university of groningen

University Medical Center Groningen

University of Groningen

Primary aldosteronism is associated with decreased low-density and high-density lipoprotein particle concentrations and increased GlycA, a pro-inflammatory glycoprotein biomarker

Berends, Annika M. A.; Buitenwerf, Edward; Gruppen, Eke G.; Sluiter, Wim J.; Bakker, Stephan J. L.; Connelly, Margery A.; Kerstens, Michiel N.; Dullaart, Robin P. F.

Published in:
Clinical Endocrinology

DOI:
10.1111/cen.13891

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2019

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):
Berends, A. M. A., Buitenwerf, E., Gruppen, E. G., Sluiter, W. J., Bakker, S. J. L., Connelly, M. A., Kerstens, M. N., & Dullaart, R. P. F. (2019). Primary aldosteronism is associated with decreased low-density and high-density lipoprotein particle concentrations and increased GlycA, a pro-inflammatory glycoprotein biomarker. *Clinical Endocrinology*, *90*(1), 79-87. https://doi.org/10.1111/cen.13891

Copyright
Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the “Taverne” license. More information can be found on the University of Groningen website: https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment.

Take-down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): http://www.rug.nl/research/portal. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

Received: 29 August 2018 | Revised: 17 October 2018 | Accepted: 23 October 2018

DOI: 10.1111/cen.13891

ORIGINAL ARTICLE

WILEY

Primary aldosteronism is associated with decreased low-density and high-density lipoprotein particle concentrations and increased GlycA, a pro-inflammatory glycoprotein biomarker

Annika M. A. Berends[1] | Edward Buitenwerf[1] | Eke G. Gruppen[1,2] | Wim J. Sluiter[1] | Stephan J. L. Bakker[2] | Margery A. Connelly[3] | Michiel N. Kerstens[1] | Robin P. F. Dullaart[1]

[1]Department of Endocrinology, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands

[2]Department of Nephrology, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands

[3]Laboratory Corporation of America® Holdings (LabCorp), Raleigh, North Carolina

Correspondence
Annika M. A. Berends, Department of Endocrinology, University Medical Center Groningen, Groningen, The Netherlands.
Email: m.a.berends@umcg.nl

Abstract

Background: Primary aldosteronism (PA) may confer increased cardiovascular risk beyond effects on systemic blood pressure, but contributing mechanisms remain incompletely understood. We compared plasma (apo)lipoproteins and lipoprotein particle characteristics, GlycA, a pro-inflammatory glycoprotein biomarker of enhanced chronic inflammation, and plasma total branched-chain amino acids (BCAA), measured using nuclear magnetic resonance (NMR) spectroscopy, between patients with PA, control subjects without hypertension, subjects with untreated hypertension and subjects with treated hypertension.

Methods: Twenty PA patients were individually matched with 2819 control subjects without hypertension, 501 subjects with untreated hypertension and 878 subjects with treated hypertension participating in the PREVEND (Prevention of Renal and Vascular End-Stage Disease) cohort study with respect to age, sex, body mass index, smoking and statin use. The Vantera® Clinical Analyzer was used to determine NMR-based laboratory parameters.

Results: Total cholesterol, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), apolipoprotein (apo) B, apolipoprotein A-I (apoA-I), LDL particle and HDL particle concentrations were all decreased in PA subjects vs control subjects and subjects with untreated hypertension ($P < 0.016$). Triglycerides (TG) and triglyceride-rich lipoprotein (TRL) concentrations were lower in PA subjects vs subjects with (untreated) hypertension. GlycA was increased in PA vs the three comparator groups ($P < 0.016$). Total BCAA concentrations were unaltered in PA.

Conclusions: Primary aldosteronism is associated with lower concentrations of LDL and HDL particles and to some extent also with lower TG and TRL particle concentrations. PA is also characterized by increased GlycA levels, indicating enhanced low-grade chronic inflammation. Low HDL particle concentrations and increased GlycA could contribute to accelerated cardiovascular disease development in PA.

Berends and Buitenwerf equally contributed to the study.

 © 2018 John Wiley & Sons Ltd.

KEYWORDS
branched-chain amino acids, GlycA, high-density lipoproteins, lipid protein subfractions, low-density lipoproteins, nuclear magnetic spectroscopy, primary aldosteronism

1 | INTRODUCTION

Primary aldosteronism (PA), due to autonomous adrenal aldosterone secretion, is considered to be a rather frequent cause of endocrine hypertension.[1,2] During the past few years, evidence is accumulating that the risk of cardiovascular morbidity and mortality is increased in PA patients compared to patients with essential hypertension even when taking account of the systemic blood pressure level.[5,6] In line, a recent meta-analysis demonstrated a greater carotid artery intima media thickness, a marker of subclinical atherosclerosis, in PA patients compared to individuals with essential hypertension.[9]

Besides key roles in regulating electrolyte and fluid balance, aldosterone has been identified to exert pro-inflammatory and profibrotic effects on arterial tissue and the heart, and to enhance circulating levels of pro-inflammatory cytokines.[10,11] Moreover, PA may be associated with a higher prevalence of the metabolic syndrome (MetS).[15,16] Elevated fasting glucose levels have been equivocally reported in PA,[6,7,17,18] whereas several population-based studies have suggested an association of higher plasma aldosterone with increased prevalence of MetS or insulin resistance.[19,20] Some reports have pointed to an association of high circulating aldosterone concentrations with decreased levels of high-density lipoprotein cholesterol (HDL-C).[13,15,19] Remarkably however, plasma total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) and triglycerides (TG) were documented to be lower in PA patients compared to subjects with essential hypertension and normotensive control subjects,[6,8] although unchanged levels of TC, LDL-C and TG in PA have been found in other studies.[7,18,20,21] Additionally, HDL-C was found to decrease, whereas triglycerides were increased after unilateral adrenalectomy or mineralocorticoid receptor antagonist treatment in PA.[22] Notably, circulating high sensitivity C-reactive protein (hsCRP) levels reflecting low-grade systemic inflammation have been variably shown to be unchanged or elevated in PA patients, possibly coinciding with increased oxidative stress.[13,21,23]

