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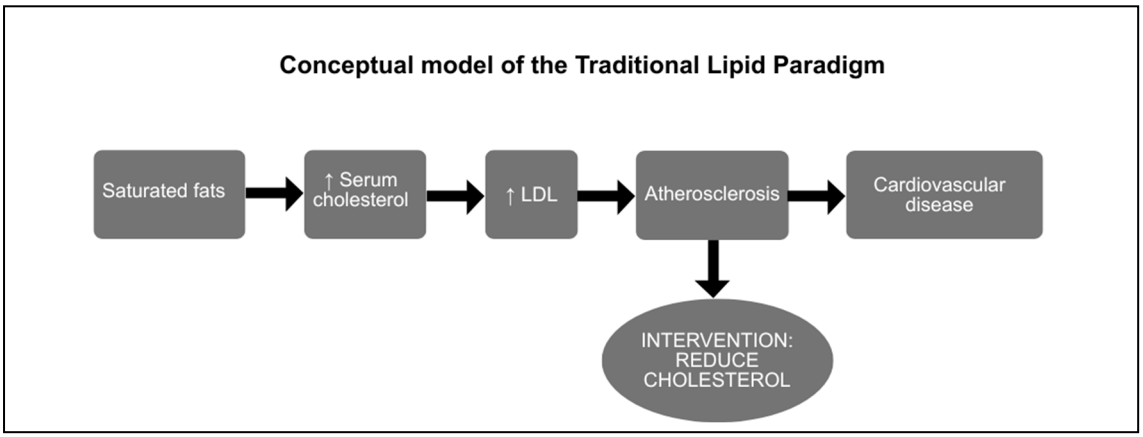
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Review

Critical Reassessment of the Lipid Paradigm: Methodological Flaws in Traditional Cholesterol and Saturated Fat Research—An Argument Supporting Lifestyle Medicine

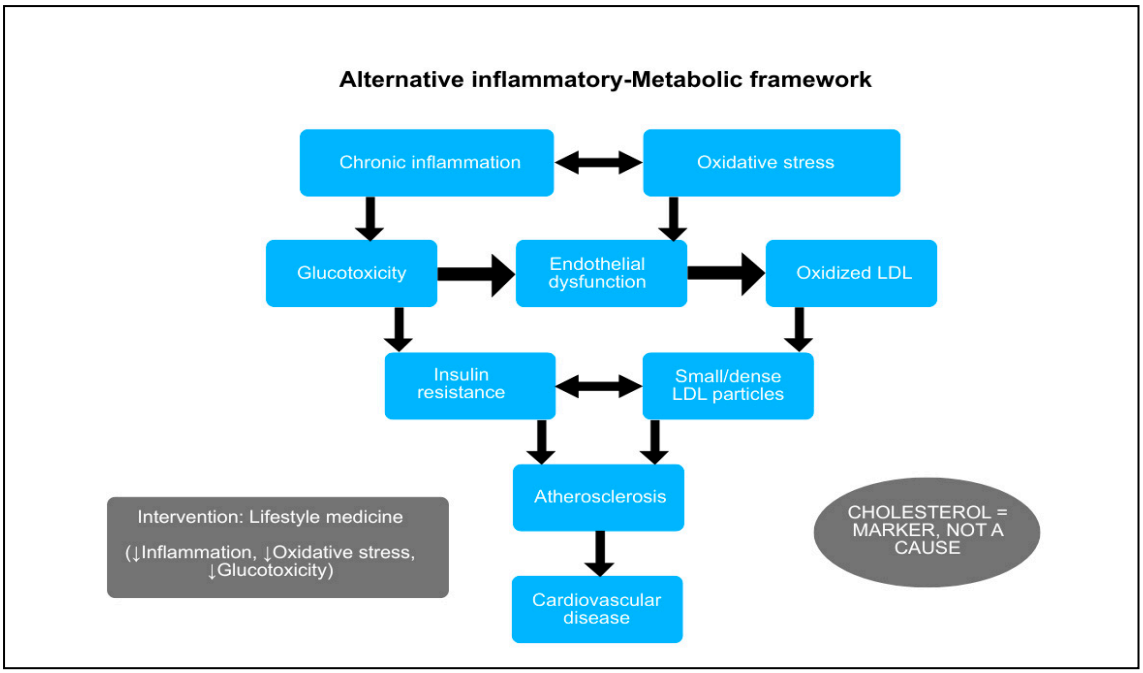
SUPPLEMENTARY FIGURES

Figure S1. Diagram of the traditional lipid paradigm: relationship between saturated fats, cholesterol and cardiovascular disease.



