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Association of Alkaline Phosphatase with Cardiovascular Disease in Patients with Dyslipidemia: A 6-Year Retrospective Study

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Abstract: Background and Aim: Serum alkaline phosphatase (ALP) activity has been associated with atherosclerotic cardiovascular disease (ASCVD). We aimed to investigate the association of ALP with ASCVD in patients with dyslipidemia. Methods: Retrospective cohort study including consecutive adults with dyslipidemia followed-up for ≥3 years (from 1999 to 2022) in the outpatient Lipid Clinic of Ioannina University General Hospital, Greece. The primary endpoint was the association between baseline ALP and incident ASCVD after adjusting for traditional risk factors (i.e. sex, age, hypertension, diabetes, smoking, dyslipidemia), baseline ASCVD and lipid-lowering treatment. ALP levels were stratified by tertiles as follows: low:<67 U/L, middle: 67-79 U/L, high: ≥79 U/L. Results: Overall, 1178 subjects were included; 44% were males and their median age was 57 years (range 49-65). During a 6-year median follow-up (interquartile range IQR: 4-9), 78 new ASCVD events (6.6%) occurred. A statistically significant association between baseline ALP levels and incident ASCVD was demonstrated (Odds Ratio, OR: 6.99, 95% Confidence Interval, CI: 2.29-21.03, p=0.001). Subjects in the highest ALP tertile had the highest odds for ASCVD when compared with those in the lowest tertile (OR: 2.35; 95% CI: 1.24-4.41, p=0.008). Conclusions: The present study demonstrated that ALP is associated with ASCVD development in patients with dyslipidemia.

Keywords: alkaline phosphatase; cardiovascular disease; cholesterol; statins

1. Introduction

Atherosclerotic cardiovascular disease (ASCVD) stands out as a leading cause of global mortality, underscoring the critical role of modifiable risk factors in its occurrence and associated mortality [1]. Among these factors, elevated levels of low-density lipoprotein cholesterol (LDL-C) have been identified as a major contributor to ASCVD [2]. The proven benefits of LDL-C reduction through existing lipid-lowering regimens have significantly impacted both primary and secondary prevention efforts [2]. Despite the successes of current treatments, a noteworthy proportion of optimally managed patients continue to face the risk of cardiovascular events and mortality, suggesting the existence of residual cardiovascular risk [3]. Emerging evidence from retrospective and prospective studies, encompassing populations with and without established cardiovascular disease (CVD), indicates that baseline serum alkaline phosphatase (ALP) levels might serve as a valuable predictive marker for overall mortality and cardiovascular risk [4–8]. It is particularly intriguing that recent research has explored the potential of drug-induced reduction in ALP to mitigate cardiovascular risk in individuals with ASCVD [9]. A notable example is a phase 2, placebo-controlled trial involving apabetalone, conducted with 795 patients already diagnosed with ASCVD and undergoing statin therapy. This trial demonstrated a significant reduction in major adverse cardiovascular events (MACE) when ALP levels were lowered by 1 standard deviation (1-SD) after

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24-26 weeks of treatment, with a hazard ratio (HR) of 0.64 (95% confidence interval, CI: 0.46-0.90, p=0.009) [9].

Against this backdrop, our study aims to delve into the potential role of ALP as a predictive biomarker for ASCVD and as a promising therapeutic target in patients with dyslipidemia receiving lipid-lowering treatment. Our investigation specifically involves scrutinizing the association between baseline ALP levels and the incidence of ASCVD, while meticulously adjusting for traditional risk factors such as sex, age, hypertension, diabetes, smoking, dyslipidemia, as well as baseline ASCVD and ongoing lipid-lowering treatments. This comprehensive approach seeks to unravel the intricate interplay between ALP levels and cardiovascular outcomes, offering insights that could potentially refine risk stratification and inform targeted therapeutic interventions in the realm of cardiovascular health.

2. Materials and Methods

This retrospective cohort study involved the comprehensive analysis of 1178 Caucasian patients of Hellenic origin diagnosed with dyslipidemia. The participants were enrolled from the Outpatient Lipid Clinic of the University Hospital of Ioannina, Greece, and had a minimum follow-up duration of three years, spanning the period between 1999 and 2022. The study protocol obtained ethical approval from the local Institutional Ethics Committee, ensuring compliance with established ethical standards. Additionally, informed consent was diligently obtained from all participating patients.

