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Original Research

Fibrinogen and plasma clot properties are associated with apolipoprotein B and apolipoprotein B-containing lipoproteins in Africans

Daniel Bruwer, MSc; Zelda de Lange-Loots, PhD; Marlys L. Koschinsky, PhD; Michael B. Boffa, PhD; Marlien Pieters, PhD*

Centre of Excellence for Nutrition, Faculty of Health Sciences, North-West University, Potchefstroom, South Africa (Drs Bruwer, de Lange-Loots and Pieters); SAMRC Extramural Unit for Hypertension and Cardiovascular Disease, Faculty of Health Sciences, North-West University, Potchefstroom, South Africa (Drs de Lange-Loots and Pieters); Department of Physiology & Pharmacology and Robarts Research Institute, Schulich School of Medicine & Dentistry, University of Western Ontario, London, Ontario, Canada (Dr Koschinsky); Department of Biochemistry and Robarts Research Institute, Schulich School of Medicine & Dentistry, The University of Western Ontario, London, Canada (Dr Boffa)

KEYWORDS
African;
Apolipoprotein B;
Lipoprotein(a);
Fibrinogen;
Fibrinolysis

BACKGROUND: Case-control, intervention and laboratory studies have demonstrated a link between apolipoprotein B (ApoB)-containing lipoproteins and clot structure and thrombosis. There is, however, limited evidence on a population level.

OBJECTIVES: We determined the cross-sectional relationship between lipoprotein(a) [Lp(a)], low-density lipoprotein cholesterol (LDL-C), and ApoB with fibrinogen and plasma clot properties in 1462 Black South Africans, a population with higher fibrinogen and Lp(a) levels compared with individuals of European descent.

METHODS: Data were obtained from participants in the South African arm of the Prospective Urban and Rural Epidemiology study. Clot properties analyzed included lag time, slope, maximum absorbance, and clot lysis time (turbidity). Lp(a) was measured in nM using particle-enhanced immunoturbidimetry. General linear models (GLM) were used to determine the associations between ApoB and ApoB-containing lipoproteins with fibrinogen and plasma clot properties. Stepwise regression was used to determine contributors to clot properties and Lp(a) variance.

RESULTS: GLM and regression results combined, indicated fibrinogen concentration and rate of clot formation (slope) had the strongest association with Lp(a); clot density associated positively with both Lp(a) and LDL-C; time to clot formation associated negatively with ApoB; and clot lysis time (CLT) demonstrated strong positive associations with both ApoB and LDL-C, while its association with Lp(a) was fibrinogen concentration dependent.

* Correspondence author at: Centre of Excellence for Nutrition, North-West University, Potchefstroom Campus, Private Bag X6001, Potchefstroom, 2520, South Africa.
E-mail address: marlien.pieters@nwu.ac.za (M. Pieters).
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Social media: (Z. de Lange-Loots), (M.L. Koschinsky), (M.B. Boffa), (M. Pieters)

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CONCLUSION: These findings suggest that ApoB and the lipoproteins carrying it contribute to prothrombotic clot properties in Africans on an epidemiological level and highlight potential novel prothrombotic roles for these (apo)lipoproteins to be considered for the development of targeted therapeutic approaches to address thrombotic conditions related to clot properties.

Introduction

Accumulating evidence demonstrates a close relationship between lipoprotein metabolism, coagulation, and fibrinolysis. Lipoproteins are known to modulate the expression and function of coagulation and fibrinolytic factors, transport coagulation proteins, and demonstrate a dynamic, reciprocal regulation with the fibrinolytic system.[1-3]

Increased concentrations of low-density lipoprotein cholesterol (LDL-C), representing one of the primary apolipoprotein B (ApoB)-containing lipoprotein species, are associated with hypercoagulability[4,5] and more specifically, prothrombotic clot properties—clots comprised of thinner fibers that are densely packed (less permeable), stiffer and more difficult to lyse.[6-8] Additionally, interventions known to reduce LDL-C, *e.g.*, weight loss and statin use, have been shown to improve clot properties, specifically improving lysis rates.[9-11] On the other hand, high-density lipoprotein cholesterol (HDL-C), representing a lipoprotein class containing primarily apolipoproteins A1 and A2, is associated with improved clot permeability, decreased density, and improved lysis.[7,12] Proteomic analysis of plasma clots, furthermore, reveals that the apolipoproteins of low- and high-density lipoproteins are present in plasma clots.[8,13,14]

The relationship between another ApoB-containing lipoprotein, lipoprotein(a) [Lp(a)], and plasma clot properties, specifically clot density and clot lysis, is more complex and, to date, less well understood. Evidence from case-control studies report high Lp(a) to be associated with the formation of less permeable,[15-18] more dense[16] clots. However, some studies also found no association with clot density.[17,18] Numerous observational and *in vitro* studies have reported on the relationship between Lp(a) and clot lysis time (CLT) with discordant findings.[16,19-22] Results from a Lp(a)-lowering intervention study that used an antisense oligonucleotide found no reduction in *ex vivo* CLT,[23] while an intervention that lowered a variety of ApoB-containing lipoproteins, including Lp(a), with apheresis reported a reduction in *in vivo* CLT.[24]

These inconsistencies are likely due to methodological differences between the studies including study design, analysis of endogenous Lp(a) levels *vs.* the addition of purified Lp(a) to patient samples, analytical differences in Lp(a) and CLT measurement techniques, the use of plasma *vs.* whole blood, or participant characteristics such as healthy *vs.* diseased, ethnicity, and gender.

Whether the measurement of ApoB itself is preferable to the measurement of the lipoproteins carrying it, particularly in a clinical setting, is contested.[25] To our knowledge, research that has investigated the relationship between ApoB and clot properties specifically, is limited to two case-control studies that both found ApoB to be associated with a prothrombotic clot phenotype, specifically decreased clot permeability.[13,26]

Individuals of African descent generally have higher fibrinogen[27-29] and median Lp(a) concentrations,[30-32] with the latter being attributable to higher Lp(a) levels associated with medium-sized apolipoprotein(a) [Lp(a)] isoforms in Africans. Consequently, individuals of African descent may have an increased risk of thrombotic disease.[7,33,34] However, the relationship of Lp(a) and ApoB with fibrinogen and plasma clot properties has yet to be studied in this population. Furthermore, no large observational studies have been performed to determine whether Lp(a) or ApoB concentration is associated with fibrinogen, CLT and other clot properties at a population level. Therefore, we aimed to determine the associations between two ApoB-containing lipoproteins, LDL-C and Lp(a), as well as ApoB itself, with fibrinogen concentration and plasma clot properties in individuals of African descent.