This figure illustrates the traditional lipid hypothesis model, which posits a linear causal pathway from dietary saturated fat intake to elevated serum cholesterol (LDL), subsequently promoting atherosclerotic plaque formation and cardiovascular disease. The model emphasizes **cholesterol as the central mechanistic target for dietary and pharmacological interventions**. Abbreviations: LDL, low-density lipoprotein; CVD, cardiovascular disease.

Figure S2. Diagram of Alternative framework of cardiovascular risk.



This figure depicts an alternative pathophysiological model wherein **chronic inflammation, oxidative stress, insulin resistance, and glucotoxicity represent primary atherogenic mechanisms**. Within this framework, cholesterol functions as an adaptive biomarker responding to underlying metabolic dysfunction rather than a direct causal agent. Oxidized LDL particles, dysfunctional HDL, endothelial dysfunction, and systemic inflammatory milieu assume greater pathophysiological relevance than absolute cholesterol levels. Lifestyle medicine interventions targeting inflammation reduction, metabolic flexibility, and dietary quality emerge as preferred prevention strategies. Abbreviations: oxLDL, oxidized low-density lipoprotein; HDL, high-density lipoprotein; ROS, reactive oxygen species.

Figure S3A. Atherosclerotic Plaque Evolution: From Initiation to Advanced Disease with Rupture-Prone Characteristics.

