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RESEARCH ARTICLE

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Interrelationships among platelet-activating factor and lipoprotein-associated phospholipase A_2 activity and traditional cardiovascular risk factors

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Abstract

Traditionally cardiovascular disease (CVD) risk has been assessed through blood lipids and inflammatory marker C-reactive protein (hsCRP). Recent clinical interest in novel pro-inflammatory markers platelet-activating factor (PAF) and lipoprotein-associated phospholipase A_2 (Lp-PLA_2) recognizes that vascular damage can exist in the absence of traditional risk factors. This cross-sectional study investigated the potential relationship between circulating PAF, Lp-PLA_2, hsCRP, and traditional risk factors for CVD. One hundred adults (49 ± 13 years, 31% male) with variable CVD risk were recruited. Fasting inflammatory markers PAF, Lp-PLA_2 and hsCRP and total, high-density lipoprotein (HDL), low-density lipoprotein (LDL) cholesterol, and triglycerides were measured. Blood pressure, body mass index, and waist circumference were measured. Medical and physical activity data were self-reported. Linear and multiple regressions were performed. PAF, Lp-PLA_2, and hsCRP independently correlated with several CVD risk factors. PAF was correlated significantly with risk factors in an unexpected way; there was a medium positive correlation between PAF and HDL cholesterol ($r = 0.394$, $p < 0.001$) and medium negative correlations with Total:HDL cholesterol; ($r = -0.436$, $p < 0.001$) systolic blood pressure; ($r = -0.307$, $p = 0.001$); BMI ($r = -0.381$, $p < 0.001$); and waist circumference ($r = -0.404$, $p < 0.001$). There were large positive correlations between Lp-PLA_2 and LDL ($r = 0.525$, $p < 0.001$) and non-HDL cholesterol ($r = 0.508$, $p < 0.001$). There were large positive correlations between hsCRP and Total:HDL cholesterol ($r = 0.524$, $p < 0.001$); BMI ($r = 0.668$, $p < 0.001$); and waist circumference ($r = 0.676$, $p < 0.001$). PAF, Lp-PLA_2, and hsCRP are implicated in the pathophysiology of inflammation in CVD; however, the relationships between each marker and traditional risk

Abbreviations: CVD, cardiovascular disease; hsCRP, high-sensitive C-reactive protein; Il-6, interleukin 6; Lp-PLA_2, lipoprotein-associated phospholipase A_2; PAF, platelet-activating factor; PAF AH, platelet-activating factor acetylhydrolase; TNF-α, tumor necrosis factor alpha.

factors were different suggesting they may be involved in different atherogenic pathways.

KEYWORDS

cardiovascular disease, inflammation, lipoprotein-associated phospholipase A_2, Lp-PLA_2, PAF, platelet-activating factor, risk factors

1 | INTRODUCTION

Inflammation plays a key role in the development and progression of atherosclerosis from initial insult of the endothelium to the formation of fatty streaks, plaque rupture, platelet activation, and ensuing thrombosis.[1,2] Recent research has focused on identifying biomarkers that play a pathological role in this inflammatory process that can be used to improve assessment of risk, diagnosis, and prognosis of cardiovascular disease (CVD). C-reactive protein (CRP) is a widely recognized and well-researched inflammatory marker involved in CVD. CRP has been shown to be associated with various traditional cardiovascular risk factors and may actively contribute to the development of atherosclerotic plaques and their instability, however, more research is needed to confirm this.[3–5] Increasing evidence suggests that in inflamed tissues, pentameric CRP dissociates into monomeric CRP with a distinct biological role, mediated by bioactive lipids, resulting in an aggravation of inflammation.[6] CRP has been shown to be associated with an increased risk of heart attack, stroke, sudden death and vascular disease[7]; however, there are limitations in using CRP for CVD risk prediction, due to the inability of current assays to differentiate between pentameric and the more atherogenic monomeric isoforms. In addition, CRP has a significant within-person variability requiring repeated testing to confirm levels.[8]

Two novel markers of inflammation involved in CVD identified in recent literature are platelet-activating factor (PAF) and lipoprotein-associated phospholipase A_2 (Lp-PLA_2).[9] PAF, an ether-linked glycerophospholipid, is one of the most potent inflammatory mediators in the body and plays a major role in atherosclerosis. PAF is produced by numerous cells such as platelets, endothelial cells and leukocytes and triggers an inflammatory cascade through the stimulation of numerous inflammatory mediators such as interleukin-6 (IL-6), interleukin-8, monocyte chemoattractant protein-1, and tumor necrosis factor alpha (TNF-α).[10–15] PAF triggers the release of adhesion molecules and acts as a chemoattractant to monocytes.[11,14,16] PAF increases reactive oxygen species leading to LDL oxidation, increases endothelial permeability and stimulates the differentiation of monocytes into macrophages.[17–21] PAF is associated with numerous CVDs such as, heart failure, acute myocardial infarction, coronary heart disease (CHD), and stroke[22–25] and other related inflammatory chronic diseases such as diabetes and nonalcoholic fatty liver disease where PAF stimulates hepatic lipid synthesis and causes hypertriglyceridemia.[26–28]

Lp-PLA_2 is a 50-kD, Ca^{2+}independent phospholipase (EC 3.1. 1.47) which belongs to Group VII of the superfamily of PLA2 enzymes.[29,30] Like all PLA2 enzymes it hydrolyses the *sn*-2 ester bond of glycerophospholipids, such as the acetyl group at the *sn*-2 position on PAF, which led to its original name, PAF acetylhydrolase (PAF AH). However, Lp-PLA_2 is not specific only to PAF but has a high degree of specificity toward oxidatively modified (*sn*-2) phospholipids including ether-linked glycerophospholipids.[31] A number of immune cells (macrophage, monocytes, mast cells, and T lymphocytes) produce and secrete Lp-PLA_2 into plasma where it is carried bound to LDL, HDL, and lipoprotein (a) (Lp(a))[32,33] with the majority carried on LDL, particularly small dense fractions.[34] Lp-PLA_2 associated with HDL is considered protective.[35,36] However, when associated with LDL, Lp-PLA_2 is atherogenic as it is responsible for hydrolyzing oxidized phospholipids on the surface of LDL generating pro-inflammatory by-products such as lysophosphatidylcholine and oxidized, nonesterified fatty acids.[37] These by-products mimic PAF and mediate inflammation by attracting monocytes to the area, activating leukocytes and stimulating the production of other inflammatory cytokines such as IL-6 and TNF-α. Lp-PLA_2 further promotes atherosclerosis by attracting smooth muscle cells to the intima as well as contributing to the apoptosis and necrosis of macrophages in plaque.[36,38–41] Lp-PLA_2 activity is a vascular specific marker with a low biological fluctuation[42] and studies have shown it to be an independent risk marker for CHD events, stroke, calcific aortic valve stenosis, and plaque stability.[43–46]

There is a paucity of clinical research examining PAF and CVD due to the sheer novelty of this marker. In addition, there are limitations to previous research examining Lp-PLA_2. The majority of previous research on Lp-PLA_2 measured plasma concentrations (mass) instead of enzymatic activity. Results from mass and activity assays have shown poor concordance between each other due to Lp-PLA_2 mass assays detecting only a small and variable fraction of total Lp-PLA_2.[47] Lp-PLA_2 activity is now

considered the more accurate measure as it captures a greater proportion of the Lp-PLA_2 located in LDL cholesterol[48] as only 5% of total plasma enzyme activity is associated with HDL in plasma with normal LDL levels.[49] However, there is no established cut-off for Lp-PLA_2 activity levels for identifying those at risk of CVD and different assays all have different reference intervals which limits the comparison of data across studies.[50] Another limitation in previous Lp-PLA_2 research is the inclusion of diverse ethnic groups which results in large variability in reported Lp-PLA_2 activity levels, potentially due to genetic differences.[51–53] Previous research across European, South Asian, and East Asian populations has identified five functional variants including four loss of function variants resulting in up to 64% lower levels of Lp-PLA_2 activity.[54] In addition, past studies have not excluded participants who are consuming medications known to lower Lp-PLA_2 levels such as statins, ezetimibe, fibrates, PCSK9 inhibitors, hormone replacement therapy, niacin or orlistat,[55–58] or dietary supplements such as fish oils and omega-3 fatty acids.[59,60]

To date, no study has examined the relationship between these two novel markers and traditional risk factors in a western population where strict exclusion criteria were applied. This is the first study examining PAF and Lp-PLA_2 levels in an Australian population. More evidence is needed to discern PAF's independent influence on CVD.[61] Similarly, despite Lp-PLA_2 having the potential to become an important diagnostic marker for risk prediction and prognosis of CVD, controversy about Lp-PLA_2's role in CVD exists because of inconsistent and limited research and complexity of the enzyme, and more research is needed to validate the practicability and utility of measuring Lp-PLA_2.[62]

The aim of this study was to determine the relationship between circulating PAF, Lp-PLA_2 activity, and high-sensitivity CRP (hsCRP) and traditional risk factors of CVD in a mostly Caucasian population residing in Australia. The identification of specific risk factors that most strongly associate with each inflammatory marker can allow for a better understanding of how these novel markers are involved in atherosclerosis, and tailor future CVD interventions to mitigate vascular inflammation within this population to reduce the risk of CVD.

