

Lipoprotein(a) and vascular function in pre- and postmenopausal women

by

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## Abstract

**Intro.** Lipoprotein(a) [Lp(a)] is a newly appreciated risk factor for cardiovascular disease (CVD), yet its contribution to subclinical disease, particularly vascular endothelial dysfunction, is not fully elucidated. Fibrinogen may represent an important pathway by which Lp(a) may contribute to vascular endothelial dysfunction. Additionally, menopausal status has been shown to alter both Lp(a) and FMD. We therefore assessed, in pre- and postmenopausal women, whether: 1) Lp(a) is associated with FMD, 2) Lp(a) is associated with fibrinogen, and 3) Lp(a) and FMD collectively influence CVD risk.

**Methods.** 1,379 women from the Framingham Offspring cohort at exam 7 were included in the analysis. We stratified by menopausal status (114 premenopausal, 1,265 postmenopausal), and excluded individuals with current CVD or missing data for Lp(a), FMD, or CVD.

Associations between Lp(a) and FMD were evaluated using both categorical (generalized linear) and continuous (linear regression) models. Associations between Lp(a) and fibrinogen and fibrinogen and FMD were evaluated using linear regression. Mediation analysis was performed to evaluate the mediating role of fibrinogen in the association between Lp(a) and FMD. Cox proportional hazards models evaluated the effect of Lp(a) and FMD on incident CVD risk.

**Results.** Premenopausal women with elevated Lp(a) ( $\geq 65$  mg/dL) exhibited significantly reduced FMD compared to those with lower levels. This association persisted after multivariable adjustment. In postmenopausal women, no significant difference in FMD was observed between Lp(a) groups. Lp(a) was positively associated with fibrinogen in both pre- and postmenopausal women. However, fibrinogen was not associated with FMD. Mediation analysis confirmed that the association between Lp(a) and FMD was not mediated through the effects of fibrinogen. Compared to individuals with normal FMD and low Lp(a), those with high Lp(a) alone (and

normal FMD) faced a 115% increased risk of CVD (HR 2.15), while low FMD alone (and low Lp(a)) was associated with a 43% increased risk (HR 1.43). The joint association of high Lp(a) and low FMD conferred a significant two-fold increase in CVD risk (HR 2.00; 95% CI: 1.31-3.05,  $p=0.001$ ) after adjusting for traditional cardiovascular risk factors.

**Conclusions.** Elevated Lp(a) is associated with impaired endothelial function in premenopausal women, suggesting early vascular effects prior to menopause. The assessment of Lp(a) and FMD modified CVD risk, highlighting the importance of integrating genetic, lipid, and functional vascular markers in women.

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# Chapter 1 - Introduction

## Introduction

Despite well-established and effective preventive measures, cardiovascular disease (CVD) continues to be the leading cause of morbidity and mortality worldwide [1]. Its development is not instantaneous; rather, it reflects a progressive pathophysiology beginning years before full clinical manifestation [2], shaped by a complex interplay of environmental, behavioral, and genetic factors [3], [4], [5], [6]. As cardiovascular medicine continues to evolve, the range of recognized risk factors expands, with therapeutic strategies gradually following suit.

Lipoprotein(a) [Lp(a)], a low-density lipoprotein (LDL) like particle, has recently been identified as a primary risk factor for cardiovascular disease (CVD), exhibiting greater atherogenic, prothrombotic, and proinflammatory properties than LDL cholesterol alone [7]. As such, recent work has demonstrated that elevated Lp(a) is significantly associated with atherosclerotic cardiovascular disease (ASCVD), independent of traditional cardiovascular risk factors [8], [9]. While evidence is mixed, many have linked elevated Lp(a) with adverse vascular structure and function, suggesting that Lp(a) promotes cardiovascular disease by initially impairing arterial health [10]. Despite initial findings, important gaps regarding the relationship between Lp(a) and vascular function remain.

The vascular endothelium contributes to overall vascular health, such that early stages of endothelial dysfunction are associated with an increased lifetime risk of CVD [11]. Celermajer et al. demonstrated an attenuated brachial flow-mediated dilation (FMD) response, a non-invasive measure of endothelial function, in children and adults with familial hypercholesterolemia, coronary artery disease (CAD), or current smoking status, before the development of clinically significant ASCVD [12]. Several experimental and clinical studies suggest that endothelial

dysfunction is an important risk factor for subsequent major cardiovascular events [13]. While to date, aging, smoking, hypertension, hypercholesterolemia, diabetes, physical activity, diet, and even menopausal status have been found to alter vascular endothelial function [13]; the impact of Lp(a) levels remains less understood. Early work by Wilimink et al. (2000) suggested that vascular endothelial function, measured by FMD, was inversely related to Lp(a) in postmenopausal women [14]. Conversely, Schlaich et al. demonstrated that endothelium-dependent dilation was not associated with elevated Lp(a) [15]. Given these opposing findings, there is a need to further understand the relationship between Lp(a) and endothelial function, as well as their combined influence on CVD risk.

Increased fibrinogen can impair endothelial function and may serve as an intermediate between Lp(a) and endothelial function [16]. Allen et al. examined the relationship between elevated fibrinogen and endothelial function, assessed via FMD, and observed a significant inverse association [17], [18]. This is consistent with the work of Madhu et al., who reported a significant correlation between fibrinogen and FMD in diabetic subjects [19]. In hypertensive patients, Kullo et al. found no association between FMD and Lp(a) or fibrinogen, which contrasts with the consistent findings of Allen et al. and Madhu et al. [20]. Conceptually, most studies support a link among increased fibrinogen, impaired vascular integrity, increased platelet aggregation, and an overall proinflammatory endothelial phenotype [21]. However, it is unknown if Lp(a) mediates its potential effects on endothelial function through increased fibrinogen.

Therefore, in the present study, we tested the hypothesis that an elevated Lp(a) is associated with low FMD, a measure of vascular endothelial function. Moreover, despite >90% genetic determination, menopause has been shown to alter Lp(a) [12]. Despite the known

increase in Lp(a) with menopause, its link to impaired vascular function in post-menopause women is not well established [22]. We hypothesize that relationship between Lp(a) and FMD may be different between pre- and postmenopausal women. We also tested the hypothesis that prothrombotic factors, specifically fibrinogen, mediate the relationship between Lp(a) and FMD. Lastly, we assessed the combined influence of Lp(a) and endothelial function on cardiovascular disease risk. We hypothesized that Lp(a) and FMD have independent and conjunctive associations with incident CVD.



## **Chapter 2 - Literature Review**

### **Endothelial Function**

#### **Overview of the Endothelium**

Endothelial cells (ECs) are linearly aligned within the vascular lumen, forming a smooth interface between the blood and the tunica media. This inner lining maintains hemostasis by maintaining arterial tone and structural integrity, inhibiting cellular adhesion and proliferation, and exerting potent anti-inflammatory effects [23]. The endothelium is extremely dynamic, and its structure and function are highly specific to the persistent hemodynamic environment (including vessel size, location, and type) and acute hemodynamic changes (altered tangential and shear stress, metabolic/chemical) [24].

EC morphology will mimic the direction of blood flow [25], [26]. In regions of the vasculature with laminar flow, EC's are subjected to a consistent frictional force (shear stress) promoting linear EC alignment and maintenance of key functional and antiatherogenic pathways [25]. Where flow is disturbed, such as bifurcations and curvatures, EC's experience a lower, oscillatory shear stress. Exposure to this disruptive flow pattern increases EC turn over and misalignment [26]. These morphological changes go hand in hand with the development of endothelial dysfunction - where vascular regulation is impaired and the atherogenic process begins.

#### **Regulation of Vascular Tone and Blood Flow**

A healthy arterial endothelium is essential for sensing mechanical and metabolic stimuli and transducing these signals into specific physiologic outputs [27]. In health, this reactivity allows for the tight regulation of blood flow and the maintenance of vascular homeostasis. When

subjected to acute increases in shear stress, ECs undergo mechanical deformation. This hemodynamic change is sensed and transmitted through the cytoskeleton, altering its structural conformation and ultimately modulating gene expression [24], [27]. The primary physiological outcome of this mechanotransduction is the release of vasoactive factors that regulate vascular tone and vessel diameter.

Disruption of the endothelial layer compromises signaling pathways, impairing vasoregulation and promoting atherosclerotic plaque development at sites of vascular vulnerability [26]. Endothelium-derived relaxing factors (EDRFs) cause vascular smooth muscle relaxation, to accommodate increased blood flow [27]. Nitric oxide (NO) is the most well-characterized EDRF; however, prostacyclin and other endothelial-derived hyperpolarization factors (EDHF) can contribute to vasodilation. Importantly, the proportional release of vasoactive mediators is species, age, and vascular bed specific [28].

NO is synthesized by nitric oxide synthase (NOS), which catalyzes the conversion L-arginine and O<sub>2</sub> into L-citrulline and NO [29]. Endothelial nitric oxide synthase, eNOS, is the primary isoform within the endothelial lining and is promptly activated by multiple shear-stress induced pathways [30]. Once produced, NO rapidly diffuses from the endothelium to the vascular smooth muscle, where it activates soluble guanylate cyclase (sGC). This activation increases the conversion of guanosine triphosphate (GTP) to cyclic guanosine monophosphate (cGMP), triggering protein kinase G (PKG) signaling. PKG reduces intracellular calcium availability and limits actin–myosin crossbridge formation, resulting in vascular smooth muscle relaxation [31].