Detailed cardiovascular histories and relevant medication profiles were meticulously documented for each patient. A detailed documentation of patient demographic and clinical characteristics was conducted, focusing on sex, age, smoking, and concomitant diseases, with a specific emphasis on CVD and cardiovascular risk factors.

Clinical and laboratory data were meticulously gathered to unravel the intricate relationships between cardiovascular health, laboratory parameters, and clinical attributes. Blood pressure measurements adhered to the European Society of Cardiology (ESC) / European Society of Hypertension (ESH) guidelines, utilizing a validated upper-arm cuff BP measurement device and an appropriate cuff size.10 Fasting plasma glucose (FPG) levels were obtained from morning blood samples after an overnight fast of at least 8-12 hours. A comprehensive lipid profile, including Total Cholesterol (TC), Triglycerides (TG), High-Density Lipoprotein Cholesterol (HDL-C), and LDL-C, underwent meticulous analysis. LDL-C levels were calculated using the Friedewald formula, considering TG levels below 400 mg/dL (4.5mmol/L). Additional laboratory assessments included a complete blood count, creatinine, urea, electrolytes, liver enzymes, creatine kinase (CK), thyroid function, and urine analysis. Renal function was estimated using the estimated Glomerular Filtration Rate (eGFR) calculated with the CKD-EPI (CKD Epidemiology Collaboration) formula, utilizing creatinine results calibrated to isotope dilution mass spectrometry.

Cardiovascular and metabolic conditions were comprehensively defined, encompassing a spectrum of ASCVD, including Coronary Heart Disease (CHD), stroke, Peripheral Artery Disease (PAD), and carotid stenosis >50%. Precise diagnostic criteria were established for hypertension, diabetes, and impaired fasting glucose, adhering to ESC/ESH and ESC/EASD guidelines.11 Diagnosis of Chronic Kidney Disease (CKD) was contingent upon observing a decline in eGFR <60 ml/min/1.73 m^2 in two periodical examinations over at least a 3-month timeline.

Concomitant therapy, with a particular focus on lipid-lowering drugs such as statins, ezetimibe, PCSK9 inhibitors, fibrates, colesevelam, and n-3 fatty acids, was diligently recorded. The intensity of statin therapy was classified as 'high,' 'moderate,' and 'low' based on the anticipated average LDL-C lowering effect.

Alkaline phosphatase (ALP) levels were categorized into tertiles ('low' <67 U/L, 'middle' 67-79 U/L, 'high' ≥79 U/L), offering nuanced insights into ALP concentrations within the study population. This stratification aimed to facilitate a comprehensive analysis of the potential association between ALP and cardiovascular outcomes.

Statistical Analysis

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Continuous variables underwent normality testing using the Kolmogorov-Smirnov test, and logarithmic transformations were applied if necessary. Parametric data are presented as mean ± standard deviation (SD), while non-parametric data are expressed as median [interquartile range (IQR)]. Categorical values are presented as frequency counts and percentages. Paired sample t-tests, both parametric and non-parametric, were employed to examine changes in numeric variables during the follow-up period within each group.

Pearson's and Spearman's correlation coefficients were utilized to explore the relationship between ALP changes and other variables. Multivariate logistic regression analysis was conducted to investigate the impact of ALP changes on the development of ASCVD, adjusting for potential confounding factors. Associations with ASCVD outcomes are reported as odds ratios (OR) with accompanying 95% CI. Significance was set at $p<0.05$, and all analyses were conducted using the Statistical Package for Social Sciences (SPSS) v21.0 software (SPSS IBM Corporation, Armonk, New York, USA). The rigorous methodology employed in this study aimed to provide robust insights into the intricate relationships between ALP, dyslipidemia, and ASCVD incidence within this specific cohort.

3. Results

A total of 1178 subjects were enrolled in this study, representing a diverse cohort with various demographic and clinical characteristics. Among the participants, 44% were male, with a median age of 57 years (range, 49-65). Notably, 32% of the cohort were smokers, and the prevalence of hypertension, diabetes, and chronic kidney disease was observed in 61%, 11%, and 10% of the subjects, respectively. Furthermore, 16% of the total sample had established ASCVD, and 271 individuals were on statin therapy at the initiation visit.