Despite ongoing interest in pro-atherogenic alterations in metabolic and inflammatory processes conveyed by PA, little is currently known about the relationship of PA with possible alterations in lipoprotein particle characteristics. In this regard, it is relevant that measurement of lipoprotein particle concentrations by nuclear magnetic resonance (NMR) spectroscopy may provide additional insight into the prediction of cardiovascular disease beyond conventional lipid measures.[24,25] Of further note, using the same NMR spectroscopy-based technique, an assay, designated GlycA, has been developed, which represents a composite marker of five abundant inflammatory glycoproteins.[26,27] GlycA is strongly correlated with hsCRP, and has the advantage of being less variable within individual subjects compared to hsCRP.[26,27] GlycA has been shown to be elevated in MetS,[29,30] and predicts newly developed type 2 diabetes mellitus (T2DM),[31] as well as cardiovascular disease.[32] The same NMR spectroscopy-based technique enables measurement of plasma concentrations of total branched-chain amino acids (BCAA), that is valine, leucine and isoleucine, which may be implicated in the pathogenesis of insulin resistance and other pathologies.[33,34]

We initiated the present study to determine the extent to which (apo)lipoproteins, lipoprotein particle concentrations, GlycA and BCAA, as determined by NMR spectroscopy, were altered in individuals with PA, compared to non-hypertensive control subjects, subjects with untreated hypertension and subjects with medically treated hypertension. The latter three groups of subjects were participants in the Prevention of Renal and Vascular End-stage Disease (PREVEND) cohort study, representing a Dutch cohort recruited from the general population of the city of Groningen, the Netherlands.

2 | SUBJECTS AND METHODS

2.1 | Participants

All studies were performed in the University Medical Center Groningen, the Netherlands.

The PA group consisted of 23 subjects with biochemically confirmed PA, that is elevated plasma aldosterone-renin ratio and non-suppressible 24-hour urinary aldosterone excretion after a 3-day salt loading test.[35] Venous blood samples were obtained at the beginning of an adrenal venous sampling (AVS) procedure for subtype classification. The subjects included in this PA group were enrolled between 2008 and 2015. The PA group originally consisted of 12 men and 11 women. Three patients used metformin because of T2DM, four patients used a statin and two patients were current smokers. The three diabetic patients were excluded from the analysis in order to avoid bias due to concomitant effects of diabetes status on lipoprotein characteristics and GlycA levels.[27,31] The clinical and routine laboratory characteristics of the 20 PA patients used for analysis are shown in Table 1. In the PA group, the plasma renin concentration (PRC) was 2.5 [1.9-2.7] (ng/L), serum aldosterone 570 [382-843] (pmol/L) and the aldosterone/PRC ratio 244 [123-407] (pmol/ng). The 24-hour urinary aldosterone excretion after a 3 day salt loading test was (nmol/24 h) 78 [43-110] (upper limit of normal <37.6 nmol/24 h).[36] Based on the AVS procedure, 12 patients were diagnosed with a unilateral aldosterone producing adenoma and 8 with bilateral adrenal hyperplasia.

TABLE 1 Clinical and laboratory characteristics of primary aldosteronism patients and control groups

	Primary aldosteronism (n = 20)	Subjects without hypertension (n = 3876)	Subjects with untreated hypertension (n = 644)	Subjects with treated hypertension (n = 944)
Age (y)	52.4 ± 12.2	49.1 ± 10.2	58.9 ± 11.2	60.9 ± 10.2**
Sex (men/women)	9/11	1781/2095	385/259	494/450
Systolic blood pressure (mm Hg)	154 ± 29	120 ± 6***	152 ± 12	136 ± 21**
Diastolic blood pressure (mm Hg)	90 ± 13	72 ± 4***	84 ± 8*	77 ± 9***
BMI (kg/m^2)	27.7 ± 4.2	25.7 ± 3.8**	27.8 ± 4.4	28.4 ± 4.3
Current smoking (yes/no)	2/18	1186/2690	147/497	223/721
Statin use (yes/no)	1/19	131/3745	46/598	239/705
eGFR (mL/min/1.73 m^2)	90.5 ± 19.7	97.2 ± 13.8	89.2 ± 14.5	85.9 ± 14.0

Data are given in means ± SD or in numbers.
ARR, aldosterone-renin ratio; BMI, body mass index; eGFR, estimated glomerular filtration rate; PRC, plasma renin concentration; SLT, salt loading test.
P-values compared to the primary aldosteronism group:
*P < 0.016; **P < 0.01; ***P < 0.001. *P*-values <0.016 are considered significant.

The blood material used for the present analysis was obtained in the fasting state during routine medical care. Consequently, the studies that were performed in the PA patients were exempted for approval of the medical ethics committee according to the Dutch Medical Research Involving Human Subjects Act.

In order to compare data from the PA group with appropriate comparator groups, three groups of participants in the PREVEND study were selected: (a) control subjects without hypertension; (b) subjects with untreated hypertension; and (c) subjects with treated hypertension. The latter group was included to take account of potential confounding due to antihypertensive treatment. The PREVEND study investigates vascular and renal damage among inhabitants of the city of Groningen, the Netherlands, in a predominantly white population. Details of the study have been described elsewhere.[28,31,32,37] In short, after exclusion of subjects with insulin-treated diabetes and pregnant women, all subjects with a urinary albumin concentration ≥10 mg/L were invited to participate (n = 7768), of whom 6000 accepted. In addition, a random sample of 2592 individuals with a urinary albumin concentration <10 mg/L was included. These 8592 subjects (aged 28-75 years) completed the baseline survey (1997-1998). The second screening round, during which the blood samples were obtained for the present study, took place between 2001 and 2003 (n = 6894). Plasma lipids, (apo)lipoproteins, lipoprotein particle concentrations, lipoprotein sizes, the inflammation marker, GlycA and BCAA were measured at this second screening round of the PREVEND prospective cohort study in 5526 subjects of whom samples of sufficient quality and quantity were available. We excluded PREVEND participants with T2DM and subjects with a compromised kidney function as indicated by an estimated glomerular filtration rate (eGFR) <60 mL/min/1.73 m^2. First, differences between the PA group and the three separate comparator groups were determined. Second, PA patients were individually matched with subjects from the comparator groups to correct for potential confounders. For each PA patient, we individually matched control subjects without hypertension, subjects with untreated hypertension (ie, blood pressure >140/90 mm Hg without the use of antihypertensive drugs) and subjects with treated hypertension (defined as the use of any antihypertensive drug regardless of actual blood pressure), from the PREVEND cohort study with respect to sex, age (within 5 years), body mass index (BMI; within 5 kg/m^2), current smoking (yes/no) and statin use (yes/no). Note that as a consequence of the individual matching procedure, control subjects without hypertension and subjects with (untreated) hypertension could be included more than once in the comparisons with the individual PA patients. Characteristics of the PA group and the three comparator groups from the PREVEND cohort are presented in Table 1. In total, the 20 PA patients were matched with 2819 control subjects without hypertension, 501 untreated hypertensive subjects and 878 subjects with treated hypertension.