2 | MATERIALS AND METHODS

2.1 | Study design and setting

This study used a cross-sectional design and was carried out on the Gold Coast, Queensland, Australia. A convenience sampling technique was used, and participants were recruited through non-health community settings such as sporting clubs, surf life savings clubs, fitness centers, council libraries, community centers, and through social media and online/email methods to obtain a representative community sample of healthy adults at varying risk of CVD. Recruitment began in February 2021 and samples were collected over four 2-week periods beginning May 3, 2021 to April 14, 2022.

This study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the Bond University Human Research Ethics Committee (approval DR03194).

2.2 | Study population and sample size

Eligible participants included adults between 18 and 70 years old who were classified as either higher or lower risk of CVD. To be classified as higher risk of CVD participants had to either have confirmed type 2 diabetes OR have two or more of the following risk factors for CVD: systolic blood pressure ≥140 mm Hg or diastolic ≥90 mm Hg or receiving medication for high blood pressure; total cholesterol ≥5.2 mmol/L; LDL cholesterol ≥4.1 mmol/L; HDL cholesterol <=1 mmol/L; family history of premature CHD (≤60 years); or excess weight (BMI ≥25 kg/m^2). To be considered lower risk, participants had to report having no existing chronic disease or be on any routine medication, be below the cut-offs listed for higher risk individuals for blood pressure, cholesterol, BMI, and have no family history of premature CHD.

Participants who reported a history of angina, myocardial infarction, peripheral vascular disease, congenital heart disease, or stroke were excluded. In addition, current smokers and those taking certain medications or supplements known to impact measurements of PAF and/or Lp-PLA_2 including statins, niacin, fenofibrate, ezetimibe, orlistat, omega 3, fish oil supplements, and hormone replacement therapy were excluded. Any participant reporting Asian or African ethnicity were excluded. All participants provided written informed consent before participating in the study. With 100 participants, there was an 80% power to detect a correlation between outcomes and explanatory variables of 0.4 or greater assuming a level of significance of 5%. A correlation of 0.4 is a medium effect size for a correlation according to Cohen.[63]

2.3 | Data collection

Data were collected through face-to-face visits held at the Bond Institute of Health and Sport, Bond University,

Queensland, Australia and included anthropometric, clinical, and biochemical measurements.

Anthropometric data were measured in the fasting state with light clothing but without shoes. Standing height was measured to the nearest 0.1 cm using a wall mounted stadiometer. Weight was measured with a calibrated digital scale to the nearest 0.1 kg. Waist circumference was measured six times, three times at the umbilicus and three at minimum waist.[64] BMI was calculated as (weight (kg)/height (m^2).

Blood pressure was measured with a Creative Medical PC-900 Pro Vital Signs monitor in the nondominant arm, seated, with a clinical cuff and measured in triplicate, 2 min apart. The first measurement was disregarded and the second and third measurements were averaged.[65]

Information on age, sex, medical history, menopausal status, medication and supplement intake, smoking status, and alcohol consumption was self-reported from questionnaires completed during the study visit.

Physical activity was measured using the World Health Organization (WHO) Global Physical Activity Questionnaire.[66] Participant's metabolic equivalent (MET) minutes per week were calculated based on the participant responses from 16 questions assessing time spent physically active during work, travel, and recreation in addition to sedentary time. Physical activity levels were categorized into tertiles based on WHO's physical activity recommendation where 0 = low MET <600 min/week, 1 = moderate MET ≥600 to <1500 min/week, and 2 = high MET ≥1500 min/week.

2.4 | Assessment of biomarkers

Fasting blood samples were collected in EDTA tubes and centrifuged within 30 min of collection at 4°C and 1.3 relative centrifugal force (rcf) for 15 min. Plasma was aliquoted and immediately frozen at −80°C until assays were performed.

Lipids were measured with Afinion™ point of care machine (Abbott, Australia) within 10 min of blood collection. Measurements included LDL, HDL, triglycerides, non-HDL cholesterol, and Total:HDL cholesterol. All values that exceeded detection range of the instrument were recorded as the value at the top of detection range.

Lp-PLA_2 activity was determined using a commercial colorimetric assay (Cayman Chemical Co, USA) with 2-thio-PAF as substrate. Briefly, Lp-PLA_2 hydrolyzes the acetyl thioester bond at the *sn*-2 position of the substrate, 2-thioPAF, creating free thiols which are detected using 5,5′dithio-*bis*-(2-nitrobenzoic acid). Absorbance was measured at 412 nm using an OMEGA Fluostar microplate plate reader. High sensitivity CRP was assayed by Queensland Health, Pathology Queensland. PAF was measured using a PAF ELISA assay based on competitive-ELISA detection method (Assay Genie, Ireland), according to the manufacturer's protocol. Briefly, microtiter plates were provided pre-coated with PAF. During the reaction, PAF in the samples or standards competed with a fixed amount of target on the solid-phase supporter for sites on biotinylated detection antibody. Excess conjugate and unbound sample or standard were washed from the plate, HRP-streptavidin enzyme and TMB substrate were added, and color change was measured spectrophotometrically at 450 nm on an OMEGA Fluostar microplate plate reader.

Absolute CVD risk scores were calculated for each participant. The CVD risk score is a composite score that combines multiple traditional cardiovascular risk factors into a single risk score which was developed by the National Vascular Disease Prevention Alliance based on the Framingham Risk Equation.[67] The CVD risk score assesses traditional risk factors such as age, gender, smoking status, blood pressure, total cholesterol, HDL, diabetes status, and existence of left ventricular hypertrophy.

2.5 | Data analysis

All data were analyzed using SPSS version 28.0.0.0(190) (SPSS Inc., Chicago, USA). Data were assessed for normality using Q-Q plots. Variables that were not normally distributed (PAF, hsCRP, triglycerides) were log transformed prior to analysis. Independent t tests were performed on normally distributed variables to test for differences between males and females, higher risk and lower risk, and year of data collection. Mann–Whitney *U* tests were performed for non-normally distributed variables. Chi-squared tests were performed on categorical data.

Linear associations between markers of inflammation (log PAF, Lp-PLA_2, and log hsCRP) and risk factors were estimated using linear regression and reported as Pearson's correlations. Subgroup analysis was performed by level of risk of CVD (high or low). To compare associations in the two risk groups, a test for interaction was conducted. This was achieved by creating a new variable for each of the traditional risk factors by multiplying the risk factor by the level of risk (0 for low or 1 for high) which was then entered into a regression model with the risk factor and level of risk as main effects and markers of inflammation as the dependent variable.

Multiple linear regression was performed with log PAF, Lp-PLA_2, and log hsCRP as dependent variables and independent variables that included measures of dyslipidemia, including HDL and LDL cholesterol, and triglycerides, systolic and diastolic blood pressure, waist circumference, physical activity levels and year of data collection. Checks for multicollinearity were conducted using variance inflation factor and tolerance indices and revealed no variables were highly correlated.