A detailed examination of baseline characteristics, as presented in Table 1, revealed significant insights. Notable variations were observed across the three groups, particularly concerning smoking status and lipid-lowering therapy. The lowest tertile of alkaline phosphatase (ALP) exhibited a higher percentage of smokers ($p<0.001$), while a larger proportion of patients in this group were on lipid-lowering therapy ($p<0.05$).

Laboratory variables also demonstrated differences (Table 2), with higher mean values of calcium and direct bilirubin found at the middle and lower tertiles of ALP, respectively ($p<0.05$).

Table 1. Baseline characteristics of study participants.

		Tertiles of Alkaline Phosphatase			
	Total Sample	**Low**	**Middle**	**High**	**p**
N	1178	383	398	397	
Sex (male), %	44	49	42	43	NS
Age, yrs	57 (49-65)	55 (47-64)	57 (51-66)	58 (49-66)	NS
Follow-up, years	6 (4-9)	5 (3-8)	5 (4-8)	8 (5-12)	NS
Smoking, %	32	40	27	29	<0.001
Hypertension, %	61	59	59	64	NS
Diabetes, %	11	9	11	12	NS
Atherosclerotic Cardiovascular Diseaser, %	16	15	15	18	NS
Chronic kidney disease, %	10	9	10	10	NS
Systolic blood pressure, mmHg	140 (125-150)	134 (120-150)	138 (126.5-150)	140 (130-160)	NS
Diastolic blood pressure, mmHg	85 (80-93)	83 (78-91)	84 (78-90)	90 (80-95)	NS

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Fasting plasma glucose, mg/dL	96 (88-106)	93 (87-103)	96 (88-106)	97 (89-109)	NS
Total cholesterol, mg/dL	250 (212-286)	246 (205-271)	248 (212-289)	257 (222-301)	NS
Triglycerides, mg/dL	129 (94-186)	121 (89-171)	130.5 (92-189.5)	137 (100-194)	NS
High-density lipoprotein cholesterol, mg/dL	52 (44-62)	52 (45-63)	54 (45.5-65)	50 (42-60)	NS
Low-density lipoprotein cholesterol, mg/dL	166.2 (131.8-195.4)	161 (125.5-187.7)	164.3 (130-193.2)	173 (141-207.6)	NS
Alkaline Phosphatase, IU/L	67 (54-90)	50 (44-54)	67 (63-72)	112 (90-169)	<0.001*
Lipid-lowering therapy, %	23	26	24	18	<0.05*
Antihypertensive therapy, %	46	44	45	48	NS
Antidiabetic therapy, %	7	8	9	8	NS

Tertiles of alkaline phosphatase were defined as the following: low: <67 U/L, middle: 67-79 U/L, high: ≥79 U/L.

In a fully adjusted model accounting for age, sex, hypertension, diabetes, smoking, baseline ASCVD, triglycerides, HDL-C, LDL-C, and lipid-lowering therapy, ALP levels demonstrated a significant association with higher incident ASCVD (OR: 6.99; 95% CI: 2.29-21.03, p=0.001; Table 3). This finding remained significant for the highest ALP tertile, with an OR of 2.35 (95% CI: 1.24-4.41, p=0.008).

Consistent results were obtained in less-adjusted analytical models, both in a crude analysis and an adjusted model (Models 1 and 2, Table 3). The association between ALP levels as a continuous variable and incident ASCVD was significant in both models (OR: 8.67; 95% CI: 3.21-23.38, p<0.001 and OR: 9.10; 95% CI: 3.22-25.75, p<0.001, respectively). Furthermore, a similar significant association was observed for the highest tertile of ALP within both analytical models (OR: 2.79; 95% CI: 1.54-5.04, p=0.001 and OR:2.72; 95% CI: 1.49-4.98, p=0.001, respectively).

Table 2. Baseline laboratory variables.