2.2 | Procedures and data ascertainment

Body mass index was calculated as weight (in kg) divided by height squared (in metre). Smoking status was categorized as current (yes/no). In the PA subjects with PA, blood pressure was on the right arm in the supine position using a sphygmomanometer during routine patient care. In PREVEND participants, blood pressure was measured on the right arm, in the supine position, every minute for 10 minutes with an automatic Dinamap XL Model 9300 series device (Johnson-Johnson Medical, Tampa, FL, USA). Hypertension was defined as a systolic blood pressure (SBP) >140 mm Hg and/or a diastolic blood pressure (DBP) >90 mm Hg (untreated hypertension group), or the use of antihypertensive drugs irrespective of the actual blood pressure (treated hypertension group). T2DM was defined as a fasting

plasma glucose level >7.0 mmol/L, a non-fasting plasma glucose level >11.1 mmol/L, self-report of a physician diagnosis and/or the use of glucose lowering drugs, retrieved from a central pharmacy registry. Information on self-reported medication was combined with information from a pharmacy-dispensing registry, which has complete information on drug usage/consumption of >95% of subjects participating in PREVEND.[28,31,32] All participants were instructed to let venous blood being drawn after an overnight fast. Blood samples were taken after 15 minutes rest.

2.3 | Laboratory methods

Ethylenediaminetetraacetic acid (EDTA) anti-coagulated venous blood samples were prepared by centrifugation at 1400 × *g* for 15 minutes and stored at −80°C until assay. Plasma samples were sent frozen to LabCorp, Morrisville, NC, USA, for testing.

Nuclear magnetic resonance spectra were acquired on a Vantera® Clinical Analyzer from EDTA plasma samples as previously described for the *NMR LipoProfile*® test at LabCorp (Morrisville, NC, USA).[38,39] The *NMR MetaboProfile* analysis, which reports lipoprotein particle concentrations and sizes, as well as concentrations total BCAA, that is the sum of valine, leucine, and isoleucine, was performed using the recently developed LP4 lipoprotein profile deconvolution algorithm. Linear regression of the lipoprotein subclass signal areas against serum lipid and apolipoprotein levels measured chemically in a large reference range study population (n = 698) provided the conversion factors to generate NMR-derived concentrations of TC, TG, LDL-C and HDL-C, apolipoprotein B (apoB) and apolipoprotein A-I (apoA-I). The inter-assay precision for these parameters ranges from 1.4% to 6.2%. NMR-derived concentrations of these parameters are highly correlated ($r \geq 0.95$) with those measured by standard methods.

The diameters and inter-assay precision (% coefficients of variation [CV]) for the lipoprotein classes reported by the LP4 algorithm are triglyceride-rich lipoprotein particles (TRL-P; 24-240 nm; 6.4%), LDL particles (LDL-P; 19-23 nm; 1.5%) and HDL particles (HDL-P; 7.4-12.0 nm; 2.4%). Of note, LDL-P and HDL-P generated from the LP4 algorithm have been calibrated to be closer to the absolute concentrations of LDL and HDL particles. Consequently, the LDL and HDL particle concentrations are expected to be on average higher and lower, respectively, than those reported by the previous LP2 and LP3 algorithms. Mean TRL, LDL and HDL particle sizes are weighted averages derived from the sum of the diameter of each subclass multiplied by its relative mass percentage.

The GlycA signal was quantified as previously described.[26] Briefly, the GlycA NMR signal originates from highly mobile protons of the *N*-acetylglucosamine moieties located on the carbohydrate side-chains of circulating acute phase proteins (ie, α1-acid glycoprotein, haptoglobin, α1-antitrypsin, α1-antichymotrypsin and transferrin). The signals from these *N*-acetyl methyl group protons were used to calculate the concentrations of GlycA (μmol/L). The intra-assay and inter-assay CVs for the GlycA assay are 1.9% and 2.6%, respectively.[26,28] Details for the quantification of the BCAA have been previously reported.[34,40] The inter-assay CVs for the BCAA measurements are 3.1% for valine, 5.9% for leucine 14.1% for isoleucine and 3.2% for total BCAA.[34]

Plasma aldosterone was assayed with a competitive fixed-time solid-phase Radioimmunoassay (RIA) as described (Coat-a-Count®; Siemens Medical Solutions Diagnostics, Erlangen, Germany).[36] PRC was measured with an immunoradiometric renin assay (Renin III Generation®; Cisbio, Codolet, France).[36] Other biochemical tests were performed by routine laboratory procedures. eGFR was calculated applying the creatinine-based Chronic Kidney Disease Epidemiology Collaboration Equation.[41]

2.4 | Statistical analysis

IBM SPSS software (version 23.0 IBM Corp, Armonk, NY, USA) was used for data analysis. Data are given in means ± SD, medians (interquartile ranges) or in numbers (percentages). The difference in various variables in the PA group compared to the matched control subjects without hypertension, subjects with untreated hypertension and subjects with treated hypertension was expressed as differences in SD-scores (*Z*-scores). Since we performed three independent comparisons, the threshold for statistical significance was, therefore, set at two-sided *P*-values <0.05/3 = <0.016.

