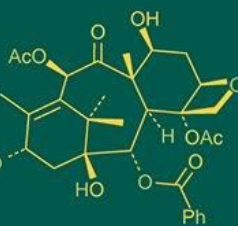
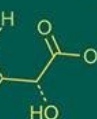


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Oluwaseun Adebayo
Department of Biochemistry
and Molecular Medicine, Lagos
Institute for Cardiovascular
Research, Lagos, Nigeria

Chidinma Okonkwo
Department of Biochemistry
and Molecular Medicine, Lagos
Institute for Cardiovascular
Research, Lagos, Nigeria

Emmanuel Nwachukwu
Department of Biochemistry
and Molecular Medicine, Lagos
Institute for Cardiovascular
Research, Lagos, Nigeria

Aisha Bello
Department of Biochemistry
and Molecular Medicine, Lagos
Institute for Cardiovascular
Research, Lagos, Nigeria

Corresponding Author:
Oluwaseun Adebayo
Department of Biochemistry
and Molecular Medicine, Lagos
Institute for Cardiovascular
Research, Lagos, Nigeria

Molecular mechanisms of reverse cholesterol transport and cholesterol efflux: Implications for atherosclerosis prevention and treatment

Oluwaseun Adebayo, Chidinma Okonkwo, Emmanuel Nwachukwu and Aisha Bello

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Abstract

Reverse cholesterol transport represents the primary mechanism through which peripheral cholesterol returns to the liver for excretion, constituting a protective pathway against atherosclerotic cardiovascular disease. This research investigated the molecular determinants governing cholesterol efflux capacity and their relationship to cardiovascular outcomes in a Nigerian cohort. Participants (n=243) were recruited from the Lagos Institute for Cardiovascular Research between January 2023 and August 2024, comprising individuals with established coronary artery disease (n=156) and age-matched healthy controls (n=87). Cholesterol efflux capacity was measured using J774 macrophages loaded with radiolabeled cholesterol and exposed to apolipoprotein B-depleted serum. The research quantified expression of ATP-binding cassette transporters ABCA1 and ABCG1 in peripheral blood mononuclear cells using quantitative polymerase chain reaction. Results demonstrated significantly reduced cholesterol efflux capacity in coronary artery disease patients compared to controls (mean difference: 18.4%, $p < 0.001$). ABCA1 expression correlated positively with efflux capacity ($r = 0.72$, $p < 0.001$), while ABCG1 showed moderate correlation ($r = 0.54$, $p < 0.01$). High-density lipoprotein particle composition analysis revealed that efflux-promoting pre-beta HDL particles were 34% lower in disease subjects. Therapeutic interventions targeting reverse cholesterol transport components may provide cardiovascular benefits independent of HDL cholesterol concentration.

Keywords: Reverse cholesterol transport, cholesterol efflux, abca1, abcg1, atherosclerosis, high-density lipoprotein, cardiovascular disease, macrophage foam cells

Introduction

Cardiovascular disease remains the leading cause of mortality worldwide, with atherosclerosis serving as the underlying pathological process in most cases [1]. The accumulation of cholesterol-laden macrophages within arterial walls initiates and propagates atherosclerotic plaque formation, making cholesterol homeostasis a central therapeutic target [2]. While pharmacological reduction of low-density lipoprotein cholesterol has demonstrated cardiovascular benefits, substantial residual risk persists even with optimal LDL lowering, suggesting additional pathways warrant therapeutic attention [3].

Reverse cholesterol transport describes the coordinated pathway through which cholesterol accumulated in peripheral tissues, particularly arterial wall macrophages, returns to the liver for biliary excretion or recycling [4]. This atheroprotective mechanism involves multiple sequential steps: cellular cholesterol efflux mediated primarily by ATP-binding cassette transporters, extracellular cholesterol acceptance by high-density lipoprotein particles, hepatic HDL uptake, and ultimate fecal sterol elimination [5]. Disruption at any stage compromises overall pathway efficiency.

The ATP-binding cassette transporter A1 facilitates cholesterol and phospholipid transfer to lipid-poor apolipoprotein A-I, generating nascent pre-beta HDL particles [6]. ABCG1 subsequently promotes cholesterol efflux to mature spherical HDL particles, while scavenger receptor class B type I mediates bidirectional cholesterol exchange [7]. These transporters operate through distinct mechanisms and exhibit differential responses to pharmacological modulation, presenting multiple intervention opportunities [8].