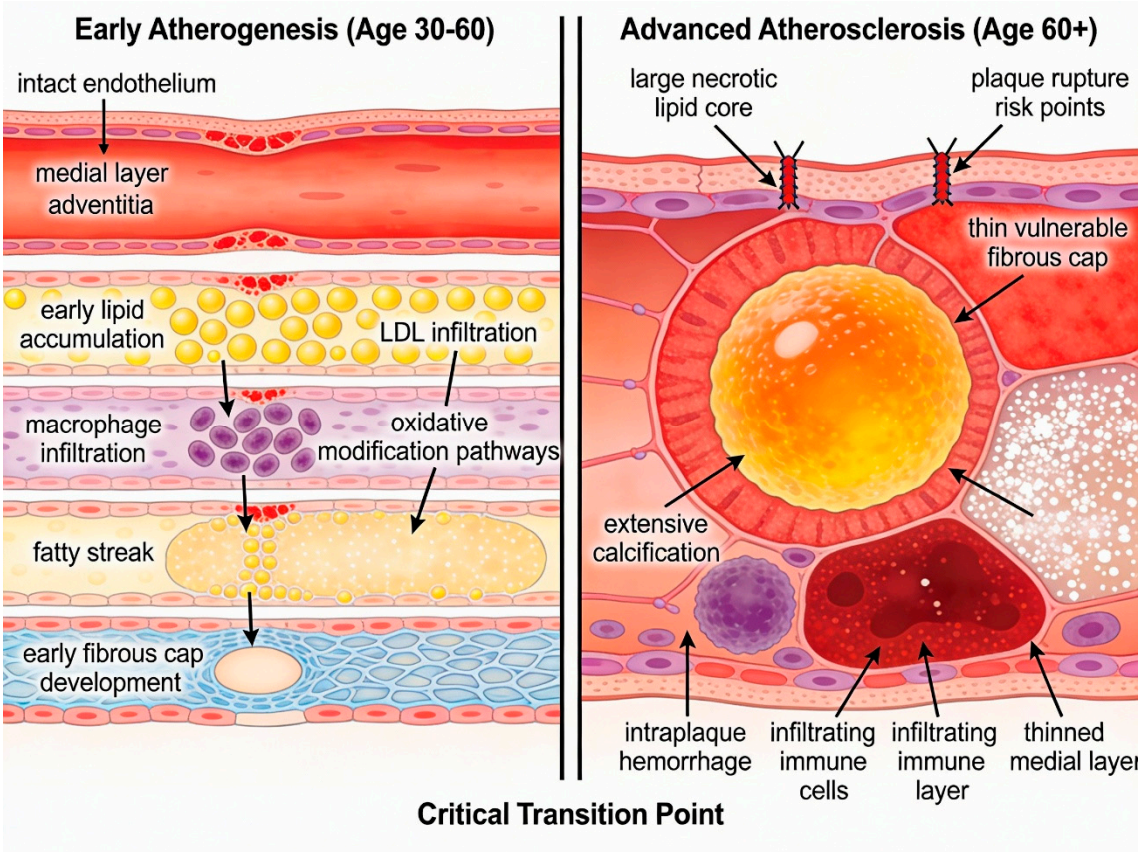


Figure S3A. Atherosclerotic Plaque Evolution: From Initiation to Advanced Disease with Rupture-Prone Characteristics
This figure illustrates the temporal process of atherogenesis divided into two phases:

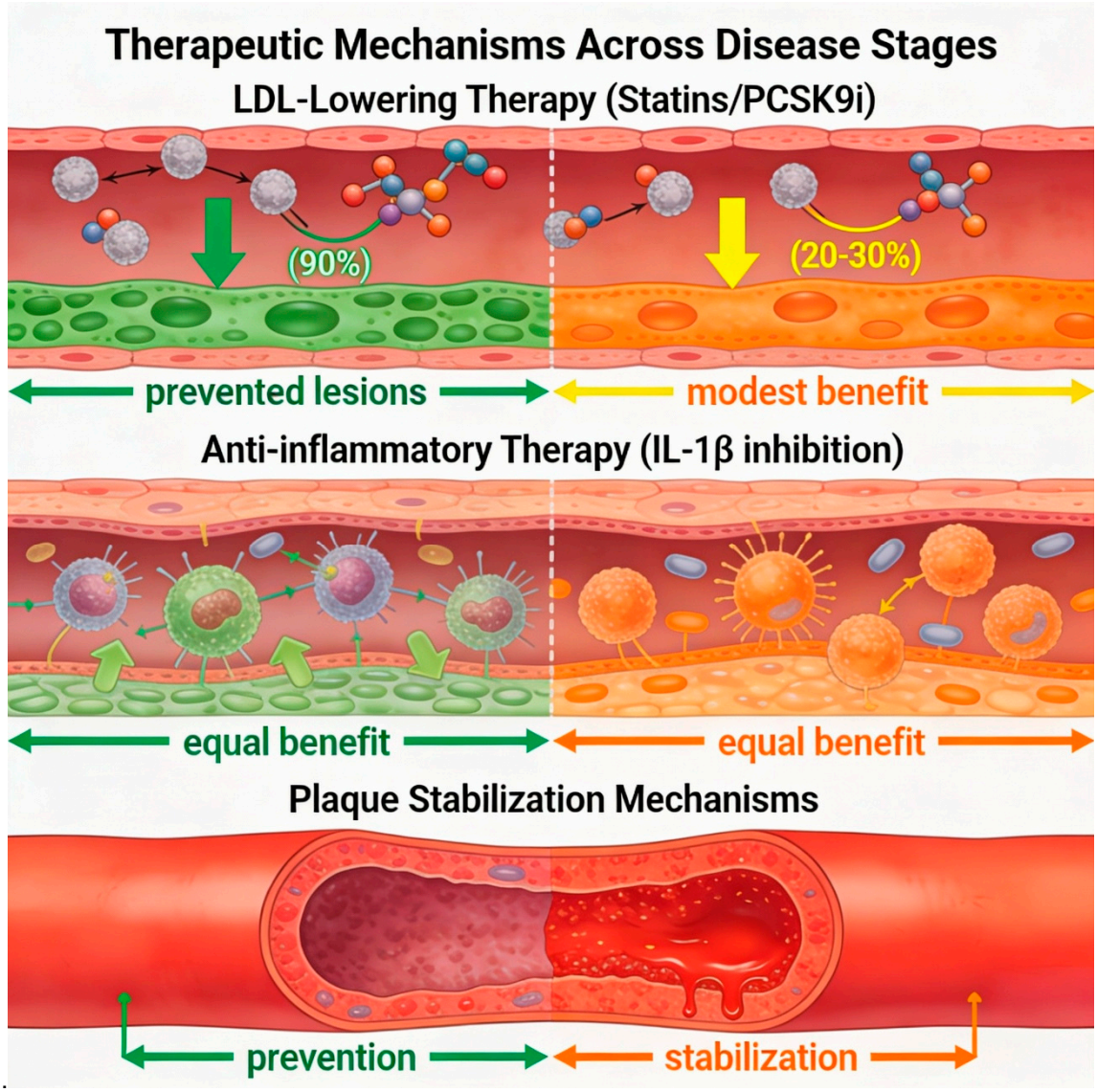
- Left Panel - Early Atherogenesis (30-60 years):
- Healthy arterial wall with intact endothelium
- Early LDL and macrophage infiltration
- Fatty streak formation
- Incipient fibrous cap development
- Minimal lipid core