The endothelial-dependent vasodilatory response to shear stress is dependent on NO bioavailability. NO bioavailability refers to the fraction of NO that results in smooth muscle

relaxation; bioavailability is governed by both the rate of enzymatic production by NOS and the rate of consumption through interactions with reactive oxygen species (ROS) [29], [30].

Reductions in NO bioavailability, due to eNOS uncoupling and or NO scavenging, impair vasoreactivity. Impaired vascular regulation is a hallmark of multiple disease states (ex. coronary artery disease, familial hypercholesterolemia, diabetes, etc.) and those with common cardiovascular risk factors [2], [26], [32].

### **Endothelial Control of Hemostasis and Thromboregulation**

A healthy endothelium displays essential antithrombotic characteristics. The smooth monolayer regulates molecular exchange between the blood and adjacent tissues, balancing procoagulant and anticoagulant processes. Signaling molecules released from the endothelium, such as NO and prostacyclin ( $\text{PGI}_2$ ), not only act within the tunica media, but can bind to platelets within the blood stream to inhibit wall adhesion and cell aggregation [33]. Additionally, heparin-like molecules on the EC surface bind to antithrombin-III to inactivate clotting factors  $\text{IX}_a$ ,  $\text{X}_a$ ,  $\text{XI}_a$ ,  $\text{XII}_a$ , and II (thrombin) [33].

Endothelial injury disrupts this antithrombotic environment, leading to diminished NO and  $\text{PGI}_2$  production and the loss of essential membrane-bound proteins [33]. The endothelium reacts to the insult by increasing endothelin-1 release and vasoconstriction. At the site of injury, von Willebrand factor is expressed and binds to the glycoprotein Ib (GP1b) on platelets [34]. This interaction propagates the release of cytokines, such as thromboxane A2 ( $\text{TxA}_2$ ), serotonin, and adenosine diphosphate (ADP), from adhered platelet granules, promoting vasoconstriction, platelet adhesion, and further aggregation [35], [36].

The extrinsic clotting cascade is activated when endothelial damage directly exposes tissue factor (factor III) to the bloodstream. This complex activates factor X, where intrinsic and

extrinsic pathways coverage. Activated factor X,  $X_a$ , cleaves factor II, prothrombin, into thrombin, which subsequently converts soluble fibrinogen into insoluble fibrin monomers. These insoluble monomers begin to cross-link forming a fibrin mesh [36]. Factor XIII stabilizes the newly formed platelet plug, adding structure to the now embedded thrombus. Individuals with elevated fibrinogen concentrations exhibit a higher propensity for this pathway, correlating with increased cardiovascular risk [18].

To combat unnecessary clot formation, endothelial cells release fibrinolytic substances such as tissue plasminogen activator (tPA). tPA is a plasma protease that converts plasminogen to plasmin, the enzyme primarily responsible for clot degradation [35], [36]. To balance the fibrinolytic actions of tPA endothelial cells produce plasminogen activator inhibitor (PAI-1) [35], [36]. PAI-1 can form a complex with tPA disabling its ability to convert plasminogen to plasmin [35], [36].

## **Determinants and Modifiers of Endothelial Function**

There are numerous determinants and modifiers of endothelial function, and diverse stimuli can drive acute or chronic, physiological, or pathological endothelial adaptations. Aging is a known cause of vascular dysfunction due to changes in gene expression, cellular senescence, oxidative stress, inflammation, and reduced NO bioavailability [37], [38]. In this state the endothelium expresses a maladaptive phenotype, compromising functionality. The vasculature loses its reactivity, elasticity, and becomes more vulnerable to disease [39]. Although chronological aging is inevitable, the rate of vascular decline can be significantly modified by optimizing lifestyle and cardiometabolic risk factors [40]. Among classic CVD risk factors, physical inactivity is a major, independent risk factor for cardiovascular disease. Regular

physical activity has consistently shown to improve endothelial function through increased shear stress, which stimulates endothelial nitric oxide synthase (eNOS) and NO production. [40]. The response to exercise is systemic and induces several beneficial physiological actions. It can also be an antioxidant, promote angiogenesis, and reduce chronic inflammation [40].

To translate the importance of cardiovascular health, to physicians and patients alike, the AHA established a score derived from 8 key metrics: sleep, physical activity, diet quality, body mass index, blood lipids, blood glucose, blood pressure, and nicotine exposure. Research has indicated that favorable Life's essential 8 scores (LE8) lead to favorable vascular outcomes [41]. These risk factors independently and together represent actionable targets for improving and or maintaining vascular health.

## **Assessment of Endothelial Function**

### **Conceptual Basis of Flow-Mediated Dilation**

Flow-mediated dilation (FMD) is a research technique used to non-invasively measure conduit artery endothelial function [42]. This technique began with a 1992 publication [32], quantifying endothelial function in smokers, children with familial hypercholesterolemia (FH), and patients diagnosed with coronary artery disease (CAD). They found lower FMD in each group compared to controls — implicating impaired endothelial function preceding atherosclerotic cardiovascular disease development (ASCVD). Despite the ability to detect early changes in vascular function, FMD remains primarily a research tool which requires strict procedural adherence to produce interpretable data [43]. Different groups employing this technique have established their own idiosyncrasies, limiting data synchronization and result comparisons among the research community.

## **Physiological and Molecular Mechanisms Underlying FMD**

The mechanism by which increased shear stress induces conduit artery vasodilation entails transformation of the mechanical signal (the shear stimulus) into a chemical output (EDRF's); the NO pathway is accepted as the most prominent endothelial-dependent mechanism for FMD when following recommended guidelines [43]. Initially increased flow – intrinsically increasing shear stress - physically deforms the endothelial cell membrane, and signal transmission reaches the internal scaffolding of the cell [44]. As a result, endothelial nitric oxide synthase (eNOS) is activated, catalyzing the conversion of L-arginine to L-citrulline, producing NO. NO then diffuses into the vascular smooth muscle (VSM), binding to soluble guanylate cyclase (sGC) and eliciting the conversion of guanosine triphosphate (GTP) to cyclic guanosine monophosphate (cGMP). Interactions between cGMP and Protein Kinase G (PKG) limit crossbridge formation, causing vascular relaxation [28].

## **Methodological Considerations in FMD Assessment**

To measure FMD, a cuff is placed on the arm and inflated above systolic blood pressure (SBP) to occlude arterial inflow. Measurement in other peripheral conduit arteries, such as the superficial femoral artery (SFA), has also been conducted and validated [45]. An ultrasound probe is placed upstream of the pressurized cuff to measure changes in brachial artery diameter and flow. Measures made distal to the cuff are impacted by vasodilatory metabolites and therefore don't represent an isolated endothelial-dependent response to shear stress [46]. To measure a primarily endothelial-dependent response, vessel diameter should be measured upstream of the cuff with a 5-minute period of occlusion [43]. Occlusion decreases downstream vascular resistance resulting in a hyperemic response upon cuff release. Reactive hyperemia

increases conduit artery flow and therefore shear stress. The strength of the shear stimulus, represented by the area under the curve (AUC), and health of the vasculature dictate the %FMD [47]. A stronger stimulus should lead to greater flow and diameter changes [48].

## **Lipoprotein(a)**

### **Overview and Structural Characteristics of Lp(a)**

Cardiovascular disease (CVD) is the leading cause of morbidity and mortality [1], [49]. Recently, Lipoprotein(a) has been identified as an independent, causal risk factor for vascular disease; Lp(a) structurally resembles LDL but can be distinguished by an apolipoprotein(a) tail covalently bound to apolipoproteinB-100 [22]. Unlike LDL, Lp(a) is ~90% genetically determined, causing levels to remain consistent throughout the lifespan and resistant to most lifestyle changes [50], [51]. Lp(a) has been known to fluctuate in some conditions such as pregnancy, menopause, acute illness, post-myocardial infarction, and kidney or liver disease [52].

### **Roles in Pathology**

Lp(a)'s atherogenic, prothrombotic, inflammatory, and stenotic properties, marking it a hot topic in contemporary CVD research. Lp(a) possesses the full cholesterol-carrying capacity of LDL yet appears more atherogenic, as it more readily penetrates and remains in the arterial wall and is the preferential carrier of oxidized-phospholipids (OxPLs) [7], [53], [54]. This combination of arterial retention and presence of increased OxPLs makes Lp(a) a potent catalyst for foam cell formation, vascular inflammation, and ASCVD development [55].

Structurally, the apo(a) tail impacts thrombotic pathways due to its genetic similarities to plasminogen [56]. Lp(a) binds to fibrinogen and to endothelial surfaces to compete with plasminogen and tissue plasminogen activator (tPA), thereby impairing plasmin generation and fibrinolysis [56].

