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Cardiovascular Reviews

Focus and Scope

Cardiovascular Reviews publishes peer-reviewed research articles across basic, translational, and clinical cardiovascular medicine. The journal aims to enhance insight into cardiovascular disease mechanisms and the prospects for innovation. The Journal covers all topics within cardiology and cardiovascular biology with an emphasis on studies that challenge the status quo of treatments, at the molecular, sub-cellular, cellular, organ, and organism level, and of clinical proof-of-concept and translational studies and practices in cardiovascular care or facilitate the translation of scientific advances into the clinic as new therapies or diagnostic tools. Manuscripts are expected to provide a significant contribution to the field with relevance for cardiovascular biology and diseases.

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The Impact Analysis of Different Lipid-Lowering Regimens and Major Adverse Cardiovascular Events in ACS Patients after PCI

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Abstract: *Background and Objective:* Intensive lipid-lowering therapy serves as a core intervention to improve the long-term prognosis of patients with acute coronary syndrome (ACS) after percutaneous coronary intervention (PCI). Currently, evidence-based clarification is still required for the efficacy discrepancies and applicable populations of three first-line lipid-lowering regimens: high-intensity statin monotherapy, statin plus ezetimibe, and statin plus proprotein convertase subtilisin/kexin type 9 inhibitor (PCSK9i). This study aimed to systematically evaluate the impacts of these three regimens on major adverse cardiovascular events (MACE) in patients with ACS after PCI. *Methods:* We collected relevant randomized controlled trials (RCTs) and high-quality cohort studies on the three first-line regimens published from 2016 to 2022 worldwide. A random-effects model was adopted to analyze the risk of MACE in post-PCI ACS patients, and the therapeutic effects and sources of heterogeneity across different regimens were assessed. *Results:* Thirty-four studies with a total of 50,537 patients were finally included. The baseline characteristics were generally comparable between groups, and minor inter-group differences did not affect the analysis. (1) High-intensity statin significantly reduced the risk of MACE (RR = 0.52, 95% CI [0.37, 0.72], $p < 0.0001$), with pronounced benefits in Asian populations but no significant benefit in Western populations. (2) Statin combined with ezetimibe lowered the MACE risk (RR = 0.59, 95% CI [0.40, 0.87], $p = 0.007$), and the benefit was more significant in Asian populations. (3) Statin combined with PCSK9i reduced the MACE risk (RR = 0.80, 95% CI [0.72, 0.89], $p < 0.0001$) with low heterogeneity, and the benefit was more prominent in Asian populations and on the basis of moderate-intensity statin. Only the high-intensity statin group presented a positive Egger test, which was a false positive driven by heterogeneity, and the pooled results were stable. *Conclusion:* All three intensive lipid-lowering regimens can reduce the risk of MACE in post-PCI ACS patients, with more significant benefits observed in Asian populations. Statin combined with PCSK9i shows a stable risk-reduction effect.

Keywords: Acute coronary syndrome; Percutaneous coronary intervention; Lipid-lowering regimen; Major adverse cardiovascular events

1. Introduction

Acute coronary syndrome (ACS) is an acute cardiovascular event caused by the rupture/erosion of unstable atherosclerotic plaques in the coronary arteries, leading to subsequent coronary thrombosis and myocardial ischemia ^[1,2]. With the widespread adoption of percutaneous coronary intervention (PCI) treatment, the mortality rate during the acute phase of ACS patients has significantly decreased. However, the high incidence of major adverse cardiovascular events (MACE) after surgery remains a core issue affecting long-term prognosis.

Multiple studies have confirmed a clear dose-response relationship between serum low-density lipoprotein cholesterol (LDL-C) and atherosclerotic events ^[3]. Therefore, early and sufficient reduction of LDL-C in ACS-PCI patients remains the cornerstone of secondary prevention ^[1,4]. Current guidelines recommend intensive lipid-lowering therapy based on statins, with common strategies including high-intensity statins, moderate-intensity statins combined with cholesterol absorption inhibitors, or combined with PCSK9 inhibitors (PCSK9i) ^[5]. However, differences in the effectiveness of various regimens in improving prognosis and differences in real-world adherence significantly impact regimen selection ^[3].

Therefore, this study focuses on three first-line initial intensive lipid-lowering regimens currently prioritized by clinical guidelines and supported by the most robust evidence (high-intensity statins, statins combined with cholesterol absorption inhibitors, and statins combined with PCSK9i). It systematically compares their effects on postoperative MACE in ACS-PCI patients through a meta-analysis. The determination of statin intensity and dosage strictly adheres to the definitions outlined in the Chinese Guidelines for Lipid Management (2023) ^[6].

2. Materials and methods

2.1. Inclusion criteria (All must be met for inclusion)

The inclusion criteria are as follows:

- (1) Study population: ACS patients who have undergone PCI and meet the diagnostic criteria for ACS (referencing the 2023 ESC Guidelines for Acute Coronary Syndromes ^[2]);
- (2) Interventions: High-intensity statins, statins combined with cholesterol absorption inhibitors, statins combined with PCSK9i;
- (3) Language: Chinese or English;
- (4) Outcome measures: Clear definition of MACE and the number of events reported;
- (5) Study type: Randomized controlled trials (RCTs) or high-quality cohort studies;
- (6) Follow-up duration: Not limited, but must be clearly reported for subgroup analysis.

2.2. Exclusion criteria (Exclusion if anyone is met)

The exclusion criteria are as follows:

- (1) Study population does not meet requirements (including stable coronary artery disease patients with no separate ACS data extraction or patients not undergoing PCI);

- (2) Failure to report clinical prognosis indicators such as MACE;
- (3) Extremely low-quality literature (sample size < 50 cases, no control group, chaotic data reporting);
- (4) Low-quality secondary research or reviews without original data;
- (5) Non-clinical secondary research without original data.

2.3. Search strategy

Literature was searched on Wanfang Medical Network, PubMed, CNKI, and Web of Science from January 2016 to December 2022. Chinese search terms included: “acute coronary syndrome,” “acute myocardial infarction,” “acute ST-segment elevation myocardial infarction,” “acute non-ST-segment elevation myocardial infarction,” “unstable angina,” “percutaneous coronary intervention,” “statins,” “ezetimibe,” “hybutimibe,” “PCSK9i,” “cholesterol absorption inhibitors,” etc.

English search terms included: “Acute Coronary Syndrome,” “Acute Myocardial Infarction,” “ST Elevation Myocardial Infarction,” “Non-ST Elevated Myocardial Infarction,” “Angina, Unstable,” “Percutaneous Coronary Intervention,” “Angioplasty, Balloon, Coronary,” “Hydroxymethylglutaryl-CoA Reductase Inhibitors,” “Ezetimibe,” “Hybutimibe,” “PCSK9 Inhibitors,” “Prognosis,” “Treatment Outcome,” “Cardiovascular Diseases,” “Patient Safety,” “Cholesterol Absorption Inhibitor.” Search terms were flexibly replaced and combined according to the characteristics of each database.

2.4. Literature screening and quality assessment

Two researchers independently screened the retrieved literature based on inclusion and exclusion criteria, initially reading titles and abstracts for preliminary screening, and then reading the full text of potentially eligible studies to decide on inclusion. Disagreements were resolved through discussion between the two researchers or by a third researcher. RCTs were assessed for bias risk using the RoB 2.0 tool recommended by the Cochrane Collaboration, while cohort studies were evaluated for methodological quality using the NOS ^[7,8].

2.5. Data extraction

The following data were extracted from the literature: study name, total sample size (intervention group/control group), intervention group/control group regimens, follow-up duration, definition of MACE events, baseline gender ratio, baseline age, and baseline LDL-C levels. For studies with multiple intervention groups or control groups (multi-arm studies), corresponding analysis cohorts were included separately according to different lipid-lowering regimens to avoid duplication.

2.6. Statistical analysis methods

Meta-analysis was performed using RevMan 5.3 software and Rstudio 2025. The outcome measure MACE, a binary variable, was analyzed using risk ratio (RR) and 95% confidence interval (CI) as effect analysis statistics. A Mantel-Haenszel (M-H) random-effects model was used for pooled analysis, with I^2 values < 25%, 25%–75%, and >75% indicating low, moderate, and high heterogeneity, respectively. Sensitivity analysis was conducted by excluding studies one by one, and subgroup analysis was performed based on background statin intensity, geographic population, and follow-up duration. Publication bias was assessed using funnel plots combined with Egger’s linear regression test. For multi-arm studies sharing the

same control group, the control group sample size and event count were split proportionally according to the number of comparison groups, as recommended by Cochrane, to avoid duplicate inclusion of the same control group; this treatment did not alter the total sample size or event count and had a negligible impact on pooled effect estimates.

3. Results

3.1. Literature search results and basic information of included studies

A total of 2,345 articles were retrieved according to the search strategy, including 533 from Wanfang Medonline, 1,345 from CNKI, 355 from PubMed, and 112 from Web of Science. The detailed screening process is shown in **Figure 1**. A total of 34 studies were finally included^[9-42], of which 31 were RCTs^[9-16,18-23,25-28,30-42] and 3 were cohort studies^[17,24,29]. Due to insufficient numbers of relevant studies and high data heterogeneity, regimens such as cholesterol absorption inhibitors combined with PCSK9i, statins combined with Hybutimibe, and statins combined with cholesterol absorption inhibitors plus PCSK9i (triple therapy) were not included. A total of 50,537 patients were enrolled, including 24,818 in the experimental groups and 25,719 in the control groups. The shared control groups from multi-arm studies (Ran_2017^[13], Yuan_2018^[16], Xinyu Feng_2019^[34]) were split according to prespecified methods in all subsequent analyses^[13,16,32]. The basic characteristics of the included studies are shown in **Table 1**.

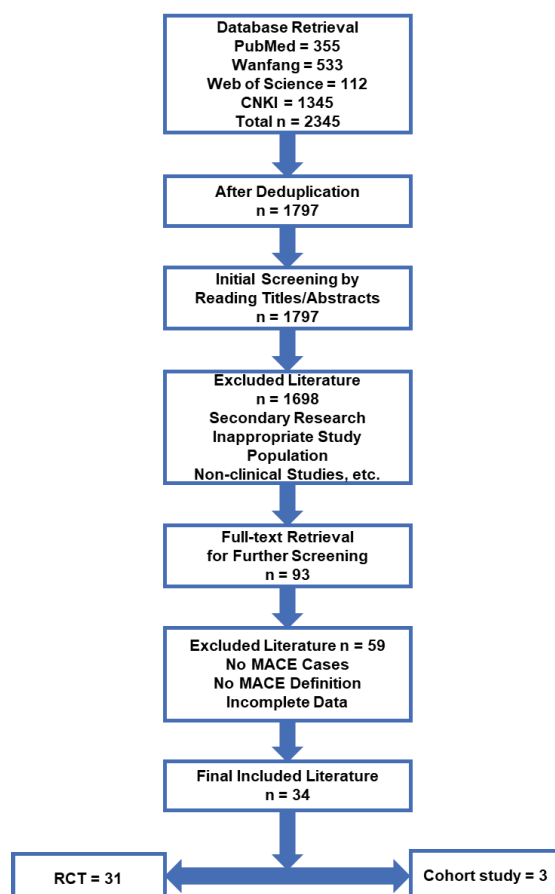


Figure 1. PRISMA flow diagram.

Table 1. Basic characteristics of included studies

Included Study	Total Sample Size (Intervention/Control)	Intervention Group Regimen	Control Group Regimen	MACE Definition	Follow- up Duration
Berwanger_2018 ^[25]	4191 (2087/2104)	Atorvastatin 80 mg/d	Atorvastatin 40 mg/d	①②③④	12 months
Guo_2017 ^[14]	92 (47/45)	Rosuvastatin 20 mg/d	Rosuvastatin 10 mg/d	①②④	12 months
He_2020 ^[9]	192 (97/95)	Atorvastatin 80 mg/d, Rosuvastatin 20 mg/d, Simvastatin 40 mg/d	Atorvastatin 40 mg/d, Rosuvastatin 10 mg/d, Simvastatin 20 mg/d	①③④ ⑤⑩	6 months
Im_2018 ^[10]	2000 (1000/1000)	Atorvastatin 40 mg/d	Atorvastatin 20 mg/d	①②③④ ⑧⑨⑩	12 months
Li_2017 ^[15]	120 (60/60)	High-intensity Atorvastatin	Moderate-intensity Atorvastatin	①②⑤⑥	12 months
LiuQ_2019 ^[11]	265 (133/132)	Atorvastatin 40 mg/d	Atorvastatin 20 mg/d	①②④	12 months
LiuZ_2016 ^[12]	798 (400/398)	High-intensity Atorvastatin	Moderate-intensity Atorvastatin	①②④	12 months
Yuan_2018 ^{[16] a}	88 (41/47b)	High-intensity Statin	Moderate-intensity Statin	①②③ ④⑤	12 months
Ran_2017 ^{[13] a}	83 (41/42b)	High-intensity Rosuvastatin	Moderate-intensity Rosuvastatin	①②③ ④⑤	12 weeks
Furtado_2022 ^[18]	17073 (8510/8563)	Evolocumab + High- intensity Statin	High-intensity Statin	①②③ ④⑤	2.2 years
Koskinas_2019 ^[19]	308 (155/153)	Evolocumab + High- intensity Atorvastatin	High-intensity Atorvastatin	①②③ ④⑦⑩	8 weeks
Leucker_2020 ^[20]	57 (30/27)	Evolocumab + High- intensity Statin	High-intensity Statin	①②③⑤	30 days
Mehta_2022 ^[21]	68 (38/30)	Alirocumab + High- intensity Statin	High-intensity Statin	①②③⑦	6 weeks
Okada_2022 ^[22]	102 (52/50); final analysis 98 (49/49)	Evolocumab + Moderate-intensity Pitavastatin	Moderate-intensity Pitavastatin	①	4 weeks
Räber_2022 ^[23]	300 (148/152); final analysis 265 (130/135)	Alirocumab + High- intensity Rosuvastatin	High-intensity Rosuvastatin	④	52 weeks
Schwartz_2018 ^[30]	18924 (9462/9462)	Alirocumab + High- intensity Statin	High-intensity Statin	①②③⑤	Median 2.8 years
Xu_2021 ^[17]	334 (96/238)	Evolocumab + Moderate-intensity Statin	Moderate-intensity Statin	②④	12 weeks
Zhang_2022 ^[24]	1564 (414/1150)	Evolocumab + Moderate-intensity Statin	Moderate-intensity Statin	①②③④⑤	18 months
Yuan_2018 ^{[16] a}	85 (38/47b)	Moderate-intensity Statin + Ezetimibe	Moderate-intensity Statin	①②③④⑤	12 months
Sun_2021 ^[27]	171 (81/90)	Rosuvastatin 20 mg/d + Ezetimibe	Rosuvastatin 20 mg/d	①②③④⑤	3 months
Dai_2017 ^[29]	173 (55/115)	Moderate-intensity Statin + Ezetimibe	Moderate-intensity Statin	①②③④	6 months
Deng_2021 ^[26]	90 (42/48)	Moderate-intensity Atorvastatin + Ezetimibe	Moderate-intensity Atorvastatin	②④⑤	12 months
Hagiwara_2017 ^[31]	1734 (864/857)	Moderate-intensity Pitavastatin + Ezetimibe	Moderate-intensity Pitavastatin	①②③④⑤	Median 3.86 years
Hibi_2018 ^[28]	128 (50/53)	Moderate-intensity Pitavastatin + Ezetimibe	Moderate-intensity Pitavastatin	①②④	10 months

Jianhua Li_2022 ^[32]	76 (38/38)	Atorvastatin 40 mg/d	Atorvastatin 20 mg/d	②④	1 month
Jinghua Wei_2020 ^[33]	200 (100/100)	Atorvastatin 40 mg/d	Atorvastatin 20 mg/d	①②⑤⑦	1 month
Xinyu Feng_2019 ^{[34] a}	70 (35/35b)	Rosuvastatin 20 mg/d	Rosuvastatin 10 mg/d	①②③④⑤	6 months
Yi Jin_2019 ^[35]	100 (50/50)	Atorvastatin 40 mg/d	Atorvastatin 20 mg/d	①②⑦	1 month
Honghe Liu_2020 ^[36]	80 (40/40)	Atorvastatin 40 mg/d	Atorvastatin 20 mg/d	①②④⑩	1 month
Zhixian Chen_2020 ^[40]	120 (60/60)	Rosuvastatin 10 mg/d + Ezetimibe	Rosuvastatin 10 mg/d	②④⑤	12 months
Chuanxi Zhang_2022 ^[37]	70 (35/35)	Rosuvastatin 10 mg/d + Ezetimibe	Rosuvastatin 10 mg/d	②⑤⑥⑦	12 months
Xinyu Feng_2019 ^{[34] a}	69 (34/35b)	Rosuvastatin 10 mg/d + Ezetimibe	Rosuvastatin 10 mg/d	①②③④⑤	6 months
Jingjing Cai_2022 ^[38]	500 (250/250)	Evolocumab + Moderate-intensity Statin	Moderate-intensity Statin	①②④⑤⑦	12 weeks
Juan Zhao_2022 ^[41]	120 (60/60); final analysis 115 (57/58)	Alirocumab + Rosuvastatin 10 mg/d	Rosuvastatin 10 mg/d	①②④	6 months
Liuqing Yang_2022 ^[39]	207 (103/104); final analysis 198 (98/100)	Evolocumab + Atorvastatin 20 mg/d	Atorvastatin 20 mg/d	①②④	10 months
Hua Yang_2020 ^[42]	65 (32/33)	Evolocumab + Atorvastatin 20 mg/d	Atorvastatin 20 mg/d	①②④⑦	6 months
Ran_2017 ^{[13] a}	84 (42/42b)	Ezetimibe + Moderate-intensity Rosuvastatin	Moderate-intensity Rosuvastatin	①②③⑤⑦	12 weeks

Note: In the definition of MACE, ① cardiovascular death, ② non-fatal myocardial infarction, ③ non-fatal stroke/TIA, ④ unplanned coronary revascularization, ⑤ recurrent angina, ⑥ malignant arrhythmia, ⑦ heart failure, ⑧ renal function deterioration, ⑨ peripheral artery intervention, and ⑩ rehospitalization for cardiac events.

High-intensity statins: Atorvastatin 40-80 mg; Rosuvastatin 20 mg.

Moderate-intensity statins: Atorvastatin 10-20 mg; Rosuvastatin 5-10 mg; Pitavastatin 1-4 mg.

a indicates that the study is a multi-arm study, with different interventions compared separately as independent analysis units included in the meta-analysis, and the shared control group has been split according to the preset method; b indicates the sample size of the shared control group in the multi-arm study.

3.2. Quality assessment of included studies

Among the 34 included studies, there are 31 RCTs and 3 cohort studies ^[9-42]. Among the RCTs, a few studies have uncertain risks in terms of random sequence generation, outcome measurement, or completeness of reporting, but the overall risk of bias is low. The quality assessments of the cohort studies all scored above 7 points, indicating a generally high quality of the literature (**Table 2** and **Table 3**).

Table 2. Risk of bias in included RCTs

Study	Randomization process	Deviations from intended intervention	Missing outcome data	Outcome measurement	Reporting bias	Overall risk of bias
Berwanger_2018 ^[25]	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Guo_2017 ^[14]	Some concerns	Some concerns	Some concerns	Some concerns	Some concerns	Some concerns
He_2020 ^[9]	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Im_2018 ^[10]	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Li_2017 ^[15]	Some concerns	Low risk	Low risk	Some concerns	Some concerns	Some concerns
LiuQ_2019 ^[11]	Some concerns	Low risk	Low risk	Some concerns	Some concerns	Some concerns
LiuZ_2016 ^[12]	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Furtado_2022 ^[18]	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Koskinas_2019 ^[19]	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Leucker_2020 ^[20]	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Mehta_2022 ^[21]	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Okada_2022 ^[22]	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Räber_2022 ^[23]	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Schwartz_2018 ^[30]	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Yuan_2018 ^[16]	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Sun_2021 ^[27]	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Deng_2021 ^[26]	Low risk	Low risk	Low risk	Some concerns	Some concerns	Some concerns
Hagiwara_2017 ^[31]	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Hibi_2018 ^[28]	Low risk	Low risk	Low risk	Some concerns	Some concerns	Some concerns
Jianhua Li_2022 ^[32]	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Jinghua Wei_2020 ^[33]	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Xinyu Feng_2019 ^[34]	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Yi Jin_2019 ^[35]	Some concerns	Low risk	Low risk	Low risk	Low risk	Some concerns
Honghe Liu_2020 ^[36]	Some concerns	Low risk	Low risk	Low risk	Low risk	Some concerns
Zhixian Chen_2020 ^[40]	Low risk	Some concerns	Low risk	Low risk	Low risk	Low risk
Chuanxi Zhang_2022 ^[37]	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Jingjing Cai_2022 ^[38]	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Juan Zhao_2022 ^[41]	Low risk	Some concerns	Low risk	Low risk	Low risk	Some concerns
Liuqing Yang_2022 ^[39]	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Hua Yang_2020 ^[42]	Low risk	Some concerns	Low risk	Low risk	Low risk	Some concerns
Ran_2017 ^[13]	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk

Table 3. Risk of bias in included cohort studies

Study ID	Study Population Selection (0-4 points)	Comparability (0-2 points)	Exposure/Outcome Measurement (0-3 points)	Total Score (0-9 points)	Quality Grade (≥ 7 = High Quality)
Xu_2021 ^[17]	4 points	2 points	2 points	8 points	High quality
Zhang_2022 ^[24]	3 points	2 points	2 points	7 points	High quality
Dai_2017 ^[29]	4 points	2 points	2 points	8 points	High quality

3.3. Meta-analysis

3.3.1. High-intensity statin therapy reduces the incidence of MACE in ACS patients after PCI

A total of 14 studies were included, with comparable baseline characteristics between groups (all $p > 0.05$), as detailed in **Table 4** ^[9–16,25,32–36]. Analysis using the Mantel-Haenszel (M-H) random-effects model (**Figure 2**) revealed that, compared to moderate-intensity statin therapy, high-intensity statin treatment significantly reduced the risk of MACE in ACS patients undergoing PCI (RR = 0.52, 95% CI [0.37, 0.72], $p < 0.0001$), although moderate heterogeneity was observed ($I^2 = 70\%$, $p < 0.0001$).

After excluding each study one by one, the pooled RR remained stable between 0.47 and 0.57 (all $p < 0.05$), indicating robust results (**Table 5**). After excluding the study by Berwanger_2018, the heterogeneity I^2 decreased to 42%, suggesting that this study may be the primary source of heterogeneity ^[25]. However, the pooled effect remained stable, supporting the conclusion of the benefits of high-intensity statin therapy in ACS patients undergoing PCI.

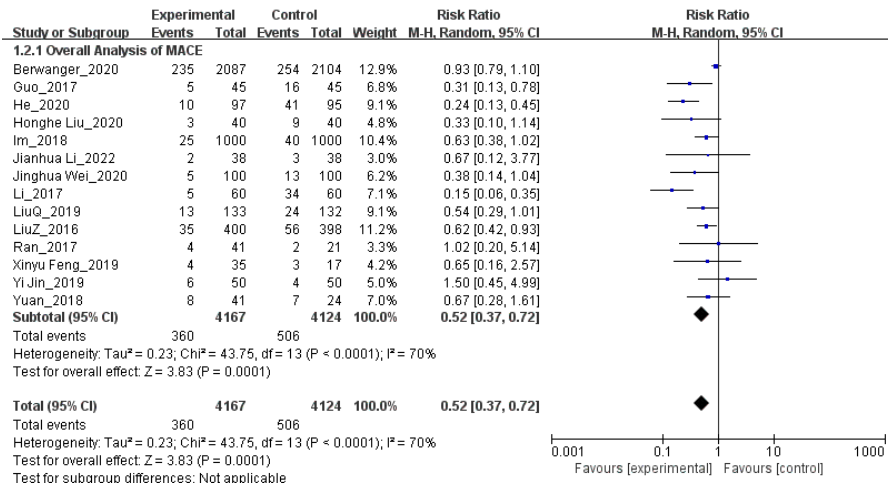


Figure 2. Forest plot of the impact of high-intensity statin therapy on major adverse cardiovascular events (MACE) in ACS patients after PCI. Note: The analysis was conducted using a random-effects model.