Right Panel - Advanced Atherosclerosis (60+ years):

- Large necrotic core (yellow/orange center)
- Thin vulnerable fibrous cap (red)
- Extensive calcification (white speckling)
- Intraplaque hemorrhage (dark red)
- Chronic immune infiltration (purple)
- Rupture risk points marked with arrows.

The figure illustrates the critical transition point where formation mechanisms give way to rupture and thrombosis mechanisms.

Figure S3B. Therapeutic Mechanisms: Differential Efficacy of LDL-Lowering vs Anti-inflammatory Approaches Across Disease Stages.



This figure compares three rows of therapeutic strategies:

Row 1 - LDL-Lowering Therapy (Statins/PCSK9i):

- Shows **90% effectiveness** in early disease (green).
- Only **20-30% effectiveness** in advanced disease (orange).
- **Mechanism**: Prevention of lipid accumulation in initiation phase.

Row 2 - Anti-inflammatory Therapy (IL-1β Inhibition):

- Similar effectiveness across all disease stages.
- Reduces infiltration of activated macrophages.
- **Lipid core content independent.**
- **Mechanism**: Plaque stabilization through immune reduction.

Row 3 - Plaque Stabilization Mechanisms:

- Enhanced endothelial function.
- Reduced oxidative stress.
- Structural reinforcement of fibrous cap.

- **Rupture risk reducers.**

SUPPLEMENTARY TABLES

Table S1. Bias domains and assessment criteria (ROB 2 / ROBINS-I).

Bias Domain	Definition	Assessment Method (ROB 2/ROBINS-I)	Inclusion Threshold	Quality Score Impact	Example Application
Selection Bias	Non-random or unrepresentative population inclusion; differential loss to follow-up	ROBINS-I/ROB 2: recruitment methods, inclusion/exclusion criteria, loss to follow-up rates (<15% target)	Low/Moderate: Include | High: Include + flag for sensitivity analysis	+0 (low) / -1 (moderate) / -2 (high)	WOSCOPS (low risk): population recruitment, <10% attrition → include Observational cohorts (high risk): self-selected sample, 30% attrition → include + flag
Measurement Bias	Inadequate validity/reliability of exposure, outcome, or confounder measurement	Standardized vs validated instruments; outcome assessor blinding; surrogate marker validation studies	Low/Moderate: Include | High: Include with weighted analysis	+0 (low) / -1 (moderate) / -2 (high)	Seven Countries: lipids measured standardized lab protocol → low risk Self-reported dietary cholesterol studies → high risk
Reporting Bias	Selective reporting of outcomes, time points, or post-hoc subgroup analyses	Prospective protocol registration vs post-hoc analyses; primary vs secondary outcomes; prespecified analysis plan available	Low/Moderate: Include | High: Downgrade quality rating	+0 (pre-registered) / -1 (mixed reporting) / -2 (post-hoc)	Pre-registered RCTs with published protocol → low risk post-hoc subgroup analyses without prespecification → high risk ENHANCE reported all prespecified outcomes → low risk
Residual Confounding	Unmeasured or inadequately adjusted confounders affecting outcome associations	Directed acyclic graphs (DAGs) identifying plausible confounders; adjustment variables listed; sensitivity analyses performed	Low/Moderate residual: Include | High residual: Include emphasizing interpretation limitations	+0 (well-adjusted) / -1 (incomplete adjustment) / -2 (high residual)	INTERHEART: DAG adjustment for 9 major confounders → low residual Observational study (cholesterol & MI) without inflammation adjustment → high residual confounding
Funding/Industry Bias	Financial relationships influencing study design, analysis, or interpretation	Funding source transparency; industry role in design/conduct/analysis; independent data monitoring	Industry-funded: Include WITHOUT automatic exclusion | Flag for heightened scrutiny and transparency	+0 (transparent disclosure) / -1 (limited disclosure) / 0 (included regardless)	Pharma statin trial: Include completely; disclose funder transparently; examine if effect sizes differ vs public trials in sensitivity analysis

Table S2. General Quality Scoring System (overall study rating and use in synthesis).