Together, these are some of many compounding actions thought to underlie the molecule's pathogenic effects. In particular, its OxPL content plays a central role: it upregulates endothelial adhesion molecules and pro-inflammatory cytokines, facilitates monocyte recruitment and transmigration, and accumulates within the arterial wall, where it promotes plaque progression and eventually calcification [55].

### **Determinants of Lp(a) Levels**

Apo(a) is encoded by the autosomal, codominant *LPA* gene located on chromosome 6q26-27 [57]. The 2026 Guideline on the Management of Dyslipidemia considered Lp(a) levels >50mg/dL or 125nmol/L elevated and discusses a consistent increase in relative ASCVD risk compared to those with low Lp(a) [58]. Approximately 20% of the population experience elevated Lp(a) – predisposing them to higher CVD risk [59], [60]. Median concentrations vary significantly by sex and racial subgroups; A study with 460,506 participants found medians of 19, 75, 31, and 16 nmol/L, for Caucasians, African Americans, South Asians, and Chinese respectively [8]. However, incremental increases in Lp(a) resulted in comparable increases in risk among different subgroups [8].

Polymorphisms in distinct folded protein structures called Kringle domains can cause ~30-70% variance in phenotypic Lp(a) levels among individuals with different ethnic backgrounds [61]. The number of Kringle IV-2 (KVI<sub>2</sub>) repeats dictates particle size, and particle

size, ultimately determines plasma concentrations [62]. Fewer KIV2 repeats results in smaller apo(a) isoforms, and higher Lp(a) plasma concentrations and vice versa, greater KIV2 copies result in larger apo(a) isoforms, and lower plasma concentrations [62]. Variance in the number of Kringle repeats changes apo(a) liver synthesis and release. Larger apo(a) isoforms tend to spend more time in the cell's endoplasmic reticulum, resulting in lower serum concentrations [61].

## **Chapter 3 - Methods**

### **Study Population**

Women from the seventh examination cycle (1998-2001) of the Framingham Offspring Study, a continuation of the Framingham Heart Study (FHS), were eligible for analysis in the present investigation (n=2,935). Details regarding the FHS sampling, methodology, and results can be found in Kannel's 1979 report [63]. Participants were excluded due to a history of cardiovascular disease (n=119) or incomplete data for Lp(a), FMD, and CVD status (n=1,437). This resulted in a total sample of 1,379 women for investigation. All procedures were approved by Boston Medical Center's Institutional review board, and informed consent was obtained prior to starting the study.

### **Lp(a) and Lipid Assessment**

Participants were asked to fast for 12hrs before their visit. Blood was drawn into 0.1% EDTA tubes, centrifuged for 30mins at 2,500 rpm, and stored at -70°C. Routine lipid measurements including total cholesterol (TC), triglycerides (TG), HDL and LDL, were made with an Abbott Diagnostics ABA-200 analyzer and Abbott A-Gent enzymatic reagents. LDL-C was calculated using the Friedwald equation ( $\text{LDL-C} = \text{total cholesterol} - \text{HDL-C} - \text{triglycerides}/5$ ). Corrected LDL-C was calculated as  $\text{LDL-C} - 0.3 \times \text{Lp(a)}$  [64]. Lp(a) values were measured via chemiluminescent ELISA and reported as mg/dL. Samples were analyzed in triplicate, yielding an inter-assay CV of 12.1% and an intra-assay CV of 6.4%.

## **Assessment of Endothelial Function**

Flow mediated dilation was assessed in all participants using a Toshiba #SSH-140A ultrasound system with a 7.5- MHz linear array transducer. Participants were asked to abstain from smoking, caffeine, food, and vigorous activity before their visit. Statins and hypertensive medications were continued as prescribed. Blood pressure measurements and routine paperwork were completed prior to starting the protocol. Set up entailed placing a blood pressure cuff (Hokanson AG101) 3mm distal to the antecubital fossa on the right forearm. The ultrasound probe was placed above the cuff on the brachial artery. During the first 2-minutes, baseline vessel diameter and blood velocity were recorded using the ultrasound's B-mode and pulse-wave Doppler settings respectively. After ensuring proper baseline imaging was acquired, a brachial artery cuff was inflated to either 200mmHg or 30mmHg above SBP. This was maintained for 5 mins, occluding blood flow downstream of the cuff. Immediately following cuff release, 5 beats of pulse-wave Doppler images were saved. B-mode images were carried out for a 2-minute post-occlusion recovery period. Following data acquisition, quantitative measures were processed with automated edge detection software, in which FMD was calculated and reported as % change  $(D_f - D_b / D_b) \times 100 = \text{FMD\%}$  and diameter change from baseline. Blinded personnel performed both the data collection and analysis.

## **Fibrinogen**

Fibrinogen measurements were made from antecubital blood draws after an overnight fast. Samples were housed in 3.8% trisodium citrate, centrifuged, and stored for further analysis. Immunoprecipitation and the clauss method [65] were used to determine concentrations.

## **CVD Outcome Measures**

The criteria for major CVD events were defined as fatal or nonfatal myocardial infarction; angina pectoris; intermittent claudication; congestive heart failure; cerebrovascular accident, including stroke, transient ischemic attack, and atherothrombotic brain lesion; or related CVD death in the absence of the previous occurrence by any of these diseases. All events were obtained from medical records, clinical evaluation, or autopsy record and validated by Framingham Endpoint Review Committee or physician review. Follow-up time for each person was calculated as the number of years between their Lp(a) measurement and the first occurrence of CVD or censoring at the last available follow-up.

## **Covariates**

Covariates were assessed at the clinical evaluation during the 7th examination cycle. Smoking status, hypertension, hyperlipidemia, and diabetic medication use were identified through self-report and patient's medical records. Diabetes was defined as a fasting glucose level  $\geq 126$  mg/dl or the current use of oral hypoglycemic agents or insulin [66]. Hypertension was defined as a SBP  $\geq 140$  mmHg, diastolic blood pressure (DBP)  $\geq 90$  mmHg, or the current use of antihypertensive medication [67]. Other possible confounding variables when exploring the relationship between Lp(a) and FMD were extracted and are detailed within the statistical analysis.

## **Statistical Analysis**

To test our hypothesis that Lp(a) is inversely related to FMD, we conducted a multistep analysis. First, we used multiple imputation by chained equations to address missing covariates

values. 10 imputed data sets were generated in STATA, following multiple imputation estimation procedures, with Rubin's rules used to establish the final, pooled imputed values [68].

For our analyses, we considered Lp(a) as both a categorical and continuous variable, which was log transformed to account for the rightward skew of the data. Continuous variables are presented as mean  $\pm$  standard deviation or median (interquartile range). We were then able to group Lp(a) into quintiles. Consistent with previous publications, we defined high Lp(a) as values within the upper quintile ( $\geq 65$  mg/dL) and all other participants were considered to have low Lp(a) [PMID: 11058721]. To assess differences in the relationship between Lp(a) and FMD in pre- and postmenopausal women, we defined those who reported the absence of a menstrual cycle for  $\sim 1$  year prior to exam 7 as postmenopausal. Descriptive characteristics were compared between pre- and postmenopausal women using independent t-tests. Stratification allowed for comparisons of endothelial function, measured by FMD, between our high and low Lp(a) and pre- and postmenopausal groups.

We utilized a multistep analysis to test the hypothesis that Lp(a) is inversely related to FMD and mediated by fibrinogen (**Figure 2**). All primary analyses were conducted independently in pre- and postmenopausal women. The relationship between Lp(a) and FMD was assessed using generalized linear models with a gaussian distribution (Path A). Our primary outcome was brachial artery flow-mediated dilation, expressed as the natural log-transformed variable to improve normality. We constructed three models to adjust for potential confounders: (1) an unadjusted model; (2) an age-adjusted model; and (3) a fully adjusted model for age, diabetes status, body mass index, SBP, antihypertensive medication, smoking status, and corrected LDL-C. In the second step (Path B), we evaluated the relationship between Lp(a) and fibrinogen via linear regression analysis in (1) an unadjusted model; (2) an age-adjusted model;

and (3) a fully adjusted model for age, diabetes status, body mass index, SBP, antihypertensive medication, smoking status, and corrected LDL-C. For the third step (Path C) we evaluated the relationship between fibrinogen and FMD via linear regression analysis in (1) an unadjusted model; (2) an age-adjusted model; and (3) a fully adjusted model for age, diabetes status, body mass index, SBP, antihypertensive medication, smoking status, and corrected LDL-C. For the final step, we performed a formal mediation analysis to determine whether fibrinogen mediates the association of Lp(a) with FMD. Natural direct effects (NDE), natural indirect effects (NIE), and total effects (TE) were estimated. All mediation analyses included age, diabetes status, body mass index, SBP, antihypertensive medication, smoking status, and corrected LDL-C as covariates.