		Tertiles of Alkaline Phosphatase			
	Total Sample	**Lower**	**Middle**	**Highest**	**p**
Estimated glomerular filtration rate, mL/min/1.73 m²	80.7 (70.57-91.79)	83 (72.24-92.35)	80.79 (71.98-90.51)	78.63 (68.53-92.21)	NS
Aspartate aminotransferase, IU/L	21 (18-25)	21 (18.25)	22 (18-26)	21 (18-25)	NS
Alanine aminotransferase, IU/L	22 (17-29)	21 (16-28)	22 (17-29)	22 (17-29)	NS
Gamma-glutamyltranspetidase, IU/L	18 (13-28)	18 (12-25.5)	18 (13-28)	20 (14-30)	NS
Total bilirubin, mg/dL	0.6 (0.5-0.8)	0.7 (0.5-0.8)	0.6 (0.5-0.8)	0.6 (0.5-0.8)	NS
Direct bilirubin mg/dL	0.1 (0.08-0.13)	0.11 (0.09-0.14)	0.1 (0.08-0.13)	0.09 (0.07-0.12)	<0.05

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Hemoglobin, g/dL	14 (13.2-14.9)	14.1 (13.2-15.1)	13.9 (13.1-14.9)	13.9 (13.1-14.6)	NS
Calcium, md/dL	9.7 (9.4-10)	9.7 (9.4-10)	9.76 (9.4-10)	9.6 (9.4-9.9)	<0.05
Phosphate, mg/dL	3.3 (3-3.7)	3.2 (2.9-3.6)	3.3 (3-3.8)	3.4 (2.95-3.7)	NS
Albumin, g/dL	-	4.4 (4.3-4.6)	4.4 (4.2-4.6)	4.5 (4.3-4.7)	NS

Tertiles of alkaline phosphatase were defined as the following: lowest: <67 U/L, middle: 67-79 U/L, highest: ≥79 U/L.

Table 3. Association between alkaline phosphatase and incident atherosclerotic cardiovascular disease.

	Model 1	**Model 2**	**Model 3**
Alkaline Phosphatase (continuous variable)*	8.67 (3.21-23.38), p <0.001	9.10 (3.22-25.75), p <0.001	6.99 (2.29-21.03), p=0.001
Alkaline Phosphatase tertiles			
Low	Reference	Reference	Reference
Middle	1.15 (0.58-2.27), p=0.687	1.13 (0.57-2.25), p=0.725	1.19 (0.59-2.39), p=0.621
High	2.79 (1.54-5.04), p=0.001	2.72 (1.49-4.98), p=0.001	2.35 (1.24-4.41), p=0.008

Associations are expressed as odds ratios (95% confidence intervals). Model 1: Crude analysis; Model 2: adjusted for sex and age; Model 3: adjusted for sex, age, hypertension, diabetes, smoking, baseline atherosclerotic cardiovascular disease, triglycerides, high- and low-density lipoprotein cholesterol and lipid-lowering therapy. * Alkaline Phosphatase (continuous variable) was logarithmically transformed.

4. Discussion

The present study demonstrated that ALP is associated with ASCVD development in patients with dyslipidemia. These robust associations, consistently observed across various analytical models, underscore the potential predictive role of ALP levels, particularly in its highest tertile, in incident ASCVD. Our fully adjusted model, considering an array of demographic and clinical factors, further emphasizes the strength of this association, providing valuable insights into the link between ALP and cardiovascular outcomes in individuals with dyslipidemia.

Our study's prospective design aligns with existing research, particularly a comprehensive 10-year cohort involving 6974 subjects from the general population [8]. This seminal investigation revealed a compelling and independent association between serum ALP activity and ASCVD, underscoring the significance of ALP as a potential cardiovascular risk marker.[8] The HR of 1.34 (95% CI: 1.14-1.56, p<0.001) indicates a substantial increase in ASCVD risk associated with elevated ALP levels [8]. The interconnectedness of ALP and CRP was further illuminated in a study involving 4155 subjects over 20 years old, hinting at a shared biological pathway between these two parameters [12–14]. Expanding the scope to a prospective study involving 3381 men aged 60 to 79 years, the association between elevated ALP levels and adverse cardiovascular outcomes became more pronounced [15]. The heightened risk of CHD (HR: 1.90; 95% CI: 1.40-2.56, p<0.0001), CVD mortality (HR:1.72; 95% CI: 1.27-2.32, p<0.0001), and overall CVD events (HR:1.73; 95% CI: 1.39-2.16, p<0.0001) in elderly men with elevated ALP levels reinforces the potential utility of ALP as a prognostic marker in this demographic [15]. Crucially, this association retained significance even after meticulous adjustment for a spectrum of cardiovascular risk factors and CRP levels, with an adjusted HR of 1.19 (95% CI: 1.05-1.34, p=0.007) [15]. This adjustment accounts for confounding variables and affirms the robustness of the association [15]. Furthermore, the broader landscape of observational studies strengthens the case for ALP as a predictor of cardiovascular outcomes [5], [16]. A systematic review and meta-analysis of 24 observational studies, encompassing 147,634 patients, provided compelling evidence for a positive association between serum ALP activity and total mortality in individuals