TABLE 1 Demographic and clinical characteristics of study subjects

Characteristics	Mean ± SD or *N* (%) or median (IQR range)				Mean ± SD or *N* (%) or median (IQR range)		
	Total ***n*** **= 100**	**Male** ***n*** **= 31**	**Female** ***n*** **= 69**	***p*-Value**[a]	**Higher risk of CVD** ***n*** **= 68**	**Lower risk of CVD** ***n*** **= 32**	***p*-Value**[a]
Age, years[b]	49 ± 13	46 ± 13	50 ± 13	0.120	53 ± 13	38 ± 14	**<0.001**
Race, Caucasian *n* (%)	92 (92)	25 (86)	67 (94)		65 (96)	27 (84)	
Male *n* (%)					21 (31)	10 (31)	
BMI, kg/m^2[b]	28.3 ± 6.5	27.41 ± 5.0	28.65 ± 7.2	0.729	30.65 ± 6.4	23.19 ± 2.7	**<0.001**
Waist circumference (cm) umbilicus[b]	95.8 ± 6.7	95.99 ± 12.60	95.70 ± 18.40	0.526	102.36 ± 15.40	81.83 ± 9.15	**<0.001**
Type 2 diabetes diagnosis %	4 (4)	3 (10)	1 (1)	-	4 (6)	0 (0)	-
Physical activity METs tertiles	1.41 ± 0.65	1.61 ± 0.72	1.32 ± 0.83	0.193	1.28 ± 0.84	1.69 ± 0.65	0.193
n (%) low PA	20 (20)	4 (13)	16 (23)	-	17 (25)	3 (9)	-
n (%) medium PA	19 (19)	4 (13)	15 (22)	-	15 (22)	4 (13)	-
n (%) high PA	61 (61)	23 (74)	38 (55)	-	36 (53)	25 (78)	-
SBP, mm Hg	119 ± 13.1	119 ± 13	119 ± 13	0.883	124 ± 12.41	110 ± 8.71	**<0.001**
DBP, mm Hg	73 ± 8.50	74 ± 10.42	73 ± 7.56	0.783	76 ± 7.69	67 ± 7.07	**<0.001**
Total cholesterol, mmol/L	5.68 ± 1.32	5.59 ± 1.13	5.71 ± 1.41	0.678	6.02 ± 1.32	4.94 ± 1.01	**<0.001**
HDL cholesterol, mmol/L	1.84 ± 0.48	1.61 ± 0.37	1.94 ± 0.49	**<0.001**	1.69 ± 0.47	2.14 ± 0.36	**<0.001**
LDL cholesterol, mmol/L[c]	3.16 ± 1.11	3.28 ± 1.00	3.11 ± 1.17	0.493	3.55 ± 0.97	2.34 ± 0.95	**<0.001**
Non HDL cholesterol, mmol/L	3.84 ± 1.38	3.98 ± 1.11	3.77 ± 1.49	0.481	4.33 ± 1.26	2.81 ± 1.03	**<0.001**
Triglycerides, mmol/L[b]	1.40 ± 0.82	1.56 ± 0.79	1.33 ± 0.84	0.055	1.58 ± 0.91	1.02 ± 0.38	**<0.001**
PAF, ng/ml[b]	7.96 (3.89–16.77)	9.95 (4.31–15.33)	6.45 (3.81–18.90)	0.814	4.84 (3.24–14.57)	13.27 (9.59–21.63)	**<0.001**
Lp-PLA$_2$, nmol/min/ml	14.91 ± 4.29	16.98 ± 4.90	13.98 ± 3.65	**<0.001**	15.30 ± 4.42	14.09 ± 3.94	0.19
hsCRP, mg/L[b,c]	0.96 (0.49–2.98)	0.93 (0.41–2.1)	1.1 (0.5–3.14)	0.392	1.79 (0.64–3.80)	0.56 (0.22–1.01)	**<0.001**

Abbreviations: BMI, body mass index; DBP, diastolic blood pressure; HDL, high density lipoprotein; hsCRP, high sensitivity Creactive protein; LDL, low density lipoprotein; Lp-PLA2, lipoprotein-associated phospholipase A2; mg/L, milligrams per liter; mm Hg, millimeters of mercury; mmol/L, millimoles per liter; ng/L, nanograms per liter; nmol/min/ml, nanomoles per min per milliliter; PA, physical activity; PAF, platelet activating factor; SBP, systolic blood pressure; SD, standard deviation. Bolded *p*-values denotes statistical significance.

[a]Independent *t* test performed $p < 0.05$ represents significant difference.

[b]Mann–Whitney *U* test performed $p < 0.05$ represents significant difference.

[c]$n = 99$.

3 | RESULTS

3.1 | Clinical characteristics

A total of 100 participants were recruited and attended a study data collection visit. Demographic and clinical characteristics represented as a total cohort, males and females, and individuals at higher versus lower CVD risk are shown in Table 1. The mean age was 49 (range 20–69) years and 92% of the cohort was Caucasian.

The mean BMI was 28.3 ± 6.5 kg/m^2. Mean waist circumference of males was 95.9 ± 12.6 cm and for females 95.7 ± 18.4 cm. Four participants had type 2 diabetes. Mean systolic blood pressure was 119 ± 13 mm Hg and mean diastolic blood pressure was 73 ± 8.5 mm Hg. Mean level of HDL cholesterol was 1.84 ± 0.48 mmol/L and was significantly higher in females compared to males (1.94 ± 0.49 vs. 1.61 ± 0.37 $p < 0.001$). Mean LDL cholesterol was 3.16 ± 1.11 mmol/L and triglycerides were 1.40 ± 0.82 mmol/L. Although not an exclusion criterion, none of the participants reported taking PCSK9 inhibitor medications.

3.2 | Inflammatory marker results

3.2.1 | Platelet-activating factor

Median PAF level was 7.96 (3.89–16.77) ng/ml (Table 1). No significant difference was seen between males and females. Median PAF was higher for those at lower risk of CVD compared to those at higher risk (13.27 [9.59–

TABLE 2 Pearson's correlations between inflammatory markers and traditional risk factors of cardiovascular disease

	Log PAF		Lp-PLA$_2$		Log hsCRP	
Risk factor $n = 100$	***r***	***p*-Value**	***r***	***p*-Value**	***r***	***p*-Value**
Log hsCRP, mg/L	−0.261	**0.005**	0.047	0.643	-	-
Lp-PLA$_2$, nmol/min/ml	−0.032	0.376	-	-	-	-
Lipids						
Total cholesterol, mmol/L	−0.152	0.065	0.435	**<0.001**	0.284	**0.002**
LDL-C, mmol/L	−0.211	**0.018**	0.525	**<0.001**	0.338	**<0.001**
HDL-C, mmol/L	0.394	**<0.001**	−0.262	**0.004**	−0.413	**<0.001**
Log triglycerides, mmol/L	−0.281	**0.002**	0.284	**0.002**	0.455	**<0.001**
Non HDL-C, mmol/L	−0.283	**0.002**	0.508	**<0.001**	0.413	**<0.001**
Total:HDL-C, mmol/L	−0.436	**<0.001**	0.468	**<0.001**	0.524	**<0.001**
Blood pressure						
Systolic, mm Hg	−0.307	**0.001**	0.177	**0.039**	0.366	**<0.001**
Diastolic, mm Hg	−0.215	**0.016**	0.196	**0.025**	0.408	**<0.001**
BMI, kg/m^2	−0.381	**<0.001**	0.113	0.131	0.668	**<0.001**
Waist circumference, cm	−0.404	**<0.001**	0.162	**0.053**	0.676	**<0.001**
Physical activity level, MET minutes	0.097	0.168	0.005	0.481	−0.314	**0.001**
CVD risk score	−0.246	**0.007**	0.330	**<0.001**	0.245	**0.007**

Abbreviations: BMI, body mass index; DBP, diastolic blood pressure; HDL, high density lipoprotein; hsCRP, high sensitivity C- reactive protein; LDL, low density lipoprotein; Lp-PLA2, lipoprotein-associated phospholipase A2; mg/L, milligrams per liter; mm Hg, millimeters of mercury; mmol/L, millimoles per liter; ng/L, nanograms per liter; nmol/min/ml, nanomoles per min per milliliter; PA, physical activity; PAF, platelet activating factor; SBP, systolic blood pressure; SD, standard deviation. Bolded *p*-values denotes statistical significance.

21.63] ng/ml vs. 4.84 [3.24–14.57] ng/ml, $p < 0.001$). A significant difference was seen between blood samples taken in 2021 (3.3 ± 1.66 ng/ml) compared to samples taken in 2022 (19.82 ± 12.95 ng/ml), $t(85) = -13.96$, $p < 0.001$ with a large effect size (eta squared 0.665).

There was a significant medium positive correlation between PAF and HDL and significant medium negative correlations with Total:HDL cholesterol, systolic blood pressure, BMI, and waist circumference (Tables 2). Subgroup analysis comparing higher to lower CVD risk groups found no evidence of a difference in correlations between any of the explanatory variables and dependent variable logPAF at the 5% level (Table 3).

As shown in Table 4, multiple regression showed the date of data collection was the only statistically significant variable (at the 5% level) contributing to the model.

3.2.2 | Lipoprotein-associated phospholipase A$_2$

As shown in Table 1, mean circulating levels of Lp-PLA$_2$ activity were 14.91 ± 4.29 nmol/min/ml and were significantly higher in males than females (16.98 ± 4.90 nmol/min/ml vs. 13.98 ± 3.65 nmol/min/ml, $p < 0.001$). There was no significant difference in Lp-PLA$_2$ activity between those at lower risk versus higher risk of CVD. There was no significant difference between blood samples taken in 2021 (14.62 ± 3.65 nmol/min/ml) compared to samples taken in 2022 (15.16 ± 4.78 nmol/min/ml), $t(98) = -6.32$, $p = 0.529$.

There was a significant large positive correlation between Lp-PLA$_2$ and LDL and non-HDL cholesterol (Table 2). There was a significant medium positive correlation with total cholesterol and Total:HDL cholesterol. There was a medium positive correlation between Lp-PLA$_2$ and absolute CVD risk score.