Table 4. Baseline characteristics table of the included studies

Included Study	Age (years) ¹		Male (%)				Inter-group Comparability
	Intervention	Control	Intervention	Control	Intervention	Control	
Berwanger_2018 ^[25]	61.7 (11.3)	61.9 (11.7)	1581/2031 (75.8)	1525/2104 (72.5)	2.87 (1.10)	2.84 (1.06)	All $p > 0.05$
Guo_2017 ^[14]	57.8 (6.4)	62.3 (3.7)	20/47 (42.56)	26/45 (57.78)	Not reported	Not reported	LDL-C not reported; all other variables $p > 0.05$
He_2020 ^[9]	67.28 (7.51)	69.06 (7.81)	49/97 (50.52)	44/95 (46.32)	3.11 (1.4)	3.13 (1.4)	All $p > 0.05$

Honghe Liu_2020 ^[36]	Not reported	Not reported	21/40 (52.50)	22/40 (55.00)	4.11 (0.72)	4.07 (0.84)	Described in text: “No difference in age between groups”; all other variables $p > 0.05$
Im_2018 ^[10]	64 (12)	64 (12)	714/1000 (71.4)	701/1000 (70.1)	3.08 (1.16)	3.09 (1.12)	All $p > 0.05$
Jianhua Li_2022 ^[32]	48.75 (4.38)	48.57 (4.26)	20/38 (52.63)	19/38 (50)	4.66 (1.28)	4.65 (1.22)	All $p > 0.05$
Jinghua Wei_2020 ^[33]	60.03 (6.74)	58.76 (6.69)	58/100 (58)	55/100 (55)	3.50 (0.32)	3.49 (0.30)	All $p > 0.05$
Li_2017 ^[15]	61.65 (10.32)	61.68 (10.28)	32/60 (53.33)	33/60 (55)	2.74 (0.75)	2.75 (0.77)	All $p > 0.05$
LiuQ_2019 ^[11]	58.4 (15.7)	60.5 (16.1)	97/133 (72.9)	94/132 (71.2)	3.8 (1.1)	3.7 (0.9)	All $p > 0.05$
LiuZ_2016 ^[12]	61.8 (10.1)	62.5 (11.2)	293/400 (73.3)	284/398 (71.4)	3.4 (0.9)	3.4 (1.1)	All $p > 0.05$
Ran_2017 ^[13] *	60.5 (10.0)	60.6 (6.7)	30/41 (73.2)	31/42 (73.8)	3.65 (0.91)	3.65 (0.85)	All $p > 0.05$
Xinyu Feng_2019 ^[34] *	61 (8)	61 (8)	28/35 (80)	26/35 (74)	2.19 (0.78)	2.19 (0.78)	All $p > 0.05$
Yi Jin_2019 ^[35]	55.9 (9.1)	56.3 (8.7)	29/50 (58)	31/50 (62)	2.97 (0.46)	2.96 (0.48)	All $p > 0.05$
Yuan_2018 ^[16] *	Not reported	Not reported	Not reported	Not reported	3.30 (0.88)	3.08 (0.74)	Described in text: “No difference in age or gender” between groups

Note: 1. Age and LDL-C data are presented as “mean (standard deviation)” or “median (interquartile range)”, depending on the original reporting format. 2. The study reports the median and interquartile range (or range). 3. Baseline LDL-C values were converted from mg/dL to mmol/L (conversion factor: 1 mg/dL = 0.0259 mmol/L).

This table only includes comparisons related to high-intensity statin therapy; * indicates that the study is a multi-arm trial, and its control group is shared with other comparisons.

Table 5. Summary of sensitivity analysis results for high-intensity statin therapy

Excluded Study	Pooled RR [95% CI]	p -value(Effect)	I ²	p -value (Heterogeneity)
Original pooled	0.52 [0.37, 0.72]	< 0.0001	70%	0.0001
Berwanger_2018 ^[25]	0.47 [0.35, 0.64]	< 0.00001	44%	0.05
Guo_2017 ^[14]	0.54 [0.38, 0.76]	0.0004	70%	< 0.0001
He_2020 ^[9]	0.56 [0.41, 0.78]	0.0004	61%	0.002
Honghe Liu_2020 ^[36]	0.53 [0.37, 0.75]	0.0003	72%	< 0.0001
Im_2018 ^[10]	0.50 [0.34, 0.74]	0.0004	72%	< 0.0001
Jianhua Li_2022 ^[32]	0.51 [0.36, 0.72]	0.0002	73%	< 0.0001
Jinghua Wei_2020 ^[33]	0.53 [0.37, 0.75]	0.0003	72%	< 0.0001
Li_2017 ^[15]	0.57 [0.42, 0.78]	0.0002	60%	0.003
LiuQ_2019 ^[11]	0.51 [0.35, 0.74]	0.0004	72%	< 0.0001
LiuZ_2016 ^[12]	0.50 [0.34, 0.74]	0.0006	72%	< 0.0001
Ran_2017 ^[13]	0.50 [0.35, 0.71]	0.0001	72%	< 0.0001
Xinyu Feng_2019 ^[34]	0.51 [0.36, 0.73]	0.0002	73%	< 0.0001
Yi Jin_2019 ^[35]	0.49 [0.34, 0.69]	< 0.0001	72%	< 0.0001
Yuan_2018 ^[16]	0.50 [0.35, 0.72]	0.0002	73%	< 0.0001

Subgroup analysis based on the geographical regions of the study populations revealed the following results (**Figure 3**):

(1) In the “Western” group (1.3.1 in **Figure 3**, comprising 2 studies ^[10,25]), no significant benefit of high-intensity statins in reducing MACE was observed (RR = 0.82, 95% CI [0.57, 1.18], $p = 0.29$), with moderate heterogeneity within the group;

(2) In the “Asian” group (1.3.2 in **Figure 3**, comprising 12 studies), high-intensity statins significantly reduced the risk of MACE in Asian patients with ACS undergoing PCI (RR = 0.45, 95% CI [0.32, 0.64], $p < 0.00001$), with moderate heterogeneity within the group ^[9,11–16,32–36]. There was a statistically significant difference between subgroups ($p = 0.02$, $I^2 = 80.9\%$), suggesting that geographical region is an important source of overall heterogeneity.

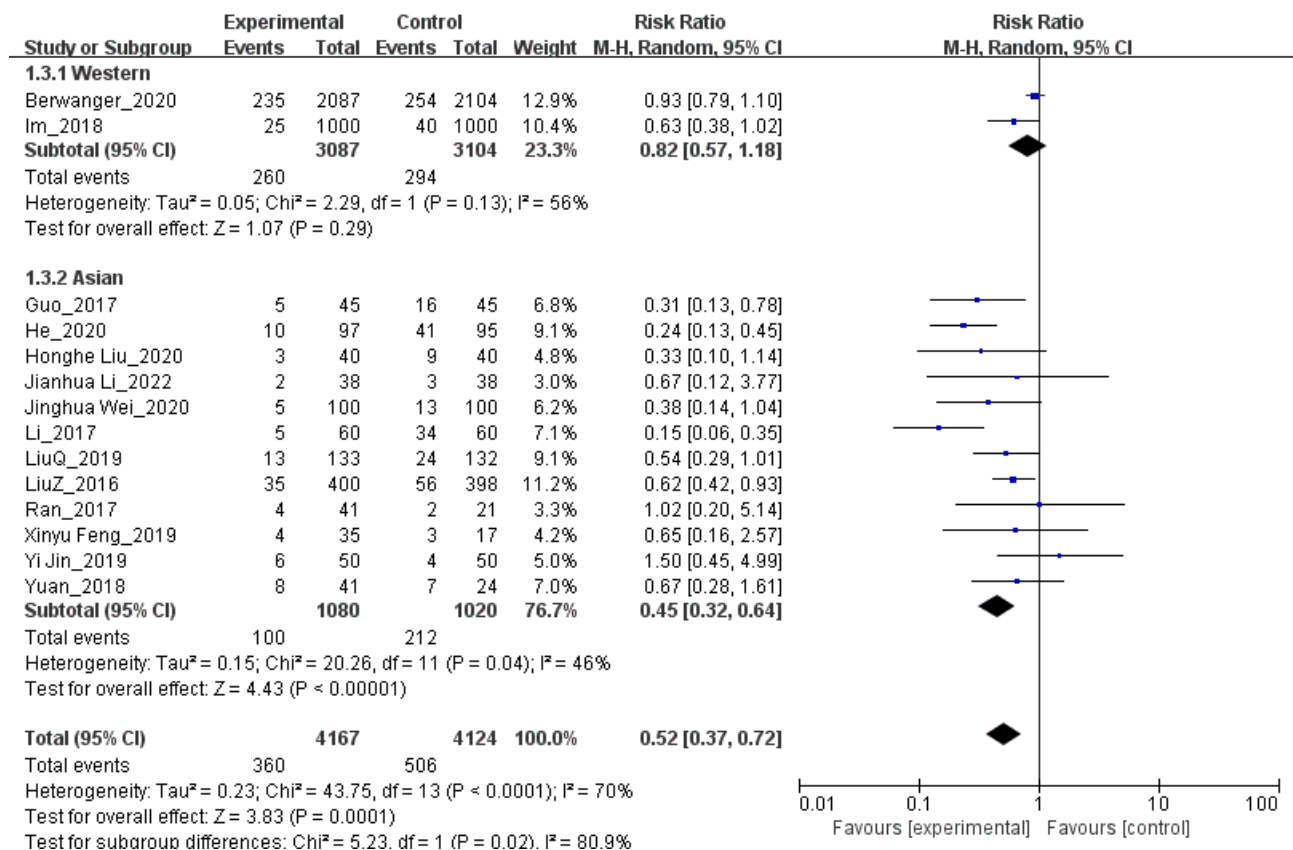


Figure 3. Subgroup analysis forest plot of the intervention effects of high-intensity statins among populations from different regions. Note: A random-effects model was used for the analysis.

The funnel plot is generally symmetrical, with studies primarily distributed around the pooled effect size (RR = 0.52). The vast majority of studies fall within the funnel boundaries of the 95% confidence interval. However, the results of the Egger test suggest the presence of publication bias ($t = -2.55$, $p < 0.05$) (**Figure 4**).

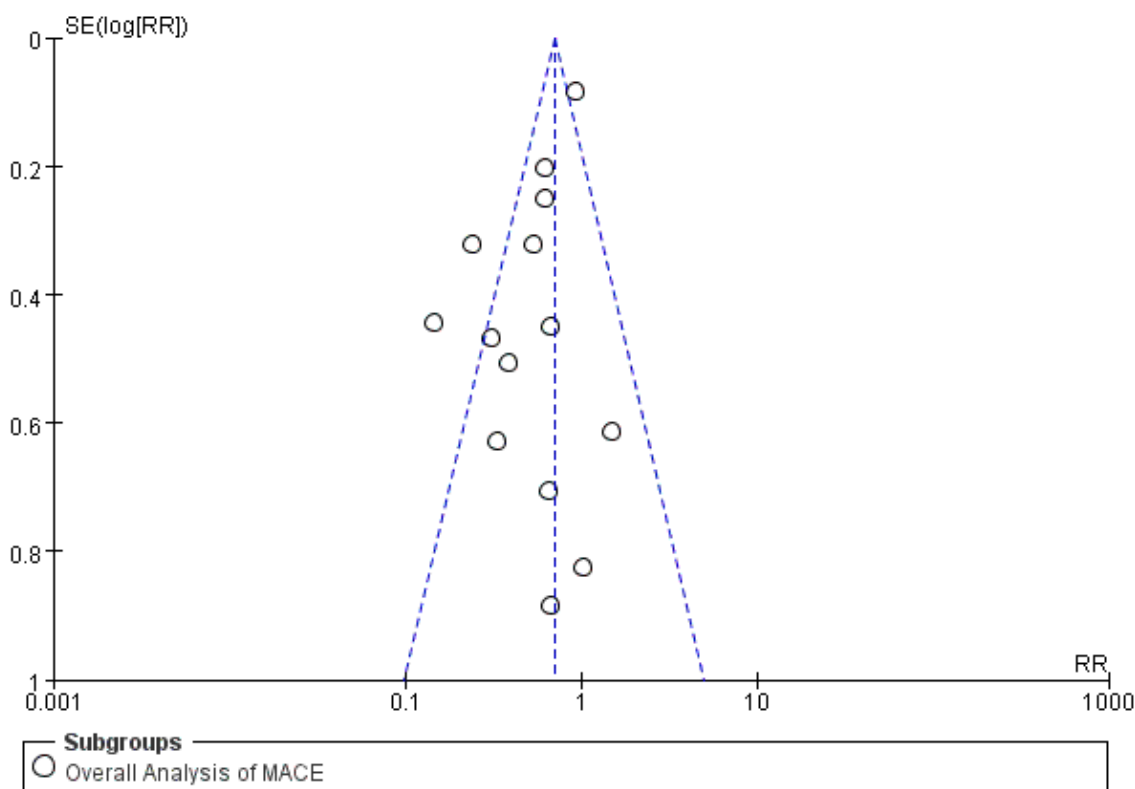


Figure 4. Funnel plot for the high-intensity statin group. Note: This funnel plot was drawn based on a fixed-effects model, while a random-effects model was used for the actual pooled analysis.

3.3.2. Statin combined with ezetimibe reduces the incidence of major adverse cardiovascular events (MACE) in patients with acute coronary syndrome (ACS) after percutaneous coronary intervention (PCI)

Ten studies were included, with specific baseline characteristics detailed in **Table 6** ^[13,16,26–29,31,34,37,40]. Apart from the statistically significant differences in baseline LDL-C levels between groups in the studies by Dai_2017 and Yuan_2018 (both $p < 0.001$), the overall comparability among the other studies was notable ^[16,29]. Considering that these differences were only present in a minority of studies and that the overall sample size of the included studies was large, it was deemed that these differences did not significantly impact the overall pooled effect. Therefore, overall comparability between groups was maintained. The analysis using the Mantel-Haenszel (M-H) random-effects model (**Figure 5**) revealed that compared to statin monotherapy, the combination of statin and ezetimibe significantly reduced the risk of MACE in patients with ACS undergoing PCI (RR = 0.59, 95% CI [0.40, 0.87], $p = 0.007$), with moderate heterogeneity among studies ($I^2 = 57\%$, $p = 0.01$).

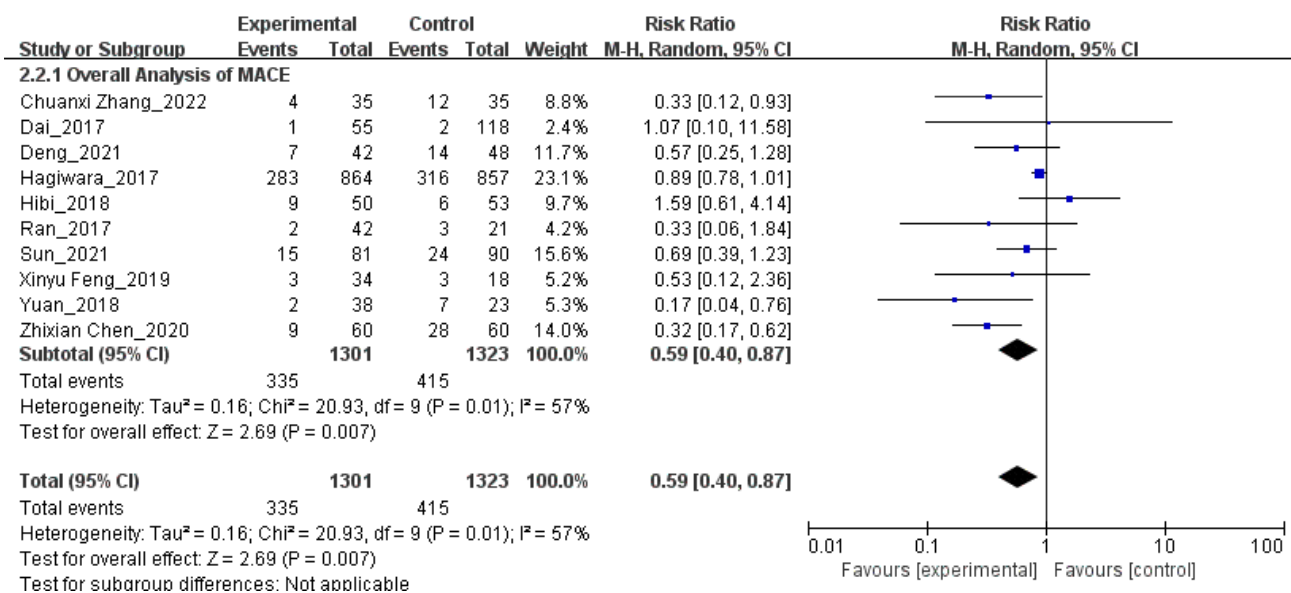


Figure 5. Forest plot of the impact of statin combined with ezetimibe therapy on MACE in ACS patients after PCI.

Note: A random-effects model was used for the analysis.

Table 6. Baseline characteristics of the included studies

Included Study	Age (years) ¹		Male(%)		Baseline ^{1,3} LDL-C (mmol/L)		Comparability Between Groups
	Intervention	Control	Intervention	Control	Intervention	Control	
Chuanxi Zhang_2022 ^[37]	56.28 (9.39)	57.52 (10.03)	21/35 (60.00)	25/35 (71.43)	3.20 (0.51)	3.15 (0.48)	All <i>p</i> > 0.05
Dai_2017 ^[29]	60.02 (9.81)	60.58 (9.04)	41/55 (74.5)	94/118 (79.7)	3.21 (0.95)	2.60 (0.85)	Baseline LDL-C <i>p</i> < 0.001; all other variables <i>p</i> > 0.05
Deng_2021 ^[26]	Not reported	Not reported	Not reported	Not reported	2.55 (0.79)	2.70 (1.41)	Baseline LDL-C <i>p</i> > 0.05; age and sex not reported
Hagiwara_2017 ^[31]	65.7 (11.7)	65.5 (11.9)	661/857 (77.1)	639/864 (74.0)	3.49 (0.76)	3.51 (0.78)	All <i>p</i> > 0.05
Hibi_2018 ^[28]	63 (10)	63 (12)	41/82 (50.0)	41/77 (53.2)	3.18 (0.83)	3.26 (0.85)	All <i>p</i> > 0.05
Ran_2017 ^[13] *	60.4 (8.2)	60.6 (6.7)	32/42 (76.2)	31/42 (73.8)	3.65 (0.70)	3.65 (0.80)	All <i>p</i> > 0.05
Sun_2021 ^[27]	61.74 (8.78)	64.08 (10.45)	55/81 (67.9)	63/90 (70.0)	2.95 (0.85)	2.94 (1.06)	All <i>p</i> > 0.05
Xinyu Feng_2019 ^[34] *	59 (9)	61 (8)	24/34 (70.59)	26/35 (74.29)	2.61 (0.84)	2.59 (0.88)	All <i>p</i> > 0.05
Yuan_2018 ^[16] *	Not reported	Not reported	Not reported	Not reported	3.92 (1.05)	3.08 (0.74)	Baseline LDL-C <i>p</i> < 0.001; authors stated “no differences in age and sex”
Zhixian Chen_2020 ^[40]	66.35 (13.42)	68.12 (16.27)	49/60 (81.67)	52/60 (86.67)	2.76 (0.63)	2.75 (0.56)	All <i>p</i> > 0.05

Note: 1. Age and LDL-C data are presented as “mean (standard deviation)” or “median (interquartile range)” depending on the original reporting format. 2. The study reports median and interquartile range. 3. Baseline LDL-C was converted from mg/dL to mmol/L (conversion factor: 1 mg/dL = 0.0259 mmol/L). This table includes only comparisons related to statin combined with ezetimibe; * indicates that the study is a multi-arm

trial, and its control group is shared with other comparisons.

After excluding studies one by one, the combined relative risk (RR) fluctuated between 0.52 and 0.68 (all $p < 0.05$), indicating robust results (**Table 7**). After excluding the study Hagiwara_2017, the overall heterogeneity decreased from 57% to 32% ($p = 0.16$); similarly, excluding the study Zhixian Chen_2020 resulted in a significant reduction in overall heterogeneity to 37% ($p = 0.12$), suggesting that these two studies are the primary sources of heterogeneity ^[31,40]. However, the combined effect size remained statistically significant after their exclusion.

Table 7. Summary of sensitivity analysis results for statin combined with ezetimibe therapy

Excluded Study	Pooled RR [95% CI]	<i>p</i> -value (Overall Effect)	I ²	<i>p</i> -value for Heterogeneity
Original pooled	0.59 [0.40, 0.87]	0.007	57%	0.01
Chuanxi Zhang_2022 ^[37]	0.62 [0.42, 0.93]	0.02	55%	0.02
Dai_2017 ^[29]	0.58 [0.39, 0.86]	0.007	62%	0.007
Deng_2021 ^[26]	0.58 [0.38, 0.90]	0.01	60%	0.01
Hagiwara_2017 ^[31]	0.52 [0.35, 0.79]	0.002	32%	0.16
Hibi_2018 ^[28]	0.53 [0.35, 0.79]	0.002	58%	0.01
Ran_2017 ^[13]	0.60 [0.40, 0.90]	0.01	60%	0.01
Sun_2021 ^[27]	0.56 [0.35, 0.89]	0.01	61%	0.008
Xinyu Feng_2019 ^[34]	0.59 [0.39, 0.88]	0.01	61%	0.008
Yuan_2018 ^[16]	0.64 [0.45, 0.92]	0.02	52%	0.03
Zhixian Chen_2020 ^[40]	0.68 [0.48, 0.97]	0.03	37%	0.12

Subgroup analyses were conducted based on background statin intensity, geographic region of the study population, and follow-up duration, with the results shown in detail in **Figures 6, 7, and 8** as follows:

(1) In the “moderate-intensity background statin” group (2.3.1 in **Figure 6**, encompassing 9 studies), the combination of ezetimibe with moderate-intensity statins significantly reduced the risk of major adverse cardiovascular events (MACE) in patients with ACS undergoing PCI (RR = 0.56, 95%CI [0.35, 0.89], $p = 0.01$), with moderate heterogeneity within the group ^[13,16,26,28,29,31,34,37,40],

(2) In the “high-intensity background statin” group (2.3.2 in **Figure 6**, involving 1 study), no significant benefit was observed with the addition of ezetimibe (RR = 0.69, 95%CI [0.39, 1.23], $p = 0.21$) ^[27]. The p -value for the test of differences between subgroups was 0.56 ($I^2=0\%$), indicating no statistically significant impact of background statin intensity on treatment efficacy; however, the limited number of studies in the high-intensity statin subgroup and the consequent limited statistical power necessitate cautious interpretation of the results.

(3) In the “Western” subgroup (2.3.3 in **Figure 7**, involving 1 study), the addition of ezetimibe did not significantly reduce the risk of MACE in Western patients with ACS undergoing PCI (RR = 1.59, 95%CI [0.61, 4.14], $p = 0.34$) ^[28];

(4) In the “Asian” subgroup (2.3.4 in **Figure 7**, including 9 studies), the combination of ezetimibe reduced the risk of MACE by 47% in Asian patients with ACS undergoing PCI (RR = 0.53, 95%CI [0.35, 0.79], $p = 0.002$), with moderate heterogeneity within the subgroup^[13,16,26,27,29,31,34,37,40]. The p-value for the test of differences between subgroups was 0.04, with $I^2 = 76.8\%$, suggesting that geographic region is the primary source of overall heterogeneity, and that Asian patients with ACS undergoing PCI derive significantly greater clinical benefit from statin-ezetimibe combination therapy than their Western counterparts; however, the limited number of studies in the Western group means that these results should be considered as reference only.

(5) In the “ ≥ 12 months follow-up period” group (2.3.5 in **Figure 8**, involving 4 studies), the addition of ezetimibe significantly reduced the risk of MACE in patients with ACS undergoing PCI when the follow-up duration was ≥ 12 months (RR = 0.48, 95% CI [0.24, 0.98], $p = 0.04$), with high heterogeneity within the subgroup^[16,26,31,40];

(6) In the “ <12 months follow-up period” group (2.3.6 in **Figure 8**, including 6 studies), the addition of ezetimibe did not significantly reduce the risk of MACE when the follow-up duration was < 12 months (RR = 0.68, 95% CI [0.42, 1.08], $p = 0.10$), with low heterogeneity within the subgroup^[13,27-29,34,37]. The difference between subgroups was not significant ($p = 0.44$, $I^2 = 0\%$), and follow-up duration is not currently considered the primary source of heterogeneity.