3 | RESULTS

Table 2 shows plasma lipids and (apo)lipoproteins, lipoprotein particle concentrations and sizes, GlycA and BCAA in the PA group (n = 20) in comparison with three groups of unmatched control subjects without hypertension (n = 3876), subjects with untreated hypertension (n = 644) and subjects with treated hypertension (n = 944). Plasma TC and LDL-C were lower in the PA group compared to the three comparator groups. In line with lower LDL-C, also LDL-P and apoB were lower in the PA group compared to all comparator groups. HDL-C was lower in the PA group compared to the normotensive and untreated hypertensive control groups as was apoA-I. HDL-P was lower in the PA group compared to all control groups. Lipoprotein particle sizes were not different between the groups. GlycA was higher in the PA group compared to the control group of subjects without hypertension. The total BCAA concentration was not different in the PA group compared to the three comparator groups, although leucine was lower in PA vs (untreated) hypertension.

Table 3 demonstrates the comparison between the PA group and three matched groups of control subjects without hypertension (n = 2819), subjects with untreated hypertension (n = 501) and subjects with treated hypertension (n = 878). Plasma TC and LDL-C were lower in the PA group vs the three comparator groups. HDL-C was also decreased in the PA group, although the difference with the group of subjects with treated hypertension did not reach significance. In line with lower LDL-C and HDL-C, respectively, apoB and apoA-I were also lower in the PA group. Consistent with lower LDL-C and apoB, LDL-P

TABLE 2 Distribution of lipoprotein, branched-chain amino acids and GlycA in the control groups

	Primary aldosteronism (n = 20)	Subjects without hypertension (n = 3876)	Subjects with untreated hypertension (n = 644)	Subjects with treated hypertension (n = 944)
Total cholesterol (mmol/L)	3.99 ± 0.94	4.97 ± 0.89***	5.23 ± 0.76***	5.05 ± 0.90***
LDL-C (mmol/L)	2.22 ± 0.75	2.92 ± 0.76***	3.11 ± 0.75***	2.99 ± 0.75***
HDL-C (mmol/L)	1.15 ± 0.03	1.62 ± 0.32**	1.31 ± 0.32*	1.29 ± 0.31
Triglycerides (mmol/L)	1.04 ± 0.70	1.19 ± 0.76	1.51 ± 1.12**	1.46 ± 1.00**
apoB (g/L)	0.71 ± 0.21	0.89 ± 0.23***	0.97 ± 0.23***	0.93 ± 0.22***
apoA-I (g/L)	1.17 ± 0.24	1.33 ± 0.22**	1.31 ± 0.23*	1.28 ± 0.23
TRL-P (nmol/L)	134.57 ± 68.28	148.58 ± 62.44	171.61 ± 71.07**	163.81 ± 63.25*
LDL-P (nmol/L)	1171.55 ± 359.21	1468.88 ± 373.43***	1612.56 ± 385.24***	1555.58 ± 371.27***
HDL-P (μmol/L)	18.50 ± 3.04	21.29 ± 2.67***	21.42 ± 2.75***	20.91 ± 2.83***
TRL size (nm)	44.56 ± 5.68	44.97 ± 8.05	47.81 ± 9.50	47.81 ± 9.23
LDL size (nm)	20.88 ± 0.54	21.13 ± 0.51	20.97 ± 0.58	20.93 ± 0.58
HDL size (nm)	9.08 ± 0.64	9.01 ± 0.46	8.89 ± 0.45	8.91 ± 0.44
GlycA (μmol/L)	389.50 ± 69.43	340.98 ± 57.30***	361.02 ± 60.24	369.67 ± 60.92
Total BCAA (μmol/L)	377.00 ± 78.55	369.46 ± 69.10	395.94 ± 76.25	394.52 ± 76.63
Valine (μmol/L)	212.65 ± 40.96	203.15 ± 35.97	215.84 ± 39.14	215.69 ± 38.01
Isoleucine (μmol/L)	49.75 ± 17.43	42.00 ± 14.28	47.04 ± 17.40	45.85 ± 17.40
Leucine (μmol/L)	114.40 ± 29.89	124.32 ± 26.23	133.06 ± 28.41**	132.97 ± 29.94**

Data are given in mean ± SD.
apo, apolipoprotein; BCAA, branched-chain amino acids; HDL, high-density lipoprotein; HDL-C, HDL cholesterol; HDL-P, HDL particles; LDL low-density lipoproteins, LDL-C, LDL cholesterol; LDL-P, LDL particles; TRL, triglyceride-rich lipoproteins, TRL-P, TRL particles.
P-values compared to the primary aldosteronism group:
*$P < 0.016$; **$P < 0.01$; ***$P < 0.001$. *P*-values <0.016 are considered significant.

was decreased in the PA group. Furthermore, consistent with lower HDL-C and apoA-I, HDL-P was decreased in the PA group as well. However, neither LDL nor HDL size was altered in PA patients. Plasma TG concentrations were lower in the PA group vs the group of subjects with untreated hypertension, whereas TRL-P was decreased in PA vs subjects with untreated and treated hypertension. TRL size was also decreased in PA subjects vs subjects with treated hypertension.

GlycA was increased in the PA group vs the three comparator groups (Table 4). Plasma total BCAA were not different in the PA group vs the three comparator groups, although PA patients showed an increase in isoleucine vs the subjects with treated hypertension, whereas leucine was lower in PA patients vs control subjects without hypertension and subjects with untreated hypertension (Table 4).