Subgroup analysis comparing higher to lower CVD risk groups found no evidence of a difference in correlations between any of the explanatory variables and dependent variable Lp-PLA$_2$ at the 5% level with the exception of absolute CVD risk score (Table 3).

As shown in Table 5, multiple regression identified HDL, LDL, and date of data collection to be the statistically significant variables contributing to the model.

TABLE 3 Pearson's correlations between inflammatory markers and traditional risk factors of cardiovascular disease, stratified by risk

	Log PAF lower risk	Log PAF higher risk		Lp-PLA2 lower risk	Lp-PLA2 higher risk		Log hsCRP lower risk	Log hsCRP higher risk	
Risk factor	***r***	***r***	***p* for interaction**	***r***	***r***	***p* for interaction**	***r***	***r***	***p* for interaction**
Lipids									
Total cholesterol, mmol/L	0.011	0.003	0.977	0.522	0.389	0.353	0.199	0.130	0.737
LDL-C, mmol/L	−0.002	−0.006	0.982	0.644	0.497	0.622	0.248	0.126	0.687
HDL-C, mmol/L	0.074	0.313	0.314	−0.329	−0.198	0.459	−0.182	−0.313	0.621
Log triglycerides, mmol/L	−0.075	−0.181	0.681	0.274	0.252	0.745	0.150	0.424	0.298
Non HDL-C, mmol/L	−0.015	−0.113	0.671	0.625	0.482	0.350	0.258	0.251	0.971
Total:HDL-C, mmol/L	−0.040	−0.297	0.375	0.628	0.431	0.07	0.297	0.390	0.970
BP									
Systolic, mm Hg	0.004	−0.159	0.535	0.295	0.081	0.285	0.117	0.222	0.749
Diastolic, mm Hg	0.057	−0.048	0.661	0.088	0.177	0.680	0.255	0.262	0.661
BMI, kg/m^2	−0.284	−0.202	0.471	0.258	0.017	0.222	0.290	0.643	0.818
Waist circumference, cm	−0.331	−0.205	0.487	0.372	0.043	0.103	0.264	0.664	0.239
PA level in met minutes	−0.083	0.085	0.492	0.091	−0.001	0.686	−0.242	−0.304	0.755
CVD risk score	0.113	−0.096	0.552	0.433	0.329	**0.049**	−0.116	0.093	0.518

Abbreviations: BMI, body mass index; DBP, diastolic blood pressure; HDL, high density lipoprotein; hsCRP, high sensitivity C- reactive protein; LDL, low density lipoprotein; Lp-PLA2, lipoprotein-associated phospholipase A2; mg/L, milligrams per liter; mm Hg, millimeters of mercury; mmol/L, millimoles per liter; ng/L, nanograms per liter; nmol/min/ml, nanomoles per min per milliliter; mmol/L, PA, physical activity; PAF, platelet-activating factor; SBP, systolic blood pressure; SD, standard deviation.

TABLE 4 Standard multiple regression analysis of relationship between various cardiovascular risk factors and logPAF in the subjects

Variable	Standardized coefficients	*p*-Value
HDL cholesterol	−0.001	0.989
LDL cholesterol	0.032	0.623
Log triglycerides	−0.139	0.072
Systolic BP	−0.004	0.968
Diastolic BP	0.087	0.305
Waist circumference	−0.098	0.280
Physical activity	−0.060	0.316
Data collection date	0.802	**<0.001**

Note: Bolded *p*-values denotes statistical significance.

TABLE 5 Standard multiple regression analysis of relationship between various cardiovascular risk factors and Lp-PLA_2 activity in the subjects

Variable	Standardized coefficients	*p*-Value
HDL cholesterol	−0.291	**0.007**
LDL cholesterol	0.545	**<0.001**
Log triglycerides	0.087	0.434
Systolic BP	0.109	0.396
Diastolic BP	−0.039	0.746
Waist circumference	−0.191	0.144
Physical activity	0.033	0.705
Data collection date	0.277	**0.004**

Note: Bolded *p*-values denotes statistical significance.

3.2.3 | High-sensitivity C-reactive protein

Median hsCRP levels were 0.96 (0.49–2.98) mg/L (Table 1). No significant difference was seen between males and females; however, hsCRP levels were higher in those at high risk of CVD 1.79 (0.64–3.80) mg/L compared to those at low risk 0.56 (0.22–1.01) mg/L, $p < 0.001$.

As shown in Table 2, there was a significant large positive correlation between hsCRP and Total:HDL cholesterol, BMI and waist circumference. There was a medium negative correlation between hsCRP and HDL cholesterol and physical activity, and a medium positive correlation with LDL cholesterol, log triglycerides, non-HDL cholesterol, and systolic and diastolic blood pressure.

Subgroup analysis comparing higher to lower CVD risk groups found no evidence of a difference in correlations between any of the explanatory variables and dependent variable loghs CRP at the 5% level (Table 3).

TABLE 6 Standard multiple regression analysis of relations of various cardiovascular risk factors to logCRP in the subjects

Variable	Standardized coefficients	*p*-Value
HDL cholesterol	−0.053	0.552
LDL cholesterol	0.083	0.330
Log triglycerides	0.036	0.362
Systolic BP	−0.161	0.165
Diastolic BP	0.124	0.259
Waist circumference	0.596	**<0.001**
Physical activity	−0.165	**0.037**
Data collection date	0.029	0.738

Note: Bolded *p*-values denotes statistical significance.

Multiple regression showed waist circumference and physical activity to be the only statistically significant variables contributing to the model (Table 6).

3.3 | Correlations between inflammatory markers

There was a small negative correlation between PAF and hsCRP but no significant correlation between PAF and Lp-PLA_2 or between Lp-PLA_2 and hsCRP (Table 2).

4 | DISCUSSION

This cross-sectional study investigated the association between PAF, Lp-PLA_2, and CRP and traditional CVD risk factors in 100 adults with varying levels of risk of CVD in an Australian population. It is the first such study that included strict exclusion criteria, and analyzed PAF and Lp-PLA_2 activity in a broadly Caucasian population, outside of Greece.[68] Correlations between PAF, Lp-PLA_2, and CRP levels and risk factors for CVD were examined including blood pressure, lipids, BMI, waist circumference, and physical activity levels.

Although a key finding from this study was that several traditional cardiovascular risk factors are related to circulating levels of both novel and traditional inflammatory markers, there was also an unexpected finding with PAF. Notably, levels of PAF significantly differed based on the year of data collection. The blood samples collected in 2022 coincided with the Omicron variant COVID-19 outbreak in Australia, and a boost in vaccination rates with adenovirus vector and mRNA vaccines

which may explain these unexpected results. Although vaccination status was not collected, the Gold Coast region had a vaccination rate of 90.9% for first dose and 88.5% for second dose of the vaccine by the end of January 2022 (unchanged by April 2022), the timeframe for 2022 data collection.[69] Both the adenovirus-vector and mRNA vaccines increase inflammation and platelet activation following COVID-19 vaccination.[70] Individuals who had recently had their second vaccination were found to have an 83% decrease in EC_{50} PAF levels (a measure of concentration that induces 50% of maximal aggregation) in platelet rich plasma in response to PAF as a agonist, with this increase in platelet sensitivity hypothesized to be attributed to an enhancement of PAF production and/or upregulation of PAF receptor expression.[71] The addition of the SARS-CoV-2 spike protein to a U-937 cell line has been shown to cause a two-fold increase in intracellular PAF levels[71] and lipidomic analysis of in vitro human cells demonstrated a 3.5-fold increase in PAF levels when infected with coronavirus.[72] Despite temperature checks before proceeding with data collection, it is possible some of the participants in the 2022 batch for the current study may have had COVID-19, or were recovering from COVID-19, resulting in elevated levels of PAF.

Surprisingly, mean PAF was higher in participants at lower risk of CVD compared to those at higher risk. In contrast, people with atherosclerosis and CHD have been reported to have significantly higher PAF than healthy controls.[24,73] However, these studies examined adults diagnosed with CVD compared to the "higher risk" (but without a diagnosis of CVD) group in the current study. The different findings are most likely explained by confounding from COVID-19 vaccination or disease, as the majority of the lower risk group were recruited in 2022 whereas 65% of the higher risk group were recruited in 2021 when vaccination and diagnosed COVID-19 rates were low.

Another surprising finding was the lack of significant difference in mean Lp-PLA_2 levels between those at higher risk versus lower risk of CVD, in contrast to previous research[57] and also potentially due to COVID-19 vaccination or infection. Although the relationship between COVID-19 vaccination and Lp-PLA_2 levels is unknown, patients infected with COVID-19 have been reported to have significantly higher levels of plasma Lp-PLA_2 compared to healthy controls.[74] Although *t* tests found no significant difference between blood samples taken in 2021 and 2022, the multiple regression analysis found that data collection date was a statistically significant variable contributing to the model for Lp-PLA_2 in the current study.