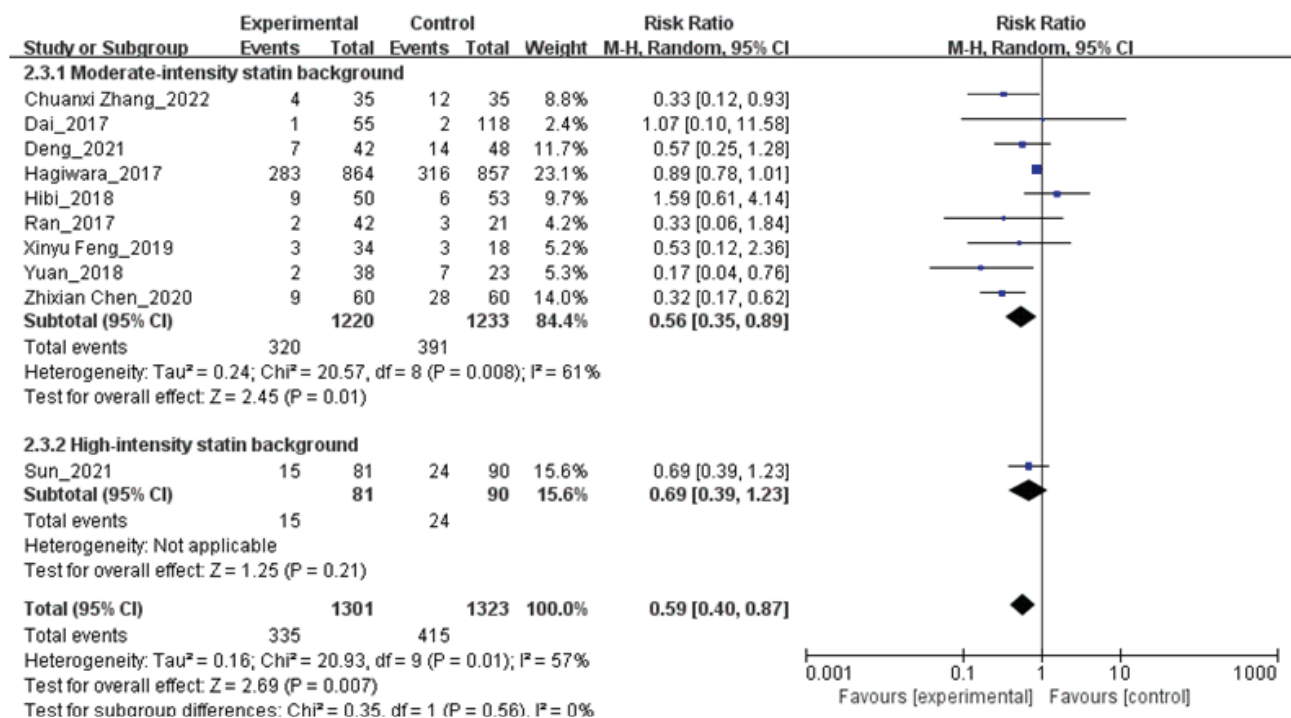


Figure 6. Forest plot of subgroup analysis on the intervention effects of statin combined with ezetimibe across different background statin intensities. Note: A random-effects model was used for the analysis.

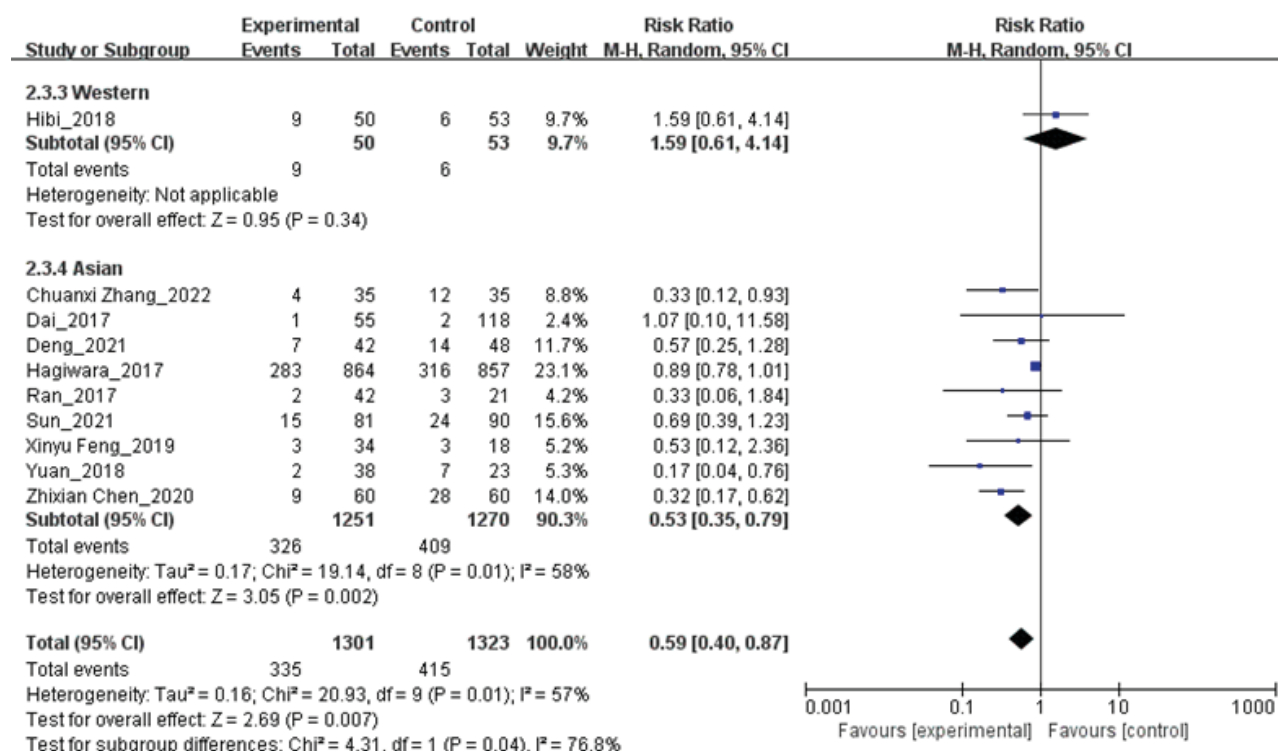


Figure 7. Forest plot of subgroup analysis on the intervention effects of statin combined with ezetimibe across populations from different geographical regions. Note: A random-effects model was employed for the analysis.

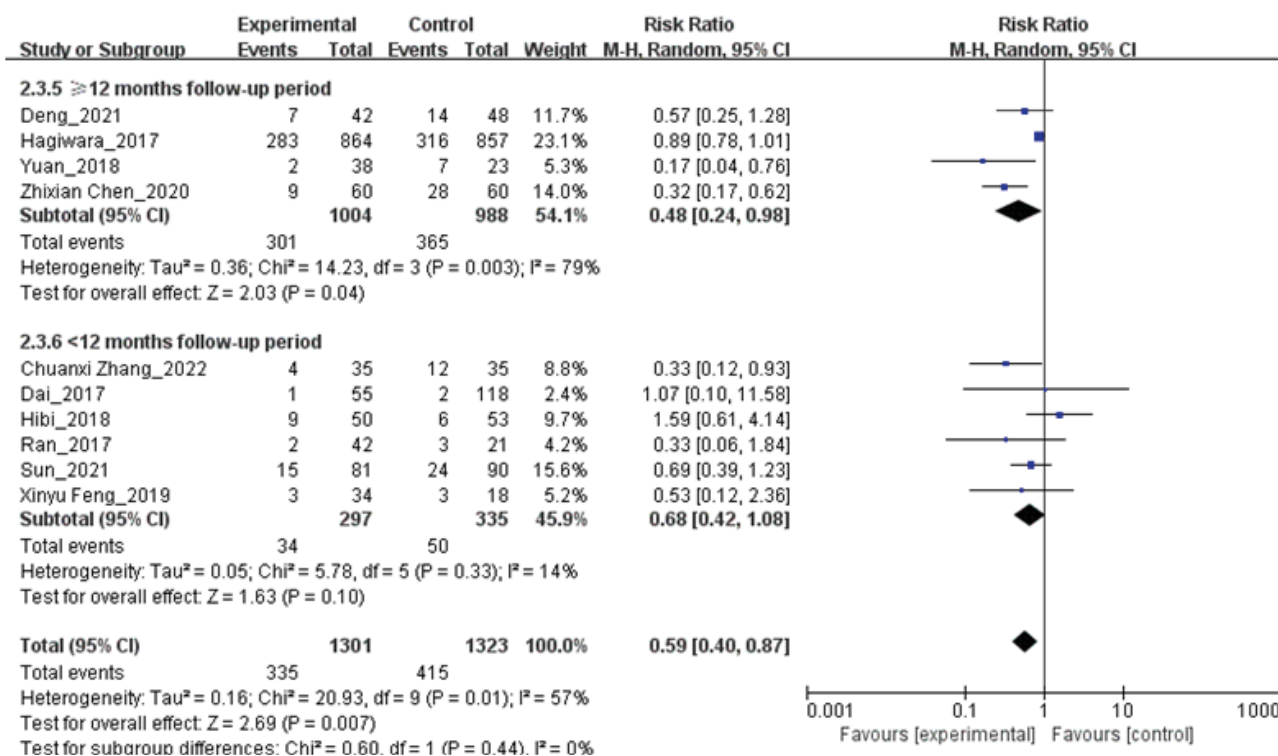


Figure 8. Forest plot of subgroup analysis on the intervention effects of statin combined with ezetimibe across different follow-up durations. Note: A random-effects model was used for the analysis.

In the funnel plot, the studies are roughly symmetrically distributed around the pooled effect size (RR = 0.59), with only a very small number (2 studies) falling outside the 95% confidence interval. Egger's test indicates no significant publication bias among the included studies ($t = -2.23$, $p = 0.06$) (**Figure 9**).

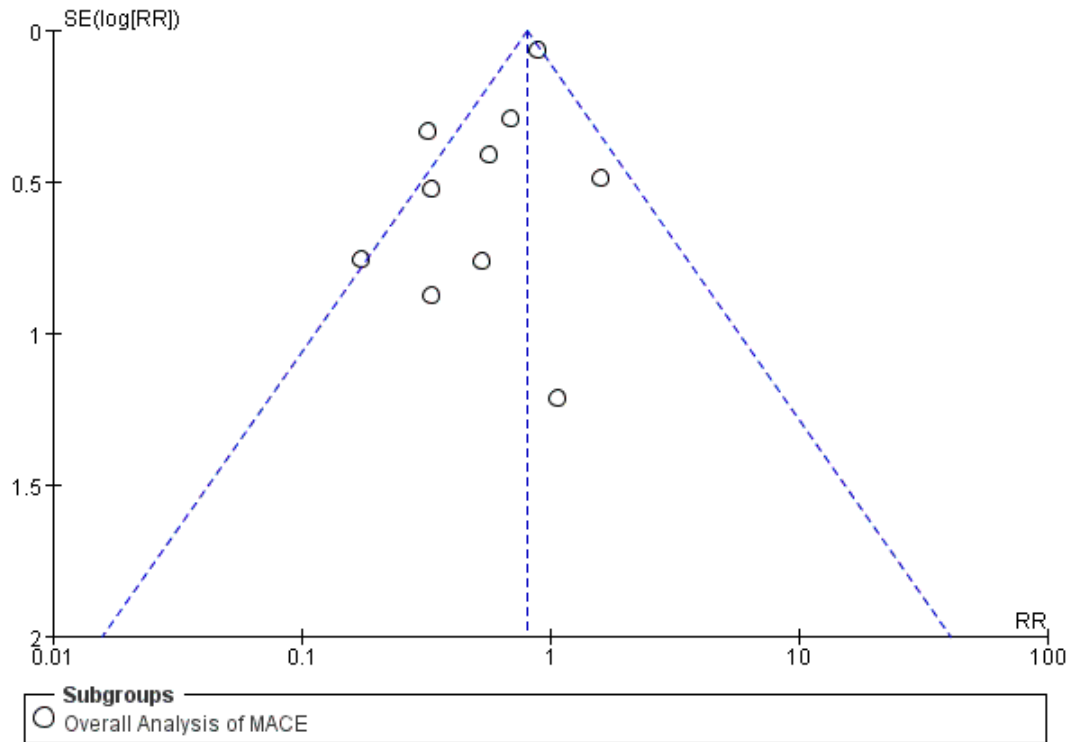


Figure 9. Funnel plot of the statin combined with ezetimibe group. Note: This funnel plot was drawn based on a fixed-effects model, while a random-effects model was employed for the actual pooled analysis.

3.3.3. Statin combined with PCSK9i can significantly reduce the incidence of MACE in ACS patients after PCI

A total of 13 studies were included [17–24,33,38,39,41,42]. In 9 studies, there were no statistically significant differences in core baseline characteristics such as age, gender, and baseline LDL-C between the two groups ($p > 0.05$) [18,19,22,24,33,38,39,41,42]. Only in the studies by Leucker 2020 and Mehta 2022 were there statistically significant differences in gender proportions ($p = 0.03$) [20,21]. In the studies by Räber 2022 and Xu 2021, there were statistically significant differences in baseline LDL-C levels ($p < 0.05$), as detailed in **Table 8** [17,23]. These differences were limited to single indicators, and there was no imbalance in multiple core indicators, indicating good overall comparability between groups. The analysis using a random-effects model revealed (**Figure 10**) that compared to statin monotherapy, statin combined with PCSK9i significantly reduced the risk of MACE in ACS-PCI patients (RR = 0.80, 95% CI [0.72, 0.89], $p < 0.0001$), with low heterogeneity ($I^2 = 25\%$, $p = 0.19$).

Table 8. Baseline characteristics of included studies

Study	Age (years)		Male (%)		Baseline LDL-C (mmol/L)		Baseline LDL-C (mmol/L)
	Intervention	Control	Intervention	Control	Intervention	Control	
Furtado 2022 ^[18]	622 (55, 68)	622 (55, 68)	Not reported	Not reported	2.37 (2.06, 2.80)	2.37 (2.06, 2.80)	The original text described “no differences in age, sex, and baseline LDL-C between the two groups”
Hua Yang_2020 ^[42]	61.00 (10.00)	58.00 (9.00)	23/32 (71.88)	21/33 (63.64)	2.80 (1.20)	2.80 (1.10)	All p > 0.05
Jingjing Cai_2022 ^[38]	59.08 (11.67)	61.00 (10.77)	198/250 (79.20)	212/250 (84.80)	3.77 (0.91)	3.07 (0.80)	All p > 0.05
Juan Zhao_2022 ^[41]	59.12 (10.74)	60.39 (11.23)	34/57 (59.65)	31/58 (53.45)	2.88 (0.71)	2.83 (0.71)	All p > 0.05
Koskinas_2019 ^[19]	60.5 (12.00)	61.0 (10.70)	128/155 (83.00)	123/153 (80.00)	3.61 (1.00)	3.42 (0.94)	All p > 0.05
Leucker 2020 ^[20]	55 (13.00)	55 (13.00)	22/30 (73.00)	11/27 (41.00)	2.37 (0.91)	2.32 (1.06)	p = 0.03 for sex; all other variables p > 0.05
Liuqing Yang_2022 ^[39]	59.65 (3.05)	59.30 (4.46)	60/98 (61.20)	68/100 (68.00)	3.73 (0.96)	3.78 (0.65)	All p > 0.05
Mehta_2022 ^[21]	61.37 (11.04)	63.63 (10.38)	27/38 (71.05)	28/30 (93.33)	2.97 (1.09)	2.87 (0.90)	p = 0.03 for sex; all other variables p > 0.05
Okada 2022 ^[22]	66.4 (13.1)	63.4 (14.0)	43/52 (82)	47/50 (94)	3.12 (0.79)	3.22 (0.87)	All p > 0.05
Räber_2022 ^[23]	58.4 (10.0)	58.6 (9.4)	124/148 (83.8)	119/152 (78.3)	4.01 (0.80)	3.91 (0.94)	All p > 0.05 except baseline LDL-C
Schwartz 2018 ^[30]	58.5 (9.3)	58.6 (9.4)	7072/9462 (74.7)	7090/9462 (74.9)	2.38 (0.8)	2.38 (0.8)	All p > 0.05
Xu_2021 ^[17]	56.53 (10.84)	58.91 (10.350)	73/96 (76.04)	175/238 (73.53)	3.67 (0.59)	3.34 (0.82)	All p > 0.05 except baseline LDL-C
Zhang_2022 ^[24]	62.1 (10.9)	62.2 (10.0)	258/414 (62.3)	681/1150 (59.2)	3.34 (0.73)	3.26 (0.77)	All p > 0.05

Note: 1. Age and LDL-C data are presented as “mean (standard deviation)” or “median (interquartile range),” depending on the format of the original report. 2. The study report provides the median and interquartile range. 3. Baseline LDL-C values were converted from mg/dL to mmol/L (conversion factor: 1 mg/dL = 0.0259 mmol/L).

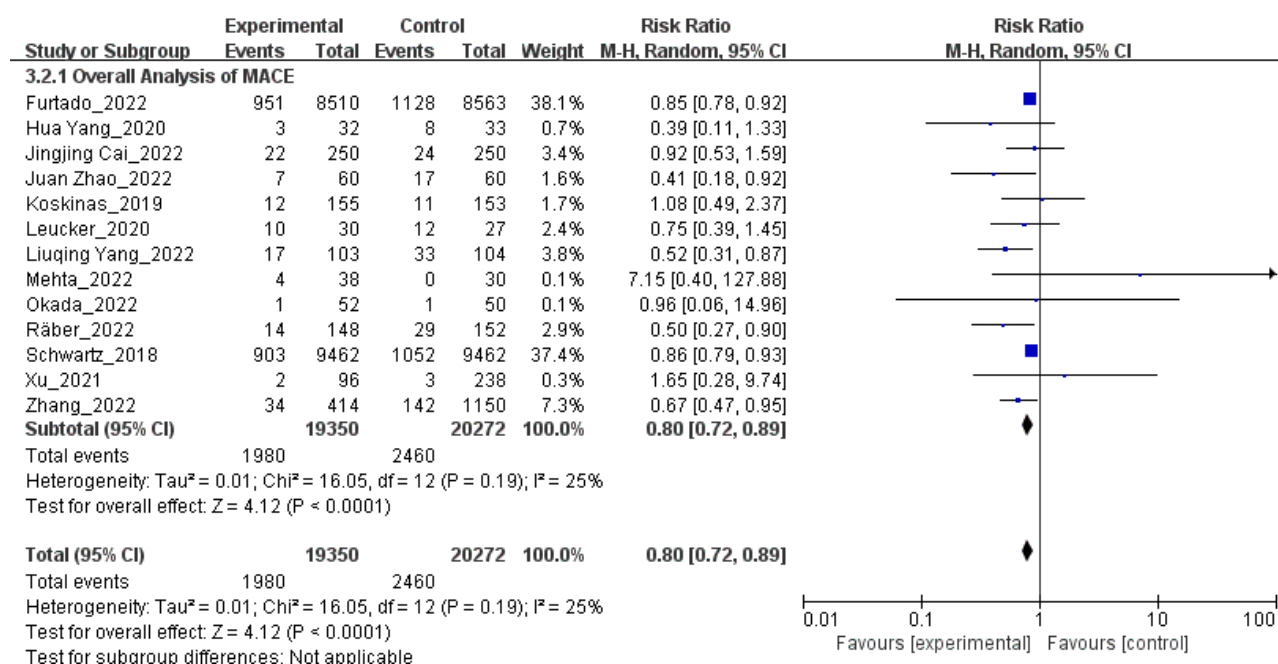


Figure 10. Forest plot depicting the impact of statin combined with PCSK9i therapy on major adverse cardiovascular events (MACE) in patients with acute coronary syndrome (ACS) after percutaneous coronary intervention (PCI). Note: A random-effects model was employed for the analysis.

Following sensitivity analysis, where studies were excluded one by one (**Table 9**), the pooled relative risk (RR) remained stable between 0.72 and 0.83, with heterogeneity maintained at a low level, indicating robust results.

Table 9. Summary of sensitivity analysis results for statin combined with PCSK9i therapy.

Excluded Study	Pooled RR [95% CI]	p-value (Overall Effect)	I ²	Heterogeneity p-value
Original pooled	0.80 [0.72, 0.89]	< 0.0001	25%	0.19
Furtado 2022 ^[18]	0.72 [0.60, 0.88]	0.001	31%	0.15
Hua Yang_2020 ^[42]	0.81 [0.73, 0.90]	< 0.0001	24%	0.20
Jingjing Cai_2022 ^[38]	0.79 [0.71, 0.89]	< 0.0001	31%	0.14
Juan Zhao_2022 ^[41]	0.82 [0.75, 0.90]	< 0.0001	16%	0.29
Koskinas_2019 ^[19]	0.79 [0.71, 0.89]	< 0.0001	30%	0.15
Leucker 2020 ^[20]	0.80 [0.71, 0.89]	< 0.0001	31%	0.14
Liuqing Yang_2022 ^[39]	0.83 [0.76, 0.90]	< 0.0001	14%	0.31
Mehta_2022 ^[21]	0.81 [0.73, 0.89]	< 0.0001	21%	0.24
Okada 2022 ^[22]	0.80 [0.71, 0.89]	< 0.0001	31%	0.14
Räber_2022 ^[23]	0.82 [0.76, 0.90]	< 0.0001	16%	0.29
Schwartz 2018 ^[30]	0.72 [0.60, 0.88]	0.001	29%	0.16
Xu_2021 ^[17]	0.80 [0.72, 0.89]	< 0.0001	29%	0.16
Zhang_2022 ^[24]	0.82 [0.73, 0.91]	0.0002	24%	0.21

Subgroup analyses were conducted based on background statin intensity and geographical population, and the results were as follows:

- (1) In the group with “moderate-intensity statin background” (Section 3.3.1 in **Figure 11**, comprising 7

studies), combination therapy significantly reduced the risk of major adverse cardiovascular events (MACE) (RR = 0.64, 95% CI [0.51, 0.82], $p = 0.0003$), with no significant heterogeneity observed among the studies [17,22,24,38,39,41,42],

(2) In the group with “high-intensity statin background” (Section 3.3.2 in **Figure 11**, comprising 6 studies), combination therapy also significantly reduced the risk of MACE (RR = 0.85, 95% CI [0.79, 0.91], $p < 0.0001$), with low heterogeneity^[18-21,23,33]. The test for subgroup differences yielded a p -value of 0.03 and an I^2 of 78.8%, suggesting that background statin intensity is associated with the overall heterogeneity in this study;

(3) In the “Western” population group (Section 3.3.3 in **Figure 12**, comprising 7 studies), statin combined with PCSK9i significantly reduced the risk of MACE in patients with ACS undergoing PCI (RR = 0.85, 95% CI [0.80, 0.90], $p < 0.00001$), with minimal heterogeneity within the group^[18-23,33],

(4) In the “Asian” group (Section 3.3.4 in **Figure 12**, comprising 6 studies), statin combined with PCSK9i also significantly reduced the risk of MACE (RR = 0.64, 95% CI [0.50, 0.82], $p = 0.0004$), with no heterogeneity observed within the group^[17,24,38,39,41,42]. The test for subgroup differences was significant ($p = 0.03$, $I^2 = 79.1\%$), indicating that geographical region is also an important source of heterogeneity in the efficacy of this treatment regimen.