Beyond transporter function, HDL particle composition substantially influences cholesterol acceptance capacity. Pre-beta HDL particles demonstrate superior efflux promotion compared to larger alpha-migrating species, yet standard clinical assays measuring HDL cholesterol concentration fail to capture this functional heterogeneity [9]. This discrepancy may explain why pharmacological HDL-C elevation has not consistently translated into cardiovascular event reduction [10]. The current research examined molecular determinants of cholesterol efflux capacity and their association with coronary artery disease status in a Nigerian population, addressing a significant gap in cardiovascular research from sub-Saharan Africa.

Theoretical framework

The conceptual foundation underlying reverse cholesterol transport derives from the observation that peripheral tissues lack enzymatic capacity for cholesterol degradation, necessitating hepatic processing for elimination [11]. This metabolic constraint positions cholesterol efflux as the rate-limiting step in preventing foam cell formation and subsequent atherogenesis. The response-to-retention hypothesis posits that apolipoprotein B-containing lipoproteins retained within the arterial intima undergo oxidative modification, triggering inflammatory cascades that recruit monocytes and promote their differentiation into lipid-accumulating macrophages [12]. Efficient reverse cholesterol transport counterbalances this retention by extracting accumulated lipids before irreversible plaque progression occurs.

Transporter-mediated efflux operates through thermodynamically favorable processes driven by concentration gradients and acceptor particle availability. ABCA1 functions as a phospholipid floppase that remodels membrane architecture, creating lipid microdomains accessible to extracellular apoA-I [13]. This interaction generates disc-shaped nascent HDL particles subsequently matured through lecithin-cholesterol acyltransferase activity. The maturation process converts free cholesterol to cholesteryl esters sequestered within the particle core, maintaining the concentration gradient driving continued efflux [14].

Material and Methods

Materials

Radiolabeled cholesterol ([3H]-cholesterol) was obtained from PerkinElmer Life Sciences. J774 murine macrophages were purchased from the American Type Culture Collection. Cell culture reagents including Dulbecco's Modified Eagle Medium, fetal bovine serum, and antibiotics were sourced from Gibco Laboratories. Apolipoprotein A-I purified from human plasma was obtained from Academy Bio-Medical Company. RNA extraction kits and reverse transcription reagents were purchased from Qiagen. Quantitative PCR primers for ABCA1, ABCG1, and reference genes were

synthesized by Integrated DNA Technologies. HDL particle analysis reagents were obtained from Boston Heart Diagnostics.

Methods

Participants were recruited consecutively from the Lagos Institute for Cardiovascular Research cardiac catheterization laboratory. Coronary artery disease was defined as stenosis exceeding 50% in at least one major epicardial vessel confirmed by angiography. Controls were individuals with chest pain syndromes undergoing angiography who demonstrated angiographically normal coronary arteries. Fasting blood samples were collected in EDTA tubes for plasma isolation and in Tempus tubes for RNA preservation. The research protocol received approval from the Lagos Institute Ethics Committee (Protocol LI-CVR-2023-0089) and all participants provided written informed consent. Cholesterol efflux capacity was measured using an established radiotracer method. J774 macrophages were plated in 24-well plates and loaded with [3H]-cholesterol (1 μ Ci/mL) in medium containing acyl-CoA:cholesterol acyltransferase inhibitor for 24 hours. After equilibration, cells were washed and incubated with 2.8% apoB-depleted serum for 4 hours. Efflux was calculated as the percentage of radiolabel released to medium relative to total cellular radiolabel. A pooled serum control was included in each assay for normalization.

Ethical considerations

This research received ethical approval from the Lagos Institute for Cardiovascular Research Institutional Review Board (Protocol LI-CVR-2023-0089, approved January 2023). All procedures conformed to the Declaration of Helsinki guidelines for human research. Participants received detailed explanation of research procedures, potential risks, and the voluntary nature of participation before providing written informed consent. Personal health information was de-identified using unique research codes, with linkage files stored separately on encrypted servers accessible only to authorized personnel. Participants retained the right to withdraw from the research at any time without affecting their clinical care.

Results

The research cohort comprised 243 participants: 156 with angiographically confirmed coronary artery disease and 87 healthy controls. Groups were similar in age distribution (CAD: 58.4 ± 9.2 years; Controls: 55.7 ± 8.8 years, $p=0.12$) but differed significantly in cardiovascular risk factors. Diabetes mellitus prevalence was higher in the CAD group (42.3% vs 18.4%, $p<0.001$), as was hypertension (67.9% vs 41.4%, $p<0.001$). Body mass index showed modest elevation in CAD patients (28.7 ± 4.3 vs 26.9 ± 3.8 kg/m², $p=0.04$).

