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A metabolomic index based on lipoprotein subfractions and branched chain amino acids is associated with incident hypertension

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ABSTRACT

Objective: The present study aims to evaluate the performance of the Diabetes Risk Index (DRI), a metabolomic index based on lipoprotein particles and branched chain amino acids, on the incidence of newly developed hypertension in a large community dwelling cohort.

Methods: The DRI was calculated by combining 6 lipoprotein parameters [sizes of very-low-density lipoprotein (VLDL), low-density lipoprotein (LDL) and high-density lipoprotein (HDL), concentrations of large VLDL, small LDL, and large HDL particles], and the concentrations of valine and leucine. DRI scores were estimated in 4169 participants from the PREVEND prospective cohort. Cox proportional hazards regression was used to evaluate the association of DRI scores with incident hypertension.

Results: During a median follow-up of 8.6 years, 924 new hypertension cases were ascertained. In analyses adjusted for age and sex, there was a significant association between DRI and incident hypertension with a hazard ratio (HR) per 1 SD increase of 1.45 (95% CI 1.36,1.54; $p < 0.001$). After additional adjustment for traditional risk factors, the HR remained significant (HR_{adj} 1.21, 95% CI 1.10, 1.33, $p < 0.001$). Likewise, subjects in the top quartile of DRI presented with a higher risk of hypertension (HR_{adj} 1.64, 95% CI 1.28, 2.10, $p < 0.001$). Furthermore, the net reclassification improvement assessment improved after the addition of DRI to a traditional risk model ($p < 0.001$), allowing proper reclassification of 34% of the participants.

Conclusion: Higher DRI scores were associated with an increased risk of incident hypertension. Such association was independent of traditional clinical risk factors for hypertension.

1. Introduction

Hypertension is the leading preventable risk factor for cardiovascular disease (CVD) and all-cause mortality worldwide, with a sustained increment in mortality over the last 40 years [1]. Over the same period of time, elevated blood pressure remains the risk factor with the highest disability-adjusted life-years burden [2]. Given the constant rise in hypertension incidence, improvement in the risk classification for hypertension development is needed.

Improvement in the performance of metabolite measurement platforms, such as nuclear magnetic resonance (NMR) spectroscopy, has allowed for the quantification of circulating biomarkers and exploration of their associations with incidence of hypertension [3]. Recently, the role of branched chain amino acids (BCAA) in the development of hypertension has gained attention. It has been shown that high concentrations of BCAA, quantified by means of NMR in normotensive subjects are associated with higher risk of hypertension incidence in a period of seven years [4].

Moreover, the association of insulin resistance with hypertension [5] has been further corroborated with the use of novel NMR biomarkers [6]. For instance, the lipoprotein insulin resistance index (LP-IR), a high-throughput multivariable marker of insulin resistance that combines the information from 6 lipoprotein particle parameters, has been shown to be strongly associated with insulin resistance [6, 7]. Notably high LP-IR scores have also been demonstrated to associate with elevated blood pressure in several studies, including participants from

more than 27 countries [6, 8, 9].

Considering that circulating concentrations of BCAA have also been shown to be higher in insulin resistant conditions [10–12], a new multimarker called the Diabetes Risk Index (DRI) has been developed that integrates both BCAA and LP-IR into a score that predicts incident type 2 diabetes (T2D) [9].

Hypertension and T2D are two cardiometabolic entities that overlap in the general population. These conditions share common causes, such as sedentary lifestyle; but also, pathophysiological mechanisms that underlie their development with insulin resistance being most relevant [13]. Considering that the etiologic relationship between hypertension and T2D is bidirectional [14], we hypothesized that a T2D multimarker score which combines LP-IR and BCAA would provide an enhanced clinical ability to identify individuals at higher risk of developing primary hypertension. The aim of the present study was to assess the performance of the DRI score to predict the development of primary hypertension in individuals from the PREVEND study, a large cohort of adults from the general population in The Netherlands.

2. Material and methods

Data supporting the findings of this study are available from the corresponding author on reasonable request.

2.1. Study population

Briefly, the Prevention of Renal and Vascular End-stage Disease (PREVEND) Study is a Dutch cohort drawn from the general population of the city of Groningen in the northern part of the Netherlands. Briefly, from 1997 to 1998, all residents from Groningen aged 28–75 years were invited to participate. After exclusion of subjects with insulin-treated diabetes and pregnant women, subjects with a urinary albumin concentration $\geq$10 mg/L were invited to participate, resulting in cohort of 8592 subjects (aged 28–75 years) who completed the baseline survey. The second screening, which was the starting point of the current study, took place between 2001 and 2003 (n = 6892). For the current study, subjects with prevalent hypertension at baseline (defined as systolic blood pressure of $\geq$140 mm Hg or a diastolic blood pressure of $\geq$90 mm Hg), and those with missing data of the DRI at baseline and follow-up were excluded, leaving 4169 subjects for the present analyses. Cases of participants lost to follow-up were considered as censored cases. This report follows the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guideline (Supplemental Table 1).

The PREVEND study was approved by the local medical ethics committee at the University Medical Center Groningen (approval number: MEC96/01/022). All participants provided written informed consent and all procedures were conducted according to the Declaration of Helsinki [15]. Details of the study design and recruitment have been described elsewhere [16].

2.2. Laboratory and clinical measurements

Plasma samples were obtained from participants after an overnight fast and 15 minutes of rest prior to sample collection. All blood samples were taken between 8:00 and 10:00 AM. EDTA plasma samples were prepared by centrifugation at 4°C as per manufacturer's instructions and were stored at -80 ºC until analysis.