To test the hypothesis that Lp(a) and FMD are associated with incident CVD, Lp(a) and FMD were first independently analyzed using Cox proportional hazard regression adjusted for age, diabetes status, body mass index, SBP, antihypertensive medication, smoking status, and corrected LDL-C. In this analysis FMD was analyzed as a categorical variable, with individuals classified as low FMD if their values fell in the lowest tertile of the cohort distribution, which corresponded to <3.24%. The multiplicative interaction between Lp(a) and FMD was tested. To evaluate the joint association of Lp(a) and FMD with CVD, we stratified the cohort into 4 mutually exclusive groups;  $FMD \geq 3.4\%$  and  $Lp(a) < 65 \text{ mg/dL}$  (normal FMD + low Lp(a)),  $FMD \geq 3.4\%$  and  $Lp(a) \geq 65 \text{ mg/dL}$  (normal FMD + high Lp(a)),  $FMD < 3.4\%$  and  $Lp(a) < 65 \text{ mg/dL}$  (low FMD + low Lp(a)),  $FMD < 3.4\%$  and  $Lp(a) \geq 65 \text{ mg/dL}$  (low FMD + high Lp(a)).

All statistical analyses were performed using Stata statistical software version 18.0 (StataCorp). All significance tests were two-sided, using  $p < 0.05$  as the level of statistical significance.



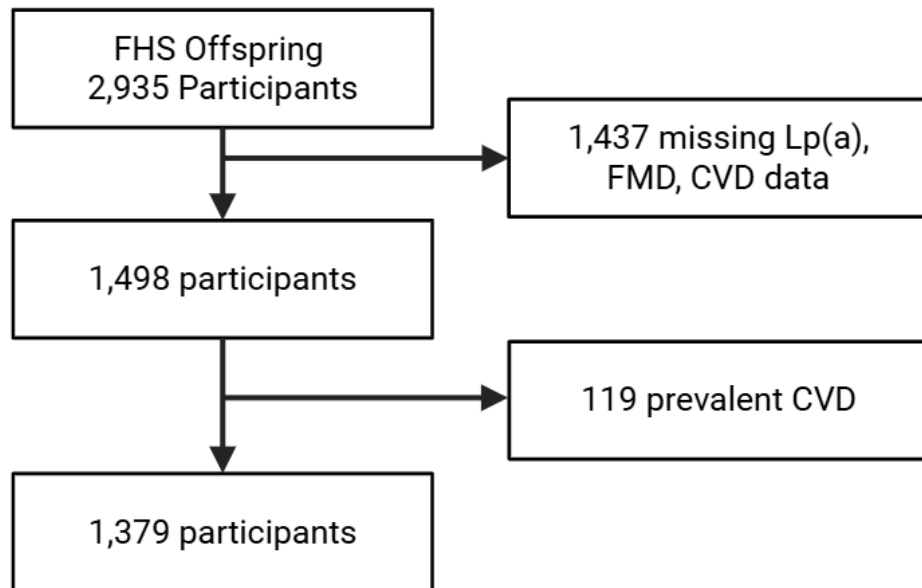


**Figure 1.** Flowchart of study participants

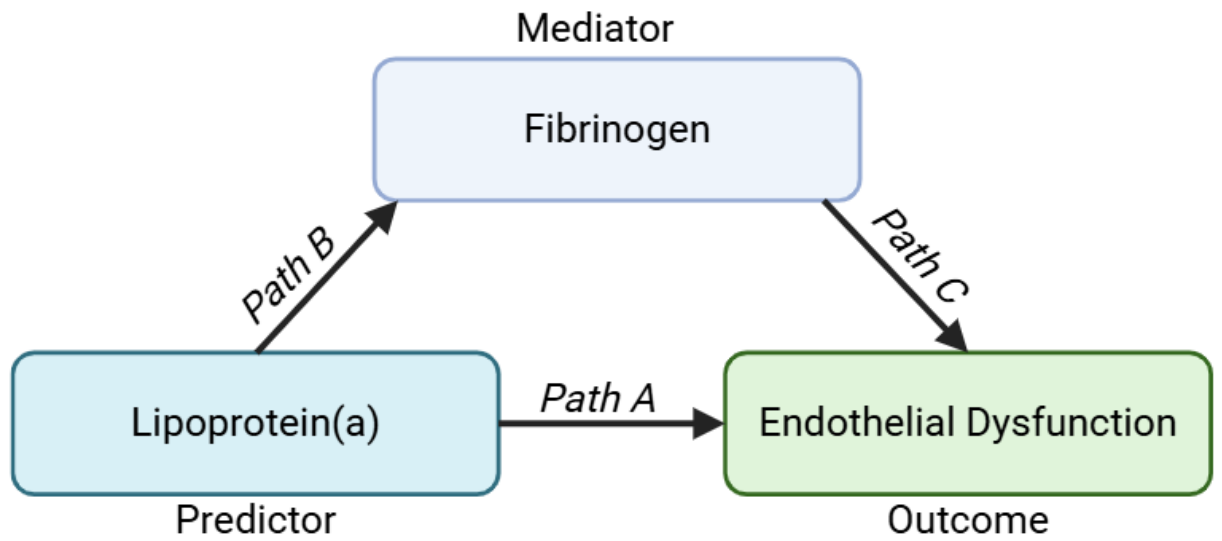


**Figure 2.** Statistical approach with a path diagram of the mediation analysis.

## Chapter 4 - Results

### Study Population

All cohort characteristics are presented in **Table 1**. Of the 1,379 women included in our study, 1,265 of them had gone through menopause at the time of exam 7. Age was significantly different between our pre- and postmenopausal strata, with averages of 50 and 61, respectively. SBP, antihypertensive medication use, statin use, Non-HDL, LDL-C, and total triglycerides were all significantly higher in postmenopausal women.

### Association of Lp(a) with FMD

Clinical characteristics between premenopausal women grouped by high ( $\geq 65\text{mg/dL}$ ) and low ( $< 65\text{mg/dL}$ ) Lp(a) were similar (**Table 2**). Importantly, a significantly lower FMD was observed in premenopausal women with high Lp(a) compared to premenopausal women with a low Lp(a) ( $p=0.019$ ) (**Figure 3**). This relationship persisted after further adjustment for diabetes status, body mass index, SBP, smoking status, hypertensive medications, and LDL-C ( $p=0.032$ ). When treated as a continuous variable in multiple linear regression, higher levels of Lp(a) correlated with lower FMD values ( $p=0.047$ ). **Figure 4A** illustrates the marginal predicted values of FMD associated with Lp(a) in our group of premenopausal women.

Postmenopausal women grouped by high or low Lp(a) levels had several significant differences in baseline clinical characteristics (**Table 3**). Those with a high Lp(a) were older, more likely to be on a statin, less likely to smoke, and had a higher LDL-C and mean SBP. After adjustment for clinically relevant risk factors, postmenopausal women showed no significant difference in FMD between our high- and low-Lp(a) groups ( $p=0.292$ ). Similarly, when

evaluated with Lp(a) as a continuous variable, using multiple linear regression, there was no significant relationship between Lp(a) and FMD ( $p=0.702$ ) (**Figure 4B**)

### **Association of Lp(a) with Fibrinogen**

In premenopausal women, Lp(a), when treated as a continuous variable in multiple linear regression, was positively correlated with fibrinogen after adjusting for covariates ( $p=0.038$ ). (**Figure 5**). Similarly, in postmenopausal women, we observed a positive correlation between Lp(a) and fibrinogen with adjustment for diabetes status, body mass index, SBP, hormone replacement therapy, smoking status, and LDL-c ( $p=0.002$ ).

### **Association of Fibrinogen with FMD**

**Figure 6** illustrates the association between fibrinogen and FMD. There was no correlation between fibrinogen and FMD in premenopausal women before or after adjustment for diabetes status, body mass index, SBP, smoking status, hypertensive medications, and LDL-C ( $p=0.171$ ). In postmenopausal women, higher fibrinogen levels were significantly associated with lower FMD in our unadjusted model ( $p<0.005$ ); however, this relationship was attenuated after adjustment ( $p=0.096$ ).

### **Mediation Analysis**

Mediation analysis in premenopausal women revealed a significant total effect of Lp(a) on FMD ( $\beta = -0.21$ ; 95%CI,  $-0.36$  to  $-0.06$ ;  $p = 0.008$ ). There was a non-significant natural indirect effect (NIE) through fibrinogen ( $\beta = -0.24$ ; 95%CI,  $-0.08$  to  $0.34$ ;  $p=0.4$ ), but a significant natural direct effect (NDE) ( $\beta = -0.19$ ; 95%CI,  $-0.36$  to  $0.017$ ;  $p=0.03$ ). These findings suggest that elevated Lp(a) is associated with impaired FMD independent of fibrinogen.

In postmenopausal women, mediation analysis revealed a non-significant total effect of high Lp(a) on FMD ( $\beta = -0.04$ ; 95%CI,  $-0.13$  to  $-0.04$ ;  $p=0.3$ ). A non-significant natural indirect effect (NIE) through fibrinogen ( $\beta = -0.003$ ; 95%CI,  $-0.14$  to  $0.007$ ;  $p=0.5$ ), and a non-significant natural direct effect (NDE) ( $\beta = -0.04$ ; 95%CI,  $-0.13$  to  $0.05$ ;  $p=0.4$ ) were also observed.