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with normal renal function [5]. The pooled risk ratio (RR) of 1.57 (95% CI: 1.27-1.95) suggests a significant correlation between elevated ALP levels and increased mortality risk in this population [5]. A meta-analysis of four prospective studies, involving 33,727 participants, contributed further weight to the association between ALP and ASCVD [4]. Reporting an 8% (95% CI: 3-14%) higher risk of ASCVD per 1-standard deviation increase in baseline ALP activity, this meta-analysis underscores the consistency of findings across diverse study populations and settings [4]. Therefore, the wealth of evidence presented across various studies, including our own, emphasizes the importance of ALP as a potential biomarker for ASCVD risk assessment. The nuanced exploration of ALP's association with cardiovascular outcomes, especially in the context of inflammation and aging, enriches our understanding of its clinical relevance. Future research should delve deeper into the mechanistic underpinnings of this association and explore the potential of ALP as a target for interventions aimed at reducing cardiovascular risk.

The intricate pathophysiology associated with alkaline phosphatase (ALP) extends beyond a mere biomarker, revealing its multifaceted involvement in cardiovascular health. Conditions characterized by heightened oxidative stress, such as chronic kidney disease (CKD), have been identified as contributors to increased ALP activity [17]. This elevation in ALP activity is implicated in various deleterious processes that collectively impact cardiovascular health [17]. One notable consequence of enhanced ALP activity is the potential induction of vascular calcification, a pathological process associated with the deposition of calcium salts in the vascular walls [17–21]. This phenomenon is linked to arterial stiffness and endothelial dysfunction, factors that collectively contribute to the progression of atherosclerosis and increased cardiovascular risk [17–21] The intricate balance of tissue mineralization is disrupted through pyrophosphate inhibition, an imbalance that can further exacerbate vascular calcification and compromise overall vascular health [16]. Moreover, ALP's involvement in interactions with inflammatory cytokines introduces an additional layer of complexity to its role in cardiovascular pathophysiology [22]. The promotion of vascular smooth muscle cell calcification and inflammation by these interactions underscores the intertwined nature of inflammatory processes and vascular health [23]. These cascading effects can contribute to the progression of atherosclerosis, a key player in the development of cardiovascular diseases. In the context of atherosclerosis, ALP emerges as a potential participant in the destabilization of atherosclerotic plaques [24]. The structural integrity of these plaques is crucial, and any factor contributing to their destabilization can elevate the risk of adverse cardiovascular events. ALP's involvement in this process adds to its significance as a potential mediator of cardiovascular risk [24].

Human ALP, comprising four distinct isoenzymes, can be dissected through chromatography techniques [6]. Among these isoenzymes, tissue-nonspecific ALP (TNALP) takes precedence, constituting over 90% of circulating ALP and emerging as the most abundant isoenzyme in the human body [6]. TNALP, in turn, encompasses two critical isoforms: bone-specific and liver-specific TNALP, each characterized by distinct enzymatic activities and localizations within the body [6]. This intricate composition underscores the need for a nuanced exploration of ALP's various forms and their individual contributions to cardiovascular health [6]. Despite the complexity of ALP's isoenzyme composition, the majority of studies have traditionally focused on investigating the association of serum circulating ALP with cardiovascular disease (CVD) and all-cause mortality without delving into the specific contributions of each isoenzyme. This presents an avenue for future research to unravel the distinct roles played by these isoforms in cardiovascular health, potentially revealing novel insights into the mechanisms underpinning ALP's association with adverse cardiovascular outcomes.