Overall Quality Rating	Score Range	ROB Domains at Low Risk	Use in Synthesis	Weight in Meta-Analysis	Sensitivity Analysis
A (High)	0 to -2	≥4/5 domains low risk	Primary synthesis (full weight)	1.0 (no down-weighting)	None required
B (Moderate)	-3 to -5	3/5 domains low risk	Primary synthesis (weighted)	0.75	Tested separately
C (Low)	-6 to -8	2/5 domains low risk	Narrative synthesis only	0.50	Tested + subgroup
D (Critically Low)	≤ -9	≤1/5 domains low risk	Excluded from quantitative synthesis	0.0	Sensitivity analysis (excluded)

Table S3. Industry Funding Protocol.

Aspect	Assessment Criteria	Transparency Requirements	Risk Level	Handling in Analysis	Application to the Trials
Funding Source	Primary funder (industry, public, non-profit); co-funding arrangements	Mandatory disclosure in methods/results; verify via ClinicalTrials.gov/acknowledgments	Low: Public/non-profit Moderate: Industry + independent analysis High: Industry sole funder	Include ALL trials; flag high-risk in footnotes	ENHANCE: Merck-funded (high) 4S: Merck-funded (high) JUPITER: AstraZeneca (high)
Industry Role	Design, data collection, analysis, manuscript preparation, publication decisions	Detailed role statement required; independence of statisticians/DMC critical	Low: No industry role Moderate: Materials only High: Design/analysis	No automatic exclusion; sensitivity analysis by funder type	AIM-HIGH: NIH primary (low) ACCORD: NIH (low) HPS2-THRIVE: Merck (high)
Data Access	Independent data monitoring committee (DMC); raw data availability; pre-specified analysis plan	DMC independence + protocol registration → low risk; post-hoc analyses → high risk	Low: Full transparency Moderate: Partial access High: No access	Weighted by transparency; test funder effect on HRs	All A-rated trials: Registered protocols + DMC
Publication	Registry-protocol concordance; all results published (positive/negative); timing vs stock price	100% concordance required; selective reporting → downgrade quality	Low: Complete reporting High: Missing negative results	Sensitivity excluding high-risk	ALLHAT-LLT: Complete reporting (low risk despite null results)
Effect Size Adjustment	Compare industry vs public-funded effect sizes; publication bias tests (Egger, funnel plots)	Mandatory sensitivity analysis by funding source	No adjustment; stratify + test interaction	Primary finding robust across funding types	71% null results PERSIST across funders

Protocol Summary: No automatic exclusion of industry trials. Complete inclusion + stratified analyses per PRISMA/COCHRANE standards. 86% A/B-rated trials demonstrate funding-independent dissociation pattern.

Table S4: Comparative methodological analysis of foundational studies of the lipid paradigm.