Materials and methods

Study population

The Prospective Urban and Rural Epidemiology (PURE) study is a large multinational observational cohort study that investigates how socio-demographic, lifestyle and established risk factors relate to non-communicable disease in low-, middle-, and high-income countries.[35] The data reported here are the cross-sectional baseline data collected in 2005 from a total of 2010 randomly selected PURE participants from the North West Province of South Africa. Power analysis revealed that a sample of approximately 2000 participants would be needed to perform a meaningful analysis.[36] Inclusion criteria were apparently healthy men and women older than 30 years of age. Exclusion criteria were any self-reported prior cardiovascular event, acute illness, pregnancy, or lactation. This study has ethical approval from the North-West University Health Research Ethics Committee (04M10; 2 September 2004) and was performed per the revised Dec-

laration of Helsinki. Written informed consent was obtained from all participants prior to data collection.

Socio-demographic data

Information relating to socio-demographic and lifestyle behaviors (such as alcohol, tobacco, hormonal contraceptive, and medication use) was collected by trained field workers using validated, standardized questionnaires.

Anthropometric and blood pressure data

All anthropometric measures were performed according to the International Society for the Advancement of Kinanthropometry (ISAK) guidelines.[37] Brachial artery blood pressure (BP) was measured with an Omron HEM-757 automatic digital blood pressure monitor (Omron Healthcare, Kyoto, Japan). Hypertension was defined as the use of antihypertensive medication, systolic BP $\geq$ 140 mm Hg, or diastolic BP $\geq$ 90 mm Hg according to the 2018 European Society of Cardiology / European Society of Hypertension guidelines.[38]

Blood processing

Blood samples were collected from fasting (overnight) participants by registered nurses, between 7:00 AM and 11:00 AM. For the measurement of lipids, ApoB, Lp(a), albumin, interleukin-6 (IL-6), and urea, blood was collected in tubes without anticoagulant. Blood was collected in sodium citrate tubes for the measurement of total and γ' fibrinogen, plasma clot properties, and plasminogen activator inhibitor-1 activity ($PAI\text{-}1_{act}$), and in ethylenediamine tetra-acetic acid (EDTA) tubes for hemoglobin A1c (HbA1c) measurement. Samples were centrifuged for 15 min at 2000 x g within 30 min of collection and the serum/platelet-poor plasma was snap frozen and stored at –80 °C until analysis.

Laboratory analysis

Exposure variables: Serum lipids—triglycerides, HDL-C, and total cholesterol—were measured using the Konelab20i™ auto-analyser (Thermo Fischer Scientific, Vantaa, Finland). LDL-C was calculated using the Friedewald–Levy–Fredrickson formula, as described by Friedewald *et al*.[39] Lp(a) concentration was measured in nM using particle-enhanced immunoturbidimetry with an automated Cobas Integra 400 plus analyzer (Tina-quant Lp(a) Gen.2, Roche, Basel, Switzerland). ApoB was measured with a multiplex magnetic bead-based immunoassay (Merck Millipore, Darmstadt, Germany) on a Luminex 200 system (Luminex, Austin, TX, United States).

Outcome variables: Total fibrinogen concentration was measured using a modified Clauss method on the Dade Behring BCS coagulation analyzer (Multifibrin *U test* Dade Behring, Deerfield, IL, USA). Fibrinogen γ' was measured with an enzyme-linked immunosorbent assay according to the method of Uitte de Willige.[40] A 2.G2.H9 mouse monoclonal coating antibody against the human γ' sequence from Santa Cruz Biotechnology (Santa Cruz, CA) was used for antigen capture and a goat polyclonal horseradish peroxidase–conjugated antibody against human fibrinogen from Abcam was used for development (Cambridge, MA). Fibrinogen γ' was reported as the relative concentration (%) of total fibrinogen. Plasma clot properties—lag time (time required for fibrin fibers to attain the appropriate length to allow lateral aggregation as well as activation of the coagulation cascade), slope (rate of lateral aggregation), maximum absorbance (MA) (clot density), and CLT—were measured with a modified global fibrinolytic potential turbidity assay.[41] Clotting was triggered by addition of tissue factor (TF) and recalcification, and lysis triggered by the addition of exogenous tissue plasminogen activator (tPA). The TF and tPA concentrations were slightly modified for comparable CLTs of approximately 60–100 min in control plasma. Final concentrations were 125 x diluted TF (Dade Innovin, Siemens Healthcare Diagnostics Inc., Marburg, Germany), 17 mM $CaCl_2$, 100 ng/mL tPA (Actilyse, Boehringer Ingelheim, Ingelheim, Germany) and 10 mM phospholipid vesicles (Rossix, Mölndal, Sweden). Turbidity was measured using a Multiskan Ascent spectrophotometer (Thermo Labsystems, Philadelphia, PA, USA) at an absorbance of 405 nm, and curves were generated and analyzed using Origin® software, version 8.5 (Origin lab®, 2010) (Online Supplement Figure 1 and 2).

Covariates included in statistical models: $PAI\text{-}1_{act}$ was quantified using an indirect enzymatic technique (Spectrolyze PAI-1, Trinity Biotech, Bray, Ireland). Albumin and urea concentrations were measured using the Konelab20i™ auto-analyzer (Thermo Fischer Scientific, Vantaa, Finland). HbA1c was measured using the d-10 Haemoglobin Testing System (Biorad, Hercules, California, USA). IL-6 was quantified via ultra-sensitive immunoassays (Elecsys 2010, Roche, Basel, Switzerland).

Statistical analysis

Statistical analyses were performed using SPSS® version 28 (IBM Corp, 2022). Type-I error was set at α=0.05. Normality was tested using the Shapiro–Wilk test, histograms, and Q-Q plots. Continuous variables that were not normally distributed are expressed as median [quartile 1; quartile 3], and continuous variables with a normal distribution as mean (95% confidence interval [CI]). Continuous variables that were not normally distributed were log-transformed to improve model fit.