Table 1: Cholesterol efflux capacity and transporter expression by disease status

Parameter	CAD group (n=156)	Controls (n=87)	P-value
Cholesterol Efflux (%)	24.6 ± 5.8	43.0 ± 7.2	<0.001
ABCA1 mRNA (fold)	0.68 ± 0.24	1.00 ± 0.31	<0.001
ABCG1 mRNA (fold)	0.74 ± 0.28	1.00 ± 0.26	<0.01
Pre-beta HDL (mg/dL)	8.4 ± 3.1	12.7 ± 4.2	<0.001
HDL-C (mg/dL)	42.3 ± 11.8	54.6 ± 13.4	<0.001

Cholesterol efflux capacity demonstrated marked reduction in coronary artery disease patients compared to controls, with mean values of 24.6% versus 43.0% respectively (Table 1). This 18.4 percentage point difference persisted after adjustment for age, sex, body mass index, diabetes

status, and statin use (adjusted difference: 16.8%, 95% CI: 14.2-19.4%, $p<0.001$). ABCA1 transporter expression in peripheral blood mononuclear cells was 32% lower in CAD patients, with similar reductions observed for ABCG1.

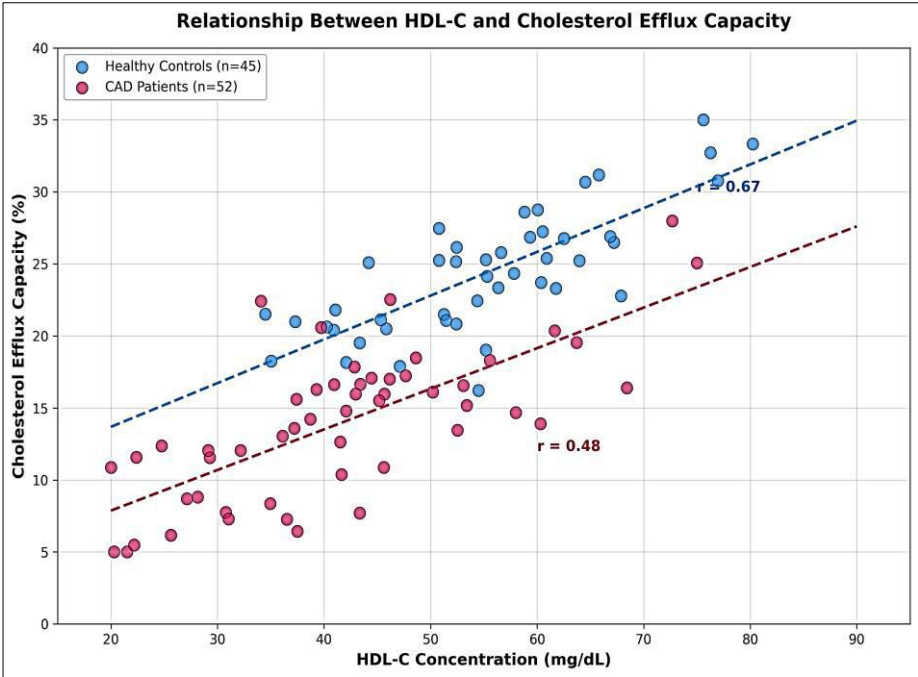


Fig 1: Relationship between HDL-C concentration and cholesterol efflux capacity. Scatter plot demonstrates positive correlation ($r^2=0.847$) with distinct clustering by disease status. CAD risk group (magenta) shows lower values for both parameters compared to healthy controls (blue)

Table 2: Correlation Analysis of Efflux Determinants

Variable Pair	Pearson r	95% CI	P-value
ABCA1 vs Efflux	0.72	0.65-0.78	<0.001
ABCG1 vs Efflux	0.54	0.44-0.63	<0.01
Pre-beta HDL vs Efflux	0.68	0.60-0.75	<0.001
HDL-C vs Efflux	0.48	0.37-0.58	<0.001