2.3. DRI calculations

The DRI scores were determined in EDTA plasma samples using a Vantera® Clinical Analyzer (Labcorp, Morrisville, NC), a fully automated, high-throughput, 400 MHz proton (^{1}H) NMR spectroscopy platform, as previously described [17, 18]. Briefly, the LP-IR scores were calculated using 6 NMR-measured lipoprotein parameters: the weighted average sizes of very-low- density lipoprotein, low-density lipoprotein

and high-density lipoprotein (HDL), along with concentrations of large very-low-density lipoprotein, small low-density lipoprotein, and large HDL particles. LP-IR scores (0–100) reflect the magnitude of insulin resistance in individual patients and exhibit strong associations with homeostatic model assessment of insulin resistance (HOMA-IR) and the glucose disposal rate (GDR) assessed by hyperinsulinemic-euglycemic clamp, and have been shown to reflect both peripheral and hepatic insulin resistance on glucose metabolism [7]. BCAA concentrations were measured using the same fully automated, high-throughput, NMR platform; in a standalone assay that has been optimized to quantify only the BCAA concentrations. Plasma samples were prepared on board the instrument and automatically delivered to the flow probe in the NMR spectrometer's magnetic field. The validation of the use of NMR for quantification of BCAA has been previously described [19]. Coefficients of variation for inter- and intra-assay precision ranged from 1.8-6.0, 1.7-5.4, 4.4-9.1, and 8.8-21.3%, for total BCAA, valine, leucine, and isoleucine, respectively. BCAA quantified from the same samples using NMR and LC-MS/MS were highly correlated (r^2 = 0.97, 0.95 and 0.90 for valine, leucine and isoleucine) [19].

DRI scores were determined using the following equation: DRI = 0.0167 [LP-IR] + 1.907 [ln (valine + 2 × leucine)]. DRI scores vary from 1–100 score, with the highest scores denoting the highest risk of progression to T2D. The coefficients of variation for intra- and inter-assay precision ranged from 3.9%–6.4% and 2.7%–7.9% for LP-IR and DRI, respectively.

Total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), triglycerides (TG), insulin, serum creatinine, and serum cystatin C were measured using standard protocols, which have been previously described [20, 21]. Urinary albumin excretion (UAE) was measured as described in two 24-hour urine collections and the results were averaged for analysis [20, 21]. Fasting plasma glucose was measured by dry chemistry (Eastman Kodak, Rochester, New York). Estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) combined creatinine-cystatin C equation [22].

Height and weight were measured with the participants standing without shoes and heavy outer garments. Body-mass index (BMI) was calculated by dividing weight in kilograms by height in meters squared.

2.4. Blood pressure measurement and ascertainment of hypertension

Participants were followed from the date of the baseline visit until end of follow-up. At both visits of each examination, blood pressure was assessed on the right arm in supine position, every minute for 10 and 8 minutes, respectively, with an automatic Dinamap XL Model 9300 series device. The mean of the last 2 recordings from each visit was used. The procedure has been previously described [23].

Use of antihypertensive medications was ascertained by a questionnaire at each examination and was complemented by information from a pharmacy-dispensing registry. For this study, incident hypertension was defined as hypertension that occurred after baseline, which included systolic blood pressure of $\geq$140 mm Hg, a diastolic blood pressure of $\geq$90 mm Hg, or the newly recorded use of antihypertensive drugs, in concordance with the recent Guidelines for the management of arterial hypertension from the European Society of Cardiology & European Society of Hypertension [24]. Antihypertensive medication use, for the definition of hypertension, included 5 second-level Anatomical Therapeutic Chemical codes: C02 (antihypertensives), C03 (diuretics), C07 (β-blockers), C08 (calcium channel blockers), and C09 (inhibitors of the renin-angiotensin system). Initiation of blood pressure lowering medication according to the central pharmacy registry follow-up data was complete as of 1 January 2011.

2.5. Statistical analyses

Normally distributed data were presented as mean and standard

deviation, whereas skewed data were expressed as median and interquartile range. Categorical data were presented as number and percentage. Linear trends across DRI quartiles were determined using ANOVA for normally distributed data, Kruskal-Wallis test for skewed distributed data, and χ^2 test for categorical variables. Skewed data were log-transformed when appropriate. Baseline associations between characteristics and DRI scores were analyzed through univariable and multivariable regression analysis.

For the prospective analysis, cumulative Kaplan-Meier curves for incidence of hypertension during follow-up according to quartiles of DRI were plotted. Time-to-event Cox proportional hazards models were used to compute hazard ratios (HRs) and 95% confidence interval (CI) of hypertension incidence among the 4169 participants with full information at baseline. HRs were calculated in models adjusted for age, sex, systolic blood pressure, history of T2D, tobacco and alcohol consumption, BMI, systolic blood pressure, glucose, insulin, total cholesterol, HDL-C, triglycerides, UAE and eGFR. The proportionality of hazards assumption was tested through the evaluation of independence between scaled Schoenfeld residuals with time for each variable and for every model as a whole [25]. Two-sided *p*-values < 0.05 were considered to be significant.