4 | DISCUSSION

The present study is the first to compare a comprehensive set of (apo)lipoproteins, lipoprotein particle characteristics and other metabolic variables between PA patients, normotensive control subjects and subjects with treated and untreated hypertension, using novel NMR spectroscopy-based methodology. The application of such techniques enables measurement of lipoprotein particle concentrations, as well as GlycA, a pro-inflammatory glycoprotein biomarker, and BCAA obtained from the same NMR spectra without the need to suppress protein and other signals.[26,34,38,39,42]

Previous studies on conventional circulating lipoprotein levels have variably reported lower or unchanged levels of plasma TC, LDL-C and TG, in addition to decreased or unaltered HDL-C in PA compared to healthy subjects or subjects with essential hypertension.[6,8,18,20,21] In one of these studies, lower TC, LDL-C and TG levels in PA patients compared to normotensive and hypertensive control subjects were suggested to be in part attributable to measurement of lipoproteins in the non-fasting state of the control subjects included.[3] Here, we show that plasma TC, LDL-C, HDL-C, apoB, apoA-I, LDL particle and HDL particle concentrations in the fasting state are all decreased in PA vs control subjects and subjects with untreated hypertension, with most of these (apo) lipoprotein variables also being decreased compared to subjects with treated hypertension. In addition, plasma TG and TRL particle concentrations were decreased in PA compared to several comparator groups. The decrements in LDL and HDL particle concentrations did not result in changes in the mean particle diameter of these lipoproteins, supporting the possibility that PA may result in a general reduction in these lipoprotein fractions. The mechanisms responsible for these PA-associated changes in lipoprotein metabolism are unclear at present. Circulating lipoproteins

TABLE 3 Differences in plasma lipids and (apo)lipoproteins, lipoprotein particle concentrations and sizes in primary aldosteronism patients compared to matched normotensive control subjects, subjects with untreated hypertension and subjects with treated hypertension

	Subjects without hypertension (n = 2819)	Subjects with untreated hypertension (n = 501)	Subjects with treated hypertension (n = 878)
Total cholesterol (mmol/L)	−1.66 (−2.60 to −0.72)***	−1.62 (−2.48 to −0.76)***	−1.51 (−2.03 to −0.98)****
LDL-C (mmol/L)	−1.12 (−1.54 to −0.69)****	−1.17 (−1.68 to −0.66)****	−1.36 (−1.91 to −0.80)****
HDL-C (mmol/L)	−0.67 (−1.20 to −0.14)*	−0.83 (−1.44 to −0.23)**	−0.59 (−1.41 to 0.23)
Triglycerides (mmol/L)	−0.23 (−0.75 to 0.29)	−0.57(−0.96 to −0.17)**	−0.31 (−0.83 to 0.21)
apoB (g/L)	−0.94 (−1.31 to −0.57)****	−1.21 (−1.82 to −0.60)***	−1.26 (−1.77 to −0.76)****
apoA-I (g/L)	−0.69 (−1.22 to −0.15)*	−0.75 (−1.37 to −0.12)*	−0.89 (−1.54 to −0.25)**
TRL-P (nmol/L)	−0.35 (−0.81 to 0.11)	−0.48 (−0.94 to −0.01)*	−0.51(−0.95 to −0.08)*
LDL-P (nmol/L)	−0.94 (−1.35 to −0.54)****	−1.15 (−1.72 to −0.58)****	−1.29 (−1.85 to −0.74)****
HDL-P (μmol/L)	−1.09 (−1.59 to −0.58)****	−1.05 (−1.66 to −0.44)***	−1.44 (−2.55 to −0.33)*
TRL size (nm)	−0.24 (−0.61 to 0.13)	−0.30 (−0.59 to −0.01)	−0.37 (−0.62 to −0.12)**
LDL size (nm)	−0.63 (−1.22 to −0.04)*	−0.37 (−0.87 to 0.13)	−0.41 (−1.03 to 0.22)
HDL size (nm)	0.44 (−0.39 to 1.28)	0.34 (−0.28 to 0.96)	0.35 (−0.30 to 1.01)

Differences of the primary aldosteronism group with three control groups are given as standard deviation scores (*Z*-scores) with corresponding 95% confidence intervals.

apo, apolipoprotein; HDL, high-density lipoprotein; HDL-C, HDL cholesterol; HDL-P, HDL particles; LDL, low-density lipoproteins, LDL-C, LDL cholesterol; LDL-P, LDL particles; TRL, triglyceride-rich lipoproteins, TRL-P, TRL particles.

P-values compared to the primary aldosteronism group:

*$P < 0.05$; **$P < 0.016$; ***$P < 0.01$ ****$P < 0.001$ are considered significant.

TABLE 4 GlycA, branched-chain amino acids (BCAA), valine, isoleucine and leucine in primary aldosteronism patients compared with matched normotensive control subjects, subjects with untreated hypertension and subjects with treated hypertension

	Comparison with control subjects without hypertension (n = 2819)	Comparison with subjects with untreated hypertension (n = 501)	Comparison with subjects with treated hypertension (n = 878)
GlycA (μmol/L)	0.93 (0.46-1.40)***	0.78 (0.21-1.39)**	0.57 (0.06-1.07)*
Total BCAA (μmol/L)	−0.13 (−0.76 to 0.51)	−0.11 (−0.62 to 0.39)	0.09 (−0.52 to 0.71)
Valine (μmol/L)	0.08 (−0.50 to 0.66)	0.10 (−0.42 to 0.61)	0.31 (−0.34 to 0.97)
Isoleucine (μmol/L)	0.36 (−0.32 to 1.04)	0.92 (−0.20 to 2.05)	0.67 (0.13 to 1.20)*
Leucine (μmol/L)	−0.69 (−1.32 to −0.06)*	−0.61 (−1.08 to −0.14)*	−0.49 (−1.07 to 0.09)

Differences of the primary aldosteronism group with three control groups are given as standard deviation scores (*Z*-scores) with corresponding 95% confidence intervals.

*$P < 0.05$; **$P < 0.016$; ***$P < 0.01$; ****$P < 0.001$ are considered significant.

may contribute adrenal steroidogenesis, but it seems unlikely that increased adrenal aldosterone production in PA would result in lower LDL and HDL particle concentrations.[35] Liver X receptors (LXR) are known to be influenced by the renin-angiotensin-aldosterone system, but it is unclear whether high aldosterone, or suppressed renin as its consequence, may directly affect lipoprotein metabolism via LXR regulation.[43]

Decreased LDL, HDL and to some extent also TRL particle concentrations are unexpected in the light of a possible increased prevalence of MetS and impaired insulin sensitivity in PA.[16,17,44] In comparison, plasma TG levels are elevated in subjects with endogenous glucocorticoid excess, which results in lower hepatic lipase activity in post-heparin plasma as well in downregulation of cholesteryl ester transfer protein, thereby at least in part maintaining HDL-C.[45,46] Taken together, the present study suggests that PA is characterized by a remarkable, if not unique, lipoprotein profile that consists of decreases in both LDL and HDL particles without affecting TRL.