Of the initial 2010 participants, those with missing data for either the exposure (Lp(a), ApoB, or LDL-C) or outcome variables (*i.e.*, fibrinogen and plasma clot properties) were excluded (Online Supplement Figure 3). Subsequent comparisons (independent *t*-tests) of the exposure and outcome variables' means between the original sample and the remaining sub-sample were performed to confirm that the exclusion of these participants did not create bias.

Independent *t*-tests were used to compare exposure variables between two groups and analysis of variance (ANOVA) with post-hoc tests (Bonferroni correction) were used for comparisons between three or more groups. Spearman correlations were performed to analyze the correlation between variables. The above-mentioned analyses were performed to identify co-variates for inclusion in linear regression analysis and general linear models (GLMs). For each clinical category, only the co-variate with the strongest association with the exposure and/or outcome variables was selected for inclusion, to minimize co-linearity. Stepwise linear regression analysis was performed to identify which exposures and covariates contributed to the variability of Lp(a) and plasma clot properties.

Consecutive GLMs were performed to evaluate the relationship between exposure variables and total and percentage γ' fibrinogen and plasma clot properties. Model 1 was unadjusted. Model 2 was adjusted for fibrinogen and performed for clot property outcomes only. Model 3 additionally adjusted for the identified covariates—sex, age, body mass index (BMI), alcohol use, tobacco use, hypertension, HbA1c, IL-6, urea, PAI-1, and albumin. Model 4 additionally adjusted for the two other exposure variables; for example, if Lp(a) was the exposure variable for a GLM, then ApoB and LDL-C were treated as covariates. Model assumptions of linearity and normality were evaluated by visual inspection of a scatter plot (independent variables against regression residuals) and a histogram (using standardized residuals), respectively. Bootstrapping was performed (using 1000 samples) if the original GLM did not meet linearity or normality requirements.

Results

Complete data were available for 1462 participants. The mean of the exposure and outcome variables did not differ significantly in this smaller dataset from the original sample of 2010 participants, indicating this group to be representative of the total study sample (not shown). The median age of the participants with a complete dataset was 48.5 years, and 62.6% were women. They had median LDL-C, ApoB and Lp(a) concentrations of 2.78 mM, 76.2 mg/dL (~1.39 μM), and 67.1 nM, respectively (Table 1, Fig. 1). While 17% of the participants used blood pressure medication, < 2% used hypoglycemic agents, and < 1% used lipid-lowering medication.

Lp(a) was positively associated with fibrinogen concentration, slope, MA, and CLT, and negatively associated with% γ' fibrinogen in the unadjusted models (Fig. 2a; Model 1; Online Supplement Table 1). Regarding clot properties, significance remained only for slope and MA after adjustment for fibrinogen concentration (Model 2). Also in the fully adjusted models, significance remained for total and % γ' fibrinogen, slope, and MA (Model 3). Additional adjustment for ApoB and LDL-C in the Lp(a) model did not significantly alter the results (Model 4).

Apart from the associations with fibrinogen and clot properties, Lp(a) also correlated with LDL-C ($r = 0.21$, $p < 0.001$), age ($r = 0.12$, $p < 0.001$), HbA1c ($r = 0.09$, $p < 0.001$), and urea ($r = 0.09$, $p < 0.001$). The cumulative percentage of Lp(a) variance attributable to the measured variables was 8.9%. LDL-C was the largest contributor, contributing 4.6% of the Lp(a) concentration variance. The second largest contributor was fibrinogen, contributing 2.8% of the variance. Urea and ApoB each contributed < 1%.

ApoB was negatively associated with lag time and positively with CLT (Fig. 2b; Model 1; Online Supplement Table 2). These associations remained after adjustment for fibrinogen (Model 2) and in the fully adjusted models (Model 3). Additional adjustment for Lp(a) and LDL-C in the ApoB model did not significantly alter the results (Model 4).

ApoB also correlated with LDL-C ($r = 0.44$, $p < 0.001$), PAI-1_{act} ($r = 0.23$, $p < 0.001$), BMI ($r = 0.21$, $p < 0.001$), HbA1c ($r = 0.13$, $p < 0.001$), urea ($r = 0.11$, $p < 0.001$), and IL-6 ($r = –0.10$, $p < 0.001$).

LDL-C was positively associated with fibrinogen concentration, MA and CLT (Figure 2c; Model 1; Online Supplement Table 3). The significant association with MA and CLT remained after adjustment for fibrinogen (Model 2) and in the fully adjusted model (Model 3). The relationship with fibrinogen, however, became non-significant in the fully adjusted model (Model 3). The covariates that significantly contributed to fibrinogen concentration in Model 3 were: IL-6 ($p < 0.001$), HbA1c ($p = 0.002$), BMI ($p = 0.002$), age ($p = 0.006$), and sex ($p = 0.04$). The association of LDL-C with MA remained significant after additional adjustment for Lp(a) and ApoB (Model 4), while fibrinogen and CLT no longer differed across the quartiles. The covariates that significantly contributed to fibrinogen concentration in Model 4 were Lp(a) ($p < 0.001$) and the same above-mentioned covariates that were significant in Model 3. The covariates that significantly contributed to CLT in Model 4 were ApoB ($p = 0.003$), tobacco use ($p = 0.005$), alcohol consumption, fibrinogen concentration, HbA1c, PAI-1_{act}, BMI and urea (all $p < 0.001$). Analyses were also performed for non-HDL-C; the results were similar to that of LDL-C (data not shown).

LDL-C also correlated with ApoB ($r = 0.44$, $p < 0.001$), urea ($r = 0.30$, $p < 0.001$), BMI ($r = 0.28$, $p < 0.001$), Lp(a) ($r = 0.21$, $p < 0.001$), HbA1c ($r = 0.18$, $p < 0.001$), PAI-1_{act} ($r = 0.12$, $p < 0.001$), age ($r = 0.10$, $p < 0.001$), and IL-6 ($r = –0.09$, $p = 0.001$).

Based on the forward stepwise regression results, Lp(a) significantly contributed to the variance in fibrinogen concentration (3.5%), % γ' fibrinogen (0.6%), slope (0.6%), and MA (1.1%) but not CLT, while ApoB contributed to lag time (0.8%), slope (0.2%) and CLT (1%) variance and LDL-C to fibrinogen concentration (0.4%), MA (0.2%), and CLT (1.1%) variance. When all three lipid exposures were combined in one model; of the three exposures, only Lp(a) remained a significant contributor to fibrinogen concentration (3.5%), % γ' fibrinogen (0.6%), slope (0.6%), and MA (1.1%). ApoB was the only lipid exposure contributing to lag

Table 1. Descriptive characteristics of the study participants ($n = 1462$).