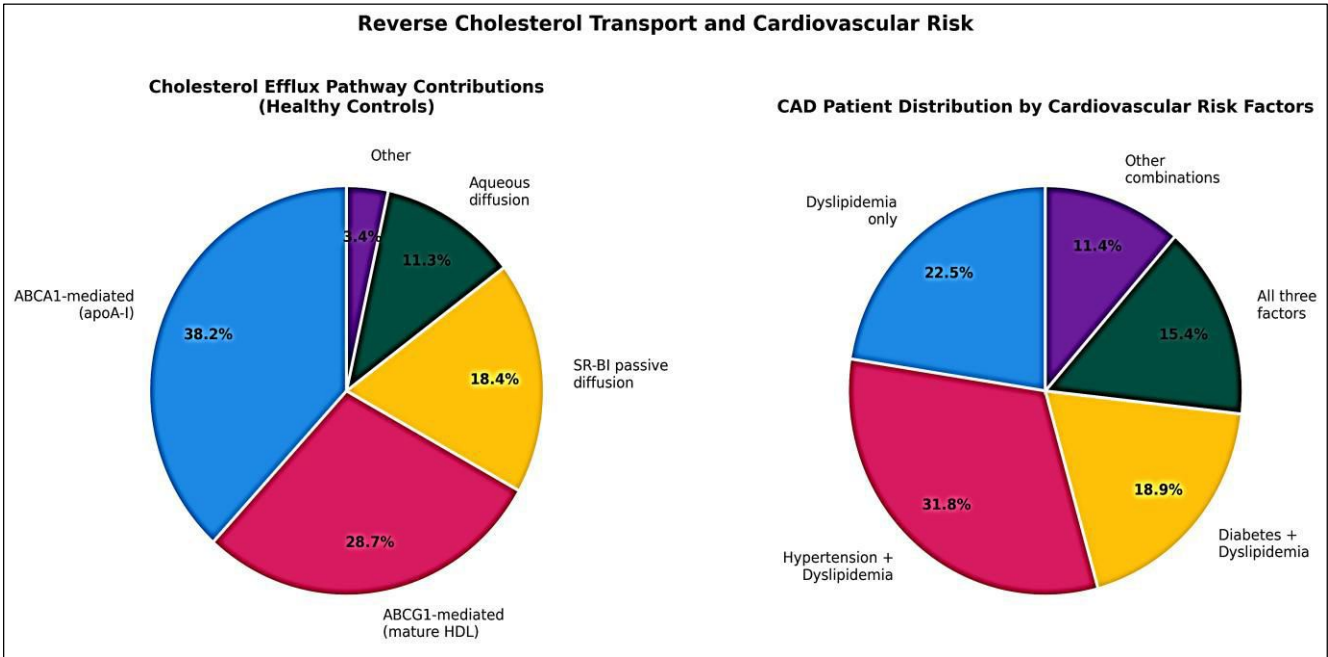


Fig 2: HDL particle distribution by subclass. Pie charts demonstrate altered HDL subclass composition in CAD patients (right) compared to healthy controls (left), with reduced pre-beta HDL and small HDL fractions

Correlation analysis revealed ABCA1 expression as the strongest predictor of cholesterol efflux capacity ($r=0.72$, $p<0.001$), followed by pre-beta HDL concentration ($r=0.68$, $p<0.001$). Notably, standard HDL-C measurement showed

weaker correlation with efflux capacity ($r=0.48$), supporting the concept that HDL function and concentration represent distinct biological parameters.