Conversely, the potential therapeutic benefits of reducing ALP through pharmacological intervention in individuals with established ASCVD have been proposed. An insightful analysis conducted across three phase 2 placebo-controlled trials involving 795 patients with diabetes and recent acute coronary syndrome has shed light on the potential cardiovascular advantages of ALP reduction. Specifically, a noteworthy finding emerged, indicating that a 1-SD reduction in ALP was associated with a marked reduction in MACE (HR: 0.64; 95% CI: 0.46-0.90, p=0.009) [9]. The focal

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agent of this investigation was apabetalone, an oral medication distinguished by its capacity to induce epigenetic modifications of gene transcription. Operating through the modification of bromodomain and extra-terminal (BET) proteins, apabetalone represents a promising avenue for targeted intervention [25]. The body of evidence, derived from both experimental models and human studies, points towards favorable effects of apabetalone on multiple facets of cardiovascular disease (CVD), encompassing atherosclerosis, inflammation, and vascular calcification [9]. Furthermore, the investigation draws parallels between the effects of apabetalone and statin therapy. Statins, renowned for their established role in lipid management, have demonstrated a positive impact on coronary and aortic valve calcification [26]. This phenomenon has been attributed to the inhibition of ALP expression, as elucidated through in vitro models [26]. The implications of statin therapy extend beyond lipid regulation, encompassing potential anabolic effects on bone tissue through intricate molecular pathways [27], [28]. These pathways include the reduction of bone turnover, as evidenced by a study demonstrating an inverse correlation between the reduction of serum plasma bone markers following statin therapy and bone mineral density [29]. However, despite the intriguing associations between statin-induced ALP reduction, bone turnover, and vascular health, the current body of evidence falls short in establishing a definitive link between statin-induced ALP reduction and a subsequent decrease in ASCVD events. Consequently, a critical gap exists in our understanding of the complex interplay between statins, ALP modulation, and cardiovascular outcomes. The paucity of data underscores the imperative for further research in this field to elucidate the nuanced relationships and potential therapeutic implications.

Study Limitations

The retrospective nature of our study introduces challenges associated with data collection. Causality inference is constrained by the observational nature of our study, preventing definitive conclusions regarding the cause-and-effect relationship between elevated ALP levels and incident ASCVD. Furthermore, our study lacks comprehensive data on inflammatory markers, such as CRP, limiting our understanding of the role of inflammation in the identified associations. Dietary habits and physical activity, recognized contributors to incident ASCVD, were not included in our study, leading to an incomplete assessment of lifestyle factors. Moreover, the study did not perform a detailed analysis of ALP isoenzymes, preventing a nuanced understanding of their contributions to ASCVD risk. Likewise, the absence of electrophoresis data limits the ability to conduct an isoform-specific analysis of ALP, hindering insights into the distinct roles of specific ALP isoforms in ASCVD. Finally, the study population primarily comprised dyslipidemic subjects, potentially restricting the generalizability of findings to diverse demographic groups.

While our study offers valuable insights into ALP and ASCVD associations, these limitations underscore the need for cautious interpretation. Future research addressing these limitations will enhance the applicability of findings in guiding clinical practice and interventions related to ALP and cardiovascular health.

5. Conclusions

The findings from the present study provide compelling evidence suggesting a potential association between elevated ALP levels and an augmented ASCVD risk. However, despite the observed association, the precise role of ALP in serving as either a reliable prognostic marker or a plausible therapeutic target remains uncertain. Further in-depth investigations are needed to delineate the intricate relationship between ALP and ASCVD risk, particularly focusing on exploring the contributions of specific ALP isoforms. A more nuanced understanding of the isoform-specific associations could elucidate the underlying mechanisms and facilitate the development of targeted interventions. All things considered, the present study serves as a steppingstone, underscoring the importance of future research endeavors to unlock the full potential of ALP as a predictive tool and potential therapeutic target in the realm of ASCVD prevention within the dyslipidemic population.

Author Contributions: Investigation, Writing - original draft PS.A.; Methodology, Writing – review & editing M.F. and E.L; Data curation, Writing – original draft AD.K. and G.A.; Validation, Writing – review & editing G.L. and H.M.; Conceptualization, Formal Analysis, Investigation, Writing – review & editing, Supervision F.B. All authors have read and agreed to the published version of the manuscript.

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