Variable	Descriptive measure
Demographic variables	
Age (years)	48.5 [42.1; 56.4]
Female sex [n (%)]	915 (62.6)
Lipid markers	
Total cholesterol (mM)	4.82 [4.02; 5.87]
Low-density lipoprotein cholesterol (mM)	2.76 [2.06; 3.62]
Triglycerides (mM)	1.07 [0.81; 1.54]
Lipoprotein(a) (nM)	67.1 [30.5; 127]
Apolipoprotein B (mg/dL)	76.2 [57.9; 102]
Hemostatic markers[*]	
Clot lysis time (min)	57.2 [50.9; 64.1]
Lag time (min)	5.02 [3.67; 6.23]
Slope ($x10^{-3}$ au/s)	6.39 [3.99; 9.33]
Maximum absorbance (Δau)	0.43 [0.34; 0.53]
Fibrinogen (g/L)	2.90 [2.30; 5.30]
γ' fibrinogen (%)	10.1 [7.12; 14.5]
Plasminogen activator inhibitor-1_{act} (U/mL)	5.12 [2.24; 8.82]
Glycemic markers	
Fasting plasma glucose (mM)	4.80 [4.30; 5.30]
Glycated hemoglobin (%)	5.50 [5.20; 5.80]
Inflammatory markers	
C-reactive protein (mg/L)	3.19 [0.96; 9.15]
Soluble urokinase plasminogen activator receptor (ng/mL)	3.47 [2.84; 4.32]
Interleukin-6 (pg/mL)	2.84 [0.75; 5.70]
Renal markers	
Creatinine (μM)	67.3 [57.4; 83.0]
Uric acid (mM)	0.38 [0.28; 0.53]
Urea (mM)	3.85 [3.00; 5.20]
Hepatic markers	
Alkaline phosphatase (U/L)	124 [97.4; 156]
Alanine transaminase (U/L)	17.4 [12.0; 25.4]
Aspartate transaminase (U/L)	26.1 [19.0; 39.2]
Gamma-glutamyl transferase (U/L)	46.0 [29.2; 88.3]
Blood pressure	
Brachial systolic blood pressure (mm Hg)	130 [116; 147]
Brachial diastolic blood pressure (mm Hg)	87.0 [78.0; 97.0]
Communicable diseases	
Human Immunodeficiency Virus status (infected) [n (%)]	255 (17.5)
Medication use	
Contraceptives (women) [n (%)]	323 (35.3)
Hypertension medication [n (%)]	249 (17.0)
Diabetes medication [n (%)]	21 (1.40)
Lipid-lowering medication [n (%)]	1 (<1.00)
Anthropometry and lifestyle	
Body mass index (kg/m^2)	22.8 [19.3; 28.3]
Alcohol use (current/previous use) [n (%)]	644 (45.4)
Tobacco use (current use) [n (%)]	825 (56.7)

Abbreviations: au, absorbance unit; g, gram; L, liter; m^2, meter squared; mg, milligram; min, minute; mL, milliliter; mM, millimole per liter; mm Hg, millimeters of mercury; n, sample size; ng, nanogram; nM, nanomole per liter; pg, picogram; s, second; U, unit; μM, micromole per liter.

Normally distributed continuous data presented as mean (95% confidence interval).

Non-normally distributed continuous data presented as median [Quartile 1; Quartile 3].

*Lag time (time required for fibrin fibers to attain the appropriate length to allow lateral aggregation as well as activation of the coagulation cascade), slope (rate of lateral aggregation), maximum absorbance (MA) (clot density).

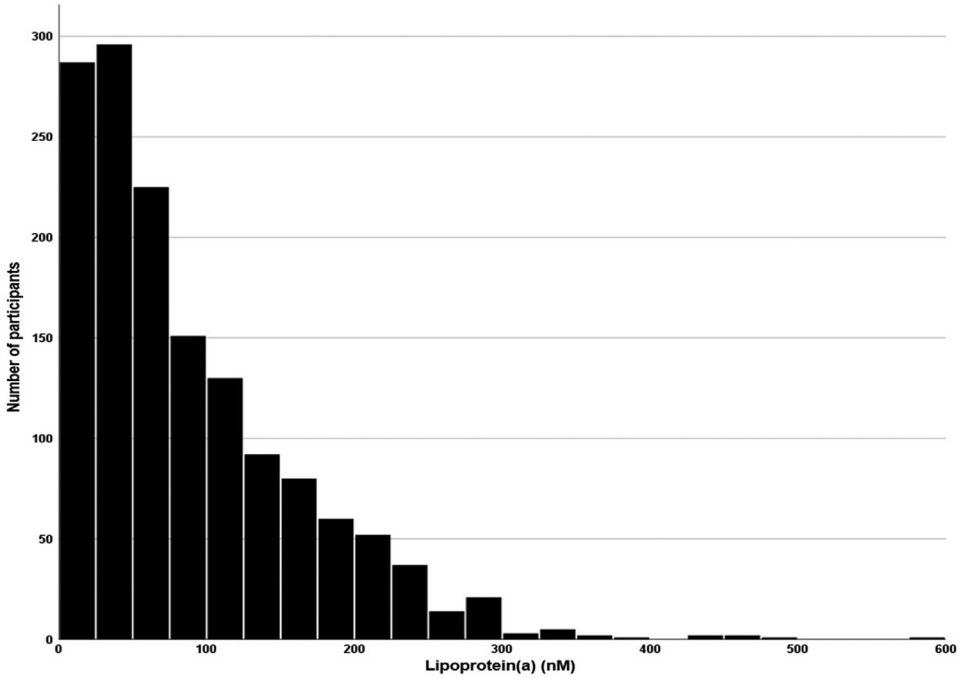

Figure 1. Distribution of lipoprotein(a) in the PURE population. Abbreviation: nM, nanomoles per liter.

time (0.9%), while both LDL-C and ApoB significantly contributed to CLT (1.1 and 0.4%, respectively). Although the reported associations were statistically significant, the contribution to variance in fibrinogen concentration and clot properties were small (0.2–3.5%).