In view of the positive association between T2D and concentrations of BCAA [26], sensitivity analyses were performed to determine whether the association was present after the exclusion of participants with prevalent T2D at baseline. Moreover, considering the potential role of BCAA as regulator of lipid metabolism [27], a sensitivity analysis was conducted after exclusion of participants taking lipid-lowering medication. Finally, to further assess the association of DRI with incident hypertension in subjects with different BMI, particularly those with normal BMI, we analyzed two separated subgroups, those with a normal BMI (<25 kg/m^2) and those with overweight and obesity (≥25 kg/m^2).

The Net Reclassification Index (NRI) and the Integrated Discrimination Improvement index (IDI) were calculated in order to evaluate whether DRI scores can improve the predictive ability of BCAA for incident hypertension. The base model included the variables: age, sex, parental history of hypertension, smoking behavior (current versus former or never smoker), according to the Framingham hypertension risk prediction model [28], as well as eGFR and albuminuria, which are predictors of incident hypertension in the general population [29, 30]. The second model included all the variables mentioned above as well as the DRI score. The NRI was calculated using predefined risk categories of hypertension development [31]: low (<10%), intermediate (10% to

Table 1
Baseline characteristics by quartiles of DRI scores in PREVEND participants (n=4169).

| | Quartiles of DRI | | | | |
	Q1 (N=1059)	Q2 (N=1056)	Q3 (N=1026)	Q4 (N=1028)	p value
Men, n (%)	162 (15.3%)	382 (36.2%)	574 (55.9%)	785 (76.4%)	< 0.001
Age, mean (SD), years	47.82 (10.01)	49.09 (10.67)	50.13 (10.54)	50.17 (9.91)	< 0.001
BMI, mean (SD), kg/m2	23.96 (3.46)	24.85 (3.26)	26.25 (3.86)	27.77 (3.98)	< 0.001
Waist circumference, mean (SD), cm	81.17 (9.67)	85.76 (9.50)	90.96 (10.12)	96.81 (10.19)	< 0.001
SBP, mean (SD), mmHg	112.72 (10.63)	115.02 (10.56)	118.72 (10.47)	122.33 (9.98)	< 0.001
DBP, mean (SD), mmHg	67.21 (7.05)	69.13 (6.99)	71.20 (6.90)	73.32 (6.76)	< 0.001
History of Cancer, n (%)	43 (4.1%)	40 (3.8%)	39 (3.8%)	44 (4.3%)	0.49
History of CVD, n (%)	10 (0.9%)	16 (1.5%)	12 (1.2%)	14 (1.4%)	0.67
History of T2D, n (%)	9 (0.8%)	11 (1.0%)	32 (3.1%)	62 (6.0%)	< 0.001
Smoking status, n (%)					< 0.001
never	363 (34.3%)	333 (31.5%)	323 (31.5%)	263 (25.6%)	
former	375 (35.4%)	408 (38.6%)	378 (36.8%)	404 (39.3%)	
<6 cig/day	65 (6.1%)	42 (4.0%)	53 (5.2%)	49 (4.8%)	
6-20 cig/day	208 (19.6%)	220 (20.8%)	213 (20.8%)	245 (23.8%)	
>20 cig/day	33 (3.1%)	43 (4.1%)	37 (3.6%)	59 (5.7%)	
Alcohol consumption, n (%)					< 0.001
No, almost never	274 (25.9%)	221 (20.9%)	230 (22.4%)	213 (20.7%)	
1-4 drinks/month	190 (17.9%)	197 (18.7%)	160 (15.6%)	164 (16.0%)	
2-7 drinks/week	356 (33.6%)	394 (37.3%)	352 (34.3%)	342 (33.3%)	
1-3 drinks/day	221 (20.9%)	216 (20.5%)	239 (23.3%)	250 (24.3%)	
4 or more drinks/day	18 (1.7%)	28 (2.7%)	45 (4.4%)	59 (5.7%)	
Lipid-lowering medication, n (%)	17 (1.6%)	19 (1.8%)	28 (2.7%)	55 (5.4%)	< 0.001
DRI score, median (IQR)	9.00 (2.00, 13.00)	23.00 (20.00, 26.00)	36.00 (33.00, 40.00)	55.00 (49.00, 63.00)	< 0.001
Glucose, median (IQR), mmol/L	4.50 (4.30, 4.80)	4.60 (4.30, 5.10)	4.70 (4.40, 5.20)	5.00 (4.50, 5.40)	< 0.001
Insulin, median (IQR), mU/L	5.80 (4.50, 7.80)	6.50 (4.90, 8.80)	7.60 (5.60, 10.30)	10.70 (7.40, 15.30)	< 0.001
Isoleucine, median (IQR), μM/L	29.72 (23.67, 36.65)	38.14 (32.15, 44.36)	44.29 (36.65, 52.06)	53.77 (45.54, 63.38)	< 0.001
Leucine, median (IQR), μM/L	90.40 (28.25)	116.68 (13.33)	129.69 (17.09)	153.74 (23.37)	< 0.001
Valine, median (IQR), μM/L	155.87 (47.69)	193.82 (22.60)	212.90 (27.79)	238.46 (31.63)	< 0.001
Total Cholesterol, mean (SD), mmol/L	5.19 (1.01)	5.21 (0.99)	5.41 (1.05)	5.67 (1.04)	< 0.001
Triglycerides, median (IQR), mmol/L	0.73 (0.58, 0.93)	0.88 (0.68, 1.10)	1.12 (0.88, 1.40)	1.74 (1.36, 2.27)	< 0.001
HDL-C, median (IQR), mmol/L	1.48 (0.36)	1.36 (0.27)	1.22 (0.26)	1.06 (0.20)	< 0.001
LP-IR score, median (IQR)	13.00 (6.00, 21.00)	24.00 (18.00, 35.00)	43.00 (34.00, 53.00)	70.50 (58.00, 82.00)	< 0.001
Large VLDL-P, median (IQR), nmol/L	1.30 (0.70, 2.15)	1.90 (1.20, 3.10)	3.60 (2.30, 5.60)	8.40 (5.50, 12.70)	< 0.001
VLDL size, mean (SD), nm	44.71 (10.49)	46.50 (7.47)	48.59 (7.66)	55.28 (8.60)	< 0.001
Small LDL-P, median (IQR), nmol/L	161.0 (0.0, 277.0)	245.5 (146.0, 364.2)	343.0 (221.2, 465.7)	574.5 (382.0, 789.2)	< 0.001
LDL size, mean (SD), nm	20.84 (3.35)	21.17 (1.80)	21.04 (1.42)	20.57 (0.90)	< 0.001
Large HDL Particles, mean (SD), μmol/L	7.49 (2.74)	6.06 (2.32)	4.50 (2.24)	2.80 (1.58)	< 0.001
HDL size, mean (SD), nm	9.58 (1.32)	9.39 (0.48)	9.02 (0.49)	8.65 (0.36)	< 0.001
eGFR, mean (SD), mL/min/1.73 m2	98.05 (14.48)	97.35 (14.27)	96.25 (14.10)	95.92 (14.29)	0.002
UAE, median (IQR), mg/24 h	7.01 (5.62, 10.20)	7.41 (5.55, 11.37)	7.64 (5.78, 11.43)	8.84 (6.21, 15.06)	< 0.001