4.1 | Lipids

The large positive correlation between Total:HDL cholesterol and medium positive correlation between LDL cholesterol and hsCRP was unexpected, and contrasts with previous research that found minimal or no correlation between these markers[75,76] except in children.[77]

The medium negative correlation between hsCRP and HDL was also unexpected, as other studies have found no correlation between these two markers.[76,78] Similarly, the medium positive correlation between PAF and HDL cholesterol seems counter intuitive, given HDL is anti-inflammatory. An interaction between PAF and COVID-19 vaccination/disease status may be one explanation. However, highly elevated HDL has been associated with inflammation and increased risk of CVD.[79] Lower levels of HDL are known to be associated with increased mortality, CHD and stroke yet pharmacological trials to raise HDL have not resulted in significant reductions in risk.[80–82] HDL at both high and low levels appear to increase CVD risk and a U-shaped curve characterizes the relationship between HDL and CVD risk.[83–85]

Upon further examination of the literature, HDL appears to become dysfunctional at higher levels resulting in inflammation and impairment of cholesterol efflux, reduction in the inhibition of adhesion molecules, and a decrease in antioxidant capacity, all functions that HDL normally undertake.[86] Dysfunctional HDL has an altered lipid core with higher levels of triglycerides and oxidized phospholipids, lower levels of cholesterol,[87] and a reduction in anti-inflammatory enzymes such as paraoxonase (PON-1) and Lp-PLA_2.[88] Functionality of HDL may be more important than circulating HDL-C levels for accurate risk prediction.[89] Notably, 75% of the participants in the current study had HDL levels above 1.5 mmol/L, and 40% had levels above 2.0 mmol/L (with 13% having values above 2.59 mmol/L, the top of the detection range of the Afinion™ instrument) which may explain the large and significant correlation with PAF. Therefore, it is unlikely that these results adequately reflect the complexity of functionality of HDL and more research is needed to elucidate the true relationship.

4.2 | Blood pressure, BMI, and WC

Results for blood pressure, BMI, and waist circumference were unsurprising, showing a strong positive correlation with hsCRP. There was a positive correlation between blood pressure and Lp-PLA_2, no correlation between Lp-PLA_2 and BMI, and only a small positive correlation with

waist circumference with previous research reporting similar results.[57,90,91] However, a strong correlation was reported between Lp-PLA_2 and central obesity in a Chinese population.[92]

The negative correlation between systolic blood pressure and PAF may be expected as animal studies show PAF induces systemic hypotension[93,94] and in humans, some biological actions of PAF include inducing vascular hyper-permeability and hypotension.[95] The current study found a medium negative correlation between PAF and both waist circumference and BMI. Interestingly, a very similar study in Greece had similar results showing a positive correlation between Lp-PLA_2 and waist circumference and a negative correlation with PAF despite the mean waist circumference being much higher in the present study, especially for females.[68]

4.3 | Physical activity levels

Physical activity levels were associated with CRP but not Lp-PLA_2 in the current study. A strong correlation between CRP and physical activity levels exists.[96–99] While there is evidence exploring the relationship between Lp-PLA_2 and physical activity, previous studies have reported mass (which is less reliable) rather than activity. However, most studies report a positive relationship between the two.[100–104] The lack of research on Lp-PLA_2 activity and physical activity, and the novel result of a lack of a correlation between physical activity and PAF highlights gaps in the literature for future research to examine.

4.4 | CVD risk score

There was a moderate positive correlation between Lp-PLA_2 and absolute CVD risk score and only small correlations seen with PAF and hsCRP. The results for hsCRP were surprising as higher CRP levels significantly correlated with the Europe SCORE risk, a risk score that estimates 10-year risk of CVD.[105]

4.5 | Correlations between each marker

In this study, there was no correlation between Lp-PLA_2 and CRP, in agreement with numerous studies,[106–110] with the exception of one study in Chinese patients with CHD.[111] Despite each marker being associated with risk factors for CVD, each appear to work independently yet complementarily to improve CVD risk prediction.

No correlation was seen between PAF and Lp-PLA_2, which is unexpected due to the relationship between PAF and its regulating enzyme, with previous literature suggesting that Lp-PLA_2 could be used as a surrogate marker for PAF.[112] However, this finding should be interpreted with caution, given the potential confounding effect of COVID-19 vaccines and possible disease at the time of data collection for some of the participants in the current study.

There was a small negative correlation between PAF and CRP in this study. Early studies found CRP inhibits PAF-induced platelet aggregation suggesting CRP may act as an early protective anti-inflammatory mechanism in acute inflammation.[113] However, CRP enhances PAF-induced inflammatory activity through binding to PAF[114] and recent research found a significant positive correlation between PAF and CRP and concluded that although hsCRP was a valuable diagnostic marker for coronary atherosclerosis, PAF was a better prognostic indicator for coronary atherosclerosis.[73]

4.6 | Strengths and weaknesses

Strengths of this study include strong exclusion criteria to minimize confounders including medication, smoking, and diagnosed CVD. Ethnicities previously reported to have lower levels of Lp-PLA_2 activity due to genetic polymorphisms were excluded, allowing a more homogenous population for analysis.

There were, however, some limitations. This was a cross-sectional study examining inflammatory levels at one point in time and thus causal relationships between the markers and CVD cannot be confirmed. Second, most participants recruited were female thus this study may not accurately reflect levels of these markers across the broader Australian population. In addition, half of the data collection was undertaken during a COVID-19 Omicron outbreak and aggressive mRNA vaccination roll out, which may have affected results.[115] Further, data on vaccination status was not collected from participants so the true relationship between an individual's PAF levels and the vaccine cannot be determined. An estimate of 13% of HDL results was made, as those participants had levels above the range of the point of care machine. There was no adjustment for multiple comparisons in the statistical analysis; hence; our results should be interpreted cautiously.

5 | CONCLUSION

In conclusion, all three markers of inflammation independently correlated with several traditional

cardiovascular risk factors in a broadly Caucasian population. A large positive correlation exists between Lp-PLA_2 and LDL while a medium positive correlation was seen between PAF and HDL. A small negative correlation between PAF and CRP was seen, and no significant correlation was shown between PAF and Lp-PLA_2. Although Lp-PLA_2 and CRP are both related to the pathophysiology of inflammation in CVD, there was no correlation with one another suggesting each marker is involved in separate atherogenic pathways. Further studies are needed to elucidate the true relationship between PAF and traditional risk factors in a non-epidemic setting.

AUTHOR CONTRIBUTIONS

Carolyn J. English and Dianne P. Reidlinger conceived the study and collected the data. Carolyn J. English and Anna E. Lohning performed the laboratory analyses. Mark Jones and Carolyn J. English analyzed the data. Carolyn J. English wrote the initial draft of the manuscript. Carolyn J. English, Dianne P. Reidlinger, Anna E. Lohning, Hannah L. Mayr and Mark Jones interpreted the data and critically reviewed and approved the final manuscript.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