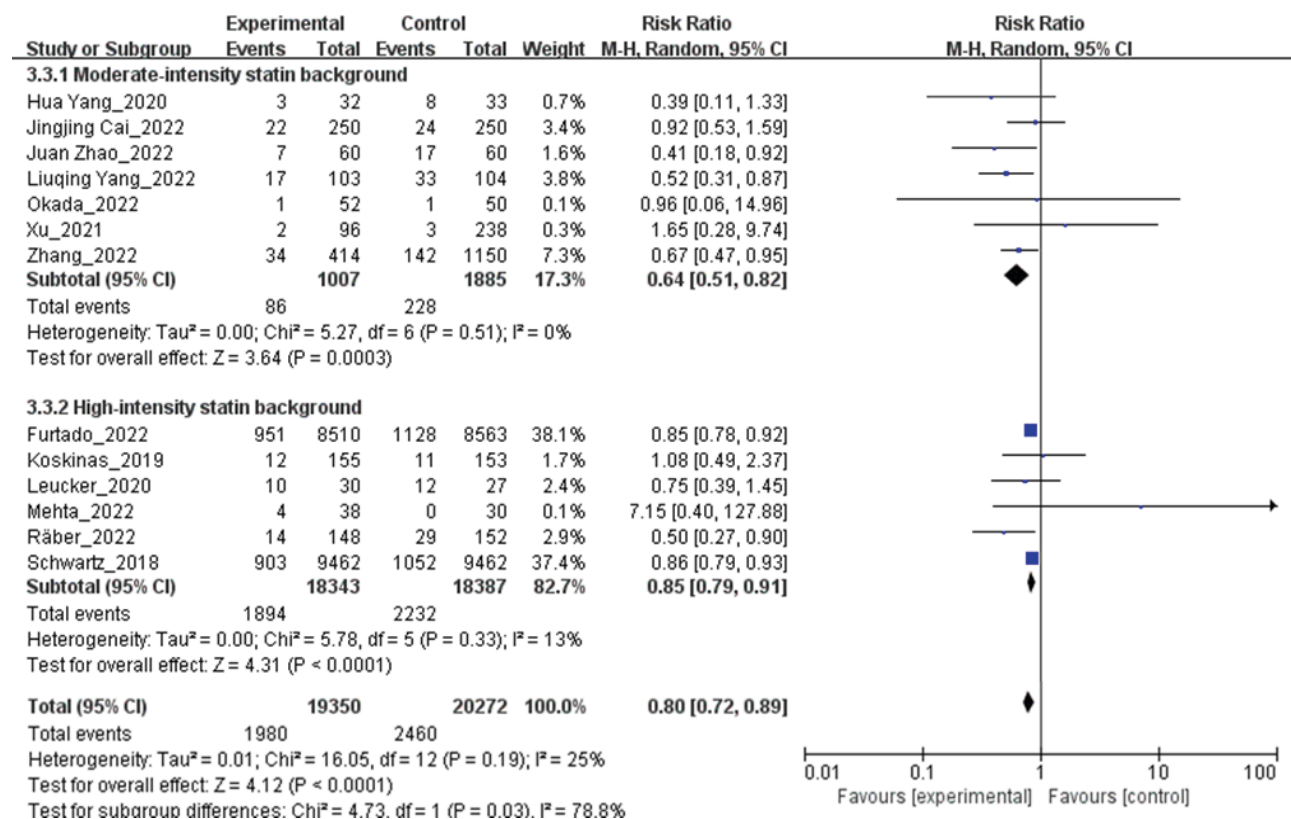


Figure 11. Subgroup analysis forest plot of the intervention effects of statins combined with PCSK9i with varying background statin intensities. Note: A random-effects model was used for the analysis.

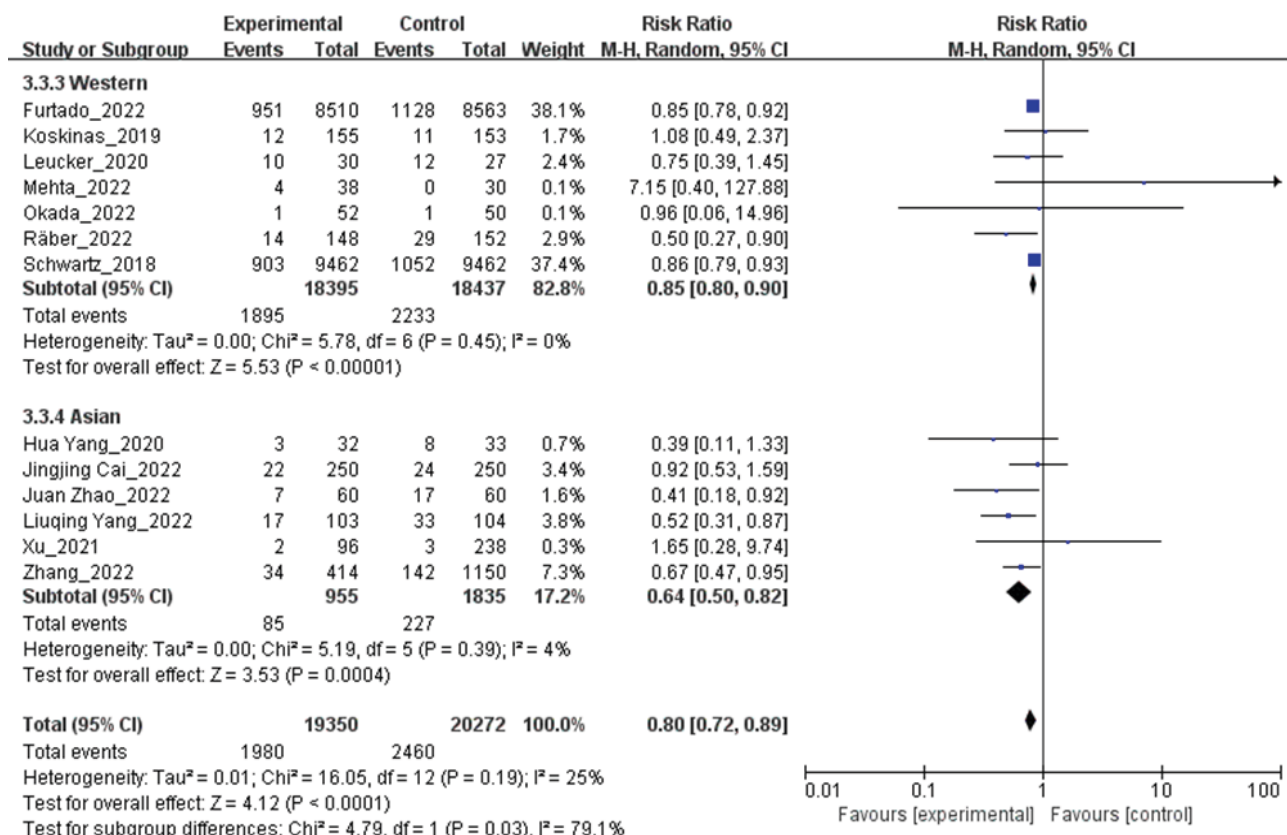


Figure 12. Forest plot of subgroup analysis on the intervention effect of statins combined with PCSK9i in populations from different regions. Note: A random-effects model was used for the analysis.

The funnel plot (**Figure 13**) in this case shows that the study points are roughly symmetrically distributed on both sides of the combined effect size. The Egger test did not reveal significant publication bias ($t = -1.18$, $p = 0.26$).

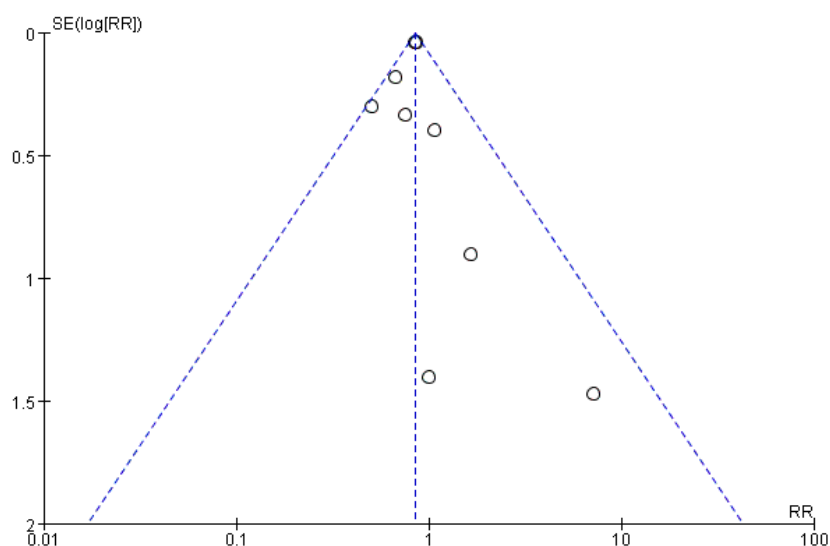


Figure 13. Funnel plot of the statin combined with PCSK9i group. Note: This funnel plot was drawn based on a fixed-effects model, while a random-effects model was used for the actual pooled analysis.

4. Discussion

This study included 34 clinical studies (31 RCTs^[9-16,18-23,25-28,30-42] and 3 high-quality cohort studies^[17,24,29]), comprising a total of 50,537 patients with ACS-PCI, to systematically evaluate the effects of three types of lipid-lowering regimens on the occurrence of MACE. Subgroup analyses and sensitivity analyses were conducted to explore the sources of heterogeneity.

4.1. High-intensity statin therapy

Compared with moderate-intensity statin therapy, this study suggests that high-intensity statin therapy can reduce the risk of MACE in patients with ACS-PCI by approximately 48%, confirming the core value of intensive lipid-lowering in secondary prevention of ACS. However, there was moderate heterogeneity, and subgroup analysis revealed that region was a key source of heterogeneity: Asian populations showed significant benefits, while no statistical difference was observed in Western populations. It is speculated that this difference is related to population genetic background, statin metabolic enzyme phenotype, baseline lipid profile, and body mass index distribution.

Sensitivity analysis indicated a substantial reduction in heterogeneity after excluding Berwanger_2018, suggesting that this large-sample Western study was the primary source of overall heterogeneity^[25]. The pooled effect remained stable after exclusion, further supporting the reliability of the conclusion regarding the benefits of intensive lipid-lowering in Asian populations. Although the Egger linear test in this subgroup suggested statistically significant publication bias, this result was not considered to be due to true publication bias based on study characteristics for the following reasons:

- (1) The funnel plot showed a roughly symmetrical distribution of studies, with the vast majority falling within the 95% confidence interval and no obvious signs of missing negative-result studies;
- (2) The included studies had a wide range of sample sizes, and extreme differences in sample size can directly interfere with the statistical power of the Egger test;
- (3) Varying follow-up times among studies and clinical heterogeneity in region, statin type, and baseline risk can inherently lead to dispersion of effect sizes, resulting in false-positive test results.

In summary, this study suggests that the positive Egger test result is more likely related to methodological and clinical heterogeneity.

4.2. Statin combined with ezetimibe therapy

Statin combined with ezetimibe significantly reduced the risk of MACE in patients with ACS-PCI, and sensitivity analysis supported the robustness of the results, consistent with conclusions from classic studies such as IMPROVE-IT^[43]. Hagiwara_2017 and Zhixian Chen_2020 were the main sources of heterogeneity; the former was a large-sample cohort study with long-term follow-up, while the latter was a single-center RCT in Asia. Differences in follow-up duration and baseline risk between the two studies directly led to dispersion of effect sizes^[31,40]. After exclusion, heterogeneity decreased, suggesting stable benefits of the combination regimen.

Subgroup studies were analyzed from three dimensions: background statin intensity, region, and follow-up time as follows:

- (1) Adding ezetimibe on the basis of moderate-intensity statin therapy provided significant benefits, while the high-intensity statin group included only one study with insufficient statistical power, so the clinical

value of this combination cannot be denied;

(2) Regional differences were also a core influencing factor, with Asian populations showing significantly better benefits than Western populations, suggesting that East Asian populations are more sensitive to additional benefits from non-statin lipid-lowering drug combination therapy. However, the Western population subgroup included only one study, so the analysis results should be interpreted with caution;

(3) In the two subgroups with different follow-up durations, the difference test showed no statistical significance. Although numerically the benefit of the combination regimen appeared more pronounced in the subgroup with follow-up duration ≥ 12 months, it cannot be concluded that follow-up duration is the key factor for the benefit.

The funnel plot in this section was symmetrical, and the Egger test showed no statistical difference, indicating high credibility of the conclusions.

4.3. Statin combined with PCSK9i therapy

The pooled results of the statin combined with PCSK9i regimen were the most stable with the lowest heterogeneity, significantly reducing the risk of MACE, highly consistent with large clinical trials such as FOURIER and ODYSSEY OUTCOMES^[30,44]. Subgroup analysis suggested that background statin intensity and region were the main sources of heterogeneity: the benefit magnitude of adding PCSK9i on the basis of moderate-intensity statin therapy was significantly higher than that of the high-intensity statin combination group, and there was no heterogeneity among studies in the former group; the benefit magnitude in Asian populations was significantly better than that in Western populations, with statistically significant differences between subgroups.

This difference may be related to differences in the treatment gradient of background lipid-lowering intensity: moderate-intensity statin therapy has limited inhibitory effects on cardiovascular events, and adding PCSK9i can bring more obvious prognostic improvements; while on the basis of high-intensity statin therapy, the intervention is close to the intensive upper limit, and the relative benefit space is compressed. In addition, the mechanism of action of PCSK9i was uniform, the dosing regimen was standardized, and most of the included studies were high-quality international multicenter RCTs with low risk of methodological bias, which also contributed to the low heterogeneity in this section. Neither the funnel plot nor the Egger test suggested publication bias, indicating reliable conclusions regarding the efficacy of this regimen.

4.4. Clinical implications

Based on the “Chinese Guideline for Lipid Management (2023)”, high-intensity statin therapy can be used as the basic regimen for Asian patients with ACS-PCI; for those intolerant to high-intensity statin therapy or requiring further intensification, moderate-intensity statin combined with ezetimibe can be preferred; for extremely high-risk patients, statin combined with PCSK9i provides more stable and potent benefits^[6].

4.5. Limitations

Limitations and shortcomings of this study are as outlined:

(1) Inconsistencies in MACE definitions, follow-up durations, and baseline characteristics among

studies;

- (2) Failure to further stratify comparisons by specific types of statins or PCSK9i;
- (3) Lack of systematic analysis of safety and adherence;
- (4) Inherent biases still present in some RCTs and cohort studies;
- (5) Cholesterol absorption inhibitor combined with PCSK9i, statin combined with bococizumab, and triple regimens were not included in the analysis due to insufficient studies.

5. Conclusion

In conclusion, all three first-line intensive lipid-lowering regimens can effectively reduce the postoperative risk of MACE in patients with ACS-PCI. Region is a key factor in efficacy heterogeneity, with Asian patients with ACS-PCI showing more significant benefits. Furthermore, statin combined with PCSK9i showed a relatively stable risk-reducing effect in this study, and the benefit was more obvious when combined with moderate-intensity statin therapy.

Disclosure statement

The authors declare no conflict of interest.

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Effects of Zhuang Medicine Longzuan Tongbi Decoction on the Expression of PDGF, IGF1 and VEGF in Rats with Left Ventricular Remodeling after Myocardial Infarction

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Abstract: *Objective:* To investigate the effects of Zhuang Medicine Longzuan Tongbi Decoction on left ventricular remodeling in rats after myocardial infarction (MI), and to explain its underlying mechanism based on expressions of PDGF, IGF1, VEGF and related inflammatory factors. *Methods:* A total of 120 SD rats were randomly divided into a sham operation group, a model group, a positive control (Shexiang Baoxin Pill) group, as well as low-, medium-, and high-dose groups of Longzuan Tongbi Decoction. The MI model was established by ligation of the left anterior descending coronary artery. Postoperatively, gavage administration was performed once daily for 6 consecutive weeks. At the end of the experiment, the infarct size and microvessel density in myocardial tissues were measured; the expression levels of PDGF, IGF1, VEGF, IL1 β and IL6 in myocardium were detected, and the serum contents of IL1 β and IL6 were determined. *Results:* Compared with the model group, all dosage groups of Longzuan Tongbi Decoction significantly reduced myocardial infarct size, elevated the number and density of microvessels in myocardial tissue, upregulated the protein expressions of PDGF, IGF-1 and VEGF in myocardium, and downregulated the levels of IL-1 β and IL-6 in myocardial tissue and serum ($P < 0.05$). In terms of promoting angiogenesis and regulating related cytokines, the medium- and high-dose groups of Longzuan Tongbi Decoction exhibited superior efficacy to the Shexiang Baoxin Pill group ($P < 0.05$), with an overall dose-dependent effect. *Conclusion:* Zhuang Medicine Longzuan Tongbi Decoction may exert synergistic multitarget effects to upregulate the expression of proangiogenic factors and suppress inflammatory responses, thereby facilitating myocardial angiogenesis and ameliorating left ventricular remodeling. Its underlying mechanism is closely associated with the regulation of angiogenesis and inflammation-related signaling pathways.

Keywords: Left ventricular remodeling; Myocardial angiogenesis; PDGF/IGF-1/VEGF; Inflammatory factors (IL-1 β /IL-6); Zhuang Medicine Longzuan Tongbi Decoction

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1. Introduction

Left ventricular remodeling (LVR) after myocardial infarction (MI) is a key pathological process driving heart failure, closely linked to impaired angiogenesis and excessive inflammation. Pro-angiogenic factors, including PDGF, IGF-1, and VEGF, promote vascular repair, while inflammatory cytokines such as IL1 β and IL6 aggravate myocardial fibrosis and ventricular dilation. In Zhuang medicine, coronary heart disease is attributed to “toxin-stasis obstructing Longlu” (blood vessels). Longzuan Tongbi Decoction (LZTB), formulated under the principle of “detoxifying and unblocking vessels”, is used clinically for this condition, yet its effects on postMI LVR remain unexplored. This study aimed to investigate whether LZTB attenuates LVR in rats after MI and to elucidate its mechanism through the regulation of PDGF, IGF1, VEGF, and inflammatory factors.

2. Experimental materials

2.1. Animals

A total of 120 clean-grade, healthy female SD rats weighing (230 ± 20) g were purchased from the Laboratory Animal Center of Zhengzhou University (Animal Certificate No.: 410156). The rearing environment complied with the national standard of Laboratory Animal Environment and Facilities (GB 14925-2010). All rats were given free access to food and water. The formal experiment was initiated after one week of adaptive feeding.

2.2. Medicines and reagents

Longzuan Tongbi Decoction (composed of *Radix Toddaliae Asiaticae* (Feilongzhangxue) 30 g, *Radix Kadsurea Coccineae* (Dazuan) 20 g, *Radix Alangii Chinensis* (Bajiaofeng) 15 g, *Radix Zanthoxyli* (Liangmianzhen) 15 g, *Caulis Sinomenii* (Qingfengteng) 15 g, *Rhizoma Smilacis Glabrae* (Tufuling) 15 g, *Caulis Spatholobi* (Jixueteng) 30 g, *Herba Plantaginis* (Cheqiancao) 15 g, *Rhizoma Dioscoreae Hypoglaucae* (Bixie) 15 g and *Pseudobulbus Cremastrae seu Pleiones* (Shancigu) 10 g) was purchased and decocted into extract by the Pharmacy Department of Fusui County Hospital of Traditional Chinese Medicine; 1 mL of extract is equivalent to 1 g of crude herb. Shexiang Baixin Pill (Shanghai Hutchison Pharmaceuticals Co., Ltd.; Batch No. A040214; 22.5 mg per pill); Immunohistochemistry Kits for PDGF- β , IGF-1 and VEGF (Wuhan Boster Biological Technology, Ltd); Enzyme-Linked Immunosorbent Assay (ELISA) Kits for rat interleukin-1 β (IL-1 β) and rat interleukin-6 β (IL-6 β) (Wuhan Boster Biological Technology, Ltd); DAB Staining Kit (Beijing Zhongshan Company); BCA Protein Quantification Kit (Solarbio Science & Technology Co., Ltd., Beijing, China); 1% Pentobarbital Sodium (analytical pure, Sinopharm Chemical Reagents Co., Ltd.); Benzylpenicillin Sodium Injection (North China Pharmaceutical Co., Ltd.).

2.3. Equipment

Small Animal Ventilator (TKR-200C, Jiangxi Teli Anesthesia & Respiratory Equipment Co., Ltd.); Optical Microscope (CH20BIMF200, OLYMPUS Optical Co., Ltd., Japan); Paraffin Microtome (HM340E, Carl Zeiss AG, Germany); Medical Image Analysis System (BT 2000, Version 3.6 Build 0211, Chengdu Taimeng Technology Co., Ltd.); High-Resolution Color Pathological Image Analysis System (Noesis S.A., France), Western Blot Electrophoresis and Transfer System (BioRad); Chemiluminescence Imaging System (Tanon 5200); Microplate Reader (BioTek Epoch).

3. Experimental methods

3.1. Preparation of animal model

The MI model was established according to the Laboratory Animal Science ^[1]. Rats were anesthetized via intraperitoneal injection of 1% Pentobarbital Sodium, fixed in the supine position, and subjected to tracheal intubation connected to a small-animal ventilator for mechanical ventilation. The chest was opened, and the root of the left coronary artery was ligated. The ligation site was 0.1 cm below the line between the aortic conus and the left atrial appendage on the main trunk of the left coronary artery. Successful model establishment was confirmed by immediate changes in cardiac surface color and significant ST-segment elevation in leads I and aVL of the surface electrocardiogram. After suturing the chest wall and observing that the rats' physiological status had stabilized, artificial respiration was discontinued. Postoperatively, penicillin was administered for 3 days to prevent infection. The surviving rats were randomly divided into the model group, Shexiang Baixin Pill group, high-dose group of Longzuan Tongbi Decoction, medium-dose group of Longzuan Tongbi Decoction, and low-dose group of Longzuan Tongbi Decoction. Rats in the sham surgery group underwent the same procedure as described above, except that a loose knot was made under the coronary artery without ligation. All rats were euthanized after 6 weeks for subsequent indicator detection.

3.2. Animal grouping and administration methods

A total of 120 healthy, clean-grade female SD rats weighing 230 ± 20 g were randomly divided into six groups (A, B, C, D, E, and F), with 20 rats in each group. A: normal control group; B: model group; C: Shexiang Baixin Pill group; D: low-dose group of Longzuan Tongbi Decoction (1 mL); E: medium-dose group of Longzuan Tongbi Decoction (2 mL); F: high-dose Longzuan Tongbi Decoction group (4 mL). (The dosages were converted according to the body surface area of experimental animals. The medication groups were administered via gavage at 8:00 a.m. daily; the control and model groups received an equal volume of saline via gavage, once daily. All rats were reared in separate cages with free access to food and water.

3.3. Collection and processing of myocardial tissue

After anesthetizing the rats, blood samples were collected and serum was separated for subsequent ELISA detection. The hearts were quickly removed. The aortic arch, right ventricle, atria, and auricles were excised. Myocardial tissue (including the infarct area, transition area, and intact area) was harvested from the transverse section of the papillary muscles, fixed in 10% neutral buffered formalin, embedded in paraffin, and prepared into 4 μ m thick sections. Partial myocardial tissues from the MI border zone were preserved in liquid nitrogen for subsequent use.

3.4. Observation indicators and detection methods

3.4.1. Detection of PDGF, IGF-1, and VEGF protein expression in the MI border zone

The immunohistochemical SP method was adopted to detect PDGF- β , IGF-1, and VEGF protein expression. Positive immunoreactivity was defined as the presence of brownish-yellow granular deposits within cells. The mean optical density (MOD) per field ($\times 100$) in the MI border zone was quantified using a pathological image analysis system. Six random fields were selected and measured on each section, and the average value was calculated.

3.4.2. Determination of PDGF, IGF-1 and VEGF protein expression in rats of each group

Protein was extracted from myocardial tissues. After electrophoresis and membrane transfer, the samples were incubated with antibodies against PDGF, IGF1, VEGF and β actin respectively. Protein bands were visualized via chemiluminescence, and band gray values were quantified using ImageJ software.

3.4.3. ELISA assay for serum IL-1 β and IL-6 β levels

ELISA was used to determine IL-1 β and IL-6 β levels in serum. Prior to the experiment, frozen serum samples were thawed slowly at 4°C, centrifuged, and the supernatant was collected. The procedure was performed according to the instructions provided with the rat IL-1 β and IL-6 β ELISA kits from Wuhan Boshide Biotechnology Co., Ltd. A microplate reader was used to measure the optical density (OD) values in each well.

3.5. Statistical methods

All data were analyzed using SPSS 32.0 statistical software. Measurement data were expressed as “mean $\pm$ standard deviation (SD)”; Comparisons between groups were performed using the independent samples *t*-test. Enumeration data presented as “number of cases (percentage)”; Comparisons between groups were performed using the χ^2 test. Ranked data were analyzed by the rank sum test. $P < 0.05$ was considered statistically significant.

4. Results

4.1. Comparison of platelet-derived growth factor- β (PDGF- β), insulin-like growth factor-1 (IGF-1), and vascular endothelial growth factor (VEGF) levels in the MI border zone of rats across different groups

Immunohistochemical SP staining was used to detect the expression levels of PDGF- β , IGF-1, and VEGF in the MI border zone of rats in each group (expressed as the mean gray value, MOD; lower MOD value indicates higher protein expression). The results showed that, compared with the normal control group, the MOD values of PDGF- β , IGF-1, and VEGF were significantly decreased (i.e., significantly elevated protein expression) in the model group and all treatment groups, and the differences were statistically significant ($P < 0.05$); Compared with the model group, the MOD values of PDGF- β , IGF-1, and VEGF in the Shexiang Baoxin Pill group and all dose groups of Zhuang Medicine Longzuan Tongbi Decoction were further reduced (further increased protein expression), and the differences were statistically significant ($P < 0.05$). Compared with the Shexiang Baoxin Pill group, the high- and medium-dose groups of Zhuang Medicine Longzuan Tongbi Decoction showed significantly lower MOD values of PDGF- β , IGF-1, and VEGF (significantly elevated protein expression) ($P < 0.05$), whereas the low-dose group of Zhuang Medicine Longzuan Tongbi Decoction showed no statistically significant difference in MOD values of PDGF- β , IGF-1, VEGF ($P > 0.05$), suggesting that Zhuang Medicine Longzuan Tongbi Decoction can upregulate the expression of PDGF- β , IGF-1, and VEGF, with the high- and medium-dose groups exhibiting superior upregulatory effects compared to the low-dose group. See **Table 1** for specific data.