Discussion

This is the first population-based study providing epidemiological evidence for the association of Lp(a) and ApoB with fibrinogen concentration and plasma clot properties. Of particular interest is the fact that the study was performed in an African cohort with relatively high median Lp(a) levels, allowing the investigation of associations with fibrinogen and clot properties in high-risk individuals. The data demonstrate that of the three lipid parameters, Lp(a) had the strongest association with fibrinogen concentration, also after adjustment for covariates. It was also positively associated with rate of clot formation (slope) and clot density (MA), independent of fibrinogen concentration. However, its association with CLT was eliminated after adjustment for fibrinogen concentration. ApoB on the other hand, had a strong negative association with time to clot formation (lag time) and a positive association with CLT, also after adjustment for fibrinogen, the covariates, Lp(a), and LDL-C. LDL-C associated positively with fibrinogen concentration, but the association was lost after adjustment for covariates and Lp(a). In addition, individuals with high LDL-C (Q4) had higher clot density, regardless of any adjustments and although it was positively associated with CLT in the unadjusted model, the association became less strong after adjustment for covariates and became non-significant after additional adjustment for ApoB.

Lp(a)

The independent, positive association between Lp(a) and fibrinogen in this study agrees with findings reported for participants from different racial groups who participated in the Multi-Ethnic Study of Atherosclerosis (MESA).[42] It has also been demonstrated that lowering Lp(a) using lipoprotein apheresis can lower fibrinogen by approximately 29%,[24] but whether the effect occurs specifically due to the reduction of Lp(a) or because LDL and other LDL-like particles are also removed from the blood is unclear. Lowering Lp(a) synthesis alone via treatment with an antisense oligonucleotide for apo(a), decreased fibrinogen concentration by 16.5%.[23] Although their finding was not statistically significant ($p = 0.1$)—likely due to the small sample size (ten participants received the placebo and seven the intervention)—the effect was clinically significant. Lp(a) is known to be pro-inflammatory; it carries oxidized phospholipids, and clinical interventions that lower apo(a) synthesis also lower IL-6 synthesis.[43,44] Given that IL-6 is the primary stimulus for fibrinogen production in the acute phase,[45] a possible mechanism behind the association between Lp(a) and fibrinogen is the role of Lp(a) as an inflammatory particle.[19] However, it is also possible that a common factor increases both Lp(a) and fibrinogen, as acute-phase proteins. Inhibition of IL-6 has been shown to decrease Lp(a), and there are IL-6 response elements in the LPA gene promoter.[43] Future research should aim to address the causal pathway of this relationship. Fibrinogen γ' is a common isoform of fibrinogen that contains