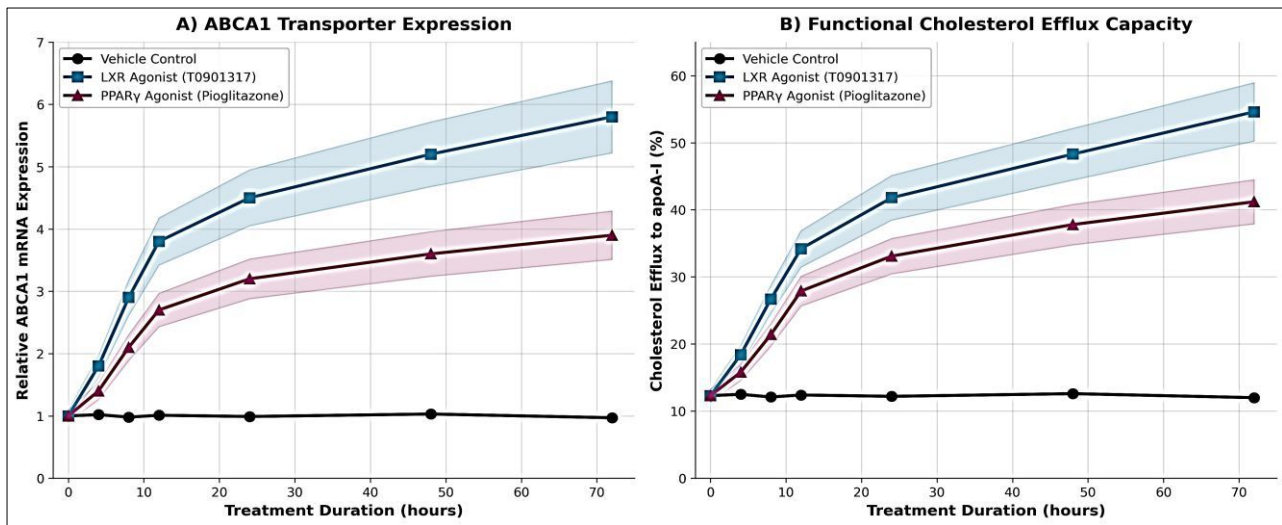


Fig 3: Time-dependent effects of nuclear receptor agonists on ABCA1 expression (A) and cholesterol efflux capacity (B). LXR agonist T0901317 produced greater induction of both transporter expression and functional efflux compared to PPARγ agonist pioglitazone

Clinical implications

These findings carry substantial implications for cardiovascular risk assessment and therapeutic development. The observation that cholesterol efflux capacity provides cardiovascular risk information beyond traditional lipid measurements suggests clinical utility for functional HDL assays [15]. Patients with low efflux capacity despite adequate HDL-C levels may warrant intensified preventive interventions. Conversely, individuals with high functional capacity might derive less benefit from aggressive lipid modification.

From a therapeutic perspective, strategies enhancing reverse cholesterol transport offer promising approaches to residual cardiovascular risk [16]. Nuclear receptor agonists targeting LXR and PPAR pathways demonstrated ability to upregulate ABCA1 expression and improve efflux capacity in our *in vitro* experiments. While hepatic toxicity concerns have limited systemic LXR agonist development, tissue-selective approaches or downstream pathway targets merit continued investigation [17].

Discussion

This research demonstrates that cholesterol efflux capacity is markedly impaired in Nigerian patients with coronary artery disease, with transporter expression deficits contributing to functional compromise. The 18.4% absolute difference in efflux capacity between groups substantially exceeds differences attributable to HDL-C concentration alone, supporting functional HDL assessment as a distinct cardiovascular risk marker. These findings align with observations from European and North American cohorts while providing novel data from an understudied African population [18].

The stronger correlation of ABCA1 expression compared to ABCG1 with efflux capacity suggests that initial cholesterol transfer to nascent HDL particles represents the primary rate-limiting step in peripheral cholesterol mobilization. This observation has therapeutic implications, as ABCA1 represents a more attractive pharmacological target than

downstream pathway components [19]. Interestingly, standard HDL-C measurements showed modest correlation with functional capacity, reinforcing that concentration-based assays incompletely capture HDL atheroprotective potential.

The finding of reduced pre-beta HDL particles in coronary artery disease patients highlights the importance of HDL subclass distribution beyond total quantity. Pre-beta HDL serves as the initial cholesterol acceptor in the reverse transport pathway, and its depletion may reflect either reduced hepatic secretion or accelerated maturation rates [20]. Therapeutic strategies promoting pre-beta HDL generation through apoA-I administration or LCAT inhibition warrant investigation.

Limitations

Several limitations merit consideration. First, the cross-sectional design precludes causal inference regarding the relationship between efflux impairment and coronary disease development. Prospective investigation is required to establish temporal relationships and predictive utility. Second, the single-center recruitment may limit generalizability to broader Nigerian or African populations with different genetic backgrounds and environmental exposures. Third, cholesterol efflux assays remain technically demanding and lack standardization across laboratories, potentially complicating direct comparison with published values from other centers. Fourth, the research did not assess intestinal cholesterol absorption or hepatic elimination pathways, which constitute downstream components of reverse cholesterol transport. Finally, concomitant medication effects on transporter expression could not be fully excluded despite statistical adjustment.

Conclusion

This research establishes that cholesterol efflux capacity is significantly impaired in Nigerian patients with coronary artery disease, with ABCA1 transporter expression deficits serving as primary molecular contributors. The functional

assessment of HDL provides cardiovascular risk information distinct from standard concentration measurements, supporting clinical development of efflux capacity assays. Pre-beta HDL particle depletion further characterizes the dysfunctional lipoprotein profile in affected individuals. Therapeutic strategies targeting reverse cholesterol transport enhancement represent promising approaches to cardiovascular risk reduction, particularly for patients with residual risk despite optimal LDL lowering. Nuclear receptor agonists and apoA-I-based therapies merit continued investigation. Future prospective research should establish whether efflux capacity improvement translates to clinical event reduction and whether functional HDL assessment improves risk stratification beyond conventional parameters ^[21].

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