Table 1. Comparison of PDGF β , IGF1 and VEGF levels in the MI border zone of rats across different groups (MOD, mean $\pm$ SD, $n = 20$)

Group	n	PDGF- β	IGF-1
Normal control group	20	32.15 $\pm$ 5.48	40.76 $\pm$ 6.52
Model group	20	71.29 $\pm$ 6.05 ¹	74.86 $\pm$ 1.231 ¹
Shexiang Baoxin pill group	20	137.58 $\pm$ 2.74 ^{1,2}	144.32 $\pm$ 2.86 ^{1,2}
Low-dose group of Zhuang medicine Longzuan Tongbi decoction	20	136.89 $\pm$ 4.12 ^{1,2}	143.95 $\pm$ 7.12 ^{1,2}
Medium-dose group of Zhuang medicine Longzuan Tongbi decoction	20	146.83 $\pm$ 7.21 ^{1,2,3}	141.54 $\pm$ 4.63 ^{1,2,3}
High-dose group of Zhuang medicine Longzuan Tongbi decoction	20	148.25 $\pm$ 7.32 ^{1,2,3}	142.17 $\pm$ 4.91 ^{1,2,3}

¹ Compared with the normal control group, $P < 0.05$

² Compared with the model group, $P < 0.05$

³ Compared with the Shexiang Baoxin Pill group, $P > 0.05$

4.2. Comparison of protein expression levels of PDGF- β , IGF-1, and VEGF in myocardial tissue of rats across different groups

Protein expression levels of PDGF β , IGF1 and VEGF in myocardial tissue of rats from each group were determined via Western Blot, quantified by the gray value of protein bands (higher gray value corresponds to higher protein expression). The results showed that, compared with the normal control group, the expressions of PDGF- β , IGF-1, and VEGF were significantly reduced in the model group, with statistically significant differences ($P < 0.05$); Compared with the model group, the expression of PDGF- β , IGF-1, and VEGF was significantly upregulated in the Shexiang Baoxin Pill Group and in all dose groups of Zhuang Medicine Longzuan Tongbi Decoction, with statistically significant differences ($P < 0.05$); Compared with the Shexiang Baoxin Pill group, the expression of PDGF- β , IGF-1, and VEGF was further upregulated in the high- and medium-dose groups of Zhuang Medicine Longzuan Tongbi Decoction, with statistically significant differences ($P < 0.05$), whereas no statistically significant differences in the low-dose group of Zhuang Medicine Longzuan Tongbi Decoction group ($P > 0.05$). These findings suggest that Zhuang Medicine Longzuan Tongbi Decoction promotes the expression of PDGF- β , IGF-1, and VEGF, with the high- and medium-dose groups exhibiting superior regulatory effects compared to the low-dose group.

4.3. Comparison of serum protein levels of IL-1 β and IL-6 β in rats across different groups

ELISA was used to measure the protein levels of IL-1 β and IL-6 β in the serum of rats from each group. The results showed that, compared with the normal control group, serum IL-1 β and IL-6 β levels were significantly elevated in the model group, with statistically significant differences ($P < 0.05$); Compared with the model group, IL-1 β and IL-6 β levels were significantly reduced in the Shexiang Baoxin Pill group and in all dose groups of Zhuang Medicine Longzuan Tongbi Decoction ($P < 0.05$); Compared with the Shexiang Baoxin Pil group, the high- and medium-dose groups of Zhuang Medicine Longzuan Tongbi Decoction exhibited further reductions in IL-1 β and IL-6 β levels ($P < 0.05$), whereas no statistically significant difference in the low dose group of Zhuang Medicine Longzuan Tongbi Decoction ($P > 0.05$). These findings suggest that the Zhuang Medicine Longzuan Tongbi Decoction can suppress the inflammatory response following myocardial infarction and reduce serum levels of inflammatory factors, with the high- and medium-dose groups exhibiting superior anti-inflammatory effects compared to the low-dose group.

5. Discussion

In Zhuang medicine theory, coronary heart disease falls under the category of “Amen,” and its pathogenesis lies in “the mutual entanglement of toxins and stasis, and the obstruction of the Longlu (blood circulation vessel in Zhuang medicine)”^[2]. Longzuan Tongbi Decoction is formulated based on the Zhuang medicine therapeutic principle of “eliminating toxins and unblocking vessels.” In this formula, *Radix Toddaliae Asiaticae* (Feilongzhangxue) serves as the monarch herb^[3]. It can dissipate stasis, stop bleeding, remove toxins and unblock collaterals, directly targeting the core pathogenesis of “toxin-stasis obstructing collaterals”, which aligns with the key therapeutic principle of “unblocking stasis in Longlu” in Zhuang medicine. *Radix Kadsurea Coccineae* (Dazuan) and *Caulis Spatholobi* (Jixueteng) act as minister herbs. *Radix Kadsurea Coccineae* (Dazuan) promotes Qi movement, activates blood circulation, unblocks collaterals and relieves pain. It reinforces the stasis-dissipating effect of the monarch herb. Following the theory that free flow of Qi ensures unobstructed blood circulation, it ameliorates Qi stagnation in the Longlu and Huolu. *Caulis Spatholobi* (Jixueteng)^[4] tonifies and activates blood, unblocks collaterals and relaxes tendons. It remedies blood deficiency in the pattern of Qi deficiency and blood stasis, and prevents damage to healthy Qi caused by excessive stasis dissipation. *Radix Alangii Chinensis* (Bajiaofeng), *Radix Zanthoxyli* (Liangmianzhen), *Caulis Sinomenii* (Qingfengteng), *Rhizoma Smilacis Glabrae* (Tufuling) and *Rhizoma Dioscoreae Hypoglaucae* (Bixie) are designated as adjuvant herbs. *Radix Alangii Chinensis* (Bajiaofeng) dispels wind, eliminates dampness, dissipates stasis and unblocks collaterals, targeting the accompanying pattern of damp-toxin combined with stasis. *Radix Zanthoxyli* (Liangmianzhen) activates blood circulation, dissipates stasis, removes toxins, and relieves swelling, further potentiating the detoxifying and stasis-resolving effects of the monarch herb.

The combined use of *Caulis Sinomenii* (Qingfengteng), *Rhizoma Smilacis Glabrae* (Tufuling) and *Rhizoma Dioscoreae Hypoglaucae* (Bixie) clears heat and drains dampness, dispels wind and relieves impediment. They eliminate damp-toxin and heat-toxin, unblock stagnated collaterals, and relieve internal accumulation of phlegm-dampness commonly seen in patients with coronary heart disease. *Herba Plantaginis* (Cheqiancao) and *Pseudobulbus Cremastrae seu Pleiones* (Shancigu) function as guide herbs. *Herba Plantaginis* (Cheqiancao) clears heat, promotes diuresis and guides pathogenic toxins downward, facilitating the excretion of toxins from the lower jiao. *Pseudobulbus Cremastrae seu Pleiones* (Shancigu) clears heat, removes toxins, dissipates abscesses and resolves masses. It enhances the overall detoxifying effect of the formula, moderates the harsh properties of *Radix Alangii Chinensis* (Bajiaofeng) and *Radix Zanthoxyli* (Liangmianzhen), and harmonizes all medicinal ingredients. The whole formula exerts the combined effects of removing toxins and dissipating stasis, promoting Qi movement and unblocking collaterals, and regulating both Qi and blood. It conforms to the traditional Chinese medicine therapeutic strategy of tonifying Qi, activating blood, removing toxins and unblocking collaterals, as well as the Zhuang medicine theory of unblocking the Longlu and Huolu and restoring synchronous movement of the three Qi. With simultaneous treatment of root cause and manifestations and balanced regulation of cold and heat nature, this formula is applicable to coronary heart disease (chest impediment and heart pain) manifested as the pattern of Qi deficiency and blood stasis accompanied by toxin-stasis obstructing collaterals. The whole prescription collectively exerts the efficacies of detoxification, dissipating blood stasis, unblocking collaterals and restoring vessels, which conforms to the Zhuang medicine therapeutic principle of “unblocking Longlu and regulating Qi and blood”. Modern research has demonstrated that left ventricular remodeling following

myocardial infarction is closely associated with insufficient angiogenesis and excessive inflammatory response ^[5,6]. PDGF, IGF-1 and VEGF are key factors regulating angiogenesis, promoting endothelial cell proliferation and vascular lumen formation ^[7,8]; whereas inflammatory factors such as IL-1 β and IL-6 β contribute to myocardial fibrosis and ventricular dilation ^[9].

Results of this study show that Longzuan Tongbi Decoction can reduce myocardial infarct size in a dose-dependent manner, increase the number and density of microvessels in the infarct border zone, synchronously upregulate the expression of PDGF, IGF-1, and VEGF in myocardial tissue, and downregulate the levels of IL-1 β and IL-6 β . This suggests that the prescription may delay the progression of post-infarction left ventricular remodeling via synergistically promoting angiogenesis and suppressing inflammatory responses. Its efficacy is superior to the positive control drug Shexiang Baoxin Pill, indicating Zhuang herbal formulas hold potential for multi-target regulation.

6. Conclusion

In conclusion, the cardioprotective effect of Longzuan Tongbi Decoction on post-myocardial infarction may be related to the regulation of angiogenesis-related factors and inflammatory signaling pathways, which interprets the modern scientific connotation of the Zhuang medicine theory of “detoxification and unblocking vessels”. Further in-depth research is required to clarify its specific molecular mechanisms and signaling pathway networks.

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Research on the Effectiveness of Teacher-Led Cardiopulmonary Resuscitation (CPR) Training for Middle School Students Using Standardized Videos under the Train-the-Trainer Model

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Abstract: *Objective:* To explore the effectiveness of teacher-led CPR training for middle school students using standardized videos under the Train-the-Trainer model and verify its non-inferiority. *Methods:* A total of 400 students from 10 second-grade classes at a middle school in Deyang City were selected from December 2024 to December 2025 and randomly assigned to an experimental group and a control group, with 200 students in each group. The experimental group was taught by teachers who had undergone standardized training using standardized videos, while the control group was taught by medical personnel using the same videos and courseware. The scores of emergency medical theory knowledge, the pass rate of CPR practical skills, and the ratings on the training attitude scale were compared between the two groups after training and three months after training. *Results:* After training, the theoretical knowledge scores and the pass rate of practical skills in the experimental group were compared with those in the control group ($P > 0.05$). The 95% confidence intervals of the scores between the two groups were entirely within the non-inferiority margin. During the three-month follow-up after training, the skill retention in the experimental group was comparable to that in the control group. Both groups of students had high ratings on the training attitude scale. *Conclusion:* Under the Train-the-Trainer model, the effectiveness of teacher-led CPR training for middle school students using standardized videos is not inferior to that of medical personnel-led training. This model can effectively alleviate the shortage of emergency training teachers in schools and is worthy of promotion and application.

Keywords: Train-the-trainer model; Standardized video; Middle school student; Cardiopulmonary resuscitation; Training effectiveness; Non-inferiority

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1. Introduction

Cardiac arrest is one of the leading causes of death among urban and rural residents in China, with approximately 544,000 out-of-hospital cardiac arrests occurring annually and a survival rate of only 1% to

5%. If bystanders perform cardiopulmonary resuscitation (CPR) promptly at the scene, the survival rate of patients can be increased by 2 to 3 times. However, less than 1% of the public in China has received CPR training, a proportion significantly lower than that in developed countries ^[1]. Adolescents, who constitute 28.4% of China’s total population, possess strong learning abilities and are receptive to first aid skills, making them a key target group for first aid training ^[1]. Developed countries have incorporated CPR education into the mandatory curriculum for primary and secondary school students. In China, however, school-based first aid training is still in its infancy, facing challenges such as a shortage of qualified instructors and inconsistent training standards. The “Train the Trainer” (TTT) model, which involves medical professionals providing standardized training to in-school teachers who then deliver instruction to students, can alleviate instructor shortages ^[2]. Nevertheless, questions arise regarding whether non-medically trained professional teachers have mastered the appropriate first aid knowledge and whether their teaching processes are standardized. Standardized video-based instruction ensures consistency in teaching content and reduces constraints on instructors ^[3]. The TTT model, through a three-tier transmission from “medical professionals to teachers to students,” effectively expands the reach of training. Teachers, as permanent school staff, have stable teaching audiences and ample instructional time, enabling the establishment of regular retraining mechanisms. This study integrates the TTT model with standardized video-based instruction, using medical professional-led instruction as a control to verify the non-inferiority of teacher-led instruction. The aim is to provide evidence-based support for constructing a replicable and scalable school-based first aid training model.

2. Materials and methods

2.1. General information

Four hundred students from ten classes in the eighth grade of a middle school in Deyang City were selected as the study subjects from December 2024 to December 2025. Using cluster randomization, the ten classes were divided into five experimental groups and five control groups, with one experimental and one control class compared at a time, totaling two classes. Details are shown in **Table 1**.

Inclusion criteria: (1) Students currently enrolled in the eighth grade of a middle school in Deyang City; (2) Aged 13 years or older with normal cognitive abilities; (3) Voluntary participation in the study with informed consent signed by legal guardians; (4) No physical disabilities affecting CPR performance.

Exclusion criteria: (1) Severe cardiopulmonary diseases or physical disabilities preventing the completion of simulated manikin operations; (2) Cognitive impairments or mental illnesses that hinder understanding of training content and cooperation with assessments; (3) Allergies to the materials of the simulated manikin.

Table 1. Comparison of baseline data between the two groups [n (%), mean ± SD]

Indicator	Experimental Group (n = 200)	Control Group (n = 200)	<i>t</i> / χ^2 value	<i>P</i> value
Age (years)	14.12 ± 0.45	14.08 ± 0.42	0.917	0.360
Gender: Male, n (%)	98 (49.00)	101 (50.50)	0.090	0.764
Gender: Female, n (%)	102 (51.00)	99 (49.50)	0.090	0.764
Previous first aid knowledge baseline score	52.34 ± 8.12	53.01 ± 7.98	0.832	0.406

2.2. Methods

The experimental group consisted of five instructors who had completed an 8-hour standardized Basic Life Support training certified by the American Heart Association and passed the assessment. These instructors delivered lectures using developed standard videos and courseware, playing the videos while simultaneously explaining key knowledge points and providing feedback and corrections during students' practical sessions. Medical personnel did not participate in direct teaching interactions but only supervised the process off-site.

The control group was taught by American Heart Association instructors with more than five years of experience in cardiopulmonary resuscitation (CPR) training. They used the same standard videos and courseware as the experimental group and followed the same training procedures and duration.

Both groups received training on the same content: the importance of out-of-hospital cardiac arrest first aid, the principles and procedures of CPR, and the indications and operation methods of automated external defibrillators (AEDs). The total training time was 2 hours, including theoretical instruction, operational demonstrations, and practical exercises. Students in each group were divided into eight subgroups, with each subgroup provided with one CPR training manikin and one AED training device to ensure that all participants conducted at least five simulated CPR + AED operations.

2.3. Observation indicators

- (1) First aid theoretical knowledge score: A theoretical knowledge test was conducted immediately after the training, with a full score of 100 points.
- (2) CPR practical skill pass rate: Practical assessments were conducted immediately after the training and three months post-training. The scoring sheet was developed based on American Heart Association standards, with a full score of 100 points, and a score of 80 or above considered passing ^[4].
- (3) Training attitude scale score: A self-made "CPR/AED Training Attitude Scale" was used, covering four dimensions: learning interest, operational confidence, willingness to provide first aid, and teaching satisfaction, with a full score of 100 points ^[5].
- (4) Non-inferiority judgment: Whether the 95% confidence interval of the difference in practical skill pass rates between groups after training was entirely within the preset non-inferiority margin.

2.4. Statistical analysis

SPSS 26.0 statistical software was used to process the study data. Continuous variables were expressed as mean + standard deviation (SD) and analyzed using *t*-tests; categorical variables were expressed as (*n*, %) frequencies and percentages and analyzed using χ^2 tests. A *P*-value < 0.05 indicated a statistically significant difference.

3. Results

3.1. Comparison of theoretical knowledge scores after training between the two groups

After training, the experimental group scored 86.54 ± 6.23 points in first aid theoretical knowledge, while the control group scored 87.12 ± 5.98 points. The comparison between the two groups showed $P < 0.05$ ($t = 0.942$, $P = 0.347$).

3.2. Comparison of practical skill pass rates after training and three months post-training

Between the two groups, the practical skill pass rate in the experimental group was higher than that in the control group after training, as shown in **Table 2**.

Table 2. Comparison of practical skill pass rates between the two groups [*n* (%)]

Time Point	Experimental Group (<i>n</i> = 200)	Control Group (<i>n</i> = 200)	Difference (%)	95% Confidence Interval	Non-Inferiority Conclusion
After training	185 (92.50)	189 (94.50)	-2.00	-5.23 to 1.23	Non-inferior
3 months later	172 (86.00)	176 (88.00)	-2.00	-6.12 to 2.12	Non-inferior

3.3. Comparison of scores on the training attitude scale between the two groups

Both groups of students scored relatively high on the overall training attitude scale, as detailed in **Table 3**.

Table 3. Scores on the training attitude scale for the two groups (mean $\pm$ SD, points)

Dimension	Experimental Group (<i>n</i> = 200)	Control Group (<i>n</i> = 200)	<i>t</i> value	<i>P</i> value
Learning Interest	22.56 $\pm$ 1.23	22.78 $\pm$ 1.18	1.812	0.071
Confidence in Operation	21.98 $\pm$ 1.56	22.12 $\pm$ 1.48	0.913	0.362
Willingness for First Aid	23.45 $\pm$ 1.34	23.56 $\pm$ 1.29	0.827	0.409
Satisfaction with Teaching	23.24 $\pm$ 1.12	23.25 $\pm$ 1.18	0.089	0.465
Total Score	91.23 $\pm$ 5.12	92.08 $\pm$ 4.89	1.673	0.095

3.4. Feedback on teaching experience from teachers

All five teachers involved in delivering the lessons completed the teaching experience feedback questionnaire. All teachers believed that the standard videos significantly aided teaching by effectively reducing lesson preparation difficulty; 80% of teachers indicated that after training, they were fully capable of completing cardiopulmonary resuscitation (CPR) teaching tasks. The overall satisfaction rating of teachers with the training model was 94.20 $\pm$ 3.15 points.

4. Discussion

The golden window for resuscitation after cardiac arrest is four minutes, and over 500,000 people die annually in China. Pre-hospital CPR can effectively improve the success rate of resuscitation, but currently, the CPR training rate among the Chinese public is less than 1%, far behind the levels in developed countries. Students are one of the most suitable groups to receive CPR training, making CPR education in schools the most effective method for cultivating citizens' first aid capabilities^[6]. However, a shortage of qualified instructors has become a critical factor hindering the promotion of first aid education in schools. The “train-the-trainer” model can expand the training effect through a three-tier transmission from “medical personnel-teachers-students,” but non-professional teachers may have uncertainties regarding the depth of medical knowledge and the standardization of teaching. Standard video instruction can unify content, standardize processes, and reduce quality fluctuations caused by differences in instructors' backgrounds^[7]. This study combined the train-the-trainer model with standard video instruction, using medical personnel teaching as a

reference to evaluate the non-inferiority of teachers' teaching effectiveness, providing empirical evidence for the scientific validity of this training model. The study showed that after training, there was no significant difference in the scores of emergency theory knowledge between the experimental group and the control group. Both groups used the same standard videos and courseware, ensuring high consistency in teaching content. Teachers in the experimental group participated in an 8-hour basic life support standardized training session before teaching and obtained certification, mastering the theoretical essence and key technical points of CPR, enabling them to correctly understand the teaching content in the videos and answer students' questions. During the teaching process, teachers used the courseware in conjunction with the videos, allowing students to learn alongside the videos, compensating for potential gaps in knowledge depth among teachers without a medical background.

The similar theoretical exam scores of students in both groups indicate that the dominant role of standard videos in knowledge transmission is sufficient to diminish differences in instructors' professional backgrounds. Regarding the pass rate of practical skills, after training, both the experimental and control groups had pass rates exceeding 90%, with the 95% confidence interval for the difference in pass rates between the two groups entirely within the predefined non-inferiority margin, proving that teachers' teaching effectiveness is not inferior to that of medical personnel. The learning of operational skills relies on correct demonstration of movements and timely error correction guidance. The standard actions for chest compressions (location, depth, frequency) and artificial respiration demonstrated by professionals in the standard videos are more standardized and reproducible than those demonstrated by any instructor on-site; teachers provide feedback and corrections to students during practice according to video standards, ensuring that each student receives the same quality of guidance ^[8].

Additionally, teachers are the teaching staff students interact with most frequently, possessing a stronger affinity and classroom management skills, enabling them to better motivate student participation and enthusiasm for practice. The follow-up pass rates for both groups three months after training decreased to varying degrees, but the decrease in the experimental group was the same as that in the control group, indicating that the skill retention rates of students trained by both methods are similar, and both require periodic reinforcement training to consolidate ^[9]. The training attitude scale results showed that both groups of students had high levels of interest in learning, confidence in performing operations, and willingness to provide first aid, with no significant differences between the two groups. In terms of teaching satisfaction, the control group was slightly higher than the experimental group, likely due to students' recognition of the authority of professional medical personnel.

However, the overall score difference was not significant, indicating that teachers can also enhance student motivation and willingness to provide first aid when teaching based on standard videos. The vividness, intuitiveness, and case-based approach of the standard videos attract students' attention and reduce the monotony of skill learning. Teachers reported that the videos significantly reduced their lesson preparation workload, standardized and made the teaching process reproducible, and increased their confidence in participating in first aid teaching. This study confirms the feasibility and effectiveness of combining the train-the-trainer model with standard videos in conducting school first aid training. Teachers who have completed short-term standardized training can independently conduct student first aid lessons. This model is no longer limited by the number of medical personnel serving as first aid instructors, providing a replicable template for broader implementation of school CPR teaching ^[10]. However, this study has some

limitations. It was conducted in only one school, lacking representativeness; it did not further analyze differences in teaching effectiveness among individual teachers; and the three-month follow-up period was insufficient to understand long-term skill retention rates. Further research is needed to address these issues.

5. Conclusion

In conclusion, under the train-the-trainer model, teachers conducting CPR training for middle school students based on standard videos achieve teaching effectiveness that is non-inferior to that of professional medical personnel. This model can effectively alleviate the shortage of first aid training instructors in schools, featuring standardization, replicability, and ease of promotion, making it a preferred option for school first aid education.

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Research on the Correlation between Eye Movement Characteristics and Cognitive Impairment in Patients with Cerebral Small Vessel Disease

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Abstract: *Objective:* To explore the eye movement characteristics of patients with cerebral small vessel disease (CSVD) and their correlation with cognitive impairment, and to validate the application value of eye-tracking technology in the early screening of CSVD-related cognitive impairment. *Methods:* A total of 81 subjects were included, with 51 in the CSVD group and 30 in the healthy control group. Cognitive assessment was conducted using the MoCA scale, and visual pairing and antisaccade tasks were detected using an AI-assisted eye-tracking system. Differences in eye movement parameters between the two groups were compared, and the correlation between eye movement indicators and cognitive scores was analyzed. *Results:* The saccade error rate in the CSVD group was significantly higher than that in the control group ($P = 0.009$). The total MoCA score, visuospatial and executive function, attention, and language function scores were significantly lower in the CSVD group than in the control group (all $P < 0.05$). The saccade error rate was significantly negatively correlated with the total MoCA score ($r_s = -0.382$, $P = 0.0005$) and visuospatial and executive function score ($r_s = -0.313$, $P = 0.005$). *Conclusion:* Patients with CSVD exhibit characteristic eye movement abnormalities, and the saccade error rate can serve as an objective biomarker reflecting their executive function and overall cognitive impairment. Eye-tracking technology provides a non-invasive, objective, and efficient new means for the early screening of CSVD-related cognitive impairment.