The GlycA signal originates from methyl groups of *N*-acetylglucosamine (GlcNAc) containing carbohydrate side-chains of several abundant glycoproteins, and GlycA measures the glycan content of proteins, not the protein concentrations as such.[26,27] Only the GlcNAc moieties in β(1 → 2) or β(1 → 6) linkage with a preceding mannose give rise to the GlycA NMR signal.[26] During the past few years, evidence has accumulated that GlycA associates with insulin resistance and MetS, predicts the development of new onset T2DM even when taking account of hsCRP levels and relates to the cardiovascular risk marker, lipoprotein-associated phospholipase A2.[27,29-31,46-48] GlycA has been shown to predict the atherosclerotic cardiovascular disease in population-based cohorts and in high-risk

patient groups, even independent of eGFR and urinary albumin excretion.[32,49-51] GlycA also predicts hospitalization for heart failure.[51] Of further relevance, one of the acute phase proteins captured in the GlycA signal, alpha-1-acid glycoprotein, has been identified as a marker of all-cause mortality in the general population.[51] In the PREVEND cohort, high GlycA levels were associated with reduced life expectancy (Gruppen et al this paper). Collectively, the present findings showing an increase in GlycA in PA consents with the notion that this condition gives rise to a state of low grade enhanced systemic inflammation.[13,16] Hence, it is plausible to postulate that low-grade systemic inflammation could contribute to the negative impact of PA on (cardiovascular) morbidity.[6,7]

The BCAA, valine, leucine and isoleucine, are essential amino acids that not only regulate protein production and catabolism but are also involved in glucose metabolism.[33,34,40,52,53] BCAA may be implicated in the development of insulin resistance by enhancing mitochondrial stress. On the other hand, insulin resistance itself could elicit higher fasting BCAA levels.[54] Using the same NMR-based technique, we recently reported elevated BCAA levels in T2DM and MetS, and observed a positive association of carotid intima media thickness with BCAA.[35] In the present study, no alterations in plasma total BCCA were observed in PA vs the various comparator populations. This makes an important contribution of abnormalities in BCAA metabolism on possible cardiometabolic abnormalities in PA unlikely.

The present study has strengths and limitations. We excluded three PA patients with T2DM. Consequently, we also excluded PREVEND subjects with T2DM. Additionally, PREVEND participants with compromised kidney function were excluded. These exclusion criteria were applied in order to circumvent bias due to diabetes- and chronic kidney disease-associated alterations in lipoprotein variables, GlycA and BCAA in the various comparator groups. Furthermore, although the PA group studied was moderately sized, these patients were individually matched with a considerable number of PREVEND participants. This approach was chosen to increase statistical power in comparing lipoprotein variables, GlycA and BCAA levels in PA patients with control subjects without hypertension and subjects with (treated) hypertension. However, it should be appreciated that we did not aim to compare the presently studied NMR-based variables between these three comparator groups. In view of the set-up of the present report, fasting plasma glucose and insulin levels were not available in PA patients, obviating the ability to demonstrate differences in insulin resistance and β-cell function between PA patients and the various comparator groups. Additionally, co-secretion of cortisol was not formally excluded in PA patients. Theoretically, cortisol co-secretion could have influenced lipoprotein profiles. Most likely, however, co-secretion of cortisol would have resulted in increased levels of LDL cholesterol and triglycerides, contrasting with our findings of significantly lower levels of LDL particle concentration and LDL-C as well as a tendency towards lower levels of triglycerides.[55,56] It should also be noted that the PA patients were of north European descent as were far most PREVEND participants. The present findings, therefore, do not necessarily hold true for non-white ethnicities. Finally, the moderate number of PA patients included in this report did not allow to provide meaningful analysis regarding possible differences in NMR-based variables between patients with a unilateral aldosterone producing adenoma and patients with bilateral adrenal hyperplasia.[57]

In conclusion, the present report demonstrates that PA is associated with decreases in plasma total cholesterol, LDL cholesterol, HDL cholesterol, apolipoprotein B and A-I, as well as in LDL and HDL particle concentrations and to some extent also with lower TG and TRL particle concentrations. This suggests that PA is featured by a rather unique lipoprotein profile. Furthermore, PA is characterized by increased GlycA levels, indicating enhanced low-grade chronic inflammation. Low HDL particle concentrations and increased GlycA could contribute to accelerated development of atherosclerotic cardiovascular disease in PA.

CONFLICT OF INTEREST

Nothing to declare.

ORCID

Annika M. A. Berends http://orcid.org/0000-0002-8440-332X

REFERENCES

1. Rossi GP, Bernini G, Caliumi C, et al. A prospective study of the prevalence of primary aldosteronism in 1,125 hypertensive patients. *J Am Coll Cardiol*. 2006;48:2293-2300.
2. Hannemann A, Wallaschofski H. Prevalence of primary aldosteronism in patient's cohorts and in population-based studies–a review of the current literature. *Horm Metab Res*. 2012;44:157-162.
3. Reincke M, Beuschlein F. Progress in primary aldosteronism: translation on the move. *Horm Metab Res*. 2015;47:933-944.
4. Käyser SC, Dekkers T, Groenewoud HJ, et al. Study heterogeneity and estimation of prevalence of primary aldosteronism: a systematic review and metaregression analysis. *J Clin Endocrinol Metab*. 2016;101:2826-2835.
5. Monticone S, D'Ascenzo F, Moretti C, et al. Cardiovascular events and target organ damage in primary aldosteronism compared with essential hypertension: a systematic review and meta-analysis. *Lancet Diabetes Endocrinol*. 2018;6:41-50.
6. Milliez P, Girerd X, Plouin PF, et al. Evidence for an increased rate of cardiovascular events in patients with primary aldosteronism. *J Am Coll Cardiol*. 2005;45:1243-1248.
7. Catena C, Colussi G, Nadalini E, et al. Cardiovascular outcomes in patients with primary aldosteronism after treatment. *Arch Intern Med*. 2008;168:80-85.
8. Reincke M, Fischer E, Gerum S, et al. Observational study mortality in treated primary aldosteronism: the German Conn's registry. *Hypertension*. 2012;60:618-624.
9. Ambrosino P, Lupoli R, Tortora A, et al. Cardiovascular risk markers in patients with primary aldosteronism: a systematic review and meta-analysis of literature studies. *Int J Cardiol*. 2016;208:46-55.
10. Farquharson CA, Struthers AD. Aldosterone induces acute endothelial dysfunction in vivo in humans: evidence for an aldosterone-induced vasculopathy. *Clin Sci (Lond)*. 2002;103:425-431.