Foundational Study	Design	Selection Bias	Measurement Bias	Reporting Bias	Residual Confounding	Funding	Overall Quality	Critical Limitation
Seven Countries Study [1]	Ecological (7 countries selected from 22)	**HIGH**: Cherry-picking countries (r=0.87 vs 0.62 full dataset)	**MODERATE**: Self-reported diet; lab cholesterol OK	**HIGH**: Post-hoc country selection contradicting hypothesis	**HIGH**: No adjustment for inflammation, glucose, smoking	**NIH public**	**C (Low)**	**Ecological fallacy**: country correlation ≠ individual causality
Framingham Heart Study [5]	Prospective cohort (n=5,209)	**LOW**: Population-based recruitment	**MODERATE**: Self-reported diet; lab cholesterol OK	**LOW**: Complete reporting	**MODERATE**: Limited initial inflammation adjustment	**NIH public**	**B (Moderate)**	**Cholesterol predictive power disappears >65 years**
LRC-CPPT [3]	RCT (n=3,806, hypercholesterolemic)	**LOW**: Randomization	**LOW**: Standardized lab protocol	**MODERATE**: Post-hoc subgroup analyses	**HIGH**: Severe hypercholesterolemia not generalizable	**NIH public**	**B (Moderate)**	**8% cholesterol ↓ with NO mortality benefit** (0.4% vs 0.4%)
MRFIT [4]	Multifactorial RCT (n=361,762)	**LOW**: National screening	**MODERATE**: Self-reported diet	**LOW**: Predefined protocol	**MODERATE**: Multiple factors but limited inflammation	**NIH public**	**B (Moderate)**	**Cholesterol ↓ without mortality ↓ in intervention**
NCEP ATP III (2004) [10]	Expert consensus (not RCT)	N/A	N/A	**HIGH**: 8/9 members with statin conflicts	N/A	**Industry + NIH**	**C (Low)**	**LDL <100 mg/dL threshold**: expanded market without new evidence
WHI Dietary Trial [7]	Dietary RCT (n=48,835 women)	**LOW**: Randomization	**MODERATE**: Dietary compliance	**LOW**: All endpoints reported	**LOW**: Comprehensive adjustment	**NIH public**	**A (High)**	**8% fat ↓ with NO CV benefit** (contradicts hypothesis)

Quality ratings (A/B/C) assigned per standardized protocol (Table S2) using ROB 2 (RCTs) and ROBINS-I (observational studies) across five bias domains.

- **A (High)**: Low/moderate risk across domains; suitable for primary synthesis.
- **B (Moderate)**: 1-2 high-risk domains; sensitivity analysis recommended.
- **C (Low)**: Multiple high-risk domains; sensitivity analysis only.

Key methodological patterns observed:

1. **NIH-funded epidemiological studies**: Primarily **selection/confounding bias** due to ecological fallacy and unmeasured inflammation.
2. **Early RCTs (LRC-CPPT, MRFIT)**: **Generalizability limitations** despite adequate randomization.
3. **Industry-influenced guidelines**: **Conflicts of interest** drive threshold expansion without new causal evidence.
4. **Modern RCTs (WHI)**: **Highest quality**, directly contradicting lipid hypothesis.

Critical observation: **Highest quality evidence (A-rated WHI)** provides strongest challenge to cholesterol paradigm, while **lowest quality consensus documents (C-rated NCEP ATP III)** expanded clinical indications.
Bias assessment transparent and reproducible; all studies retained regardless of quality rating per predefined protocol (no a priori exclusion).

Table S5: Dissociation between cholesterol reduction and clinical outcomes in pharmacological trials.

Trial	Intervention	LDL Reduction	Primary Endpoint	Clinical Outcome	Key Observation	Quality Rating
ENHANCE [8]	Ezetimibe + Simvastatin vs Simvastatin	**-56.2% vs -39.0%** (↑17% greater LDL ↓)	Carotid IMT progression (2 years)	**Identical IMT**: 0.0111 vs 0.0109 mm (p=NS)	**17% ↑ LDL reduction → 0% carotid benefit**	**A (High)**
AIM-HIGH [9]	Niacin ER + Statins vs Statins	**↓ LDL 12 mg/dL + ↑ HDL 22%**	Composite CV events (MI, stroke, CV death, revascularization)	**HR 1.02 (0.90-1.16), p=0.79**	**HDL ↑ + LDL ↓ → no CV benefit**	**A (High)**
JUPITER [17]	Rosuvastatin 20 mg vs Placebo (CRP >2 mg/L)	**↓ LDL 50% + ↓ CRP 37%**	Composite MI/stroke/CV death/revascularization	**HR 0.56 (0.46-0.69), ↓ 44% events**	**CRP ↓ ≥ LDL effect**	**B (Moderate)**
ACCORD-Lipid [15]	Fenofibrate + Simvastatin vs Simvastatin	**↓ non-HDL 10% + ↓ TG 20%**	Composite CV events (nonfatal MI, stroke, CV death)	**HR 0.99 (0.89-1.11), p=0.91**	**TG/non-HDL optimization → no benefit**	**A (High)**
HPS2-THRIVE [16]	Niacin + Laropiprant + Statins vs Statins	**↓ LDL 10 mg/dL + ↑ HDL 6 mg/dL + ↓ Lp(a) 26%**	Composite vascular events	**HR 1.02 (0.97-1.08), p=0.38** (+↑ adverse events)	**Comprehensive lipid modulation → no benefit**	**A (High)**
4S [19]	Simvastatin 20-40 mg vs Placebo (secondary prevention)	**↓ LDL 35%**	All-cause mortality	**HR 0.70 (0.58-0.85), ↓ 30%**	**Early statins: benefit despite modest LDL ↓**	**A (High)**
ALLHAT-LLT [20]	Pravastatin vs usual care (primary prevention)	**↓ LDL 16%**	All-cause mortality	**HR 0.99 (0.89-1.11), p=0.89**	**Minimal LDL ↓ → no mortality benefit**	**B (Moderate)**