### **Combined effect of Lp(a) and FMD on CVD outcomes**

A total of 314 incident CVD events were observed during a mean follow-up of  $17.1 \pm 8.6$  years. In categorical analysis, individuals with both Normal FMD and Low Lp(a) served as the referent group. Compared to this group, those with Normal FMD and High Lp(a) had more than a two-fold increased risk of CVD (HR: 2.15, 95% CI: 1.24-3.72,  $p=0.006$ ). Individuals with Low FMD and Low Lp(a) also showed an elevated risk (HR: 1.43, 95% CI: 1.07-1.91,  $p=0.02$ ). The group with both Low FMD and High Lp(a) exhibited a doubling of risk (HR: 2.00, 95% CI: 1.31-3.05,  $p=0.001$ ). All models were adjusted for traditional risk factors (**Table 4, Figure 7**).

**Table 1.** Cohort Demographics Stratified Menopause Status

|                                                                                                                                                                       | <b>All Participants</b><br>(n = 1,379) | <b>Premenopausal</b><br>(n = 114) | <b>Postmenopausal</b><br>(n = 1,265) | <b>P- value</b> |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------|-----------------------------------|--------------------------------------|-----------------|
| Age, yr                                                                                                                                                               | 60 ± 9                                 | 50 ± 3                            | 61 ± 9                               | <0.001          |
| Race %                                                                                                                                                                |                                        |                                   |                                      |                 |
| White                                                                                                                                                                 | 1,365 (99.0)                           | 114 (100)                         | 1,251 (98.9)                         | 0.3             |
| Other/Not Reported                                                                                                                                                    | 14 (1.0)                               | 0 (0)                             | 14 (1.1)                             |                 |
| Ethnicity %                                                                                                                                                           |                                        |                                   |                                      |                 |
| Non-Hispanic Black                                                                                                                                                    | 1,374 (99.64)                          | 114 (100)                         | 1,260 (99.6)                         | 0.5             |
| Hispanic/Not Reported                                                                                                                                                 | 5 (0.36%)                              | 0 (0)                             | 5 (0.4)                              |                 |
| Body mass index, kg/m <sup>2</sup>                                                                                                                                    | 27.5 ± 5.8                             | 26.8 ± 6.5                        | 27.5 ± 5.7                           | 0.2             |
| Systolic BP, mmHg                                                                                                                                                     | 125 ± 20                               | 116 ± 14                          | 126 ± 20                             | <0.001          |
| Diastolic BP, mmHg                                                                                                                                                    | 73 ± 9                                 | 74 ± 9                            | 73 ± 9                               | 0.3             |
| Antihypertensive Med, n                                                                                                                                               | 367 (26.6)                             | 12 (10.5)                         | 355 (28.1)                           | <0.001          |
| Lipid Meds, n                                                                                                                                                         | 211 (15.3)                             | 9 (7.9)                           | 202 (16.0)                           | 0.02            |
| Smoking, n                                                                                                                                                            | 186 (13.5)                             | 16 (14.0)                         | 170 (13.4)                           | 0.9             |
| Diabetes, n                                                                                                                                                           | 100 (7.3)                              | 4 (3.5)                           | 96 (7.6)                             | 0.1             |
| Total Cholesterol, mg/dl                                                                                                                                              | 207.8 ± 36.6                           | 196.2 ± 38.2                      | 208.8 ± 36.2                         | <0.001          |
| HDL, mg/dl                                                                                                                                                            | 61.5 ± 16.9                            | 62.5 ± 15.9                       | 61.4 ± 16.9                          | 0.5             |
| Non-HDL, mg/dl                                                                                                                                                        | 146.2 ± 38.1                           | 133.7 ± 38.7                      | 147.3 ± 37.8                         | <0.001          |
| Corrected LDL, mg/dl                                                                                                                                                  | 201.8 ± 37.3                           | 189.9 ± 39.5                      | 202.9 ± 36.9                         | <0.001          |
| Triglycerides, mg/dl                                                                                                                                                  | 128.6 ± 74.7                           | 98.1 ± 54.9                       | 131.4 ± 75.7                         | <0.001          |
| Lp(a), mg/dl                                                                                                                                                          | 19.7 ± 30.4                            | 21.2 ± 29.1                       | 19.6 ± 30.5                          | 0.6             |
| FMD, %                                                                                                                                                                | 3.3 ± 3.0                              | 4.3 ± 2.7                         | 3.7 ± 0.6                            | <0.001          |
| Values are mean ± SD, n(%). BP = blood pressure, HDL = high-density lipoprotein, LDL = low-density lipoprotein, Lp(a) = lipoprotein(a), FMD = flow-mediated dilation. |                                        |                                   |                                      |                 |

**Table 2.** Premenopausal Demographics Stratified Lp(a)

|                                    | <b>Low Lp(a)</b><br>(n = 98) | <b>High Lp(a)</b><br>(n = 16) | <b>P- value</b> |
|------------------------------------|------------------------------|-------------------------------|-----------------|
| Age, yr                            | 50 ± 3                       | 49 ± 3                        | 0.8             |
| Body mass index, kg/m <sup>2</sup> | 26.3 ± 5.9                   | 30.0 ± 8.6                    | 0.04            |
| Systolic BP, mmHg                  | 116 ± 15                     | 118 ± 12                      | 0.6             |
| Diastolic BP, mmHg                 | 73 ± 10                      | 75 ± 9                        | 0.6             |
| Antihypertensive Med, n            | 9 (9.2)                      | 3 (18.8)                      | 0.2             |
| Lipid Meds, n                      | 7 (7.1)                      | 2 (12.5)                      | 0.5             |
| Smoking, n                         | 12 (12.4)                    | 16 (14.0)                     | 0.2             |
| Diabetes, n                        | 3 (3.1)                      | 1 (6.3)                       | 0.5             |
| Total Cholesterol, mg/dl           | 195.9 ± 40.0                 | 198.1 ± 25.9                  | 0.8             |
| HDL, mg/dl                         | 62.7 ± 15.9                  | 61.4 ± 16.1                   | 0.8             |
| Non-HDL, mg/dl                     | 133.2 ± 40.2                 | 136.7 ± 28.6                  | 0.7             |
| Corrected LDL, mg/dl               | 192.6 ± 40.8                 | 173.4 ± 25.4                  | 0.07            |
| Triglycerides, mg/dl               | 97.2 ± 56.9                  | 103.3 ± 41.2                  | 0.7             |
| Lp(a), mg/dl                       | 11.2 ± 16.1                  | 82.2 ± 9.4                    | <0.001          |
| FMD, %                             | 4.5 ± 2.7                    | 2.8 ± 2.1                     | 0.019           |

Values are mean ± SD, n(%). BP = blood pressure, HDL = high-density lipoprotein, LDL = low-density lipoprotein, Lp(a) = lipoprotein(a), FMD = flow-mediated dilation.

**Table 3.** Postmenopausal Demographics Stratified Lp(a)

|                                    | <b>Low Lp(a)</b><br>(n = 1,120) | <b>High Lp(a)</b><br>(n = 145) | <b>P- value</b> |
|------------------------------------|---------------------------------|--------------------------------|-----------------|
| Age, yr                            | 61 ± 9                          | 63 ± 8                         | 0.02            |
| Body mass index, kg/m <sup>2</sup> | 27.4 ± 5.7                      | 28.2 ± 5.8                     | 0.2             |
| Systolic BP, mmHg                  | 125 ± 20                        | 130 ± 21                       | 0.02            |
| Diastolic BP, mmHg                 | 72 ± 10                         | 74 ± 9                         | 0.06            |
| Antihypertensive Med, n            | 316 (28.2)                      | 39 (26.9)                      | 0.7             |
| Lipid Meds, n                      | 165 (14.7)                      | 37 (25.5)                      | 0.001           |
| Smoking, n                         | 158 (14.1)                      | 12 (8.3)                       | 0.05            |
| Diabetes, n                        | 84 (7.5)                        | 12 (8.3)                       | 0.7             |
| Total Cholesterol, mg/dl           | 208.2 ± 36.6                    | 213.4 ± 33.1                   | 0.1             |
| HDL, mg/dl                         | 61.3 ± 17.0                     | 61.9 ± 16.4                    | 0.7             |
| Non-HDL, mg/dl                     | 146.8 ± 38.1                    | 151.5 ± 35.1                   | 0.2             |
| Corrected LDL, mg/dl               | 205.2 ± 36.6                    | 1855.7 ± 34.6                  | <0.001          |
| Triglycerides, mg/dl               | 208.2 ± 36.6                    | 133.0 ± 70.5                   | 0.8             |
| Lp(a), mg/dl                       | 10.2 ± 15.1                     | 92.3 ± 20.0                    | <0.001          |
| FMD, %                             | 3.3 ± 3.0                       | 3.7 ± 0.7                      | 0.1             |

Values are mean ± SD, n(%). BP = blood pressure, HDL = high-density lipoprotein, LDL = low-density lipoprotein, Lp(a) = lipoprotein(a), FMD = flow-mediated dilation.