Keywords: Cerebral small vessel disease; Cognitive impairment; Eye-tracking; Antisaccade; Biomarker

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1. Introduction

With the acceleration of global population aging, the incidence of cerebral small vessel disease (CSVD)

continues to rise, leading to approximately one-fourth of ischemic strokes and 35%–67% of vascular dementia cases, imposing a heavy disease burden on families and society ^[1,2]. CSVD-related cognitive impairment has an insidious onset and slow progression, often manifesting as mild cognitive impairment (MCI) in the early stages. If not promptly identified and intervened upon, it can easily progress to irreversible dementia. Currently, clinical cognitive assessment still relies mainly on neuropsychological scales such as MMSE and MoCA, which have limitations such as strong subjectivity, time-consuming, significant influence by educational level and audiovisual status, and high professional requirements for the examiner, making it difficult to meet the needs of large-scale, primary-level, and early rapid screening. Eye movements directly reflect core cognitive functions such as attention, executive control, working memory, and visual processing, and their abnormalities can objectively indicate corresponding brain network damage ^[1]. CSVD-induced white matter hyperintensities, lacunar infarcts, microbleeds, and other lesions often disrupt the prefrontal-subcortical inhibitory control pathway, leading to abnormalities in saccade, fixation, and antisaccade eye movement behaviors. In recent years, eye-tracking (ET) technology has demonstrated good biomarker value in the screening of MCI related to Alzheimer's disease due to its non-invasive, objective, repeatable, portable, and efficient advantages ^[3]. However, there is still a lack of sufficient evidence-based evidence regarding the specific eye movement characteristics of CSVD-MCI, their quantitative correlation with cognitive impairment, and screening efficacy. This study systematically analyzed the differences in eye movement characteristics in patients with CSVD, clarified the correlation between key eye movement indicators and cognitive domain impairment, and validated the early screening value of eye-tracking technology, providing clinical evidence for constructing a digital screening system for CSVD-related cognitive impairment.

2. Objects and methods

2.1. Study objects

This study included 51 patients with sporadic CSVD diagnosed in the Department of Neurology at Shaanxi Provincial People's Hospital from February 2024 to December 2025 as the case group; 30 healthy individuals who underwent physical examinations during the same period were selected as the control group. The study protocol was reviewed and approved by the hospital's ethics committee, and all enrolled subjects or their family members signed informed consent forms.

Inclusion criteria: (1) Age between 50 and 80 years old; (2) Met the diagnostic criteria for sporadic CSVD in the "Expert Consensus on the Diagnosis and Treatment of Cerebral Small Vessel Disease in China 2021" ^[2]; (3) Had at least 6 years of education.

Exclusion criteria: (1) Had mental disorders or reading disorders; (2) Had strabismus, amblyopia, dry eye, color vision abnormalities, or other refractive diseases affecting the test results; (3) Had significant visual fatigue within 3 days before the test; (4) Had acute stroke or transient ischemic attack within the past 3 months, or CSVD caused by metabolic, toxic, infectious, or genetic factors; (5) Had intracranial or extracranial artery stenosis > 50%, a history of large-area cerebral infarction or intracranial hemorrhage; (6) Had other neurological diseases such as Alzheimer's disease, Parkinson's disease, or intracranial tumors; (7) Had severe heart, liver, kidney, or thyroid dysfunction; (8) Had a history of alcohol/drug dependence, malignant tumor, or brain surgery; (9) Were unable to cooperate with eye movement testing.

2.2. Methods

2.2.1. Collection of general and clinical data

Detailed records were kept of the subjects' gender, age, duration of education, body mass index (BMI), and clinical information such as hypertension, type 2 diabetes, hyperlipidemia, atrial fibrillation, coronary heart disease, stroke history, smoking history, and alcohol consumption history; laboratory tests were also conducted on the subjects, including blood routine, uric acid (UA), fasting blood glucose, and homocysteine (Hcy).

2.2.2. Neuropsychological cognitive assessment

Cognitive assessment was conducted by professionally trained personnel in a quiet, distraction-free environment on a one-on-one basis. The specific assessment content was as follows: The Montreal Cognitive Assessment Scale (MoCA) was used to comprehensively evaluate the subjects' total score and dimensions such as visuospatial and executive function, attention, language function, abstract thinking, memory, and orientation.

2.2.3. Eye-tracking detection

Eye movement detection was performed using the NW-PAD-2022V1.0 intelligent eye-tracking cognitive assessment system, which relies on the front camera of an ordinary Android tablet to achieve AI-assisted eye-tracking functionality with a sampling frequency of no less than 30 Hz. During detection, the distance between the subject and the device was controlled within 0.3–0.8 m, with a positioning error of no more than 1 cm, and the entire detection process took approximately 5 minutes per session.

The specific detection tasks included two items:

- (1) Visual pairing task: During the encoding phase, 5 groups of pictures were presented sequentially, with each group displayed for 5 seconds; during the recognition phase, new and old pictures were presented in a paired manner, and relevant eye movement parameters such as fixation duration and preference ratio were recorded.
- (2) Antisaccade task: A total of 12 trials were set, requiring subjects to actively shift their gaze to the opposite side of the stimulus signal, and core detection indicators such as saccade error rate, latency, peak velocity, and gain ratio were recorded.

2.3. Statistical methods

Data analysis was performed using SPSS 27.0 software. Measurement data were expressed as mean $\pm$ standard deviation (SD) or median (quartile) [M (P25, P75)], with independent sample *t*-tests for normal distributions and Mann-Whitney U tests for non-normal distributions; count data were expressed as the number of cases (percentage), with χ^2 tests or Fisher's exact tests performed; correlation was analyzed using Spearman's rank correlation analysis. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Comparison of baseline data between the two groups

There were no statistically significant differences in gender, hypertension, diabetes, atrial fibrillation, past medical history, BMI, homocysteine, fasting blood glucose, and uric acid between the two groups (all $P > 0.05$), as shown in **Table 1**.

Table 1. Comparison of baseline data between the two groups of subjects

Item	Healthy Control Group (n = 30)	CSVD Group (n = 51)	t/χ^2	<i>P</i>
Gender (male, n, %)	2 (6.7)	26 (51.0)	0.92	0.34
Age (years)	57.63 ± 8.92	63.90 ± 7.94	3.27	0.002
Hypertension (n, %)	19 (63.3)	37 (72.5)	0.75	0.386
Type 2 diabetes (n, %)	4 (13.3)	13 (25.5)	1.69	0.194
Atrial fibrillation (n, %)	0 (0.0)	1 (2.0)	-	0.630 ¹
History of stroke (n, %)	2 (6.7)	13 (25.5)	4.45	0.035
Coronary heart disease (n, %)	2 (6.7)	7 (13.7)	0.00	0.96
Smoking history (n, %)	3 (10.7)	8 (16.3)	0.46	0.5
Drinking history (n, %)	1 (10.0)	2 (3.9)	0.84	0.36
BMI (kg/m ²)	25.02 ± 3.23	23.82 ± 2.96	1.72	0.09
Homocysteine (μmol/L)	14.74 ± 5.83	14.93 ± 5.64	0.15	0.89
Fasting glucose (mmol/L)	5.21 ± 2.06	4.86 ± 1.11	0.87	0.39
Uric acid (μmol/L)	303.74 ± 79.54	313.12 ± 76.55	0.53	0.6

Notes: (1) Due to the theoretical frequency of the atrial fibrillation indicator being less than 5, Fisher's exact test was used; (2) Smoking history: There were 28 valid cases in the healthy control group and 49 valid cases in the cerebral small vessel disease group (the rest were missing values); (3) Alcohol consumption history: There were 10 valid cases in the healthy control group (the rest were missing values); (4) Categorical variables are expressed as n (%), and the χ^2 test was used. Continuous variables are expressed as "mean ± standard deviation (SD)", and the independent samples t-test was used; (5) *P*-value less than 0.05 indicates a statistically significant difference.

3.2. Comparison of eye movement indicators between the two groups

The saccade error rate in the CSVD group was significantly higher than that in the control group ($P = 0.009$). There were no statistically significant differences between the groups in terms of latency, correct rate of error correction, saccade velocity, saccade gain, gaze duration on new/old images, total mean gaze duration, and the number of blinks during gaze (all $P > 0.05$). See **Table 2**.

Table 2. Comparison of eye movement indicators between the two groups

Variable	Healthy Control Group (n = 30)	CSVD Group (n = 51)	Statistic	<i>P</i>
Delay time (ms)	268.63 (226.37, 329.78)	314.63 (229.86, 524.08)	625	0.172
Error rate (%)	0.45 ± 0.28	0.61 ± 0.24	-2.696	0.009
Error correction accuracy (%)	0.42 (0.21, 0.59)	0.34 (0.17, 0.42)	740	0.189
Correction delay (ms)	303.53 (232.70, 349.49)	322.99 (272.88, 420.56)	506	0.229
Average velocity (pixel/ms)	1.41 (1.22, 1.65)	1.36 (1.14, 1.73)	838.5	0.475
Maximum velocity (horizontal) (pixel/ms)	2.67 (2.23, 3.52)	2.48 (2.06, 3.67)	814.5	0.632
Fixation blink count	0.00 (0.00, 0.00)	0.00 (0.00, 0.09)	634.5	0.086
Saccade gain (initial)	-0.33 (-6.93, 3.78)	0.05 (-4.55, 4.78)	721	0.671
Saccade gain (terminal)	-14.11 ± 66.71	2.75 ± 58.73	-1.186	0.239
Saccade error rate (corrected) (%)	0.91 (0.75, 1.00)	0.84 (0.65, 1.00)	844	0.429
Correct saccade latency (corrected) (ms)	369.26 (302.00, 501.75)	441.62 (312.94, 612.14)	621	0.202
Correct saccade latency (ms)	369.26 (302.00, 501.75)	441.62 (312.94, 612.14)	621	0.202
Average dwell time on new images (ms)	4512.75 (3058.55, 4958.23)	4307.20 (3214.05, 5026.00)	752	0.903
Average dwell time on old images (ms)	1832.95 (1546.35, 3328.35)	2062.70 (1451.10, 3360.00)	763	0.988

Variable	Healthy Control Group (n = 30)	CSVD Group (n = 51)	Statistic	P
Total average gaze duration (ms)	6509.35 (6213.18, 6999.53)	6596.00 (6031.50, 7040.80)	724	0.692
Fixation blink count	0.00 (0.00, 0.00)	0.00 (0.00, 0.09)	634.5	0.086

Notes: (1) Normally distributed data are presented as “mean ± standard deviation,” while non-normally distributed data are shown as “median (interquartile range).” (2) The choice of statistical method is based on the results of the Shapiro-Wilk test for normality; for normally distributed data, an independent samples *t*-test is used, whereas for non-normally distributed data, the Mann-Whitney U test is applied. (3) A statistically significant difference is defined as $P < 0.05$.

3.3. Comparison of MoCA cognitive scores between the two groups

The CSVD group had significantly lower total MoCA scores, as well as lower scores in visuospatial and executive function, attention function, and language function compared to the control group (all $P < 0.05$). There were no significant differences in abstraction function, memory function, or orientation function scores between the two groups (all $P > 0.05$).

Table 3. Comparison of MoCA scale scores in each dimension between the two groups [M(P25,P75)]

Item	Healthy Control Group (n =3 0)	CSVD Group (n = 51)	U	P
MoCA total score	26.0 (25.0, 27.0)	24.0 (21.2, 26.0)	1145.5	<0.001
Visuospatial and Executive Function score	5.0 (4.0, 5.0)	3.0 (2.0, 4.0)	1145	<0.001
Attention score	6.0 (5.0, 6.0)	5.0 (5.0, 6.0)	985.5	0.004
Language score	6.0 (6.0, 6.0)	6.0 (5.0, 6.0)	892	0.039
Abstraction score	2.0 (1.0, 2.0)	1.0 (1.0, 2.0)	843.5	0.171
Memory score	4.0 (4.0, 4.0)	4.0 (3.0, 4.0)	880	0.094
Orientation score	5.0 (5.0, 5.0)	5.0 (5.0, 5.0)	713.5	0.876

Note: The data in the table are presented as median (interquartile range), and the Mann-Whitney U test was used; a statistically significant difference is defined as $P < 0.05$. MoCA: Montreal Cognitive Assessment.

3.4. Correlation between saccade error rate and cognitive scores

Spearman rank correlation analysis revealed a moderate negative correlation between saccade error rate and total MoCA score ($r_s = -0.382$, $P = 0.0005$); a significant negative correlation with visuospatial and executive function scores ($r_s = -0.313$, $P = 0.005$); and no significant correlation with attention, language, abstract, memory, or orientation function scores (all $P > 0.05$). Additionally, there was no correlation between saccade error rate and the severity of CSVD burden ($r_s = -0.001$, $P = 0.996$). See **Tables 4, 5, and 6** for details.

Table 4. Correlation analysis between error rate and total MoCA score

Analysis Indicator	Statistical Method	Correlation Coefficient	P	Significance
Error Rate (%) vs. MoCA Total Score	Spearman	-0.382	0.0005	Yes

Note: Since the total MoCA score data exhibit a non-normal distribution ($P < 0.001$), the non-parametric Spearman rank correlation analysis method was employed.

Table 5. Correlation analysis between error rate and scores in each cognitive domain of the MoCA

Item	Correlation coefficient (r_s)	P
Visuospatial and Executive Function Score	-0.313	0.005
Attention Function Score	-0.07	0.539

Item	Correlation coefficient (r_s)	P
Language Function Score	-0.176	0.122
Abstract Function Score	-0.087	0.445
Memory Function Score	-0.113	0.321
Orientation Function Score	-0.149	0.190

Note: Spearman rank correlation analysis was applied; MoCA refers to the Montreal Cognitive Assessment; a statistically significant difference is defined as $P < 0.05$.

Table 6. Correlation analysis between eye movement error rate and severity grading of CSVD burden in the CSVD patient group

Statistical Indicator	Value
Sample size (n)	51
Correlation coefficient (r_s)	-0.001
P	0.996

Note: Spearman rank correlation analysis was used; CSVD refers to cerebral small vessel disease; a P -value < 0.05 indicates a statistically significant difference.

4. Discussion

CSVD is one of the primary vascular causes of cognitive impairment, often co-occurring with Alzheimer's disease, imposing substantial medical and economic burdens on patients' families and society, and representing a key public health issue requiring urgent attention ^[4]. Mild cognitive impairment serves as a transitional stage between normal aging and dementia, and early identification and intervention can effectively delay disease progression. Eye movement behavior, regulated by multiple cognitive-motor networks, directly reflects core cognitive functions, with abnormalities closely associated with pathological changes in brain function. Eye-tracking technology has thus emerged as a focal area of research in cognitive impairment studies. Research has confirmed that patients with CSVD exhibit specific eye movement abnormalities, primarily characterized by an increased error rate in antisaccades, which is negatively correlated with cognitive function and visuospatial executive function impairments, underscoring the value of this technology in cognitive assessment for CSVD ^[5].

Eye movement abnormalities are closely linked to structural damage in neural circuits within the brain ^[6]. This study found that the eye movement error rate in the CSVD group was significantly higher than that in the healthy control group, with no significant intergroup differences in other eye movement metrics, suggesting that eye movement abnormalities in CSVD primarily affect inhibitory control functions while sparing basic motor eye movement functions. The core mechanism lies in the fact that antisaccade tasks rely on the fronto-parietal-subcortical inhibitory control network, and typical pathological changes in CSVD are prone to damaging this pathway, leading to decreased inhibitory control function and, consequently, an increased eye movement error rate ^[7].

Cognitive function assessment revealed that the total MoCA score in the CSVD group was significantly lower than that in the healthy control group, with cognitive impairments primarily concentrated in three cognitive domains: visuospatial and executive function, attention function, and language function, while abstraction thinking, memory, and orientation functions showed no significant abnormalities. This pattern

aligns with the clinical characteristics of CSVD-related cognitive impairments^[8]. CSVD-induced white matter lesions are prone to affecting cognitive-related nerve fibers in deep brain regions and the brainstem, with the fronto-parietal network and thalamocortical pathways being particularly vulnerable to ischemic damage, whereas brain regions associated with memory and orientation functions are less affected in the early stages. This pattern is consistent with previous studies, confirming the reliability of the assessment results.

This study found that the eye movement error rate exhibited a moderate negative correlation with the total MoCA score and a significant negative correlation with visuospatial executive function scores, with no significant associations with other cognitive domains, suggesting its specific reflective value for this type of cognitive impairment. The core mechanism is that CSVD-induced damage to the fronto-parietal-subcortical network both increases the eye movement error rate and impairs visuospatial executive function, sharing a consistent pathological basis. The corrected saccade error rate results corroborate this finding, suggesting that eye movement inhibitory control indicators could serve as potential biomarkers^[9,10]. Additionally, no significant correlation was observed between the eye movement error rate and CSVD burden, likely due to the small sample size and concentrated lesion grading, necessitating further validation with larger samples.

The core value of this study lies in clarifying the application prospects of eye-tracking technology in cognitive impairment assessment for CSVD. Traditional neuropsychological scales currently relied upon in clinical practice have numerous limitations and are ill-suited for early screening needs at the grassroots level. Eye-tracking technology is non-invasive, objective, portable, and efficient, making it suitable for elderly individuals with low education levels who are at high risk. This study confirms that the eye movement error rate can serve as an objective indicator to assist traditional scales in improving early identification accuracy. Combined with multimodal approaches, it holds promise for widespread application across multiple scenarios, facilitating the achievement of “early identification, early intervention” goals.

This study also has certain limitations, manifested in the following aspects: (1) The sample size was limited, and the single-center design introduced selection bias; (2) Only a single core eye movement indicator was validated, without forming a multidimensional biomarker combination; (3) Screening performance evaluations such as sensitivity, specificity, and ROC curves were not conducted; (4) Long-term follow-up to verify prognostic value was not performed. Future research should involve larger samples, multi-center, prospective studies to establish normative data for the Chinese population, optimize AI algorithms, and promote device miniaturization and clinical translation.

5. Conclusion

In summary, patients with CSVD exhibit specific eye movement abnormalities, primarily characterized by an increased antisaccade error rate. The saccade error rate shows a significant negative correlation with the total MoCA score and visuospatial and executive function, serving as an objective biomarker reflecting CSVD-related cognitive impairment. Eye-tracking technology is non-invasive, efficient, and objective, making it suitable for early screening and dynamic monitoring of CSVD-related cognitive impairment, with significant clinical translation prospects.

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Disclosure statement

The authors declare no conflict of interest.

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Trends in the Burden of Ischemic Heart Disease in Women: An Analysis of High Body Mass Index and Low Physical Activity Factors Based on GBD 2021 Data

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Abstract: *Objective:* This study systematically assessed temporal trends in the global burden of ischemic heart disease (IHD) attributable to high body mass index (BMI) and low physical activity (PA) among women from 1990 to 2021. *Methods:* Data were obtained from the 2021 Global Burden of Disease Database, which covers disability-adjusted life years (DALYs), mortality rates, years lived with disability (YLDs), years of life lost (YLLs), and age-standardized rates (ASRs) attributable to high BMI and low PA among women. ASRs and estimated annual percentage changes (EAPCs) were calculated over 32 years. Temporal trends were analyzed by country, region, and sociodemographic index (SDI). A decomposition analysis was performed, and future predictions were made using the ARIMA time-series method to assess these trends comprehensively. *Results:* DALYs associated with IHD attributable to high BMI in women increased from 5.17 million in 1990 to 9.87 million in 2021. Deaths increased from 243,512 to 461,122, YLDs from 85,634 to 238,639, and YLLs from 5.08 to 9.63 million. However, DALYs related to female IHD caused by low PA increased from 146 to 242 million. Deaths increased from 87,707 to 152,305, YLDs from 23,564 to 54,301, and YLLs from 144 to 236 million. Although the ASRs of DALYs, mortality, and YLLs associated with high BMI and low PA have decreased, the ASR of YLDs has remained relatively stable over 32 years. This overall increase in disease burden underscores the urgency of intervention strategies to address the risk factors of high BMI and low PA. Significant regional and national differences were observed, with the burden shifting from high-SDI areas to low- and low-middle-SDI areas, a trend further amplified by population growth and aging. Although overall health inequalities have decreased, predictions indicate that the ASR of female IHD will continue to decline over the next 29 years, whereas the ASR of YLDs is

expected to increase. This indicates that although women's health awareness is increasing, more efforts are needed to maintain cardiovascular health, reduce the burden of IHD, and improve the quality of life for women. *Conclusions:* Since 1990, high BMI and insufficient PA have substantially contributed to the global burden of IHD in women, with profound variation by age, region, and sociodemographic context. These effects differ from those in men due to women's unique physiological and social roles, underscoring the need for targeted health strategies aimed at women. Nations should focus on managing BMI and promoting physical activity to reduce the IHD burden and inform public health policies to improve women's cardiovascular health and quality of life.

Keywords: Ischemic heart disease; Female; Burden of disease (GBD 2021); Estimated annual percentage change (EAPC); Decomposition analysis; Health inequality; ARIMA time series prediction

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1. Introduction

Cardiovascular diseases (CVDs) affect over 500 million individuals globally and accounted for approximately 20.5 million deaths in 2021, constituting nearly one-third of all global mortality ^[1]. According to the World Heart Federation's 2021 World Heart Report, Ischemic heart disease (IHD) is the leading cause of death globally. Therefore, IHD constitutes a major burden of cardiovascular disease on a global scale and is recognized as a substantial hindrance to sustainable development in the 21st century. Consequently, the effective prevention and management of IHD remains a critical global health priority.

A previous study demonstrated that the onset of IHD in females typically occurs approximately one decade later than in males ^[2]. These temporal gaps span approximately 20 years for major adverse cardiovascular events such as MI and sudden cardiac death. The prevalence of IHD is three times higher in women aged over 65 years than in younger women. Compared to men, women typically endure a higher burden of comorbidities and demonstrate poorer outcomes associated with IHD ^[3]. The primary behavioral risk factors for conventional IHD include unhealthy diet, elevated body mass index (BMI), inadequate physical activity (PA), tobacco use, and excessive alcohol intake. These factors substantially facilitate the progression of IHD in both genders. Previous studies have focused on risk factors such as tobacco use, elevated sodium consumption, red meat intake, and different metabolic parameters ^[4,5]. High BMI and inadequate PA are recognized as significant risk factors for IHD ^[6,7]. Numerous studies have confirmed the significant association between elevated BMI and cardiovascular diseases, with this risk further exacerbated by low levels of PA ^[8]. However, the specific effect of these two factors on the IHD burden in women, and research into their underlying mechanisms, remains insufficiently examined. Therefore, a comprehensive evaluation of the impact of elevated BMI and reduced PA on the incidence of IHD in women is crucial. This assessment is essential to clarify the underlying pathogenesis of IHD and formulate appropriate intervention strategies. The management of BMI and the promotion of PA are recognized as cost-effective and high-priority strategies for alleviating the burden of cardiovascular diseases. Consequently, it is essential to determine the global distribution of IHD burden in women due to high BMI and insufficient PA.

This study, for the first time using data from the GBD 2021, employed age-standardized rates (ASR) and estimated annual percentage change (EAPC) analyses to evaluate the evolving patterns in the IHD burden among women from 1990 to 2021 on global, national, and regional scales. The study aimed to assess the

geographical and temporal patterns of the disease burden associated with IHD among females, resulting from elevated BMI and insufficient PA across various age demographics. The impact of the sociodemographic index (SDI) and prospective future trends was also analyzed. Through a comprehensive examination of these risk determinants, we aim to provide a scientific basis for the development of targeted public health treatments and the formulation of associated health policies. This initiative seeks to enhance the women's health status, reduce healthcare costs, and enhance their quality of life.

2. Methods

2.1. Study population and data sources

We employed data from the Global Burden of Disease (GBD) 2021 database to evaluate the impact of high BMI and low PA on IHD in women. The GBD 2021 database provides detailed data on 371 diseases and injuries and 88 risk factors across 204 countries and regions and 21 geographical areas^[9,10]. In the analyses of high BMI and low PA, cases of women aged 20 to 95 and above were included. The data used in these analyses were all publicly available indicators, so no ethical approval was required.

2.2. Definitions

Body Mass Index (BMI) is used to assess overall health status. For people aged 20 and above, a BMI greater than 23 kg/m² is considered high. Low PA was assessed by weekly metabolic equivalent task (MET) minutes and included activity episodes lasting at least 10 minutes across occupational, household, transport, and leisure domains^[10]. Sociodemographic index (SDI), a composite indicator based on income, education, and fertility, was used to classify countries and regions into low, lower-middle, middle, upper-middle, and high SDI groups. The main indicators were deaths, disability-adjusted life years (DALYs), years lived with disability (YLDs), years of life lost (YLLs), and corresponding age-standardized rates (ASRs) per 100,000 population. DALYs were calculated as the sum of YLLs and YLDs. We utilized EAPC to assess the trends of diverse indicators within a specified time range, encompassing prevalence and incidence^[11].