1. Demopoulos CA, Karantonis HC, Antonopoulou S. Platelet activating factor—a molecular link between atherosclerosis theories. Eur J Lipid Sci Technol. 2003;105:705–16.
2. Pearson TA, Mensah GA, Alexander RW, Anderson JL, Cannon RO, Criqui M, et al. Markers of inflammation and cardiovascular disease. Circulation. 2003;107:499–511.
3. Castro AR, Silva SO, Soares SC. The use of high sensitivity C-reactive protein in cardiovascular disease detection. J Pharm Pharm Sci. 2018;21:496–503.
4. Haybar H, Shokuhian M, Bagheri M, Davari N, Saki N. Involvement of circulating inflammatory factors in prognosis and risk of cardiovascular disease. J Mol Cell Cardiol. 2019;132:110–9.
5. Fu Y, Wu Y, Liu E. C-reactive protein and cardiovascular disease: from animal studies to the clinic (review). Exp Ther Med. 2020;20:1211–9.
6. Thiele JR, Habersberger J, Braig D, Schmidt Y, Goerendt K, Maurer V, et al. Dissociation of pentameric to monomeric C-reactive protein localizes and aggravates inflammation. Circulation. 2014;130:35–50.
7. Kaptoge S, Di Angelantonio E, Lowe G, Pepys MB, Thompson SG, Collins R, et al. C-reactive protein concentration and risk of coronary heart disease, stroke, and mortality: an individual participant meta-analysis. Lancet. 2010;375:132–40.
8. Bower JK, Lazo M, Juraschek SP, Selvin E. Within-person variability in high-sensitivity C-reactive protein. Arch Intern Med. 2012;172:1519–21.
9. English CJ, Mayr HL, Lohning AE, Reidlinger DP. The association between dietary patterns and the novel inflammatory markers platelet-activating factor and lipoprotein-associated phospholipase A2: a systematic review. Nutr Rev. 2022;80:1371–91.
10. Triggiani M, Schleimer RP, Warner JA, Chilton FH. Differential synthesis of 1-acyl-2-acetyl-sn-glycero-3-phosphocholine and platelet-activating factor by human inflammatory cells. J Immunol. 1991;147:660–6.
11. Weyrich AS, McIntyre TM, McEver RP, Prescott SM, Zimmerman GA. Monocyte tethering by P-selectin regulates monocyte chemotactic protein-1 and tumor necrosis factor-alpha secretion. Signal integration and NF-kappa B translocation. J Clin Invest. 1995;95:2297–303.
12. Lacasse C, Turcotte S, Gingras D, Stankova J, Rola-Pleszczynski M. Platelet-activating factor stimulates Interleukin-6 production by human endothelial cells and synergizes with tumor necrosis factor for enhanced production of granulocyte-macrophage colony stimulating factor. Inflammation. 1997;21:145–58.
13. Prescott SM, Zimmerman GA, Stafforini DM, McIntyre TM. Platelet-activating factor and related lipid mediators. Annu Rev Biochem. 2000;69:419–45.
14. Tsoupras A, Lordan R, Zabetakis I. Inflammation, not cholesterol, is a cause of chronic disease. Nutrients. 2018;10:604.
15. Lordan R, Tsoupras A, Zabetakis I, Demopoulos CA. Forty years since the structural elucidation of platelet-activating factor (PAF): historical, current, and future research perspectives. Molecules. 2019;24:4414.
16. Prescott SM, McIntyre TM, Zimmerman GA, Stafforini DM. Inflammation as an early component of atherosclerosis and vascular damage. Jpn Circ J. 1996;60:137–41.
17. Handley DA, Arbeeny CM, Lee ML, Van Valen RG, Saunders RN. Effect of platelet activating factor on endothelial permeability to plasma macromolecules. Immunopharmacology. 1984;8:137–42.
18. Rouis M, Nigon F, John Chapman M. Platelet activating factor is a potent stimulant of the production of active oxygen

species by human monocyte-derived macrophages. Biochem Biophys Res Commun. 1988;156:1293–301.
19. Dentan C, Lesnik P, Chapman MJ, Ninio E. Phagocytic activation induces formation of platelet-activating factor in human monocyte-derived macrophages and in macrophage-derived foam cells. Relevance to the inflammatory reaction in atherogenesis. Eur J Biochem. 1996;236:48–55.
20. Gaut JP, Heinecke JW. Mechanisms for oxidizing low-density lipoprotein. Insights from patterns of oxidation products in the artery wall and from mouse models of atherosclerosis. Trends Cardiovasc Med. 2001;11:103–12.
21. Sukriti S, Tauseef M, Yazbeck P, Mehta D. Mechanisms regulating endothelial permeability. Pulm Circ. 2014;4:535–51.
22. Satoh K, Imaizumi T, Yoshida H, Hiramoto M, Takamatsu S. Increased levels of blood platelet-activating factor (PAF) and PAF-like lipids in patients with ischemic stroke. Acta Neurol Scand. 1992;85:122–7.
23. Chen H, Zheng P, Zhu H, Zhu J, Zhao L, El Mokhtari NE, et al. Platelet-activating factor levels of serum and gingival crevicular fluid in nonsmoking patients with periodontitis and/or coronary heart disease. Clin Oral Investig. 2010;14:629–36.
24. Zheng G-H, Xiong S-Q, Mei L-J, Chen H-Y, Wang T, Chu J-F. Elevated plasma platelet activating factor, platelet activating factor acetylhydrolase levels and risk of coronary heart disease or blood stasis syndrome of coronary heart disease in Chinese: a case control study. Inflammation. 2012;35:1419–28.
25. Detopoulou P, Fragopoulou E, Nomikos T, Antonopoulou S, Kotroyiannis I, Vassiliadou C, et al. Baseline and 6-week follow-up levels of PAF and activity of its metabolic enzymes in patients with heart failure and healthy volunteers—a pilot study. Angiology. 2013;64:522–8.
26. Kudolo GB, DeFronzo RA. Urinary platelet-activating factor excretion is elevated in non-insulin dependent diabetes mellitus. Prostaglandins Other Lipid Mediat. 1999;57:87–98.
27. Cavallo-Perin P, Lupia E, Gruden G, Olivetti C, De Martino A, Cassader M, et al. Increased blood levels of platelet-activating factor in insulin-dependent diabetic patients with microalbuminuria. Nephrol Dial Transplant. 2000;15:994–9.
28. Yin H, Shi A, Wu J. Platelet-activating factor promotes the development of non-alcoholic fatty liver disease. Diabetes Metab Syndr Obes. 2022;15:2003–30.
29. Derewenda ZS, Derewenda U. The structure and function of platelet-activating factor acetylhydrolases. Cell Mol Life Sci. 1998;54:446–55.
30. Burke JE, Dennis EA. Phospholipase A2 structure/function, mechanism, and signaling. J Lipid Res. 2009;50(Suppl): S237–42.
31. Dennis EA. Allosteric regulation by membranes and hydrophobic subsites in phospholipase A(2) enzymes determine their substrate specificity. J Biol Chem. 2022;298:101873.
32. Tselepis AD, John Chapman M. Inflammation, bioactive lipids and atherosclerosis: potential roles of a lipoprotein-associated phospholipase A2, platelet activating factor-acetylhydrolase. Atheroscler Suppl. 2002;3:57–68.
33. Tsimikas S, Tsironis LD, Tselepis AD. New insights into the role of lipoprotein(a)-associated lipoprotein-associated phospholipase A2 in atherosclerosis and cardiovascular disease. Arterioscler Thromb Vasc Biol. 2007;27:2094–9.
34. Tellis CC, Tselepis AD. Pathophysiological role and clinical significance of lipoprotein-associated phospholipase a_2 (Lp-PLA_2) bound to LDL and HDL. Curr Pharm Des. 2014;20: 6256–69.
35. Tellis CC, Tselepis AD. The role of lipoprotein-associated phospholipase A2 in atherosclerosis may depend on its lipoprotein carrier in plasma. Biochim Biophys Acta. 2009;1791: 327–38.
36. Silva IT, Mello APQ, Damasceno NRT. Antioxidant and inflammatory aspects of lipoprotein-associated phospholipase A2 (Lp-PLA2): a review. Lipids Health Dis. 2011;10:170.
37. Zalewski A, Macphee C. Role of lipoprotein-associated phospholipase A2 in atherosclerosis: biology, epidemiology, and possible therapeutic target. Arterioscler Thromb Vasc Biol. 2005;25:923–31.
38. Kohno M, Yokokawa K, Yasunari K, Minami M, Kano H, Hanehira T, et al. Induction by lysophosphatidylcholine, a major phospholipid component of atherogenic lipoproteins, of human coronary artery smooth muscle cell migration. Circulation. 1998;98:353–9.
39. Shi Y, Zhang P, Zhang L, Osman H, Mohler ER, Macphee C, et al. Role of lipoprotein-associated phospholipase A2 in leukocyte activation and inflammatory responses. Atherosclerosis. 2007;191:54–62.
40. Steen DL, O'Donoghue ML. Lp-PLA2 inhibitors for the reduction of cardiovascular events. Cardiol Ther. 2013;2:125–34.
41. Huang F, Wang K, Shen J. Lipoprotein-associated phospholipase A2: the story continues. Med Res Rev. 2020;40: 79–134.
42. Corson MA, Jones PH, Davidson MH. Review of the evidence for the clinical utility of lipoprotein-associated phospholipase A2 as a cardiovascular risk marker. Am J Cardiol. 2008;101: 41f–50f.
43. Garza CA, Montori VM, McConnell JP, Somers VK, Kullo IJ, Lopez-Jimenez F. Association between lipoprotein-associated phospholipase A 2 and cardiovascular disease: a systematic review. Mayo Clin Proc. 2007;82:159–65.
44. Chung H, Kwon HM, Kim J-Y, Yoon Y-W, Rhee J, Choi E-Y, et al. Lipoprotein-associated phospholipase A_2 is related to plaque stability and is a potential biomarker for acute coronary syndrome. Yonsei Med J. 2014;55:1507–15.
45. Hu G, Liu D, Tong H, Huang W, Hu Y, Huang Y. Lipoprotein-associated phospholipase A2 activity and mass as independent risk factor of stroke: a meta-analysis. Biomed Res Int. 2019;2019:1–11.
46. Perrot N, Thériault S, Rigade S, Chen HY, Dina C, Martinsson A, et al. Lipoprotein-associated phospholipase A2 activity, genetics and calcific aortic valve stenosis in humans. Heart. 2020;106:1407–12.
47. Topbas C, Swick A, Razavi M, Anderson NL, Pearson TW, Bystrom C. Measurement of lipoprotein-associated phospholipase A2 by use of 3 different methods: exploration of discordance between ELISA and Activity assays. Clin Chem. 2018; 64:697–704.
48. De Stefano A, Mannucci L, Tamburi F, Cardillo C, Schinzari F, Rovella V, et al. Lp-PLA(2), a new biomarker of vascular disorders in metabolic diseases. Int J Immunopathol Pharmacol. 2019;33:2058738419827154.