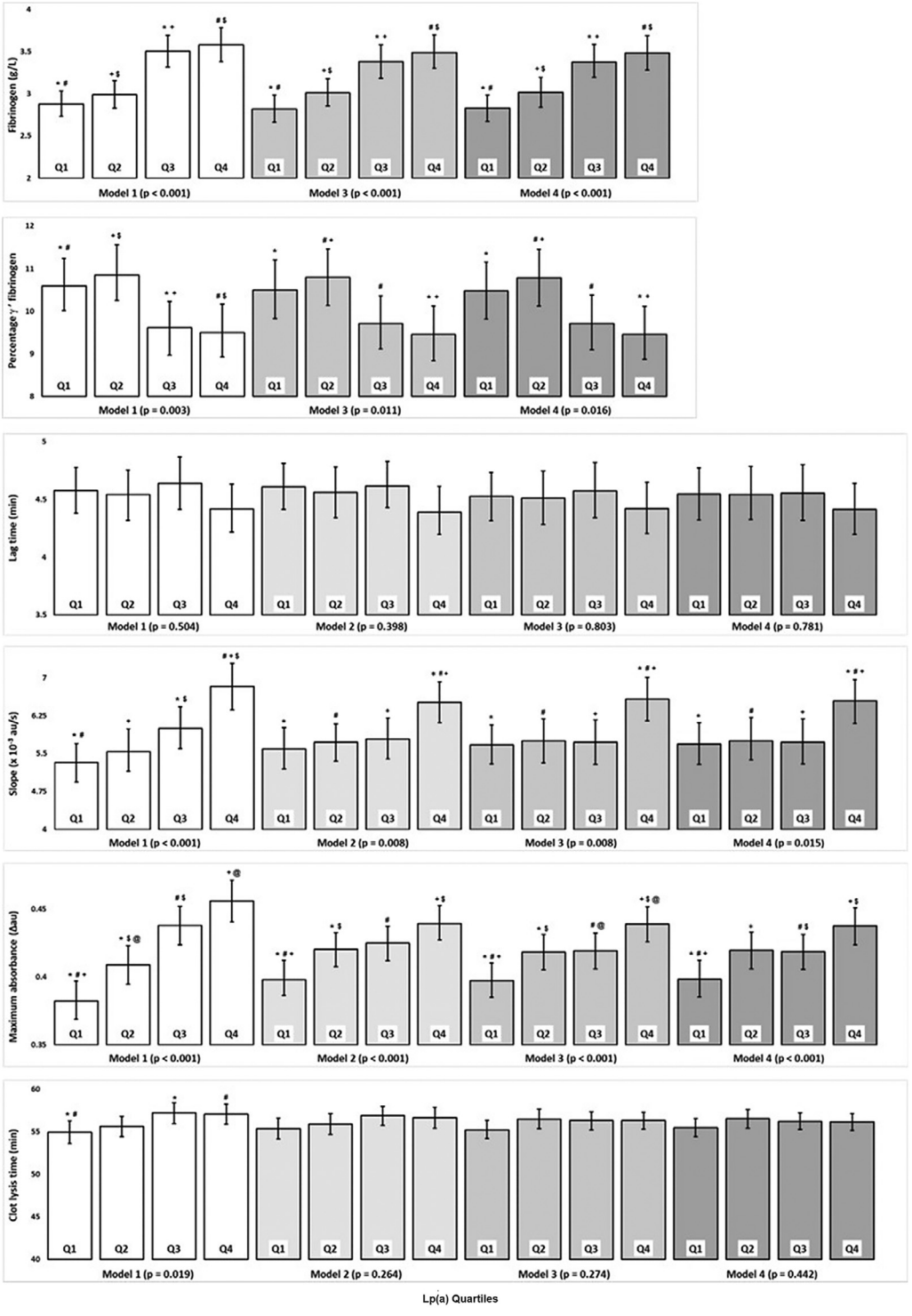

Figure 2. a: Association between lipoprotein(a) and fibrinogen and plasma clot properties. Abbreviations: au, absorbance units; g, gram; L, liter; min, minutes; p, probability of type I error; Q, quartile; s, second; Δ, delta/change in; γ', gamma prime.
Model 1: General linear models with lipoprotein(a) as the exposure and the hemostatic markers as outcomes. The estimated marginal means of the outcomes are reported across lipoprotein(a) quartiles.
Model 2: Model 1 adjusted for fibrinogen (fibrinogen and percentage γ' fibrinogen excluded as outcomes in this model).
Model 3: Model 2 adjusted for sex, age, body mass index, alcohol use, tobacco use, hypertension, glycated hemoglobin, interleukin-6, urea, plasminogen activator inhibitor-1 activity, and albumin. Fibrinogen excluded as a covariate in models where fibrinogen or percentage γ' fibrinogen is the outcome variable.
Model 4: Model 3 adjusted for apolipoprotein B and low-density lipoprotein cholesterol.
For each model, quartiles with the same symbol differ significantly from each other (*, #, +, $, and @).

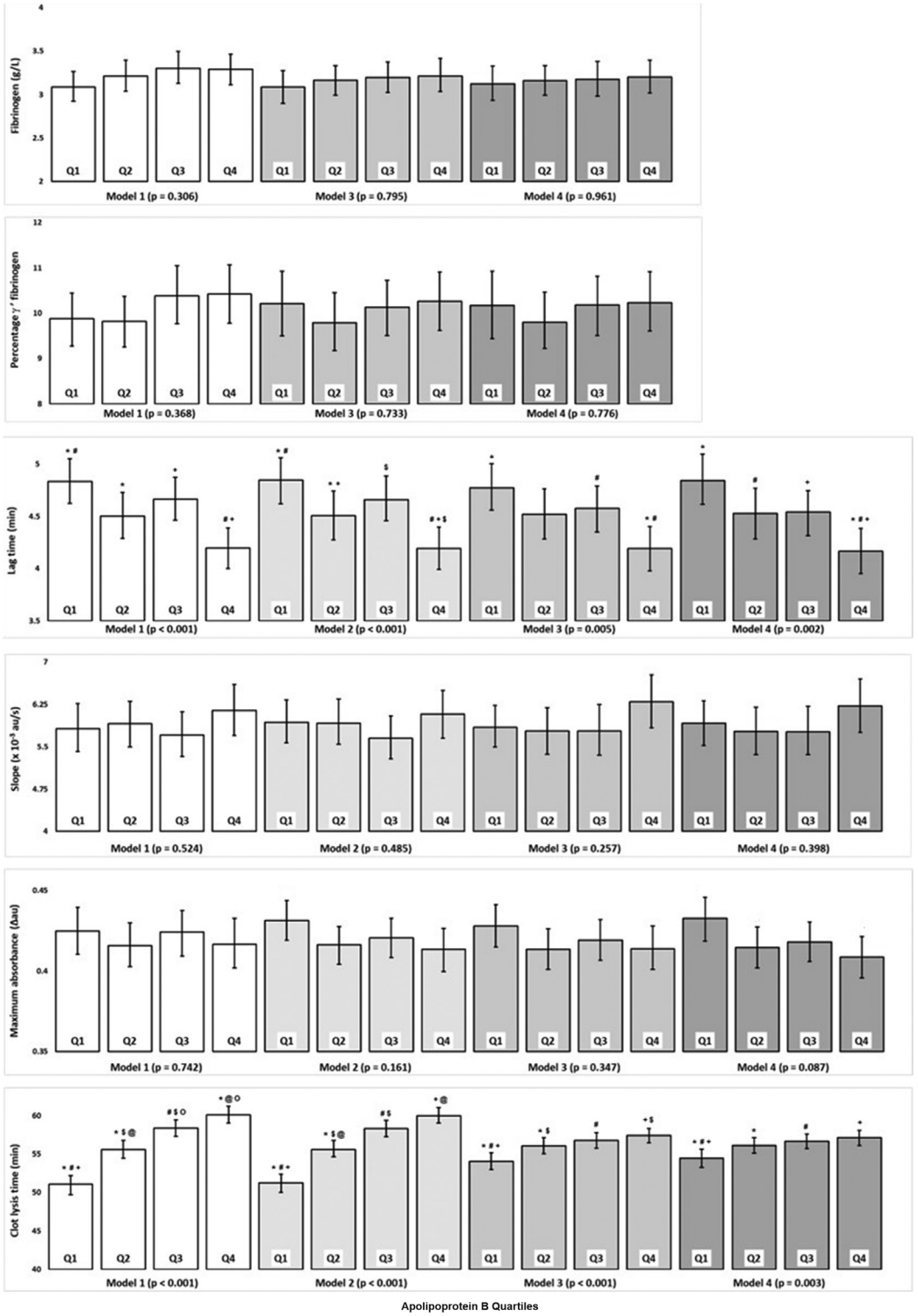

Figure 2. b: Association between apolipoprotein B and fibrinogen and plasma clot properties. Abbreviations: au, absorbance units; g, gram; L, liter; min, minutes; p, probability of type I error; Q, quartile; s, second; Δ, delta/change in; γ', gamma prime.

Model 1: General linear models with apolipoprotein B as the exposure and the hemostatic markers as the outcomes. The estimated marginal means of the outcomes are reported across apolipoprotein B quartiles.

Model 2: Model 1 adjusted for fibrinogen (fibrinogen and percentage γ' fibrinogen excluded as outcomes in this model).

Model 3: Model 2 adjusted for sex, age, body mass index, alcohol use, tobacco use, hypertension, glycated hemoglobin, interleukin-6, urea, plasminogen activator inhibitor-1 activity, and albumin. Fibrinogen excluded as a covariate in models where fibrinogen or percentage γ' fibrinogen is the outcome variable.

Model 4: Model 3 adjusted for the lipoprotein(a) and low-density lipoprotein cholesterol.

For each model, quartiles with the same symbol differ significantly from each other (*, #, +, $, and @).