11. Rocha R, Rudolph AE, Frierdich GE, et al. Aldosterone induces a vascular inflammatory phenotype in the rat heart. *Am J Physiol Heart Circ Physiol*. 2002;283:H1802-H1810.
12. Luther JM, Gainer JV, Murphey LJ, et al. Angiotensin II induces interleukin-6 in humans through a mineralocorticoid receptor-dependent mechanism. *Hypertension*. 2006;48:1050-1057.
13. Stehr CB, Mellado R, Ocaranza MP, et al. Increased levels of oxidative stress, subclinical inflammation, and myocardial fibrosis markers in primary aldosteronism patients. *J Hypertens*. 2010;28:2120-2126.
14. Zhang X, Liu J, Pang X, Zhao J, Wang S, Wu D. Aldosterone induces C-reactive protein expression via MR-ROS-MAPK-NF-κB signal pathway in rat vascular smooth muscle cells. *Mol Cell Endocrinol*. 2014;395:61-68.
15. Krug AW, Ehrhart-Bornstein M. Aldosterone and metabolic syndrome: is increased aldosterone in metabolic syndrome patients an additional risk factor? *Hypertension*. 2008;51:1252-1258.
16. Funder JW, Reincke M. Aldosterone: a cardiovascular risk factor? *Biochim Biophys Acta*. 2010;1802:1188-1192.
17. Fallo F, Veglio F, Bertello C, et al. Prevalence and characteristics of the metabolic syndrome in primary aldosteronism. *J Clin Endocrinol Metab*. 2006;91:454-459.
18. Mosso LM, Carvajal CA, Maiz A, et al. A possible association between primary aldosteronism and a lower beta-cell function. *J Hypertens*. 2007;25:2125-2130.
19. Bochud M, Nussberger J, Bovet P, et al. Plasma aldosterone is independently associated with the metabolic syndrome. *Hypertension*. 2006;48:239-245.
20. Goodfriend TL, Egan B, Stepniakowski K, Ball DL. Relationships among plasma aldosterone, high-density lipoprotein cholesterol, and insulin in humans. *Hypertension*. 1995;25:30-36.
21. Šomlóová Z, Petrák O, Rosa J, et al. Inflammatory markers in primary aldosteronism. *Physiol Res*. 2016;65:229-237.
22. Adolf C, Asbach E, Dietz AS, et al. Worsening of lipid metabolism after successful treatment of primary aldosteronism. *Endocrine*. 2016;54:198-205.
23. Grotevendt A, Wallaschofski H, Reincke M, et al. Associations of aldosterone and renin concentrations with inflammation-the Study of Health in Pomerania and the German Conn's Registry. *Endocrine*. 2017;57:298-307.
24. Otvos JD, Collins D, Freedman DS, et al. Low-density lipoprotein and high-density lipoprotein particle subclasses predict coronary events and are favorably changed by gemfibrozil therapy in the Veterans Affairs High-Density Lipoprotein Intervention Trial. *Circulation*. 2006;113:1556-1563.
25. Mora S, Otvos JD, Rifai N, Rosenson RS, Buring JE, Ridker PM. Lipoprotein particle profiles by nuclear magnetic resonance compared with standard lipids and apolipoproteins in predicting incident cardiovascular disease in women. *Circulation*. 2009;119:931-939.
26. Otvos JD, Shalaurova I, Wolak-Dinsmore J, et al. GlycA: a composite nuclear magnetic resonance biomarker of systemic inflammation. *Clin Chem*. 2015;61:714-723.
27. Connelly MA, Gruppen EG, Otvos JD, Dullaart R. Inflammatory glycoproteins in cardiometabolic disorders, autoimmune diseases and cancer. *Clin Chim Acta*. 2016;459:177-186.
28. Gruppen EG, Connelly MA, Vart P, Otvos JD, Bakker SJ, Dullaart RP. GlycA, a novel proinflammatory glycoprotein biomarker, and high-sensitivity C-reactive protein are inversely associated with sodium intake after controlling for adiposity: the Prevention of Renal and Vascular End-Stage Disease study. *Am J Clin Nutr*. 2017;104:415-422.
29. Gruppen EG, Connelly MA, Otvos JD, Bakker SJ, Dullaart RP. A novel protein glycan biomarker and LCAT activity in metabolic syndrome. *Eur J Clin Invest*. 2015;45:850-859.
30. Dullaart RP, Gruppen EG, Connelly MA, Lefrandt JD. A pro-inflammatory glycoprotein biomarker is associated with lower bilirubin in metabolic syndrome. *Clin Biochem*. 2015;48:1045-1047.
31. Connelly MA, Gruppen EG, Wolak-Dinsmore J, et al. GlycA, a marker of acute phase glycoproteins, and the risk of incident type 2 diabetes mellitus: PREVEND study. *Clin Chim Acta*. 2016;452:10-17.
32. Gruppen EG, Riphagen IJ, Connelly MA, Otvos JD, Bakker SJ, Dullaart RP. GlycA, a pro-inflammatory glycoprotein biomarker, and incident cardiovascular disease: relationship with C-reactive protein and renal function. *PLoS ONE*. 2015;10:e0139057.
33. Lynch CJ, Adams SH. Branched-chain amino acids in metabolic signalling and insulin resistance. *Nat Rev Endocrinol*. 2014;12:723-736.
34. Wolak-Dinsmore J, Gruppen EG, Shalaurova I, et al. A novel NMR-based assay to measure circulating concentrations of branched-chain amino acids: elevation in subjects with type 2 diabetes mellitus and association with carotid intima media thickness. *Clin Biochem*. 2018;54:92-99.
35. Buitenwerf E, Dullaart R, Muller Kobold AC, et al. Cholesterol delivery to the adrenal glands estimated by adrenal venous sampling: an in vivo model to determine the contribution of circulating lipoproteins to steroidogenesis in humans. *J Clin Lipidol*. 2017;11:733-738.
36. Kerstens MN, Kobold AC, Volmer M, Koerts J, Sluiter WJ, Dullaart RP. Reference values for aldosterone-renin ratios in normotensive individuals and effect of changes in dietary sodium consumption. *Clin Chem*. 2011;57:1607-1611.
37. Lambers Heerspink HJ, Brantsma AH, de Zeeuw D, et al. Albuminuria assessed from first-morning-void urine samples versus 24-hour urine collections as a predictor of cardiovascular morbidity and mortality. *Am J Epidemiol*. 2008;168:897-905.
38. Jeyarajah EJ, Cromwell WC, Otvos JD. Lipoprotein particle analysis by nuclear by nuclear magnetic resonance spectroscopy. *Clin Lab Med*. 2006;26:847-870.
39. Matyus SP, Braun PJ, Wolak-Dinsmore J, et al. NMR measurement of LDL particle number using the vantera clinical analyzer. *Clin Biochem*. 2014;47:203-210.
40. Connelly MA, Wolak-Dinsmore J, Dullaart R. Branched chain amino acids are associated with insulin resistance independent of leptin and adiponectin in subjects with varying degrees of glucose tolerance. *Metab Syndr Relat Disord*. 2017;15:183-186.
41. Inker LA, Schmid CH, Tighiouart H, et al. Estimating glomerular filtration rate from serum creatinine and cystatin C. *N Engl J Med*. 2012;367:20-29.
42. Matyus SP, Braun PJ, Wolak-Dinsmore J, et al. HDL particle number measured on the Vantera®, the first clinical NMR analyzer. *Clin Biochem*. 2015;48:148-155.
43. Maqdasy S, Trousson A, Tauveron I, Volle DH, Baron S, Lobaccaro JM. Once and for all, LXRα and LXRβ are gatekeepers of the endocrine system. *Mol Aspects Med*. 2016;49:31-46.
44. Catena C, Lapenna R, Baroselli S, et al. Insulin sensitivity in patients with primary aldosteronism: a follow-up study. *J Clin Endocrinol Metab*. 2006;91:3457-3463.
45. Taskinen MR, Nikkilä EA, Pelkonen R, Sane T. Plasma lipoproteins, lipolytic enzymes, and very low density lipoprotein triglyceride turnover in Cushing's syndrome. *J Clin Endocrinol Metab*. 1983;57:619-626.
46. Akinkuolie AO, Pradhan AD, Buring JE, Ridker PM, Mora S. Novel protein glycan side-chain biomarker and risk of incident type 2 diabetes mellitus. *Arterioscler Thromb Vasc Biol*. 2015;35:1544-1550.
47. Gruppen EG, Connelly MA, Dullaart RP. Higher circulating GlycA, a pro-inflammatory glycoprotein biomarker, relates to lipoprotein-associated phospholipase A2 mass in nondiabetic subjects but not in diabetic or metabolic syndrome subjects. *J Clin Lipidol*. 2016;10:512-518.

