. 2010), which is characterised by the thickening of the carotid intima media.

Various factors can contribute to the elevation of BCAAs and related metabolites. These factors include dietary intake of BCAAs, genetic expression of genes encoding enzymes involved in BCAA catabolism and its use in anabolic and catabolic processes.

BCAAs are essential amino acids and increased dietary intake of protein, which consists of 15–25 % BCAAs (Layman 2003), can be an important contributing factor to the elevation of BCAAs. Although ethnicity was not independently associated with the variance in either ambulatory BP or cIMT, there were significantly more Africans in the high HbA1c group. Urbanization of black South Africans resulted in changing dietary patterns, including increased consumption of animal derived foods (Vorster et al. 2005). In this study, all participants received a standardised diet the evening before blood samples were collected to control for short-term dietary differences, but long-term differences in the nutritional status might still impact on BCAA levels. However, it was previously indicated that the association between BCAAs and insulin resistance and risk for diabetes were not influenced by protein consumption (Tai et al. 2010; Wang et al. 2011).

Genetic expression of genes encoding enzymes involved in BCAA catabolism can also increase BCAA levels. It has been indicated that obesity and insulin resistance are associated with the down-regulation of BCAA metabolising enzymes in adipose tissue, with corresponding changes in BCAA levels (She et al. 2007; Pietiläinen et al. 2008). In our study, 50 % of the participants in the high HbA1c group were obese as classified by a BMI $\geq$30 (World Health Organization 1997), compared to only 25 % of the participants in the low HbA1c group. However, decreased catabolism of BCAAs was also suggested as one of the possible mechanisms accountable for the link between insulin resistance and BCAAs in non-obese male individuals of mixed ethnicity (Tai et al. 2010).

BCAA levels are also controlled by its use in anabolic and catabolic processes. The BCAAs and insulin are both involved in the activation of signalling pathways that converge at the mammalian target of rapamycin (mTOR) (Adeva et al. 2012), which integrates both extracellular and intracellular signals to regulate cell metabolism, growth, proliferation and survival (Laplante and Sabatini 2009). The anabolic effects of BCAAs on protein metabolism in both resting human muscle (Louard et al. 1990) and during recovery from endurance exercise are mediated through changes in signal transduction that involve the phosphorylation of mTOR (Blomstrand et al. 2006). Surprisingly, participants from our study in the high HbA1c group were more active as the measured total energy expenditure was significantly higher in this group and in multiple regression analyses total energy expenditure did not contribute to the variance in either ambulatory BP or cIMT.

Regardless of the mechanism(s) involved in the elevation of BCAAs, our study indicates a relationship between BCAAs and cardiovascular deterioration in a predominantly pre-diabetes group. The mechanism at work to explain the link between BCAAs and cardiovascular deterioration (ambulatory BP and cIMT) in individuals with high HbA1c levels may involve the interplay of BCAAs and insulin in the regulation of metabolism and growth promoting effects (Laplante and Sabatini 2009).

The presence of BCAAs will normally stimulate insulin secretion (Stanley et al. 1998), to maintain glucose homeostasis as part of the metabolic effects of insulin signalling. According to the BCAA overload hypothesis described by Newgard et al. (2009), the rising of circulating BCAA leads to an increased flux through catabolic pathways. Increased BCAAs may increase activation of mTOR, which is critical in the integration of cellular metabolism and growth factor signalling. Increased activation of the mTOR/S6K1 kinase pathway causes the phosphorylation of serine residues on insulin receptor substrate1 (IRS-1), with a subsequent decline in IRS-1 associated phosphatidylinositide 3-kinase (PI 3-kinase) activity. Reduced PI 3-kinase activity may result in diminished glucose uptake, glucose utilization and reduced nitric oxide synthesis (Dranzin 2006). When decreased glucose transport is sensed at the pancreatic β-cells it may result in a compensatory increase in insulin secretion (Dranzin 2006). Conversely, the mitogen-activated protein (MAP) kinase signalling pathway is not affected by insulin resistance (Cusi et al. 2000). Increased insulin secretion may lead to an excessive growth promoting signal, which stimulates various proliferative and pro-atherogenic events in vascular smooth muscle and endothelial cells (Madonna and De Caterina 2012), which are the trademarks of the pathogenic mechanisms involved in atherosclerosis.

This study has to be interpreted within the context of its limitations and strengths. This was a cross-sectional study to investigate BCAAs and its associations ambulatory BP, cIMT and CSWA in a population with high HbA1c levels, and we can therefore not infer causality. While our results were consistent after multiple adjustments, we cannot exclude residual confounding effects due to unknown factors associated with BCAAs, ambulatory BP, cIMT and CSWA. The insulin levels of our study participants are not available and insulin resistance (homeostasis model assessment score) could therefore not be quantified. In general, we conducted a well-designed study executed under controlled conditions. To our best knowledge, this is the first study to investigate associations of vascular deterioration with BCAAs in a bi-ethnic population with high HbA1c levels.

In conclusion, our results demonstrate a link between BCAAs and BP, as well as cIMT in a bi-ethnic group with elevated HbA1c. As it was previously indicated that circulating BCAAs are elevated long before any changes in insulin sensitivity could be detected (Wang et al. 2011), our results further add value by suggesting that cardiovascular deterioration may accompany or precede metabolic derangements involved in attenuated glucose handling. Future studies may shed light on underlying mechanisms that may improve the diagnostic value of elevated BCAAs.

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