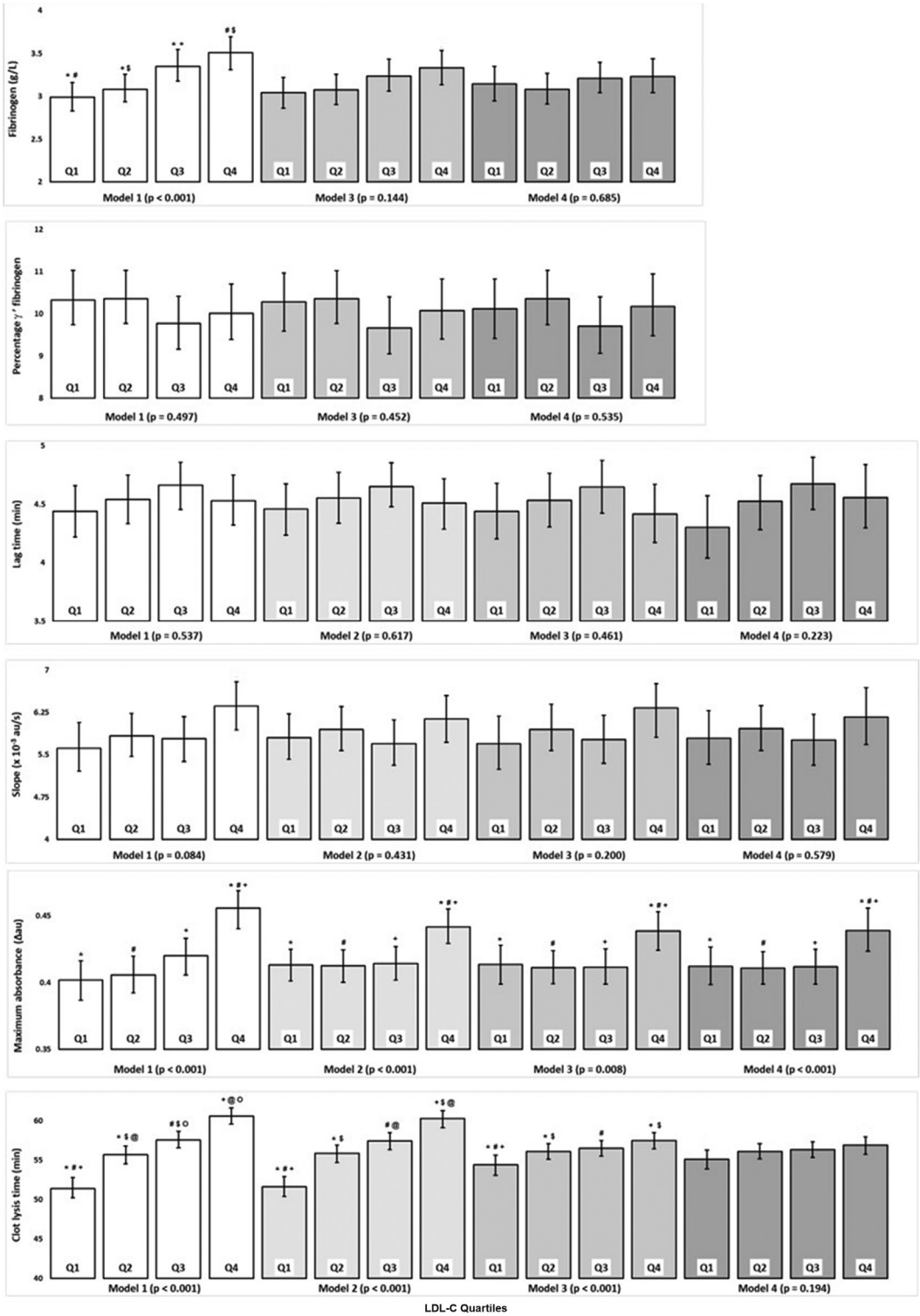

Figure 2. c: Association between low-density lipoprotein cholesterol and fibrinogen and plasma clot properties. Abbreviations: au, absorbance units; g, gram; L, liter; min, minutes; p, probability of type I error; Q, quartile; s, second; Δ, delta/change in; γ', gamma prime.
Model 1: General linear models with low-density lipoprotein cholesterol as the exposure and hemostatic markers as the outcomes. The estimated marginal means of the outcomes are reported across low-density lipoprotein cholesterol quartiles.
Model 2: Model 1 adjusted for fibrinogen (fibrinogen and percentage γ' fibrinogen excluded as outcomes in this model).
Model 3: Model 2 adjusted for sex, age, body mass index, alcohol use, tobacco use, hypertension, glycated hemoglobin, interleukin-6, urea, plasminogen activator inhibitor-1 activity, and albumin. Fibrinogen excluded as a covariate in models where fibrinogen or percentage γ' fibrinogen is the outcome variable.
Model 4: Model 3 adjusted for the lipoprotein(a) and apolipoprotein B.
For each model, quartiles with the same symbol differ significantly from each other (*, #, +, $, and @).

an altered carboxyl-terminal amino acid sequence of the γ chain, due to alternative splicing. A higher percentage of γ' fibrinogen is an inflammatory biomarker and leads to the formation of a heterogenous clot structure with larger pores, decreased protofibril packing within fibers, less stiff fibers and increased resistance to lysis.[46] In the PURE-SA-NW study, however, there was a negative association between Lp(a) and % γ' fibrinogen. This observation is likely due to the relatively strong negative association ($r = –0.53$) between fibrinogen concentration and % γ' fibrinogen, as previously reported.[47]

The positive association between Lp(a) and rate of clot formation (slope) and clot density (MA), also after adjustment for fibrinogen concentration, suggests Lp(a) may directly alter clot formation and structure. Previous case control studies also reported a positive association with clot density,[16] and the formation of less permeable clots,[15-18] although others found no associations with clot density.[17,18] Talens et al. analyzed the binding of plasma proteins to fibrin clots and found that ApoB was bound to fibrin clots.[14] It was argued that these ApoB-containing particles were likely Lp(a) particles, which bind to the clots via the lysine binding sites (LBS) on fibrin surfaces.[14] Subsequent proteomic analysis reported that apo(a) also binds to plasma clots,[8,13] supporting this argument. Increased MA, adjusted for fibrinogen, is considered a suitable proxy for the prothrombotic phenotype.[6] Therefore, a plausible additional mechanism whereby Lp(a) may exert prothrombotic effects is by binding to fibrin fibers, and in so doing increasing clot density.