**Table 4.** Joint Association of Lp(a) and FMD with CVD

|                         | <b>HR (95% CI)</b> | <b>P- value</b> |
|-------------------------|--------------------|-----------------|
| Normal FMD / Low Lp(a)  | Referent           |                 |
| Normal FMD / High Lp(a) | 2.15 (1.24-3.72)   | 0.006           |
| Low FMD / Low Lp(a)     | 1.43 (1.07-1.91)   | 0.02            |
| Low FMD / High Lp(a)    | 2.00 (1.31-3.05)   | 0.001           |

Cox proportional hazard regression models adjusted for age, diabetes status, body mass index, systolic blood pressure, antihypertensive medication, smoking status, and corrected LDL-C.

HR = Hazard ratio, Lp(a) = lipoprotein(a), FMD = flow-mediated dilation.

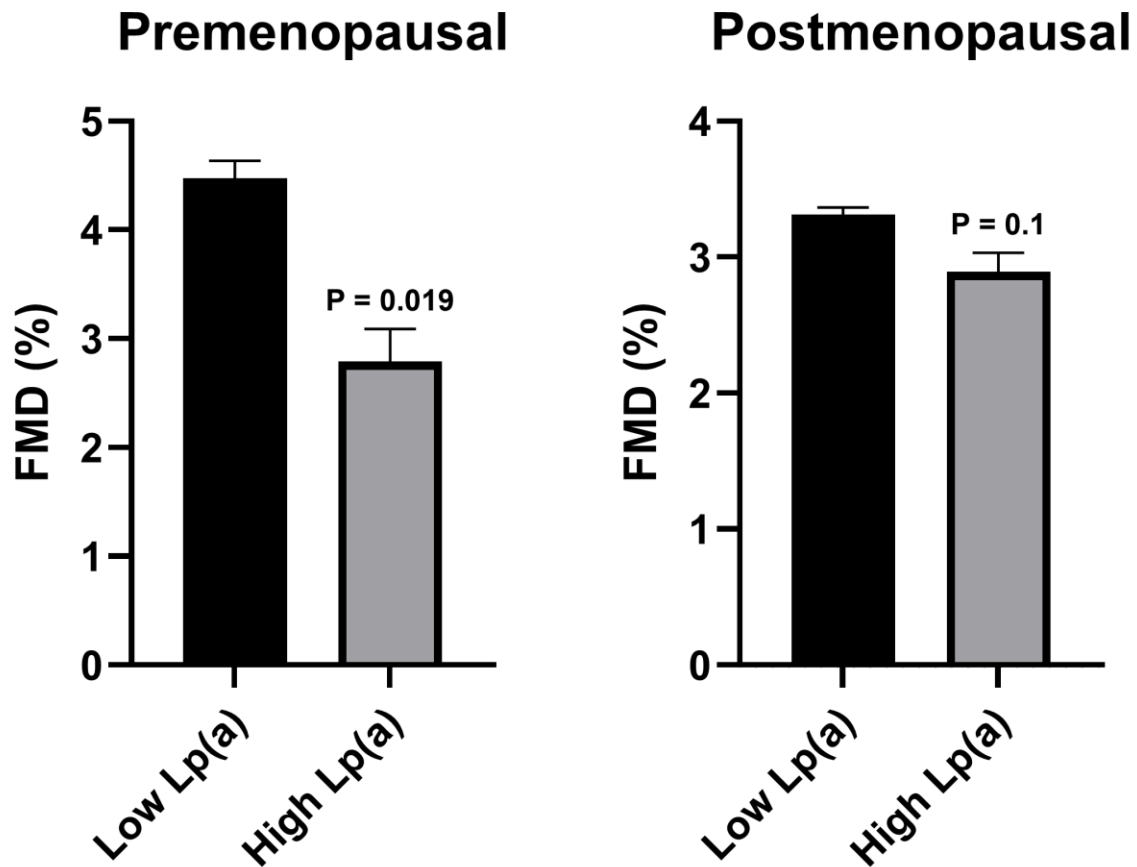


**Figure 3. The impact of low Lp(a) (grey) vs high (Black) on FMD in pre- and postmenopausal women:** Flow-mediated dilation (FMD%) is shown for individuals with Low Lp(a) (black bars) versus High Lp(a) (grey bars). (Left graph) premenopausal group. (Right graph) postmenopausal group. High Lp(a) is associated with significantly impaired endothelial function (lower FMD%) only in premenopausal women ( $p=0.019$ ). There was no significant difference in endothelial function by high and low Lp(a) in postmenopausal individuals ( $p=0.1$ ). Data are presented as mean  $\pm$  SD

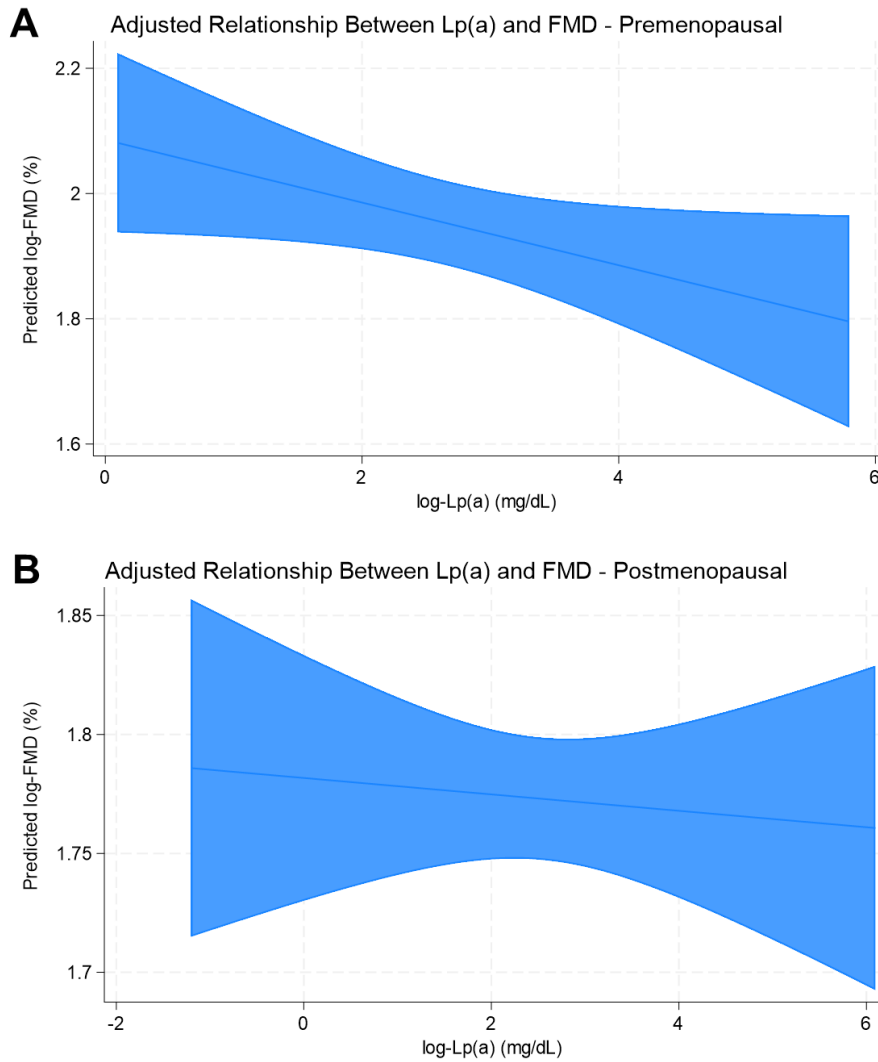


**Figure 4. Adjusted relationship between Lp(a) and FMD in premenopausal (A) and postmenopausal (B) women.** Marginal predicted values (and 95%CI) for log-transformed FMD across the observed range of log-transformed Lp(a) concentrations (log-transformed) are shown.

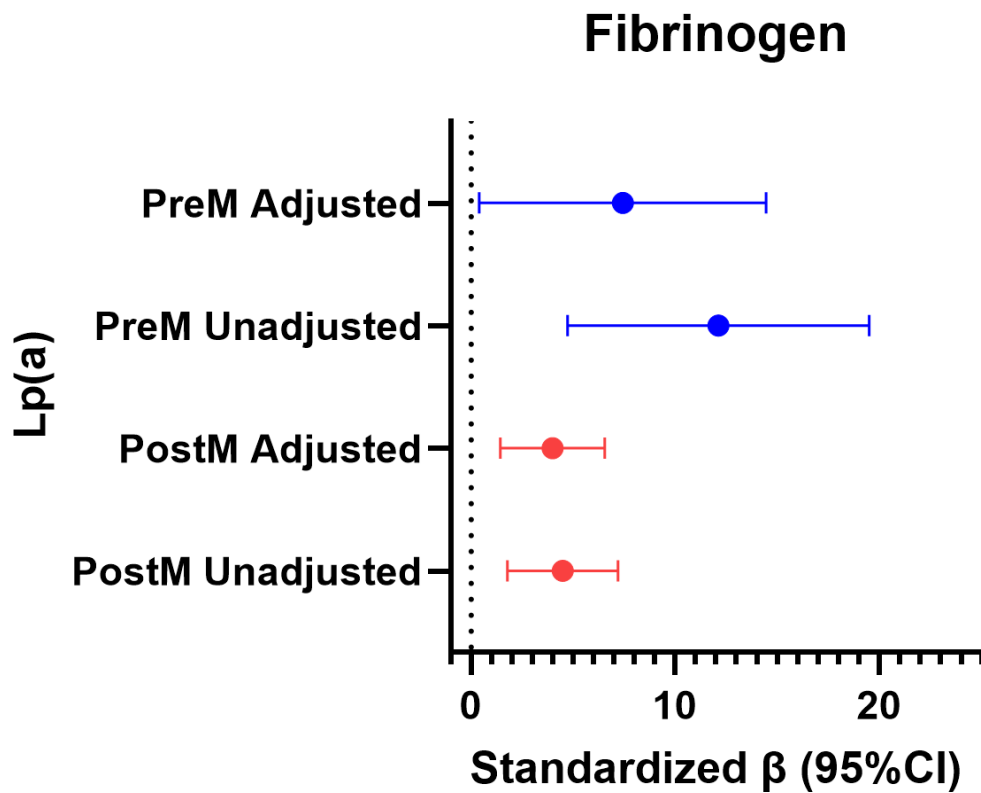


**Figure 5. Association between Lipoprotein(a) [Lp(a)] and Fibrinogen levels by menopausal status.** Results are shown for premenopausal (PreM, blue) and postmenopausal (PostM, red) groups, including both unadjusted and adjusted models for confounders.