Continuous variables are reported as mean ± standard deviation, median (IQR, interquartile range) and categorical variables are reported as percentage. p values were determined using a one-way analysis of variance for normally distributed data, Kruskal–Wallis test for skewed distributed data, and chi-square test for categorical data and represent a significant difference across the quartiles of DRI score. Abbreviations: DRI, Diabetes Risk Index; PREVEND, Prevention of Renal and Vascular End-Stage Disease; T2D, type 2 diabetes mellitus; CVD, cardiovascular disease; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglycerides; LP-IR, Lipoprotein Insulin Resistance Index; VLDL-P, very low-density lipoprotein particles; LDL-P, low-density lipoprotein particles; HDL-P, high-density lipoprotein particles.

20%), and high (≥20%).

All statistical analyses were performed with R language for statistical computing software, v. 4.0.3 (2020), (Vienna, Austria) [32].

3. Results

3.1. Baseline characteristics

Of the PREVEND participants that completed the second round of screening, 4169 subjects who did not have hypertension and had complete data available on DRI and covariates at the time of screening were included in this study. Mean baseline DRI score was 37.71 (SD=19.53) in the subjects who developed new-onset hypertension vs. 29.70 (SD=18.01) in the subjects who did not (p <0.001). Baseline characteristics, reported by quartiles of DRI scores, can be found in Table 1. Participants with higher DRI scores were more likely to be men and older. They were also more likely to have a history of T2D, be current smokers, consume more alcohol, and to be on lipid lowering medications. Those in the highest quartile of DRI scores had higher BMI, systolic and diastolic blood pressure, TC, TG, LDL-C, glucose, insulin, BCAA and lower HDL-C as well as lower eGFR and higher UAE.

3.2. Cross-sectional analysis

The associations of the concentration of DRI score with other variables of interest were evaluated with univariable and multivariable linear regression analyses. The univariable linear regression results were congruent with the trends reported in Table 1. DRI scores were positively associated with sex, age, history of T2D, smoking, alcohol consumption, lipid lowering medications, BMI, systolic and diastolic blood pressure, TC, TG, LDL-C, glucose, and BCAA. DRI was negatively associated with HDL-C (Table 2). In multivariable analyses, DRI scores were positively associated with the following variables: sex, BMI, waist circumference, diastolic blood pressure, alcohol consumption, glucose, insulin, TC, TG and inversely with HDL-C, (p <0.05) (Table 3).

3.3. Longitudinal analysis

During a median follow-up period of 8.6 years (IQR, 8.0–8.2), 924 individuals were diagnosed with new-onset hypertension, of which 122 were prescribed diuretics; 194 were prescribed β-blockers; 153 were prescribed angiotensin-converting enzyme inhibitors (ACEi) and 53 were prescribed angiotensin receptor blockers (ARBs). 171 individuals received more than 1 prescription: 46 diuretics and β-blockers, 37 diuretics and ACEi, 16 diuretics and ARBs, 47 β-blockers and ACEi, 17 β-blockers and ARBs, 8 ACEi and ARBs. 24 individuals used more than 2 antihypertensive drugs.

Cox regression analyses revealed that high DRI scores were associated with increased risk of hypertension in the crude model, with a hazard ratio (HR) per 1 SD increase of 1.45 (95% CI: 1.36, 1.54; p <0.001) (Table 3). The adjusted HR after sex and age adjustment was (HR_adj per one 1 SD increase: 1.37, 95% CI: 1.28, 1.47; p <0.001). This association remained significant in a model adjusted for BMI, systolic blood pressure, smoking, alcohol consumption, history of T2D, eGFR, and UAE (HR_adj per one 1 SD increase: 1.11, 95% CI: 1.03, 1.20; p =0.008), as well as TC, HDL-C, TG, glucose, insulin, eGFR, and UAE (HR_adj per 1 SD increase: 1.21, 95% CI: 1.10,1.33; p < 0.001) (Table 4).