49. Pantazi D, Tellis C, Tselepis AD. "SI: PAF" oxidized phospholipids and lipoprotein-associated phospholipase A2 (Lp-PLA2) in atherosclerotic cardiovascular disease: an update. BioFactors. 2022;n/a:n/a.
50. Wang D, Guo X, Hou L, Cheng X, You T, Li H, et al. Measuring lipoprotein-associated phospholipase A2 activity in China: protocol comparison and recalibration. J Clin Lab Anal. 2019; 33:e22628.
51. Brilakis ES, Khera A, McGuire DK, See R, Banerjee S, Murphy SA, et al. Influence of race and sex on lipoprotein-associated phospholipase A2 levels: observations from the Dallas Heart Study. Atherosclerosis. 2008;199:110–5.
52. Enkhmaa B, Anuurad E, Zhang W, Pearson TA, Berglund L. Association of Lp-PLA(2) activity with allele-specific Lp(a) levels in a bi-ethnic population. Atherosclerosis. 2010; 211:526–30.
53. Lee KK, Fortmann SP, Varady A, Fair JM, Go AS, Quertermous T, et al. Racial variation in lipoprotein-associated phospholipase A 2 in older adults. BMC Cardiovasc Disord. 2011;11:38.
54. Gregson JM, Freitag DF, Surendran P, Stitziel NO, Chowdhury R, Burgess S, et al. Genetic invalidation of Lp-PLA(2) as a therapeutic target: large-scale study of five functional Lp-PLA(2)-lowering alleles. Eur J Prev Cardiol. 2017; 24:492–504.
55. Packard CJ, O'Reilly DS, Caslake MJ, McMahon AD, Ford I, Cooney J, et al. Lipoprotein-associated phospholipase A2 as an independent predictor of coronary heart disease. West of Scotland Coronary Prevention Study Group. N Engl J Med. 2000;343:1148–55.
56. Oei HH, van der Meer IM, Hofman A, Koudstaal PJ, Stijnen T, Breteler MM, et al. Lipoprotein-associated phospholipase A2 activity is associated with risk of coronary heart disease and ischemic stroke: the Rotterdam Study. Circulation. 2005;111:570–5.
57. Persson M, Hedblad B, Nelson JJ, Berglund G. Elevated Lp-PLA2 levels add prognostic information to the metabolic syndrome on incidence of cardiovascular events among middle-aged nondiabetic subjects. Arterioscler Thromb Vasc Biol. 2007;27:1411–6.
58. Yokote K, Suzuki A, Li Y, Matsuoka N, Teramoto T. Pharmacokinetics and exploratory efficacy biomarkers of bococizumab, an anti-PCSK9 monoclonal antibody, in hypercholesterolemic Japanese subjects. Int J Clin Pharmacol Ther. 2019;57:575–89.
59. Gajos G, Zalewski J, Mostowik M, Konduracka E, Nessler J, Undas A. Polyunsaturated omega-3 fatty acids reduce lipoprotein-associated phospholipase A(2) in patients with stable angina. Nutr Metab Cardiovasc Dis. 2014;24:434–9.
60. Stojanovic M, Radenkovic M. Omega-3 fatty acids are capable to decrease the lipoprotein-associated phospholipase A2 blood level. Atherosclerosis. 2018;275:e237.
61. Palur Ramakrishnan AV, Varghese TP, Vanapalli S, Nair NK, Mingate MD. Platelet activating factor: a potential biomarker in acute coronary syndrome? Cardiovasc Ther. 2017;35:64–70.
62. Li J, Cao T, Wei Y, Zhang N, Zhou Z, Wang Z, et al. A review of novel cardiac biomarkers in acute or chronic cardiovascular diseases: the role of soluble ST2 (sST2), lipoprotein-associated phospholipase A2 (Lp-PLA2), myeloperoxidase (MPO), and procalcitonin (PCT). Dis Markers. 2021;2021:6258865.
63. Cohen J. Statistical power analysis for the behavioral sciences, 2nd ed. New Jersey: Lawrence Erlbaum Associates; 1988.
64. Brown RE, Randhawa AK, Canning KL, Fung M, Jiandani D, Wharton S, et al. Waist circumference at five common measurement sites in normal weight and overweight adults: which site is most optimal? Clin Obes. 2018;8:21–9.
65. O'Brien E, Coats A, Owens P, Petrie J, Padfield PL, Littler WA, et al. Use and interpretation of ambulatory blood pressure monitoring: recommendations of the British hypertension society. BMJ. 2000;320:1128–34.
66. Armstrong T, Bull F. Development of the World Health Organization Global Physical Activity Questionnaire (GPAQ). J Public Health. 2006;14:66–70.
67. National Vascular Disease Prevention Alliance. Guidelines for the management of absolute cardiovascular disease risk; 2012.
68. Detopoulou P, Nomikos T, Fragopoulou E, Stamatakis G, Panagiotakos DB, Antonopoulou S. PAF and its metabolic enzymes in healthy volunteers: interrelations and correlations with basic characteristics. Prostaglandins Other Lipid Mediat. 2012;97:43–9.
69. Australian Government Department of Health and Aged Care. COVID-19 Vaccination - Geographic vaccination rates - LGA; 2022. Available from: www.health.gov.au/resources/collections/covid-19-vaccination-geographic-vaccination-rates-lga.
70. Ostrowski SR, Søgaard OS, Tolstrup M, Stærke NB, Lundgren J, Østergaard L, et al. Inflammation and platelet activation after COVID-19 vaccines—possible mechanisms behind vaccine-induced immune thrombocytopenia and thrombosis. Front Immunol. 2021;12:779453.
71. Antonopoulou S, Petsini F, Detopoulou M, Theoharides TC, Demopoulos CA. Is there an interplay between the SARS-CoV-2 spike protein and platelet-activating factor? Biofactors. 2022;1–13.
72. Yan B, Chu H, Yang D, Sze K-H, Lai P-M, Yuan S, et al. Characterization of the lipidomic profile of human coronavirus-infected cells: implications for lipid metabolism remodeling upon coronavirus replication. Viruses. 2019;11:73.
73. Raheem SG. Investigating platelet-activating factor as a potent proinflammatory mediator in coronary atherosclerotic patients. Cell Mol Biol (Noisy-le-Grand). 2021;67:1–4.
74. Li Y, Jiang Y, Zhang Y, Li N, Yin Q, Liu L, et al. Abnormal upregulation of cardiovascular disease biomarker PLA2G7 induced by proinflammatory macrophages in COVID-19 patients. Sci Rep. 2021;11:6811.
75. Ridker PM, Rifai N, Rose L, Buring JE, Cook NR. Comparison of C-reactive protein and low-density lipoprotein cholesterol levels in the prediction of first cardiovascular events. N Engl J Med. 2002;347:1557–65.
76. Mirjanić-Azarić B, Derić M, Vrhovac M, Males-Bilić L. Correlation between C-reactive protein levels with leading risk factors for cardiovascular disease in men. Med Pregl. 2008;61: 164–8.
77. Agirbasli M, Tanrikulu A, Acar Sevim B, Azizy M, Bekiroglu N. Total cholesterol-to-high-density lipoprotein cholesterol ratio predicts high-sensitivity C-reactive protein levels in Turkish children. J Clin Lipidol. 2015;9: 195–200.
78. Arena R, Arrowood JA, Fei DY, Helm S, Kraft KA. The relationship between C-reactive protein and other cardiovascular