48. Werumeus Buning J, Dimova LG, Perton FG, Tietge U, van Beek AP, Dullaart R. Downregulation of cholesteryl ester transfer protein by glucocorticoids: a randomised study on HDL. *Eur J Clin Invest*. 2017;47:494-503.
49. Akinkuolie AO, Buring JE, Ridker PM, Mora S. A novel protein glycan biomarker and future cardiovascular disease events. *J Am Heart Assoc*. 2014;3:e001221.
50. Otvos JD, Guyton JR, Connelly MA, et al. Relations of GlycA and lipoprotein particle subspecies with cardiovascular events and mortality: a post hoc analysis of the AIM-HIGH trial. *J Clin Lipidol*. 2018;12:348-355.
51. Muhlestein JB, May HT, Galenko O, et al. GlycA and hsCRP are independent and additive predictors of future cardiovascular events among patients undergoing angiography: the intermountain heart collaborative study. *Am Heart J*. 2018;202:27-32.
52. Newgard CB. Metabolomics and metabolic diseases: where do we stand? *Cell Metab*. 2017;25:43-56.
53. Würtz P, Soininen P, Kangas AJ, et al. Branched-chain and aromatic amino acids are predictors of insulin resistance in young adults. *Diabetes Care*. 2013;36:648-655.
54. Mahendran Y, Jonsson A, Have CT, et al. Genetic evidence of a causal effect of insulin resistance on branched-chain amino acid levels. *Diabetologia*. 2017;60:873-878.
55. Boero L, Manavela M, Botta E, et al. Conditioning factors for high cardiovascular risk in patients with cushing syndrome. *Endocr Pract*. 2015;21:734-742.
56. Taskinen MR, Nikkilä EA, Pelkonen R, et al. Plasma lipoproteins, lipolytic enzymes, and very low density lipoprotein triglyceride turnover in Cushing's syndrome. *J Clin Endocrinol Metab*. 1983;57:619-626.
57. Somlóová Z, Indra T, Rosa J, et al. Have main types of primary aldosteronism different phenotype? *Physiol Res*. 2012;61:431-436.

How to cite this article: Berends AMA, Buitenwerf E, Gruppen EG, et al. Primary aldosteronism is associated with decreased low-density and high-density lipoprotein particle concentrations and increased GlycA, a pro-inflammatory glycoprotein biomarker. *Clin Endocrinol*. 2019;90:79–87. https://doi.org/10.1111/cen.13891