Similarly, Cox proportional hazard regression analysis was performed using quartiles of DRI scores. The crude model again revealed that DRI scores are associated with incident hypertension with a HR for the highest quartile of 2.74 (95% CI: 2.27, 3.32; p < 0.001). The association remained significant after adjustment for age, sex, BMI, systolic blood pressure, smoking, alcohol consumption, history of T2D, eGFR, and UAE (HR_adj: 1.28, 95% CI: 1.03, 1.60; p =0.03), as well as TC, HDL-C, TG, glucose, insulin, eGFR, and UAE (HR_adj: 1.64, 95% CI: 1.28, 2.10; p < 0.001) (Table 5). Consistently, the Kaplan–Meier curves for

Table 2

Univariate associations of DRI scores with baseline characteristics.

Characteristic	Std. β (95% CI)	p value
Men	0.99 (0.90, 1.0)	**<0.001**
Age	0.09 (0.06, 0.12)	**<0.001**
BMI	0.39 (0.36, 0.42)	**<0.001**
Waist circumference	0.54 (0.52, 0.57)	**<0.001**
SBP	0.35 (0.32, 0.38)	**<0.001**
DBP	0.33 (0.30, 0.36)	**<0.001**
History of Cancer	0.02 (-0.14, 0.18)	0.82
History of CVD	0.11 (-0.16, 0.39)	0.42
History of T2D	0.93 (0.75, 1.1)	**<0.001**
Smoking status		
former	0.14 (0.06, 0.21)	**<0.001**
<6 cig/day	0.01 (-0.14, 0.16)	0.91
6-20 cig/day	0.18 (0.09, 0.26)	**<0.001**
>20 cig/day	0.35 (0.19, 0.51)	**<0.001**
Alcohol consumption		
1-4 drinks/month	0 (-0.10, 0.10)	0.97
2-7 drinks/ week	0.04 (-0.04, 0.13)	0.29
1-3 drinks/day	0.14 (0.05, 0.23)	**0.002**
4 or more drinks/day	0.53 (0.35, 0.70)	**<0.001**
Lipid-lowering medication	0.45 (0.27, 0.63)	**<0.001**
Glucose	0.27 (0.24, 0.30)	**<0.001**
Insulin	0.41 (0.38, 0.44)	**<0.001**
Isoleucine	0.71 (0.68, 0.73)	**<0.001**
Leucine	0.99 (0.94, 1.0)	**<0.001**
Valine	0.93 (0.90, 1.0)	**<0.001**
TC	0.21 (0.18, 0.24)	**<0.001**
Triglycerides	0.62 (0.59, 0.64)	**<0.001**
HDL-C	-0.58 (-0.61, -0.55)	**<0.001**
LP-IR score	0.88 (0.86, 0.89)	**<0.001**
Large VLDL-P	0.69 (0.67, 0.71)	**<0.001**
VLDL size	0.49 (0.46, 0.52)	**<0.001**
Small LDL-P	0.65 (0.63, 0.68)	**<0.001**
LDL size	-0.21 (-0.25, -0.17)	**<0.001**
Large HDL Particles	-0.66 (-0.68, -0.63)	**<0.001**
HDL size	-0.93 (-1.0, -0.90)	**<0.001**
eGFR	-0.07 (-0.10, -0.04)	**<0.001**
UAE	0.07 (0.04, 0.10)	**<0.001**

Standardized regression coefficients are shown. Abbreviations: DRI, Diabetes Risk Index; T2D, type 2 diabetes mellitus; CVD, cardiovascular disease; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglycerides; LP-IR, Lipoprotein Insulin Resistance Index; VLDL-P, very low-density lipoprotein particles; LDL-P, low-density lipoprotein particles; HDL-P, high-density lipoprotein particles.

hypertension according to quartiles of DRI score are presented in Figure 1. The graph depicts an increased risk of hypertension events in subjects in the top quartile of DRI multimarker (p log rank < 0.001).

Notably, the association of DRI was present both in participants with and without overweight. In the subgroup of participants with BMI < 25 kg/m², DRI was associated with increased risk of hypertension in the crude model, with a HR per 1 SD increase of 1.34 (95% CI: 1.22, 1.48; p <0.001) as well as in the model adjusted for age and sex (HR_adj per 1 SD increase: 1.25, 95% CI: 1.11,1.39; p < 0.001). Likewise, In the subgroup of participants with BMI ≥ 25 kg/m², DRI was associated with increased risk of hypertension in the crude model, with a HR per 1 SD increase of 1.37 (95% CI: 1.26, 1.49; p <0.001) as well as in the model adjusted for age and sex (HR_adj per 1 SD increase: 1.33, 95% CI: 1.21,1.45; p < 0.001).

The Net Reclassification Index (NRI) was 0.13 (95% CI: 0.06, 0.20; p < 0.001), denoting that when DRI was added to the model, more subjects were correctly re-classified than with the Framingham Offspring Study risk score alone. The addition of DRI to the Framingham Offspring Study risk score allowed for the proper reclassification of 34% of subjects who developed hypertension during the follow-up. The IDI of the DRI enhanced model was 0.020 (95% CI: 0.015, 0.025), p < 0.001.