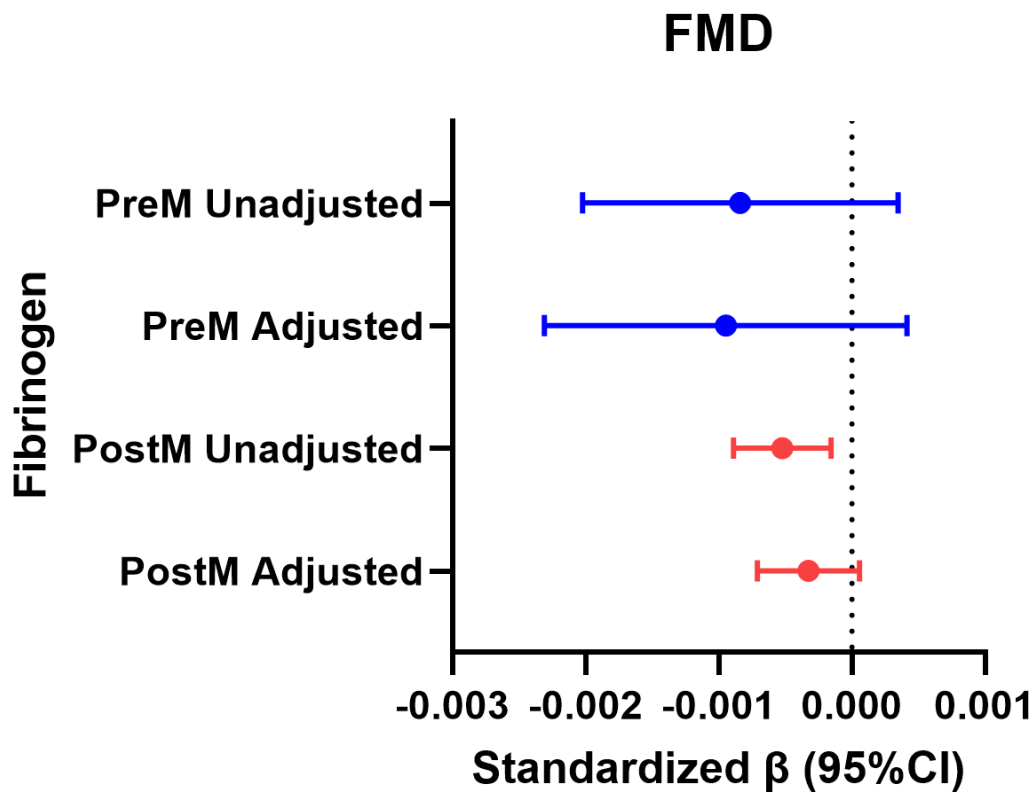


**Figure 6. Association between Fibrinogen and FMD levels by menopausal status.** Results are shown for premenopausal (PreM, blue) and postmenopausal (PostM, red) groups, including both unadjusted and adjusted models for confounders.

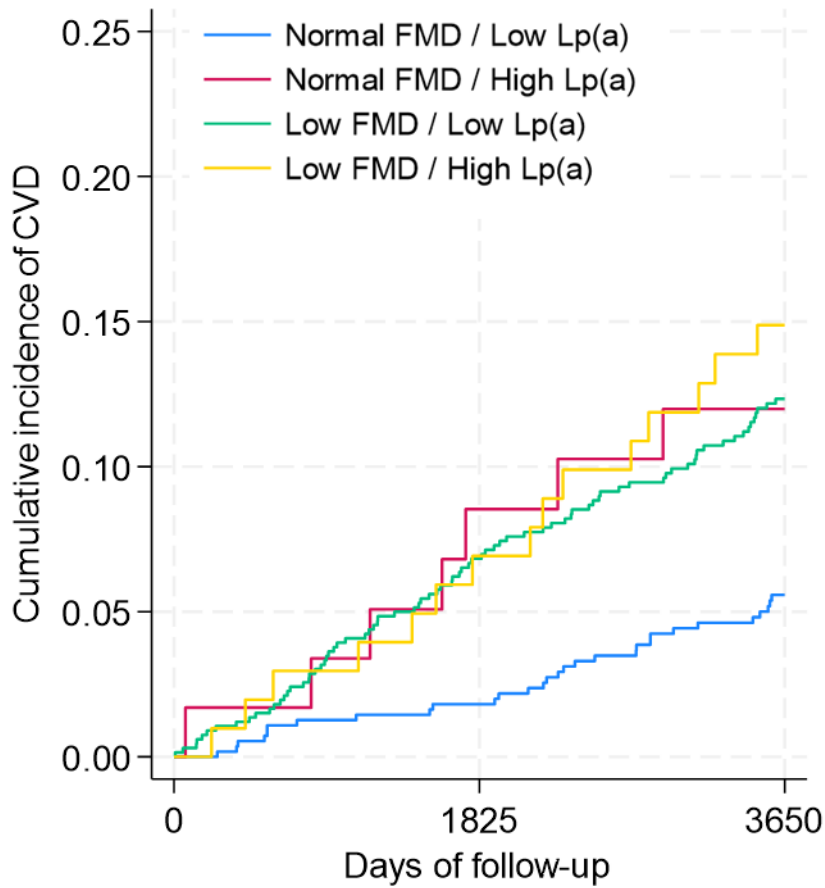


**Figure 7. Cumulative incidence of cardiovascular disease (CVD) by FMD and Lp(a) levels:** Participants with Normal FMD / Low Lp(a) (blue line) exhibited the lowest cumulative incidence of CVD. High Lp(a) or Low FMD individually increased risk to similar levels, while the presence of both risk factors (yellow line) resulted in the highest overall incidence by the end of the follow-up period.



## Chapter 5 - Discussion

Previous studies have established Lp(a) as a causal risk factor for CVD; however, the mechanisms by which elevated Lp(a) contributes to early vascular dysfunction, particularly at the level of the endothelium, remain incompletely defined [10], [69]. Endothelial dysfunction represents one of the earliest detectable steps in the atherogenic process, making it a critical target for understanding Lp(a)-mediated CVD risk. As such, the association between Lp(a), endothelial function, and CVD risk remains an area of ongoing investigation. Therefore, we sought to characterize the relationship between this genetically determined, atherogenic lipoprotein and vascular function.

Our cohort was stratified by menopausal status, which marks a robust, systemic physiological transition known to influence both Lp(a) concentrations and endothelial function [70], [71]. From this, we report several important findings in this study evaluating the relationship between Lp(a) and endothelial function, assessed via FMD. First, following stratification, we observed that premenopausal women with elevated Lp(a) levels exhibited significantly attenuated FMD compared to those with lower Lp(a). However, in contrast to our hypothesis, no significant differences in FMD were observed between high and low Lp(a) groups among postmenopausal women. To our knowledge, these data demonstrate, for the first time, a relationship between vascular endothelial function and elevated Lp(a) in premenopausal women but not in postmenopausal women. Second, while Lp(a) was associated with fibrinogen in both pre- and postmenopausal women, it did not mediate the effect of Lp(a) on FMD. These findings suggest that other pro-atherogenic and pro-thrombotic properties of Lp(a) may play a mechanistic role in mediating vascular endothelial dysfunction. Third, individuals with both elevated Lp(a) and low FMD were at significantly higher CVD risk compared to those with low

Lp(a) and normal FMD. Fourth, elevated Lp(a) remained associated with CVD risk in individuals with normal FMD, like that observed in those with low FMD and low Lp(a).

### **Association between Lp(a) and FMD**

We found that premenopausal women with high Lp(a) levels had a significantly lower FMD response compared with their low Lp(a) counterparts, indicating that high Lp(a) is associated with endothelial dysfunction specifically in women who have not yet experienced menopause. However, in our sample of postmenopausal women, this relationship was not significant. These findings extend prior work examining the relationship between Lp(a) and vascular reactivity.