2.3. Statistical analysis

By using a log-linear regression model based on ASR and calendar year, an analysis was conducted globally as well as by age group, GBD regions, countries or regions, and human development index (SDI) categories. The estimated annual percentage change rate (EAPC) was employed to reflect the temporal trend of ASR. The Das Gupta decomposition was used to separate the contribution of population growth, population aging, and epidemiological change to changes in burden^[12]. Pearson correlation was used to evaluate associations between SDI and disease burden. Health inequality was assessed by the slope index of inequality and the concentration index^[13]. Future trends were projected using autoregressive integrated moving average (ARIMA) models; stationarity, residual normality, autocorrelation, AIC, BIC, and Ljung-Box tests were used to evaluate model fit^[14]. All analyses were performed in R version 4.3.3^[15].

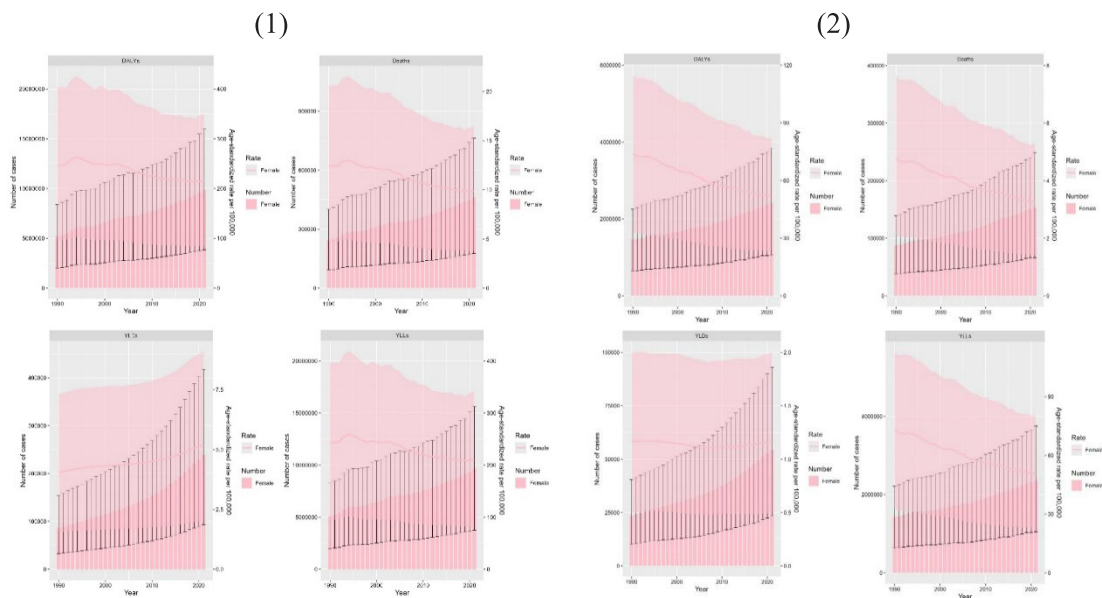
3. Results

3.1. Global trends in the burden of IHD in women due to high BMI and low PA

The absolute burden of female IHD attributable to high BMI increased substantially from 1990 to 2021.

DALYs rose from 5.17 million to 9.87 million, deaths from 243,512 to 461,122, YLDs from 85,634 to 238,639, and YLLs from 5.09 million to 9.63 million. Despite these absolute increases, the age-standardized rates of DALYs, deaths, and YLLs declined from 247.28 to 216.05, 12.43 to 9.92, and 243.24 to 210.85 per 100,000, respectively. Their EAPCs were -0.69, -0.98, and -0.72, indicating downward age-standardized trends. In contrast, the ASR of YLDs increased from 4.04 to 5.20 per 100,000, with an EAPC of 0.69, suggesting a growing nonfatal disability component.

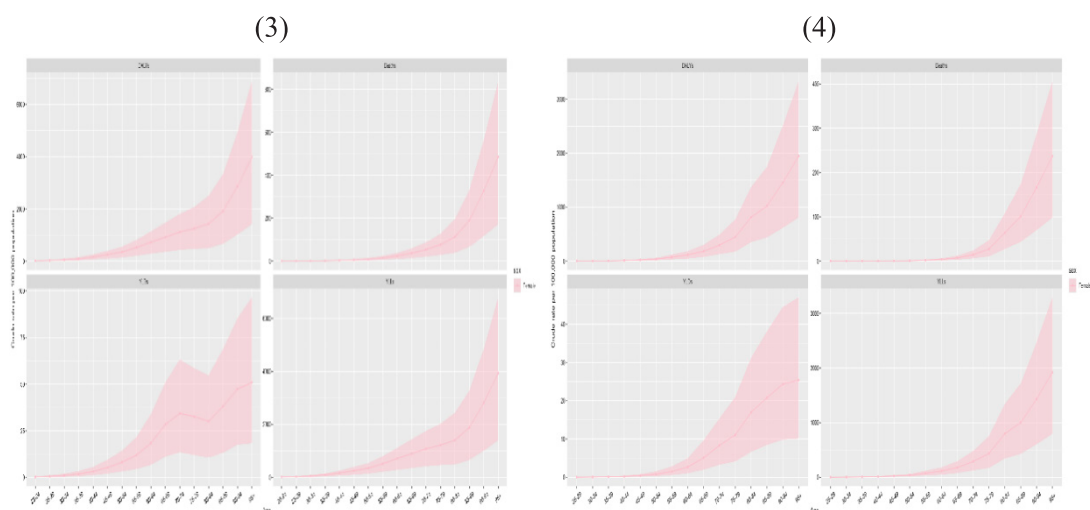
For low PA, the absolute burden also increased. DALYs rose from 1.46 million to 2.42 million, deaths from 87,707 to 152,305, YLDs from 23,564 to 54,301, and YLLs from 1.44 million to 2.36 million. However, the ASRs of DALYs, deaths, and YLLs declined from 73.85 to 51.88, 4.76 to 3.24, and 72.69 to 50.72 per 100,000, respectively, with EAPCs of -1.22, -1.29, and -1.24. The ASR of YLDs remained nearly stable, changing from 1.16 to 1.17 per 100,000. These findings indicate that mortality-related rates have improved, but population expansion, aging, and persistent disability have increased total burden (**Figures 1 and 2**).



Figures 1 and 2. Trends in number and age-standardized rates of DALYs, Deaths, YLDs, and YLLs attributable to high BMI /low PA for all ages from 1990 to 2021.

3.2. Global age trends in the burden of IHD in women due to high BMI and low PA

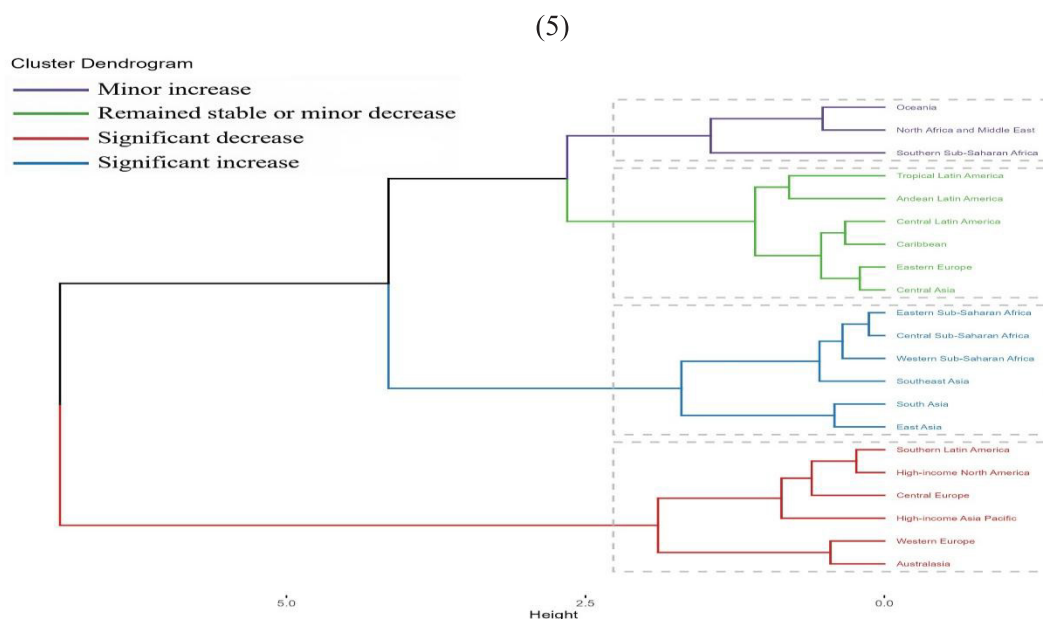
A primary prevention strategy that considers the demographic distribution of the population highlights the health concerns faced by different age groups. In 2021, the burden attributable to high BMI was concentrated among older women. DALYs, YLDs, and YLLs peaked in the 65-69-year age group, whereas deaths peaked among women aged 70-74 years. Age-standardized death, DALY, and YLL rates increased steadily with age and rose especially sharply after 80 years. YLD rates showed a decline in the 70-79-year groups and then increased again after 80-84 years. For low PA, deaths, DALYs, and YLLs were highest in the 80-84-year group, while YLDs peaked at 70-74 years. Mortality-related ASRs increased with age, particularly after 75-79 years, and YLD ASRs also rose progressively in older age groups. IHD in women attributable to elevated BMI and reduced PA presents a significant challenge to the elderly female population (**Figures 3 and 4**).



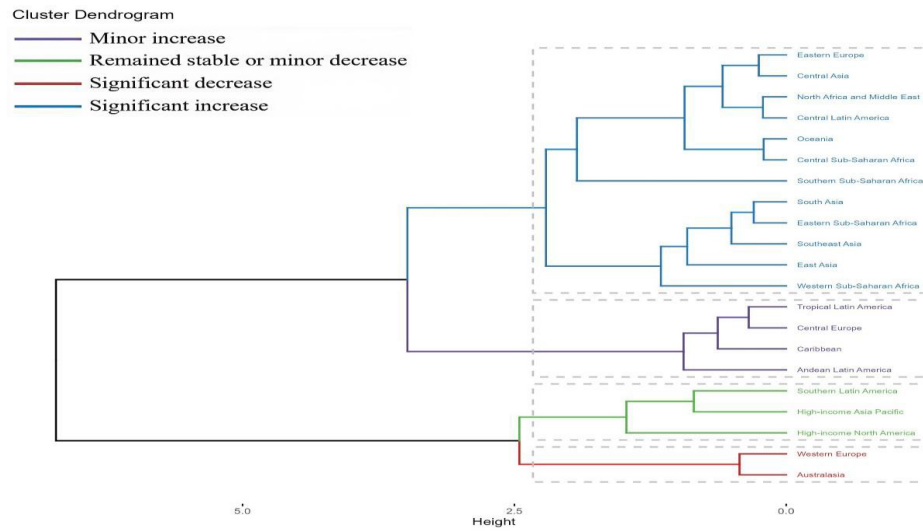
Figures 3 and 4. Trends in number and age-standardized rates of DALYs, Deaths, YLDs, and YLLs attributable to high BMI /low PA among women globally, by age group, 1990–2021.

3.3. Regional and national female IHD burden due to high BMI and low PA

The burden varied markedly across regions. For high BMI, seven of the 21 GBD regions showed increasing ASRs of DALYs and YLLs, with South Asia showing the most rapid increases in DALYs and YLLs. The ASR of deaths increased in seven regions, and the ASR of YLDs increased in 16 regions; East Asia had the largest increases in mortality and YLD rates. Australasia showed the most substantial decreases across DALYs, deaths, YLDs, and YLLs. For low PA, ASRs of DALYs and YLLs increased in three regions and death rates increased in five regions, with East Asia showing the largest increases in DALYs, deaths, and YLLs. YLD rates increased in 11 regions and rose most sharply in Western Sub-Saharan Africa, while Australasia again showed the strongest declines in all four indicators (**Figures 5 and 6**).



(6)



Figures 5 and 6. Cluster dendrogram of the Global Burden of IHD in Women Due to High BMI/low PA Across 21 Regions, 1990–2021.

At the national level, the 2021 burden showed large heterogeneity. For high BMI, the Marshall Islands had the highest ASR of DALYs, Nauru had the highest ASR of deaths, Qatar had the highest ASR of YLDs, and Egypt had the highest ASR of YLLs. Singapore had the lowest ASRs of DALYs and deaths, Ethiopia had the lowest YLD rate, and Japan had the lowest YLL rate. For low PA, Sudan had the highest ASRs of DALYs, deaths, and YLLs, whereas Tanzania had the lowest corresponding rates. The Marshall Islands had the highest YLD rate related to low PA, and Tanzania had the lowest. These national differences suggest that the combined influence of socioeconomic development, demographics, cardiometabolic risk, health-care access, and activity environments is substantial (**Figures 7 and 8**).

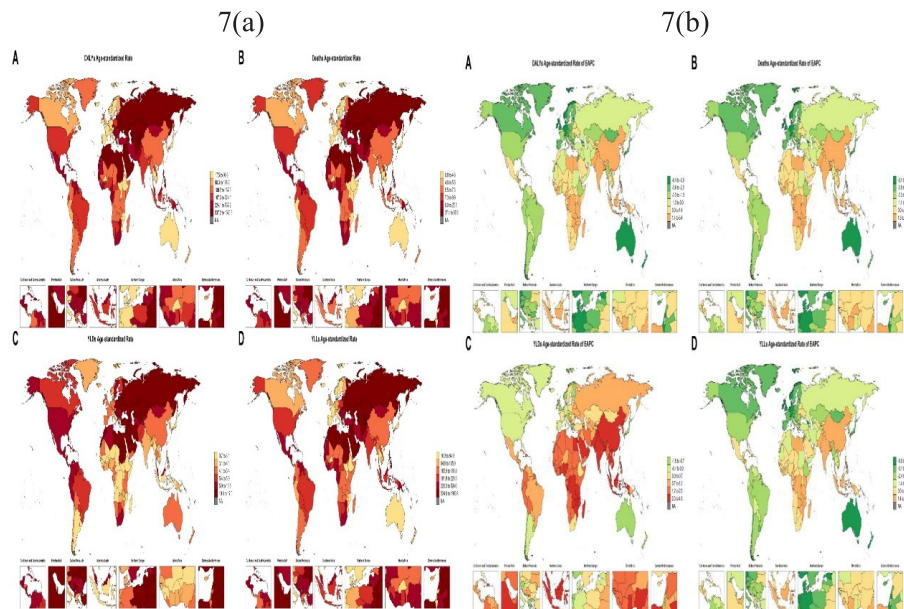


Figure 7 (a)(b). Global distribution of the burden of IHD among women and prevalence of EAPC attributable to High BMI in 2021 across 204 Countries from 1990 to 2021 (Calculated by Age-Standardized Rates of DALYs, Deaths, YLDs, and YLLs).

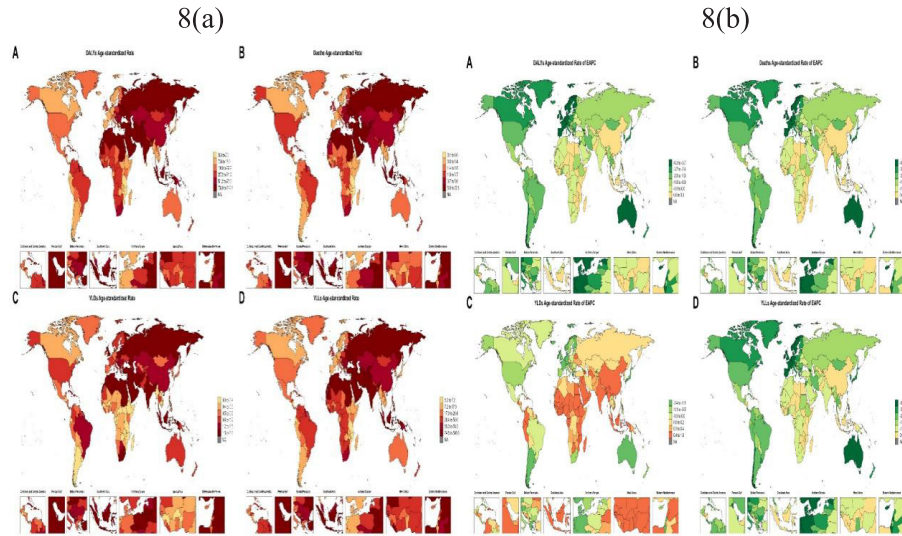
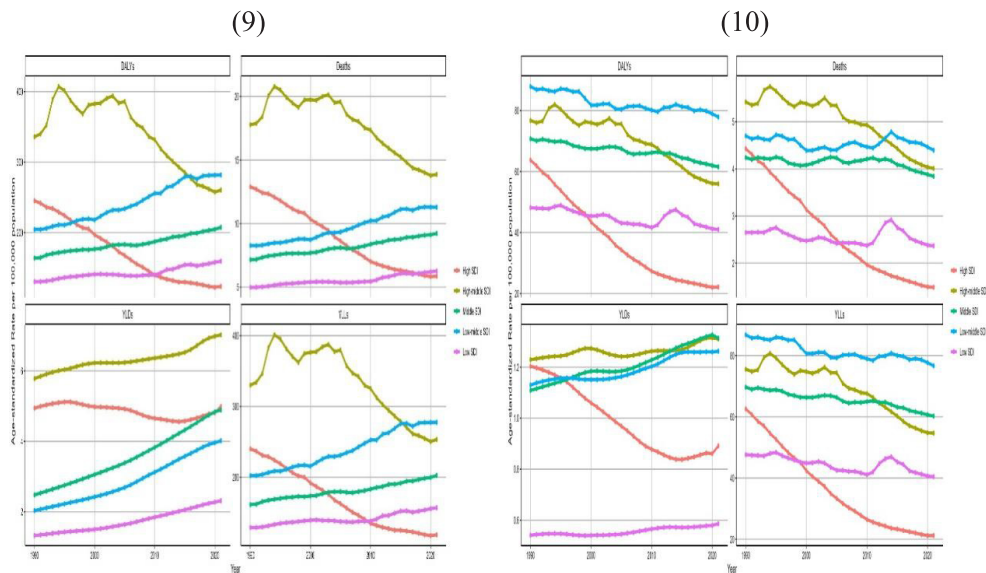


Figure 8(a)(b). Global distribution of the burden of IHD among women and prevalence of EAPC attributable to low PA in 2021 across 204 Countries from 1990 to 2021 (Calculated by Age-Standardized Rates of DALYs, Deaths, YLDs, and YLLs).Age-Standardized Rates (ASR); Estimated Annual Percentage Change (EAPC).

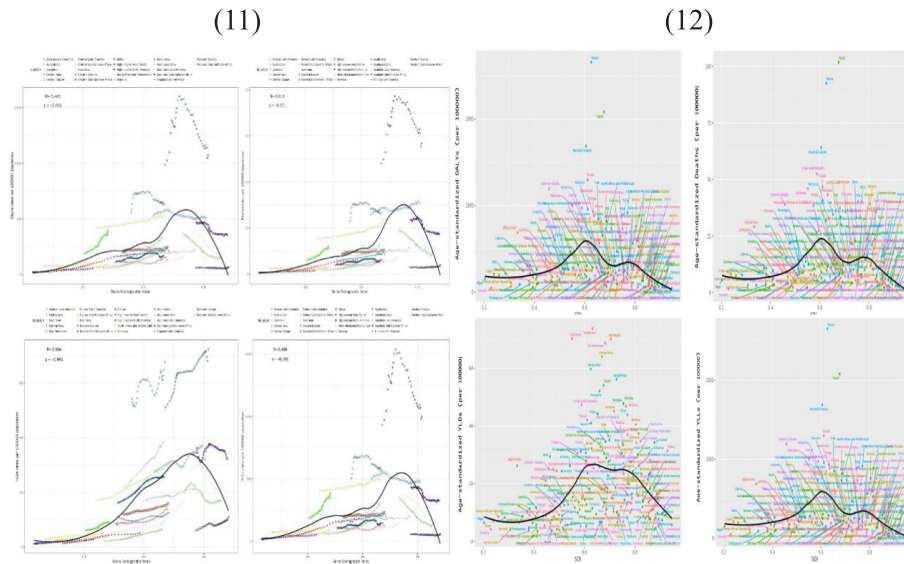
3.4. Research on SDI correlation

SDI-stratified analysis showed that high-SDI regions experienced the largest declines in mortality-related rates. For high BMI, the EAPCs for deaths, DALYs, and YLLs in high-SDI areas were -2.89, -2.52, and -2.59, respectively. Upper-middle-SDI regions also declined after approximately 2005, while lower-middle-SDI regions showed the most obvious increases. The ASR of YLDs declined slightly in high-SDI areas but increased in upper-middle-, middle-, lower-middle-, and low-SDI areas. For low PA, high-SDI regions again showed the greatest declines in death, DALY, YLL, and YLD rates. However, YLD rates increased slightly in middle- and lower-middle-SDI regions, indicating that nonfatal IHD burden is increasingly important in developing settings (**Figures 9 and 10**).

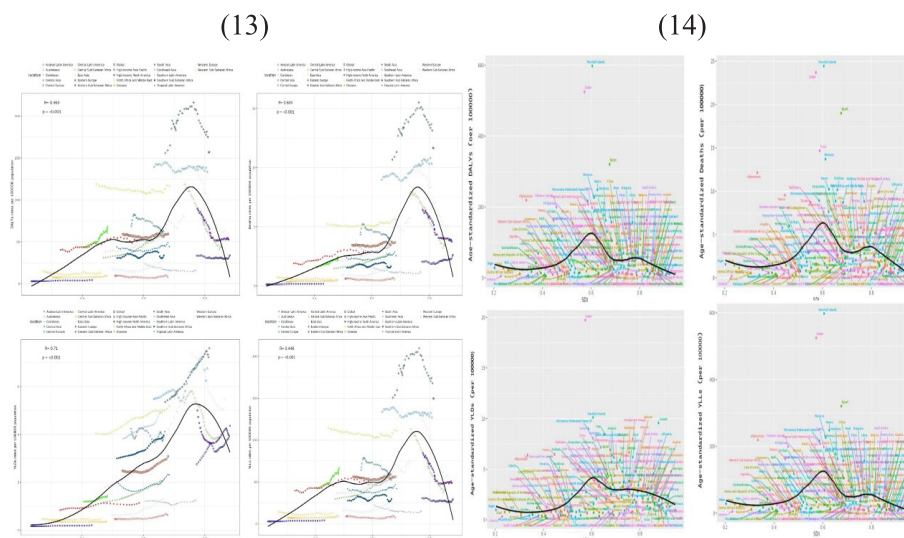


Figures 9 and 10. Time Trends in the Burden of IHD Among Women in SDI Regions Attributable to High BMI/ low PA from 1990–2021.

Across 21 regions and 204 countries, the association between SDI and IHD burden was nonlinear. For high BMI, ASRs generally followed an inverted or n-shaped relationship with SDI and declined markedly when SDI exceeded approximately 0.7. Eastern Europe, Central Asia, North Africa and the Middle East, and Oceania often had higher-than-expected burdens. The incidence of ischemic heart disease among global women due to insufficient physical activity (PA), after excluding the YLD (burden of disease) factor, is slightly higher than expected. It also presents a similar “n” shape relationship: it is more burdensome in several middle and higher development level regions. When the SDI exceeds 0.7, the correlation drops sharply. In 2021, across 204 countries, the SDI for female IHD attributable to low PA peaked at approximately 0.6, achieved a secondary peak at 0.8, and subsequently experienced a marked decline (**Figures 11 and 12** for high BMI) (**Figures 13 and 14** for low PA).



Figures 11 and 12. Relationship between age-standardized rates of DALYs, Deaths, YLDs, YLLs, and SDI levels attributable to female IHD due to high BMI in 21 regions/204 countries from 1990 to 2021. Expected values for SDI and disease rates based on all sites are shown as a black line.



Figures 13 and 14. Relationship between age-standardized rates of DALYs, Deaths, YLDs, YLLs, and SDI levels attributable to female IHD due to high BMI in 21 regions/204 countries from 1990 to 2021. Expected values for SDI and disease rates based on all sites are shown as a black line.