Considering that DRI scores are higher in men, the cox proportional hazard regression analysis analyses were performed using the sex-stratified quartiles of DRI scores. The results were similar to the non-

Table 3

Multivariable associations of DRI scores with baseline characteristics.

Characteristic	Std. β (95% CI)	*p* value
Men	0.47 (0.41, 0.52)	**<0.001**
Age	-0.03 (-0.05, 0.00)	0.05
BMI	0.11 (0.07, 0.15)	**<0.001**
Waist circumference	0.05 (0.01, 0.09)	**0.017**
SBP	0.02 (-0.01, 0.04)	0.27
DBP	0.03 (0.00, 0.06)	**0.021**
History of Cancer	-0.06 (-0.16, 0.04)	0.22
History of CVD	-0.07 (-0.25, 0.11)	0.46
History of T2D	0.08 (-0.07, 0.23)	0.31
Smoking status		
former	0.02 (-0.03, 0.06)	0.54
<6 cig/day	-0.02 (-0.11, 0.08)	0.73
6-20 cig/day	-0.02 (-0.08, 0.04)	0.49
>20 cig/day	-0.03 (-0.14, 0.07)	0.54
Alcohol consumption		
1-4 drinks/month	0.1 (0.03, 0.16)	**0.002**
2-7 drinks/ week	0.15 (0.10, 0.21)	**<0.001**
1-3 drinks/day	0.28 (0.21, 0.34)	**<0.001**
4 or more drinks/day	0.38 (0.26, 0.49)	**<0.001**
Lipid-lowering medication	0.1 (-0.02, 0.22)	0.11
Glucose	0.06 (0.03, 0.09)	**<0.001**
Insulin	0.12 (0.10, 0.15)	**<0.001**
TC	0.06 (0.04, 0.09)	**<0.001**
Triglycerides	0.35 (0.32, 0.37)	**<0.001**
HDL-C	-0.27 (-0.30, -0.24)	**<0.001**
eGFR	-0.02 (-0.04, 0.01)	0.14
UAE	0.01 (-0.01, 0.03)	0.22

Standardized regression coefficients from multivariable linear regression are shown. Abbreviations: DRI, Diabetes Risk Index; T2D, type 2 diabetes mellitus; CVD, cardiovascular disease; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides; LP-IR, Lipoprotein Insulin Resistance Index.

Table 4

Association of DRI scores with incident hypertension in the PREVEND study (n= 4169).

	DRI Per 1 SD increment	
Participants	4169	
Events	924	
	HR (95 % CI)	*p* value
Crude model	1.45 [1.36;1.54]	< 0.001
Model 1	1.37 [1.28;1.47]	< 0.001
Model 2	1.11 [1.02;1.19]	0.008
Model 2b	1.14 [1.05;1.23]	<0.001
Model 3	1.21 [1.10;1.33]	< 0.001

Data are presented as hazard ratios (HRs) with 95% CIs and *p* values.
Model 1: Model adjusted for age and sex.
Model 2: Model 1 + BMI + SBP + heart rate + T2D + smoking + alcohol consumption + eGFR + UAE.
Model 2b: Model 1 + BMI + DBP + heart rate + T2D + smoking + alcohol consumption + eGFR + UAE.
Model 3: Model 1 + TC + HDL-C + TG + glucose + insulin.
Abbreviations. BMI, body mass index; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; HDL-C, high-density lipoprotein cholesterol; SBP, systolic blood pressure; T2D, type 2 diabetes; TC, total cholesterol; TG, triglycerides; and UAE, urinary albumin excretion.

sex-stratified quartiles analyses. The crude model again revealed that DRI scores were associated with incident hypertension with a HR for the highest quartile of 2.22 (95% CI: 1.85, 2.66; $p < 0.001$). Likewise, the association remained after fully adjustment described above (HR$_{adj}$: 1.55; 95% CI: 1.24, 1.93, $p < 0.001$) (Supplemental Table 3).

The sensitivity analysis conducted in participants free from T2D (n= 4056) revealed similar results to those found in the whole cohort. In the crude model, DRI scores were associated with incident hypertension with a HR for the highest quartile of 2.72 (95% CI: 2.24, 3.30; $p <$

0.001). Similarly, the association remained after fully adjustment described above (HR$_{adj}$: 1.63; 95% CI: 1.27, 2.09, $p = 0.003$) (Supplemental Table 4).

Moreover, the sensitivity analysis conducted in subjects free from lipid lowering medication (n= 4050) revealed similar results to those found in the whole cohort. In the crude model, DRI scores were associated with incident hypertension with a HR for the highest quartile of 2.72 (95% CI: 2.24, 3.31; $p < 0.001$). Similarly, the association remained after fully adjustment described above (HR$_{adj}$: 1.65; 95% CI: 1.28, 2.13, $p < 0.001$) (Supplemental Table 5).

Finally, DRI was compared to conventional metabolic indexes such as BMI and waist circumference. With incident hypertension as outcome, DRI showed a sensitivity and specificity of 0.68 and 0.74, respectively (Area under the curve (AUC): 0.78); meanwhile BMI showed a sensitivity and specificity of 0.69 and 0.46, respectively (AUC: 0.61). Consequently, the performance of the DRI was better than that of the BMI (p-value < 0.001). In addition, waist circumference showed a sensitivity and specificity of 0.63 and 0.57, respectively (AUC: 0.63), making that the performance of DRI was better than that of the waist circumference (p-value < 0.001).