In a multiethnic cohort Wu et al. found Lp(a) levels were significantly associated with FMD, but not with nitroglycerine-induced dilation (NTG) ( $p=0.002$ ) [72]. This persisted when considering sex, indicating endothelial-dependent vascular impairments present with high levels of Lp(a) in a population with no other overt cardiovascular risk factors. Their findings provide a foundation for ongoing research into the atherogenic origins of Lp(a) and early vascular dysfunction.

Similar to our work, Wilmsink et al. investigated the association between Lp(a) and endothelial function, but in postmenopausal women only [14]. Endothelial-dependent vasodilation and endothelial-independent dilation were measured with FMD and after sublingual nitroglycerine spray. When participants were grouped by Lp(a) concentration, those in the highest quartile ( $>23.9$  mg/dL) exhibited significantly lower FMD ( $\sim 2.4\%$ ) and nitroglycerin-induced vasodilation ( $\sim 8.0\%$ ) compared with women in the lower three quartiles (mean  $5.2\%$  FMD and  $11.4\%$  nitroglycerin-induced dilation). Although limited by a relatively

small sample size ( $n = 108$ ), these findings support an association between elevated Lp(a) and impaired vascular function. However, these findings contrast with the present investigation's results in postmenopausal women, in whom no relationship was observed between Lp(a) and FMD.

Schlaich et al. further explored the impact of Lp(a) on endothelial function with strain-gauge plethysmography in a group of men and women [15]. They utilized intraarterial infusions of acetylcholine (ACh), sodium nitroprusside (SNP), and NG-nitro-L-arginine methyl ester (L-NAME), which were administered in a dose-response manner to address endothelial-dependent dilation, endothelial-independent dilation, and smooth muscle vasoconstriction, respectively [15]. After stratifying participants into tertiles by Lp(a) concentration ( $4.8 \pm 2.5$ ,  $13.0 \pm 3.0$ , and  $49.2 \pm 20.3$  mg/dL), there were no significant differences in forearm blood flow at any ACh or SNP dose, suggesting no effect of Lp(a) on endothelial function. However, there was a significantly greater vasoconstrictive response with L-NAME administration at 2 and 4  $\mu$ mol in the upper Lp(a) tertile. The authors attributed this to increased basal nitric oxide production as a compensatory mechanism to preserve vascular tone. Whether this reflects a direct interaction between Lp(a) and vasoconstrictor pathways remains uncertain. Interpretation of these findings is further constrained by the modest sample size ( $n = 57$ ).

Despite differences in methodology and outcomes, both Wilmkink et al. and Schlaich et al. support an independent relationship between higher Lp(a) concentrations and altered vascular reactivity. Our findings add to this growing body of evidence by demonstrating that premenopausal, but not postmenopausal, women with elevated Lp(a) exhibit reduced endothelium-dependent vasodilation, as reflected by the attenuated FMD. Given the well-documented decline in vascular health accompanying the menopausal transition, these findings

underscore the importance of Lp(a) as a predictive marker of cardiovascular risk in women prior to menopause. Understanding the impact of elevated Lp(a) could improve risk stratification and prompt early interventions before the onset of menopause-related endothelial decline.

### **Relationship between Lp(a) and Fibrinogen**

Recent work has established Lp(a) is an independent, causal risk factor for ASCVD [10], [69], [73]. Lp(a) most likely promotes atherogenesis through multifaceted and synergistic mechanisms linking lipid deposition, inflammation, and thrombosis. It has the greatest affinity for OxPLs among all of the apoB-100 containing lipoproteins [7]. OxPLs potentiate atherogenesis by promoting vascular inflammation, attracting monocytes, and inducing foam cell formation [54]. Beyond its atherogenic and proinflammatory properties, Lp(a) is prothrombotic and antifibrinolytic. Due to its homology with plasminogen, Lp(a) competes with plasminogen for fibrin-binding sites and attenuates plasmin generation, thereby exerting a potent antifibrinolytic effect [56]. We hypothesized that these properties may provide a mechanistic link between Lp(a) and vascular endothelial dysfunction.

The present study revealed that higher Lp(a) levels are associated with higher fibrinogen levels in both pre- and postmenopausal women. Fibrinogen is a soluble plasma glycoprotein that, when elevated, increases the risk for CVD [16]. Others have established both independent and combined effects of Lp(a) and fibrinogen on all-cause, CVD, and cancer-related mortality [74]. The hazard ratio for CVD mortality in participants with high Lp(a) and fibrinogen was 2.147 (1.483–3.109) [74]. The linear relationship between Lp(a) and FMD along with a compounded risk for CVD mortality indicate that individual presenting with both risk factors may be at high priority for intervention and adverse outcomes monitoring.

## **Association between Fibrinogen and FMD**

Fibrinogen is a primary clotting factor that, in excess, may have detrimental effects on vascular function. Previous reports show an inverse relationship between fibrinogen levels and vascular endothelial function as measured by FMD [17]. Several mechanisms may play a role including elevated blood viscosity, increased platelet aggregation, and augmented inflammatory and thrombotic activity. These traits can impact NO bioavailability, reducing vasodilatory capacity, and increasing the risk of CVD. However, contrary to our hypothesis and these earlier reports, we did not observe a significant association between fibrinogen and FMD in either premenopausal or postmenopausal women after adjustment for cardiovascular risk factors. Although an inverse relationship was observed in unadjusted analyses among postmenopausal women, this association was attenuated following multivariable adjustment, suggesting that the relationship between fibrinogen and endothelial function may be largely mediated by shared risk factors.

Our mediation analysis further revealed that the relationship between elevated Lp(a) and FMD is not mediated through fibrinogen-mediated pathways. In our premenopausal women, a significant total effect of high Lp(a) on FMD was observed, which supports our hypothesis that Lp(a) contributes to endothelial dysfunction. However, the absence of a significant natural indirect effect through fibrinogen is suggestive that fibrinogen does not meaningfully mediate this relationship. Alternative mechanisms may include other pro-inflammatory pathways but are beyond the scope of the present investigation.

## **Lp(a) and FMD as predictors of CVD**

In our work and others, Lp(a) and FMD both independently predicted incident CVD [75]. These risk factors reflect two distinct but complementary aspects of the atherogenic process, lipid deposition and vascular dysfunction. Lp(a) represents a genetically determined risk factor that contributes to lipid deposition, oxidative stress, and thrombosis, whereas FMD provides a functional assessment of endothelial health [42], [76]. Converging these independent predictors allows us to assess the compounding impact on CVD risk.

Early work by Sorensen et al. demonstrated that children with hypercholesterolemia exhibited significantly lower FMD, with Lp(a) emerging as the only lipid parameter independently associated with endothelium-dependent vasodilation [77]. In the absence of other traditional risk factors, these findings suggest that vascular endothelial dysfunction may develop early in life. Subsequent studies have reported higher Lp(a) levels in adults with FH and coronary artery disease compared to those without [70], reinforcing a potential contributory role of Lp(a) in early vascular impairment. Additional evidence links elevated Lp(a) with impaired vascular function in postmenopausal women with FH, highlighting its contribution to endothelial dysfunction within a high-risk population [77].

Despite evidence suggesting early vascular impairment in individuals with elevated Lp(a), few studies have examined the combined effects of Lp(a) and vascular function on CVD outcomes. Wang et al. demonstrated both independent and additive effects of Lp(a) and fibrinogen on CVD mortality [67].

## **Strengths and Experimental Considerations**

There are many strengths and experimental considerations that need to be made regarding this study. First, we examined the relationship between Lp(a) and endothelial function in a well-characterized cohort stratified by menopausal status, thereby allowing for the evaluation of women-specific influences on vascular health. Secondly, we used a combination of categorical and continuous modeling of Lp(a) to assess its relationship with fibrinogen, FMD, and CVD outcomes. We acknowledge that causal claims cannot be made in our current cross-sectional analyses regarding the relationship between Lp(a) levels and endothelial dysfunction. An additional limitation is the evaluation of estrogen use in the postmenopausal group. Estrogen use has been shown to decrease Lp(a) levels and increase FMD in other studies [78], [79], [80]. Future work would assess the relationship between Lp(a), FMD, fibrinogen, and CVD in pre- and postmenopausal women with and without hormone therapy.

Additionally, this cohort, composed mostly of white, middle-aged women, is not representative of the entire US population or those with more diverse backgrounds. FMD is also a technique sensitive to tester and subject errors, which could influence the results.

## **Conclusions**

In conclusion, our findings demonstrate that elevated Lp(a) is associated with impaired endothelial function, prior to menopause, suggesting that vascular dysfunction with elevated Lp(a) may play a role for early CVD pathogenesis for younger women. While fibrinogen was not independently associated with endothelial function after full adjustment in pre- or postmenopausal women, the assessment of Lp(a) and FMD provided significant prognostic value for incident CVD. These results highlight the importance of integrating predictive measures like

Lp(a) and FMD to better understand and predict cardiovascular risk. More work is needed to understand the mechanisms underlying this relationship.

## Chapter 6 - References

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