risk factors in men and women. J Cardiopulm Rehabil. 2006; 26:323–7. quiz 328–329.
79. Navab M, Anantharamaiah GM, Fogelman AM. The role of high-density lipoprotein in inflammation. Trends Cardiovasc Med. 2005;15:158–61.
80. Keene D, Price C, Shun-Shin MJ, Francis DP. Effect on cardiovascular risk of high density lipoprotein targeted drug treatments niacin, fibrates, and CETP inhibitors: meta-analysis of randomised controlled trials including 117 411 patients. BMJ: Br Med J. 2014;349:g4379.
81. Landray MJ, Haynes R, Hopewell JC, Parish S, Aung T, Tomson J, et al. Effects of extended-release niacin with laropiprant in high-risk patients. N Engl J Med. 2014;371:203–12.
82. Lincoff AM, Nicholls SJ, Riesmeyer JS, Barter PJ, Brewer HB, Fox KAA, et al. Evacetrapib and cardiovascular outcomes in high-risk vascular disease. N Engl J Med. 2017;376:1933–42.
83. Allard-Ratick M, Khambhati J, Topel M, Sandesara P, Sperling L, Quyyumi A. 50Elevated HDL-C is associated with adverse cardiovascular outcomes. Eur Heart J. 2018;39: ehy564.50.
84. Li H, Qian F, Zuo Y, Yuan J, Chen S, Wu S, et al. U-shaped relationship of high-density lipoprotein cholesterol and incidence of total, ischemic and hemorrhagic stroke: a prospective cohort study. Stroke. 2022;53:1624–32.
85. Liu C, Dhindsa D, Almuwaqqat Z, Ko Y-A, Mehta A, Alkhoder AA, et al. Association between high-density lipoprotein cholesterol levels and adverse cardiovascular outcomes in high-risk populations. JAMA Cardiol. 2022;7:672–80.
86. Kosmas CE, Martinez I, Sourlas A, Bouza KV, Campos FN, Torres V, et al. High-density lipoprotein (HDL) functionality and its relevance to atherosclerotic cardiovascular disease. Drugs Context. 2018;7:212525.
87. Salazar J, Olivar LC, Ramos E, Chávez-Castillo M, Rojas J, Bermúdez V. Dysfunctional high-density lipoprotein: an innovative target for proteomics and lipidomics. Cholesterol. 2015; 2015:296417.
88. Rosenson RS, Brewer HB Jr, Ansell BJ, Barter P, Chapman MJ, Heinecke JW, et al. Dysfunctional HDL and atherosclerotic cardiovascular disease. Nat Rev Cardiol. 2016; 13:48–60.
89. El Khoudary SR, Chen X, Nasr AN, Billheimer J, Brooks MM, McConnell D, et al. HDL (high-density lipoprotein) subclasses, lipid content, and function trajectories across the menopause transition: SWAN-HDL study. Arterioscler Thromb Vasc Biol. 2021;41:951–61.
90. Detopoulou P, Nomikos T, Fragopoulou E, Panagiotakos DB, Pitsavos C, Stefanadis C, et al. Lipoprotein-associated phospholipase A2 (Lp-PLA2) activity, platelet-activating factor acetylhydrolase (PAF-AH) in leukocytes and body composition in healthy adults. Lipids Health Dis. 2009;8:19.
91. Ntzouvani A, Giannopoulou E, Fragopoulou E, Nomikos T, Antonopoulou S. Energy intake and plasma adiponectin as potential determinants of lipoprotein-associated phospholipase A2 activity: a cross-sectional study. Lipids. 2019;54: 629–40.
92. Chen YH, Li WC, Chen YC, Yeh WC, Yu W, Hung HY, et al. The correlation between lipoprotein associated phospholipase a(2) and central overweight status. Int J Immunopathol Pharmacol. 2021;35:20587384211048562.
93. Halonen M, Palmer JD, Lohman IC, McManus LM, Pinckard RN. Respiratory and circulatory alterations induced by acetyl glyceryl ether phosphorylcholine, a mediator of IgE anaphylaxis in the rabbit. Am Rev Respir Dis. 1980;122: 915–24.
94. Tanaka S, Kasuya Y, Masuda Y, Shigenobu K. Studies on the hypotensive effects of platelet activating factor (PAF, 1-O-alkyl-2-acetyl-sn-glyceryl-3-phosphorylcholine) in rats, Guinea pigs, rabbits, and dogs. J Pharmacobiodyn. 1983;6: 866–73.
95. Nakamura T, Murata T. Regulation of vascular permeability in anaphylaxis. Br J Pharmacol. 2018;175:2538–42.
96. Albert MA, Glynn RJ, Ridker PM. Effect of physical activity on serum C-reactive protein. Am J Cardiol. 2004;93:221–5.
97. Kasapis C, Thompson PD. The effects of physical activity on serum C-reactive protein and inflammatory markers: a systematic review. J Am Coll Cardiol. 2005;45:1563–9.
98. Plaisance EP, Grandjean PW. Physical activity and high-sensitivity C-reactive protein. Sports Med. 2006;36:443–58.
99. Esteghamati A, Morteza A, Khalilzadeh O, Anvari M, Noshad S, Zandieh A, et al. Physical inactivity is correlated with levels of quantitative C-reactive protein in serum, independent of obesity: results of the national surveillance of risk factors of non-communicable diseases in Iran. J Health Popul Nutr. 2012;30:66–72.
100. Buyukyazi G, Ulman C, Taneli F, Esen H, Gozlukaya F, Ozcan I, et al. The effects of different intensity walking programs on serum blood lipids, high-sensitive C-reactive protein, and lipoprotein-associated phospholipase A2 in premenopausal women. Sci Sports. 2010;25:245–52.
101. Wooten JS, Nambi P, Gillard BK, Pownall HJ, Coraza I, Scott LW, et al. Intensive lifestyle modification reduces Lp-PLA2 in dyslipidemic HIV/HAART patients. Med Sci Sports Exerc. 2013;45:1043–50.
102. Rood B. Effects of a 12-week vigorous interval training intervention on proinflammatory markers in post-operative bariatric and obese participants. Masters Theses 640 2019.
103. Clark K, Sharp S, Womack CJ, Kurti SP, Hargens TA. Increased sedentary time and decreased physical activity increases lipoprotein associated phospholipase A(2) in obese individuals. Nutr Metab Cardiovasc Dis. 2022;32:1703–10.
104. Liu X, Zhou W, Fan W, Li A, Pang J, Chen Z, et al. The benefit of exercise rehabilitation guided by 6-minute walk test on lipoprotein-associated phospholipase A2 in patients with coronary heart disease undergoing percutaneous coronary intervention: a prospective randomized controlled study. BMC Cardiovasc Disord. 2022;22:177.
105. Cozlea DL, Farcas DM, Nagy A, Keresztesi AA, Tifrea R, Cozlea L, et al. The impact of C reactive protein on global cardiovascular risk on patients with coronary artery disease. Curr Health Sci J. 2013;39:225–31.
106. Ballantyne CM, Hoogeveen RC, Bang H, Coresh J, Folsom AR, Heiss G, et al. Lipoprotein-associated phospholipase A2, high-sensitivity C-reactive protein, and risk for incident coronary heart disease in middle-aged men and women in the Atherosclerosis Risk in Communities (ARIC) study. Circulation. 2004;109:837–42.
107. Ballantyne CM, Hoogeveen RC, Bang H, Coresh J, Folsom AR, Chambless LE, et al. Lipoprotein-associated

phospholipase A2, high-sensitivity C-reactive protein, and risk for incident ischemic stroke in middle-aged men and women in the Atherosclerosis Risk in Communities (ARIC) study. Arch Intern Med. 2005;165:2479–84.

108. Elkind MS, Tai W, Coates K, Paik MC, Sacco RL. High-sensitivity C-reactive protein, lipoprotein-associated phospholipase A2, and outcome after ischemic stroke. Arch Intern Med. 2006;166:2073–80.
109. Feng L-M, Feng G-F, Chen Y. Evaluation of lipoprotein-associated phospholipase A2 in healthy Chinese Han adult serum. Lipids Health Dis. 2014;13:6.
110. Acevedo M, Varleta P, Kramer V, Valentino G, Quiroga T, Prieto C, et al. Comparison of lipoprotein-associated phospholipase A2 and high sensitive C-reactive protein as determinants of metabolic syndrome in subjects without coronary heart disease: In search of the best predictor. Int J Endocrinol. 2015;2015:934681.
111. Cen JM, Cheng J, Xiong QY, Mei BQ, Cai WB, Yang XL. Study on the correlation between the concentration of plasma lipoprotein-associated phospholipase A2 and coronary heart disease. Chronic Dis Transl Med. 2015;1:105–9.
112. Detopoulou P, Karantonis HC, Antonopoulou S. Mediterranean diet and its protective mechanisms against cardiovascular disease: an insight into platelet activating factor (PAF) and diet interplay. Ann Nutr Disord Ther. 2015;2:1016.
113. Vigo C. Effect of C-reactive protein on platelet-activating factor-induced platelet aggregation and membrane stabilization. J Biol Chem. 1985;260:3418–22.
114. Sato A, Oe K, Yamanaka H, Yokoyama I, Ebina K. C-reactive protein specifically enhances platelet-activating factor-induced inflammatory activity in vivo. Eur J Pharmacol. 2014;745:46–51.
115. Queensland Government. Queensland Government.

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