4. Discussion

In this large prospective cohort, comprising 4169 participants, we report for the first time that higher scores of DRI, a newly developed risk algorithm based on BCAA and six lipoprotein particles parameters, are associated with incidence of hypertension. Multivariable adjusted time-to-event analyses showed that the positive association of DRI with hypertension was present after adjustment for age, sex, glucose, insulin and BMI. Additionally, we demonstrated that the Framingham Offspring Study model for hypertension risk enhanced with DRI scores improved reclassification of participants across clinical risk categories for hypertension compared to the model enriched with DRI scores.

DRI comprises the information of six lipoprotein particles parameters: the weighted average sizes of VLDL, LDL and HDL, along with concentrations of large VLDL, small LDL, and large HDL particles, as well as the concentrations of two BCAA: leucine and valine.

The components of the DRI score are closely related to the development of hypertension. A recent study has revealed an association between BCAA and incident hypertension [4]. In addition, there is extensive evidence that high BCAA concentrations induces generation of reactive oxygen species and mitochondrial dysfunction [33]; which are known to be linked to the physio-pathogenesis of hypertension [34]. Moreover, it has been reported that circulating BCAA can induce pro-inflammatory responses through the transcription factor NF-κB, resulting in the release of intracellular adhesion molecule-1 (ICAM-1) and E-selectin; hence, contributing to the development of hypertension [35].

Likewise, the HDL particles have been shown to be linked with different pathophysiology of hypertension, such as the regulation of fibrinolysis, particularly the transport of the plasmin regulator, alpha-2-antiplasmin [36], which recently had been identified as critical regulator of angiotensin II and vascular remodeling [37].

Moreover, several epidemiological studies have revealed the associations between the DRI components and hypertension. BCAA has been found to be consistently associated with prevalent hypertension in different studies [10, 38, 39]. Likewise, previous epidemiological studies have also reported the association between lipoprotein particle profiles and development of hypertension [40]. A study which involved 17,527 participants demonstrated that higher concentrations of small LDL, small HDL, and large VLDL particles were prospectively associated with incidence of hypertension in during 8 years of follow-up [41]. Furthermore, it has been reported that elevated concentrations of small dense LDL cholesterol associated with reduced blood flow and enhanced shear stress of the blood vessels, in early-stage hypertensive patients [42].

Table 5

Association of DRI scores with incident hypertension by quartiles in the PREVEND study (n= 4169).

	Q1DRI<17	Q2DRI 17-30		Q3DRI 30-45		Q4DRI >45	
Participants	1059	1056		1026		1028	
Events	155	197		228		344	
		HR (95 % CI)	*p* value	HR (95 % CI)	*p* value	HR (95 % CI)	*p* value
Crude model	(ref)	1.40 [1.13;1.73]	0.02	1.69 [1.37;2.07]	< 0.001	2.74 [2.27;3.32]	< 0.001
Model 1	(ref)	1.24 [1.00;1.54]	0.05	1.41 [1.14;1.75]	0.002	2.25 [1.82;2.77]	< 0.001
Model 2	(ref)	1.12 [0.90;1.40]	0.29	1.01 [0.79;1.23]	0.89	1.27 [1.01;1.59]	0.04
Model 2b	(ref)	1.07 [0.86;1.33]	0.55	1.05 [0.84;1.31]	0.67	1.37 [1.09;1.71]	0.007
Model 3	(ref)	1.18 [0.95;1.47]	0.13	1.22 [0.97;1.52]	0.08	1.64 [1.28;2.10]	< 0.001

Data are presented as hazard ratios (HRs) with 95% CIs and p values.

Model 1: Model adjusted for age and sex.

Model 2: Model 1 + BMI + SBP + heart rate + T2D + smoking + alcohol consumption + eGFR + UAE.

Model 2b: Model 1 + BMI + DBP + heart rate + T2D + smoking + alcohol consumption + eGFR + UAE.

Model 3: Model 1 + TC + HDL-C + TG + glucose + insulin.