In this population-based study, the crude positive association between Lp(a) and plasma CLT appears to be mediated primarily by the strong association between Lp(a) and fibrinogen concentration. While it is known that apo(a), carried on Lp(a), can impair conversion of plasminogen to plasmin due to impaired turnover of the tPA-plasminogen-fibrin catalytic complex and reduced plasmin-mediated conversion of Glu-plasminogen to Lys-plasminogen,[48,49] Lp(a) does not appear to affect plasma CLT in an epidemiological setting via this mechanism. This fibrinogen-mediated association is in agreement with intervention studies that demonstrated significant reduction in CLT in whole blood when a decrease in Lp(a) also resulted in a significant reduction in fibrinogen concentration,[24] while reduction of fibrinogen concentration to a lesser extent also did not result in reduced plasma CLTs.[23] In whole blood systems, additional mechanisms have been identified that could cause increased Lp(a) to increase CLT. These include: *i*) Lp(a)'s capacity to decrease plasminogen binding to the endothelial surface, decreasing the localization of plasminogen to the cell surface, and in turn decreasing activation by tPA, *ii*) increased activation and aggregation of platelets, *iii*) its complex formation with α2-macroglobulin, a plasmin inhibitor, and *iv*) altering other plasma clot properties (such as increased maximum absorbance) as was discussed above, resulting in increased CLT.[1,50,51] Whether this association is seen, *in vivo*, on an epidemiological level, remains to be determined.

ApoB

ApoB had a strong negative association with time to clot formation (lag time) and a positive association with CLT in this study population. Very little is known regarding the association of ApoB with clot properties. To date, two case-control studies have been performed, both reporting ApoB to be associated with decreased clot permeability.[13,26] A positive association between ApoB and CLT has also been reported,[26] although Bryk et al. found no association between clot bound ApoB and CLT.[13] Similar to other apolipoproteins, ApoB has been demonstrated to bind to plasma clots and modulate clot properties,[8,13,14] although the precise mechanisms behind this association remain to be determined. Since ApoB is present in chylomicrons, very low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL), LDL, and Lp(a) particles, it may potentially influence the relationship of plasma levels of all these lipoproteins with clot properties. The largest contributor to ApoB concentration after LDL is VLDL.[52] ApoB correlated positively with PAI-1 in this population, and CLT may consequently be increased by a VLDL-mediated increase in PAI-1, due to increased transcription of the *PAI-1* gene.[53] Additional mechanisms that may explain the association of ApoB with time to clot formation and CLT are the VLDL-mediated activation of platelets, and increased thrombin production via elevated activity of the prothrombinase complex.[53-55]

LDL-C

LDL-C was positively associated with fibrinogen concentration, clot density (MA) and CLT. In a previous substudy analysis of $n = 30$ of the PURE participants, we additionally determined fiber diameter using scanning electron microscopy and found that LDL-C positively associated with maximum absorbance independent of fiber diameter.[6] This suggests that LDL particles potentially increase internal fiber density by binding to fibrin fibers. Increased LDL-C has been reported to also be associated with stiffer clots,[56] and lower clot permeability.[57,58] Furthermore, reduction of LDL-C with statins improves clot properties.[10] One proposed mechanism for the association of LDL-C with prothrombotic clot properties is the binding of ApoB-containing particles to plasma clots,[8,13,14] as discussed above. In addition, the oxidation of lipids may furthermore influence their effects on clot structure. It has been suggested that highly oxidized LDL may directly affect the fibrin clot network by enhancing hypercoagulation and fibrinolytic resistance in comparison to non-oxidized LDL.[59]

Analysis of both GLM and regression data revealed that the crude positive association between LDL-C and fibrinogen was most significantly negated by Lp(a), IL-6, and BMI, suggesting a relationship driven by inflammatory mechanisms.[19,43,45,60,61] The positive association between LDL-C and CLT on the other hand was largely diminished by adjusting for PAI-1 and BMI. We have previously demonstrated

that the primary contributors to CLT in this population were BMI and PAI-1,[62] which also correlated with LDL-C. This points to a possible obesity-related mechanistic pathway.[63-65]

Strengths and limitations

This is the first large observational study reporting on the association of Lp(a) and ApoB with fibrinogen and clot properties at a population level. The study was furthermore performed in an African cohort, an under-studied population group, with relatively high median Lp(a) levels, thus producing novel findings in high risk individuals. Lp(a) was measured in nM using an assay relatively insensitive to the particle's size heterogeneity, thus ensuring accurate Lp(a) concentration measurement. Being a well-phenotyped study, it allowed for the identification and control of many covariates; however, residual confounding cannot be excluded. Being cross-sectional in design, causation could not be determined. This study included apparently healthy adults, and therefore the results are not generalizable and cannot be extrapolated to individuals with disease. All efforts were made to reduce selection bias; however, it is not impossible that it may have occurred in some form.

Conclusion

ApoB and ApoB-containing lipoproteins, such as LDL and Lp(a), have been reported to be associated with a prothrombotic clot phenotype. Existing studies, however, often examined these exposures separately, making it difficult to conclude if the associations are independently linked to ApoB or to specific ApoB-containing lipoproteins. Here, we evaluated ApoB, LDL-C, and Lp(a) independently as exposures and after mutual adjustment. Through this approach we were able to demonstrate that fibrinogen concentration and slope had the strongest association with Lp(a), clot density associated with both Lp(a) and LDL-C, time to clot formation associated with ApoB, and CLT demonstrated strong associations with both ApoB and LDL-C. These findings allow for the identification of novel prothrombotic roles for these (apo)lipoproteins and for the development of targeted therapeutic approaches to address thrombotic conditions related to clot properties.

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Use of AI and AI-assisted technologies statement

AI was not used in the writing process.

Ethical statement

This study has ethical approval from the North-West University Health Research Ethics Committee (04M10; 2 September 2004) and was performed per the revised Declaration of Helsinki. Written informed consent was obtained from all participants prior to data collection.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: This work was supported by the North-West University, South African National Research Foundation, Population Health Research Institute, and the South African Medical Research Council (SAMRC-RFA-EMU-1-0-2020). None of the funding bodies was involved in the design of the study, collection, analysis, or interpretation of the data or in writing of this manuscript. Opinions expressed and conclusions arrived at are those of the authors and are not to be attributed to the funding sources.

CRediT authorship contribution statement

Daniel Bruwer: Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. **Zelda de Lange-Loots:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **Marlys L. Koschinsky:** Investigation, Methodology, Software, Supervision, Writing – review & editing. **Michael B. Boffa:** Investigation, Methodology, Software, Supervision, Writing – review & editing. **Marlien Pieters:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

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Supplementary materials

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