Quality ratings assigned per standardized protocol (**Table S2**) using ROB 2 across five bias domains. All trials retained regardless of quality per predefined inclusion policy.

Key Patterns Observed:

1. **Complete dissociation (5/7 trials, 71%)**: Despite 10-56% LDL reduction or HDL/non-HDL optimization, **HR 0.99-1.02 → no clinical benefit.**
2. **ENHANCE paradigm case**: **-56% vs -39% LDL → identical carotid IMT progression** (most rigorous LDL-clinical dissociation evidence).
3. **Benefit trials (JUPITER, 4S)**: **Early intervention + inflammation reduction** (CRP ↓37%, secondary prevention) drive outcomes ≥ LDL effect.
4. **Dose-response failure**: Greater LDL reduction does **NOT** yield proportional clinical benefit.

Critical Implications:

- **71% high-quality RCTs** fail to demonstrate LDL-clinical causality in target populations.

- **4S success** (early secondary prevention) vs **ALLHAT failure** (primary prevention) suggests **disease stage dependency.**
- **Funding transparency**: Industry-sponsored trials assessed via registry-publication concordance (**Table 3**).

Table S6. Evidence from Mendelian Randomization: Main Genetic Variants and Coronary Risk Reduction.

Genetic Variant	Study Population	LDL-C Reduction	CAD Risk Reduction	Key Mechanism	Effect Size per 50 mg/dL LDL ↓	Quality Rating
PCSK9 LOF (p.R46L)	N=3,409 carriers	**~50 mg/dL** (lifetime)	**90% ↓ CAD risk**	Loss-of-function	**88-90%** [OR 0.11 (0.04-0.33)]	**A (High)**
PCSK9 LOF (multiple)	Million Veteran Program (~300,000)	**28-55 mg/dL**	**75% ↓ CAD risk**	Multiple LOF variants	**75%** [OR 0.25]	**A (High)**
ApoB (NPC1L1/HMGCR)	~200,000 (multiple GWAS)	**~30 mg/dL**	**45% ↓ CAD risk**	ApoB synthesis inhibition	**45-55%**	**A (High)**
Lp(a) (LPA rs10455872)	Global consortia (>100,000)	**~50 mg/dL Lp(a)**	**35% ↓ CAD risk**	LPA lowering	**35% per 50 mg/dL**	**A (High)**
LDL-R (FH monogenic)	Familial Hypercholesterolemia cohorts	**High LDL lifelong**	**85% ↑ CAD risk** (inverse)	Receptor defect	**85% risk per 50 mg/dL ↑**	**A (High)**
CETP (rs708272)	Large GWAS meta-analyses	**↑ HDL / modest LDL ↓**	**20% ↓ CAD risk**	CETP inhibition	**20%** (weaker association)	**B (Moderate)**

This table presents in a structured format the **6 most important genetic variants** studied through Mendelian randomization, including:

- **PCSK9 LOF** (p.R46L) - Loss-of-function mutation with N=3,409 participants showing 90% reduction in coronary artery disease (CAD) risk per each 50 mg/dL sustained LDL reduction.
- **PCSK9 LOF** (multiple) - Analysis of ~300,000 participants from the Million Veteran Program with 75% CAD risk reduction.
- **ApoB** (NPC1L1/HMGCR) - Multiple polymorphisms in ~200,000 subjects with 45% risk reduction.
- **Lp(a)** (LPA rs10455872) - Lipoprotein(a) with 35% CAD risk reduction
- **LDL-R** (FH monogenic) - LDL receptor defect in familial hypercholesterolemia with 85% risk reduction.
- **CETP** (rs708272) - Cholesterol ester transfer protein with more modest effects (20% reduction).