Abbreviations. BMI, body mass index; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; HDL-C, high-density lipoprotein cholesterol; SBP, systolic blood pressure; T2D, type 2 diabetes; TC, total cholesterol; TG, triglycerides; and UAE, urinary albumin excretion.
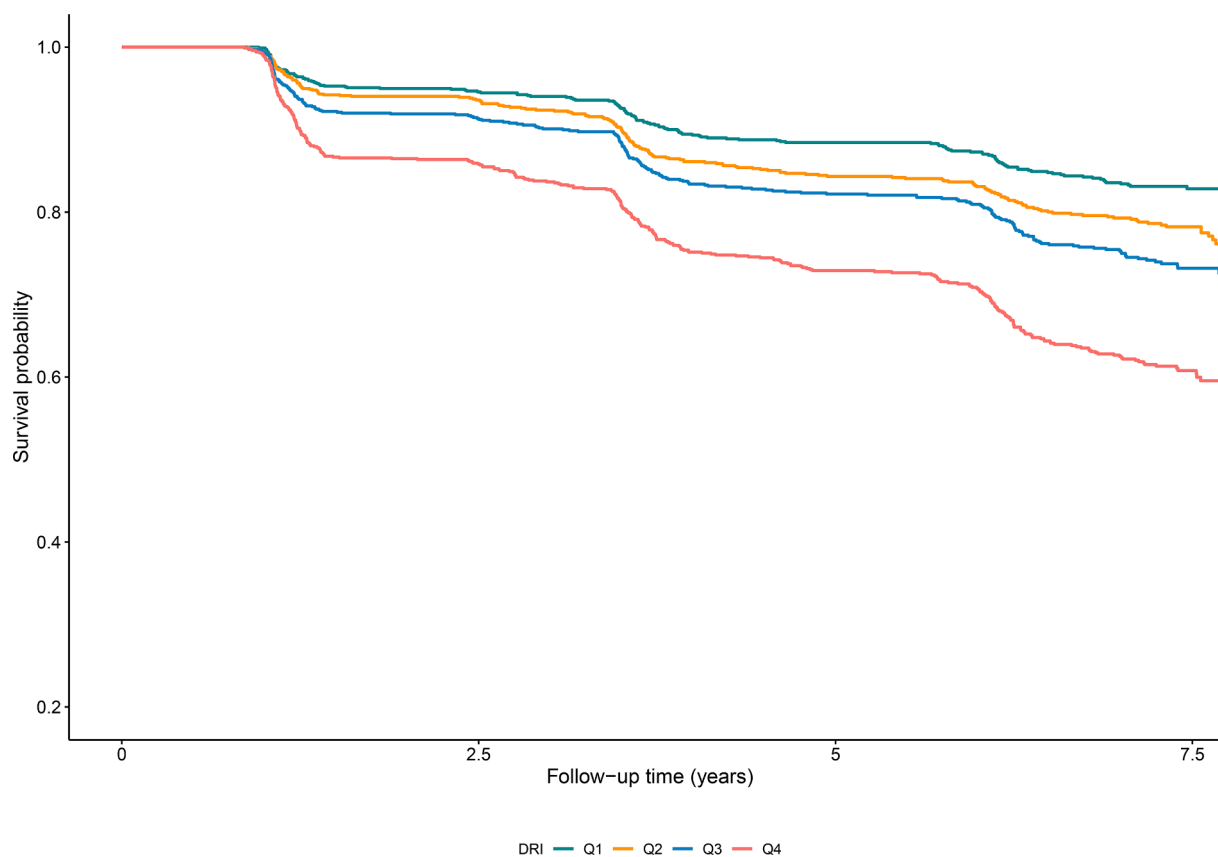

Fig. 1. Kaplan–Meier survival curves for time to T2D diagnosis according to sex-stratified quartiles of DRI, by log-rank test (p < 0.001).

In the present study we found that high scores of DRI associated with increased risk of hypertension. Interestingly, the highest quartile of DRI was comprised predominantly of men. The increased proportion of men in the highest quartile of DRI, compared to LP-IR, could be explained by the fact that both plasma concentrations of BCAA and dietary intake of BCAA-rich foods are higher in men [43, 44]. Despite the fact that there is not a general consensus about what are the main determinants of circulating BCAA, some studies suggest that such differences may at least in part be attributed to differences in dietary patterns between men and women [45]. Importantly, the association of DRI with incident hypertension remained after adjustment for sex, as well as in in sex-stratified analyses.

It has been reported that the circulating concentrations of BCAA, which are an important component of the DRI score, found to be a useful biomarker of insulin sensitivity improvement in overweight participants of a lifestyle intervention [42]. Contemplating that the global hypertension practice guidelines from the International Society of Hypertension highlights that lifestyle modification is the first line of antihypertensive treatment and may also augment the effects of pharmacologic antihypertensive treatment [46], DRI could also be an instrument to track the effects of lifestyle intervention in the context of hypertension. Further research is desirable to evaluate the usefulness of DRI on that concern.

We acknowledge several strengths of the present study. This study included a large population with a wide age range which allowed for the adjustment of the analysis with sufficient statistical power. Another strength of the present study is the implementation of a robust method of BCAA and lipoprotein particles quantification by means of NMR. The

inclusion of lipoprotein particles in the multimarker may offer several advantages, given the fact that the distribution of HDL particles is closely associated with hypertensive status. Moreover, it has been recognized that subjects with different cardiovascular risk may have indistinguishable concentrations of traditional lipids (such as LDL), but have important differences in other lipid measurements such as lipoprotein size and particle concentration [47]. To the best of our knowledge this study explores for first time the performance of a test comprising the dual factors of lipoprotein particles and BCAA in the context of hypertension risk assessment. We are also aware of the limitations of the study. First, the present study was conducted in the north of the Netherlands, and mainly comprises individuals of north European ancestry, which could limit the extrapolation of the current findings to other ethnicities. Secondly, the measurement of blood pressure was conducted in the outpatient clinic; twenty-four hour ambulatory blood pressure monitoring, which remains the gold standard for diagnosing hypertension was not performed. Furthermore, this prospective cohort study did not record physical activity and therefore, our analyses could not be adjusted for such a variable. In addition, the observational nature of the study prevents the ability to draw causal conclusions. This fact restricts the capacity to describe the underlying biological mechanisms. Moreover, even after the adjustment for several variables, residual confounding remains a limitation in observational studies.

In conclusion, this prospective cohort study showed that high score of DRI, an NMR spectroscopy-measured multimarker of lipoprotein particles and BCAA, is associated with an increased risk of developing hypertension in both men and women in the general population during extended follow-up.

Ethics approval and consent to participate

The study conforms to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the local ethics committee of the University Medical Center Groningen (approval number: MEC96/01/022). All participants provided written informed consent.

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Declaration of Competing Interests

J.L.F.-G., R.P.F.D., and S.J.L.B. declare that they have no conflict of interest. I.S., E.G., and M.A.C. are employees of LabCorp.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ejim.2021.07.002.

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