3.5. Decomposition analysis and health inequality analysis

Decomposition analysis indicated that the increase in population size and the aging demographic are recognized as primary contributors to the escalating incidence of IHD. The combined effect of high BMI and low PA makes the problem even more serious. For high BMI, population growth and aging increased global DALYs, deaths, and YLLs, whereas epidemiological changes partly offset mortality-related burden. For YLDs, epidemiological change contributed positively, consistent with the increasing nonfatal burden. By SDI category, low- and lower-middle-SDI settings were especially affected by population growth, whereas high-SDI settings showed reductions largely attributable to favorable epidemiological changes. For low PA, population growth and aging were also the dominant positive drivers of DALYs, deaths, and YLLs; epidemiological change had a negative contribution for fatal outcomes but little protective effect for YLDs (**Tables 1 and 2**)

Table 1. Changes in DALYs and Deaths by Determinants and Causes of Population Levels, 1990–2021

		DALYs					Deaths								
Cause	Location	Change due to Population-level determinants (%contribute to the total change)					Change due to Population-level determinants (%contribute to the total change)								
		Overall difference	Aging	Perc ent%	Popu lation	Perc ent%	Epidemi ological change	Perc ent%	Overall difference	Aging	Perc ent%	Popu lation	Perc ent%	Epidem iological change	
IHD attributable To High Body Mass Index	Global	46944 24.42	17646 93.13	37.59	39445 97.57	84.03	-10148 66.29	-21.62	2176 10.36	1115 38.30	51.26	1861 98.70	85.57	-801 26.60	-36.82
	Low SDI	3045 05.14	-676 9.14	-2.22	2523 48.78	82.87	5892 5.51	19.35	1032 8.60	-192.43	-1.86	847 5.70	82.06	204 5.32	19.80
	Low-middle SDI	15529 23.72	2050 03.20	13.2	9268 63.14	59.69	4210 57.33	27.11	5843 6.24	1013 0.29	17.34	3319 8.42	56.81	1510 7.52	25.85
	Middle SDI	20327 26.59	6244 79.41	30.72	10043 74.69	49.41	4038 72.49	19.87	9075 0.59	3261 9.23	35.94	4049 9.02	44.63	1763 2.34	19.43
	High-middle SDI	10149 31.59	7890 25.04	77.74	8400 49.42	82.77	-6141 42.87	-60.51	6615 3.92	5335 3.52	80.65	4455 8.71	67.36	-317 58.31	-48.01
	High SDI	-2127 18.48	4728 40.77	-22 2.28	4646 69.19	-218.44	-11502 28.44	540.73	-819 8.77	3740 3.30	-45 6.21	2679 6.94	-32 6.84	-7239 9.01	883.05
	Global	9532 73.41	5070 18.47	53.19	1154 612.19	121.12	-7083 57.26	-74.31	6459 8.03	4111 2.12	63.64	7142 0.80	110.56	-479 34.89	-74.2
	Low SDI	4575 6.05	-64 1.04	-1.4	603 63.74	131.93	-1396 6.65	-30.52	238 3.91	64.04	2.69	284 2.44	119.23	-522.56	-21.92
	Low-middle SDI	3002 63.42	7306 7.30	24.33	2866 76.67	95.48	-5948 0.56	-19.81	1672 2.84	470 4.05	28.13	1383 7.71	82.75	-181 8.92	-10.88
	Middle SDI	4957 17.03	2151 03.49	43.39	3703 36.24	74.71	-8972 2.70	-18.10	3187 0.09	1503 0.18	47.16	2047 1.59	64.23	-363 1.68	-11.40
IHD attributable to Low Physical Activity	High-middle SDI	2514 23.84	1958 02.49	77.88	2249 09.32	89.45	-1692 87.97	-67.33	2140 0.12	1710 1.65	79.91	1565 6.70	73.16	-1135 8.23	-53.08
	High SDI	-1401 95.31	1557 84.90	-11 1.12	1391 61.79	-99.26	-4351 42.01	310.38	-780 8.23	1471 1.42	-18 8.41	1038 7.07	-133.03	-3290 6.72	421.44

Table 2. Changes in YLDs and YLLs by Determinants and Causes of Population Levels, 1990–2021

Cause	Location	YLDs					YLLs								
		Change due to Population-level determinants (%contribute to the total change)					Change due to Population-level determinants (%contribute to the total change)								
		Overall difference	Aging	Perc ent%	Popul ation	Perc ent%	Epidemi ological change	Perc ent%	Popul ation	Perc ent%	Epidemi ological change				
IHD attributable To High Body Mass Index	Global	1530 04.72	3491 7.33	22.82	7985 3.94	52.19	3823 3.45	24.99	45414 19.69	1729 775.80	38.09	3864 743.63	85.10	-10530 99.74	-23.1930
	Low SDI	504 6.85	-93.18	-1.85	310 1.76	61.46	203 8.27	40.39	2994 58.29	-667 5.96	-2.23	2492 47.02	83.23	5688 7.24	19.00
	Low-middle SDI	2496 2.24	274 9.80	11.02	1137 3.22	45.56	1083 9.22	43.42	15279 61.48	2022 53.44	13.24	9154 89.92	59.92	4102 18.12	26.85
	Middle SDI	5643 9.39	1291 4.35	22.88	2033 9.30	36.04	2318 5.74	41.08	19762 87.20	6115 65.06	30.95	9840 35.39	49.79	3806 86.75	19.26
	High-middle SDI	4346 7.50	1543 9.45	35.52	1832 0.65	42.15	970 7.40	22.33	9714 64.09	7735 85.60	79.63	8217 28.76	84.59	-6238 50.27	-64.22
	High SDI	2294 1.50	1070 8.08	46.68	1232 9.32	53.74	-95.89	-0.42	-2356 59.98	4621 32.69	-196.10	4523 39.87	-191.95	-11501 32.54	488.05
	Global	30736.74	8902.39	28.96	21767.17	70.82	67.18	0.22	9225 36.67	4981 16.08	53.99	1132 845.02	122.80	-708 424.43	-76.79
	Low SDI	797.73	-3997.00	-0.50	725.54	90.95	76.18	9.55	449 58.32	-637.04	-1.42	596 38.19	132.65	-140 42.83	-31.24
	Low-middle SDI	5712.32	1142.44	20.00	3988.58	69.82	581.30	10.18	2945 51.10	719 24.86	24.42	2826 88.09	95.97	-600 61.86	-20.39
	Middle SDI	12695.14	3943.38	31.06	6940.32	54.67	1811.44	14.27	4830 21.89	2111 60.12	43.72	3633 95.92	75.23	-915 34.15	-18.95
IHD attributable to Low Physical Activity	High-middle SDI	8287.20	3196.49	38.57	4418.07	53.31	672.64	8.12	243 136.63	1926 05.99	79.22	2204 91.25	90.69	-1699 60.61	-69.90
	High SDI	3218.43	3031.80	94.20	3293.51	102.33	-3106.89	-96.53	-1434 13.74	1527 53.10	-106.51	1358 68.29	-94.74	-43203 5.12	301.25

Given the identified correlation between female IHD and the SDI, we conducted a comprehensive analysis of health disparities. Health inequality analysis suggested that the absolute burden attributable to both high BMI and low PA remained concentrated in countries with higher SDI, but the degree of inequality decreased from 1990 to 2021. This pattern indicates that the burden is gradually spreading toward lower-SDI populations as demographic growth, aging, and lifestyle transitions accelerate (**Figures 15 and 16**).

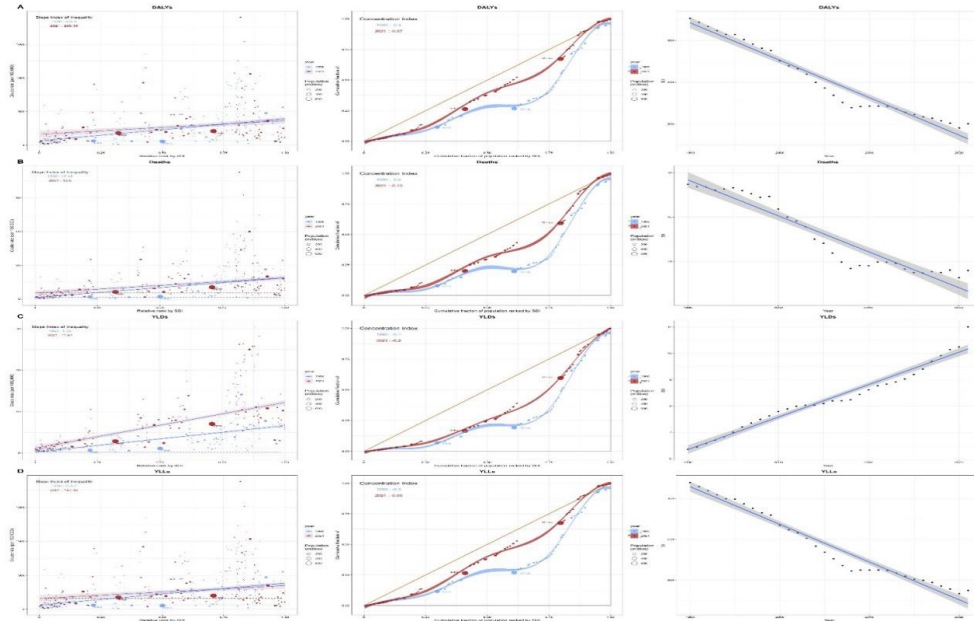


Figure 15. Absolute income-related health inequality for DALYs, Deaths, YLDs, YLLs of female IHD attributable to high BMI in SII analysis, 1990 and 2021.

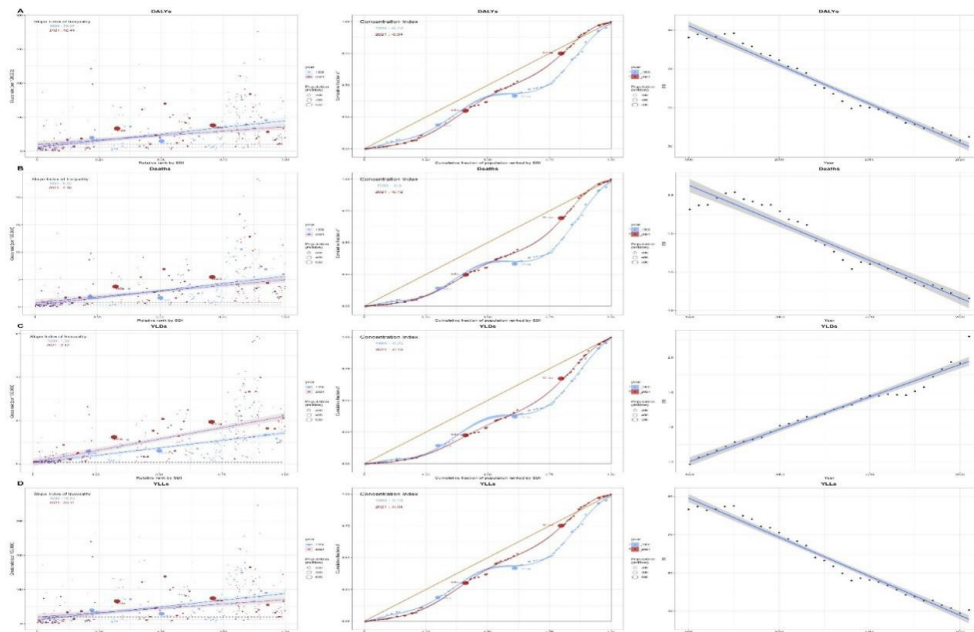
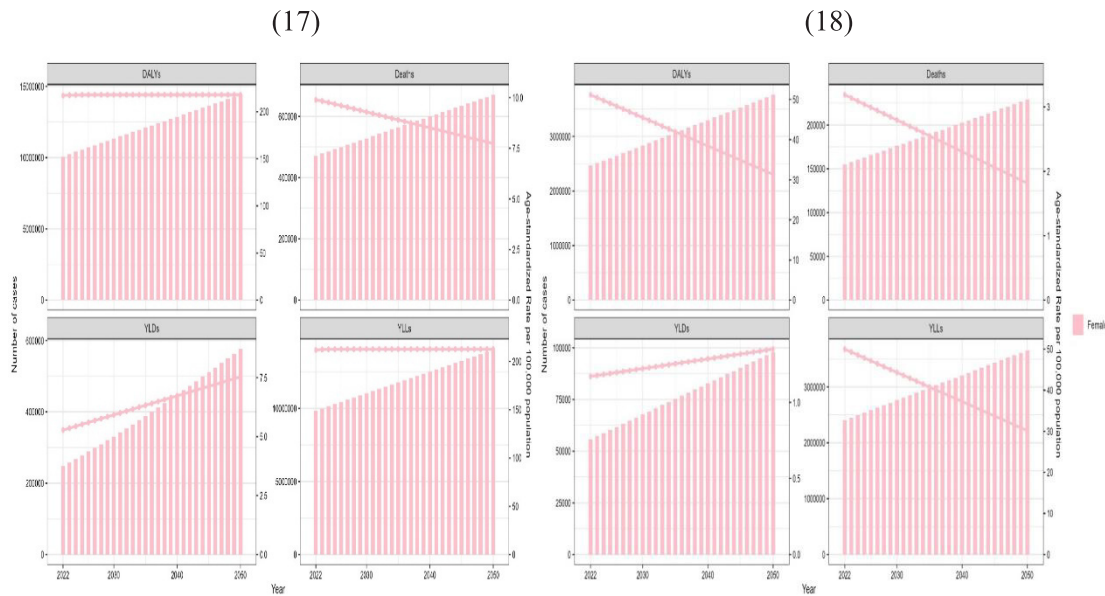


Figure 16. Absolute income-related health inequality for DALYs, Deaths, YLDs, YLLs of female IHD attributable to low PA in SII analysis, 1990 and 2021.

3.7. Projections of IHD burden in women due to high BMI and low PA over the next 29 years

We applied the auto. The ARIMA function was used to analyze global data pertaining to IHD among women, specifically due to high BMI and low PA over the period from 1990 to 2021. ARIMA projections suggested that ASRs of DALYs, deaths, and YLLs attributable to high BMI and low PA will continue to decline to 2050, while YLD rates may remain stable or increase. This indicates that the non-fatal health burden associated with ischemic heart disease (IHD) and the increasing burden of chronic diseases caused by population aging are both escalating. Therefore, public health initiatives should advance from a primary focus on mortality reduction to enhancing quality of life, disability, functional limitation, and quality of life among women with IHD will become increasingly important (Figures 17 and 18).



Figures 17 and 18. Time trends in age-standardized rates of DALYs, Deaths, YLDs, and YLLs attributable to ischemic heart disease in women due to high BMI/ low PA from 2022 to 2050. The pink dots and vertical bar represent the predicted trend and its 95% CI.

4. Discussion

This study, based on GBD 2021 data, systematically evaluated the global burden of IHD among women attributed to high BMI and insufficient PA from 1990 to 2021. The results indicated that during this period, the ASR of DALYs, mortality rates, YLDs, and YLLs of IHD among women due to high BMI and inadequate PA all exhibited an upward trend, indicating that these two major risk factors remain the focus of current IHD prevention and control in women. Our result is consistent with the latest Lancet GBD 2021 report, which identifies IHD as one of the major threats to women's health globally. Moreover, its disease burden is closely linked to obesity and physical inactivity^[16]. Nevertheless, compared to some previous studies, we further revealed the heterogeneity of disease burden distribution among different age groups, SDI regions, and countries. Particularly among middle-aged and older adult women, and in regions with moderate SDI, the effects of high BMI and low PA on IHD exhibited different age patterns and regional shifts in disease burden.

This may be associated with changes in population structure, the phase of economic development, and the specific physiological and social factors of women.

The study found that the impact of high BMI and reduced PA on the burden of IHD in women exhibited variations in age distribution: deaths and YLLs related to high BMI increased rapidly after 80–84 years, whereas indicators related to low PA began to increase significantly from 75–79 years. Additionally, the changing trend of YLDs suggests that disability associated with low PA lasts longer, particularly increasing rapidly after 70–74 years. These patterns may be associated with the sudden decrease in estrogen levels, lipid metabolism disorders, and atypical IHD symptoms during and after perimenopause in women, resulting in an increased risk of functional disability^[17]. Meanwhile, older adult women frequently have multiple chronic diseases, including cancer and diabetes, and competing risks of death may affect the age distribution of the IHD burden^[18,19]. Regarding PA, the duration of disability after IHD in women is prolonged, although the BMI factor generally contributes to the extended overall burden. Consequently, we must prioritize the primary and secondary prevention of IHD in women and highlight the importance of regular physical exercise, which can alleviate the significant economic burden associated with health care costs and mitigate its effects on mortality and overall well-being.

The innovation of this study lies in its systematic depiction of the global spatiotemporal distribution of the burden of IHD in women due to high BMI and insufficient PA, and the related differences in the SDI. Additionally, it designates areas with moderate SDI as current weak prevention and control zones. Our findings indicate that as the SDI increases, the disease burden changes in an “n” shape. In low-income areas, the problems of low-calorie intake due to insufficient food and the problems of high energy consumption caused by physical labor are less common. High-income countries have adequate resources and infrastructure to enhance healthy diets. Primary healthcare disseminates knowledge on obesity prevention and offers intervention measures, including bariatric surgery, thereby reducing the incidence of elevated BMI^[20]. However, middle-income regions have become newly susceptible areas regarding the burden of IHD due to factors such as Westernized dietary patterns, insufficient sports facilities, and the pressure of women’s social roles^[21]. Women in low-income areas face significantly reduced PA opportunities because of the shortage of sports facilities and the distribution of family responsibilities, whereas women in middle-income areas have had fewer opportunities to engage in physical activities because of extended working hours and increasing stress levels brought about by rapid economic growth^[22]. Although high SDI regions have better medical resources, the increase in white-collar occupations and reliance on electronic devices has resulted in a significant decline in PA^[23]. Therefore, we advocate for implementing a regional differentiation strategy: regions with low SDI should enhance primary medical care and community health facilities; medium SDI regions require the integration of policy and environmental transformation to foster structural health support; and in areas with high SDI, individualized risk assessment and long-term prevention mechanisms should be emphasized^[24,25].

Based on the data analysis of IHD in females and the factors of high BMI and low PA over the past 32 years, the prediction for the next 29 years (2022–2050) indicates that we should continue to enhance primary and secondary prevention efforts, promote a healthy diet and regular exercise to control weight, and reduce the risk of the disease at its source. The focus of work should be on maintaining and enhancing physical activity levels as well as enhancing cardiac rehabilitation, chronic disease management, and long-term care systems, to improve the quality of life of patients, mitigate the increase in YLDs, reduce the burden of

disability, and optimize health benefits, including morbidity and mortality reduction. Moreover, interventions aimed at reducing low physical activity are anticipated to be more effective than those addressing high BMI before 2050, as measured using DALYs.

This study has some limitations. First, the GBD data relied on the reporting systems of various countries. The data quality and completeness in regions with low SDI might be inadequate, affecting the accuracy of cross-regional comparisons. Second, BMI, as an obesity indicator, failed to differentiate between types or distribution of body fat. Future research should combine indicators such as waist-hip ratio and bioelectrical impedance to enhance assessment accuracy. Moreover, PAs' data were primarily derived from self-reported questionnaires, which introduced recall bias. In the future, smart wearable devices can be employed for objective monitoring. Multiple factors, including physiology and social factors, influence female IHD. Subsequently, methods, such as Mendelian randomization, can be employed to investigate further the causal relationships and biological mechanisms^[26]. Furthermore, this study did not analyze differences across racial groups or between urban and rural populations, nor did it include the latest GBD data from 2021. All these factors must be considered in future research.

5. Conclusion

This study demonstrated that ASR of the disease burden caused by IHD among global women decreased between 1990 and 2021, although the absolute number of cases continued to increase, indicating that the actual impact of IHD on women's health is still intensifying. Significant differences were observed among different regions and SDI levels. Women in medium-high and high SDI areas need to be vigilant about the persistent threat induced by high BMI. Women in areas with medium to low SDI are facing an increasing burden of IHD, which urgently requires more attention from health authorities and policymakers. Population growth and aging facilitate the increase in the number of IHD cases due to a larger population size and age-related risk. By 2050, the burden of IHD due to high BMI is expected to stabilize; however, it will be characterized more by chronic disability rather than premature death. Moreover, increasing women's physical activity continuously reduces IHD burden, especially helping to reduce premature death and YLLs. To address this challenge, it is essential to enhance women's health awareness, promote balanced diets, encourage regular exercise, and establish a health care support system for women at all life stages. Additionally, the medical system should be strengthened, resource allocation maximized, and collaboration among the government, communities, families, and individuals encouraged to collectively undertake targeted intervention measures to mitigate the overall burden of IHD on women globally.

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Disclosure statement

The authors declare no conflict of interest.

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Myocardial Infarction Model in Mice: Left Anterior Descending Coronary Artery Ligation

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Abstract: Background: A stable and feasible animal model for myocardial infarction (MI) is of great significance for the research on MI treatment. This study clarified the establishment process of the mouse MI model, aiming to enhance the efficiency of the modeling procedure. Method: Male C57 mice were randomly divided into a sham group and a model group, with 10 mice in each group. In the sham group, after anesthesia, the chest skin was incised to expose the heart, which was briefly exteriorized and then rapidly returned to the thoracic cavity before suturing. In the model group, MI was induced by ligation of the left anterior descending coronary artery. Subsequently, the 24-hour survival rate was recorded, heart rate, blood pressure, and electrocardiogram were measured, and myocardial infarct size was assessed by 2,3,5-triphenyltetrazolium chloride (TTC) staining. Result: After modeling, the 24-hour survival rate of mice in the model group was 70%. There was no significant difference in heart rate between the model group and the sham group, while both diastolic and systolic blood pressures were higher in the model group compared to the sham group. Compared with the sham group, the model group exhibited ST segment elevation and T wave inversion on the electrocardiogram. TTC staining revealed no infarcted areas in the hearts of the sham group, whereas distinct pale infarcted areas were observed in the model group. Conclusion: The method of inducing myocardial ischemia and necrosis by ligating the left anterior descending coronary artery of mice employed in this study proved to be a reliable approach for successfully establishing the model.

Keywords: Myocardial infarction; Mouse; Left anterior descending coronary artery ligation; Animal model; Disease research

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1. Introduction

Myocardial infarction (MI) is a severe cardiovascular disease characterized by a sudden reduction or cessation of blood flow in the coronary arteries, leading to persistent myocardial ischemia, hypoxia, and subsequent necrosis ^[1]. This condition impairs cardiac function and may result in severe complications such as arrhythmias, shock, and heart failure, posing a major threat to human health ^[2]. With the formation of an

aging society in our country, cardiovascular and cerebrovascular diseases have gradually become the main threats that seriously affect people's lives and health ^[3]. Myocardial infarction remains the leading cause of death and disability among individuals aged 50 and older ^[4]. Therefore, establishing an appropriate animal model of myocardial infarction is of great significance for studying the pathogenesis and treatment measures of MI. Various methods are available for establishing mouse models of MI, including pharmacological induction, minimally invasive techniques, and electrocoagulation. However, the most commonly employed method is ligation of the left anterior descending coronary artery. This technique is favored due to its straightforward procedure, precise vascular occlusion, and its close recapitulation of the pathological process of MI, thereby offering good potential for clinical translation. In this study, the left anterior descending coronary artery ligation method was used to establish a mouse model of MI. Heart rate, blood pressure, and electrocardiogram were measured, and TTC staining was performed to validate the model, aiming to provide a methodological foundation for future research on MI modeling.

2. Method

2.1. Animal groups

After 7 days of adaptive feeding, SPF-grade male C57 mice (6-7 weeks old) were fasted for 12 hours with free access to water. The mice were then randomly divided into the sham group and the model group using a random number table, with 10 mice in each group.

2.2. Model establishment

All surgical instruments were sterilized prior to the experimental procedures. For the sham-operated group, mice were placed in an induction chamber with 5% isoflurane for anesthesia induction. Upon reaching deep anesthesia, each mouse was positioned supine on a sterile surgical platform equipped with a respiratory mask, and anesthesia was maintained with 1.5% isoflurane. The left thoracic area was shaved and disinfected, and a sterile drape with an opening was applied. An ophthalmic scissor was used to incise the skin between the third and fourth intercostal spaces (**Figure 1A**), and a pre-placed suture was prepared. The muscle layer was bluntly dissected using forceps. With gentle compression applied to the thoracic cavity using the left hand and curved forceps held in the right hand, the intercostal space with the most pronounced cardiac pulsation was identified, and the ribs were rapidly spread to exteriorize the heart. The heart was immediately returned to the thoracic cavity, and air was evacuated from the chest. The skin incision was sutured and disinfected again. Mice were then placed on a heating pad until spontaneous breathing resumed and they recovered from anesthesia.

For the MI model group, anesthesia, skin preparation, disinfection, and heart exteriorization were performed as described above. The heart was gently lifted to visualize the left anterior descending coronary artery, located between the left atrial appendage and the pulmonary artery (**Figure 1B**). Ligation was performed near the apex of the heart. The ribs were then spread with curved forceps to facilitate returning the heart to the thoracic cavity, followed by air evacuation and subsequent procedures as described above (**Figure 1C**), thereby completing model establishment.

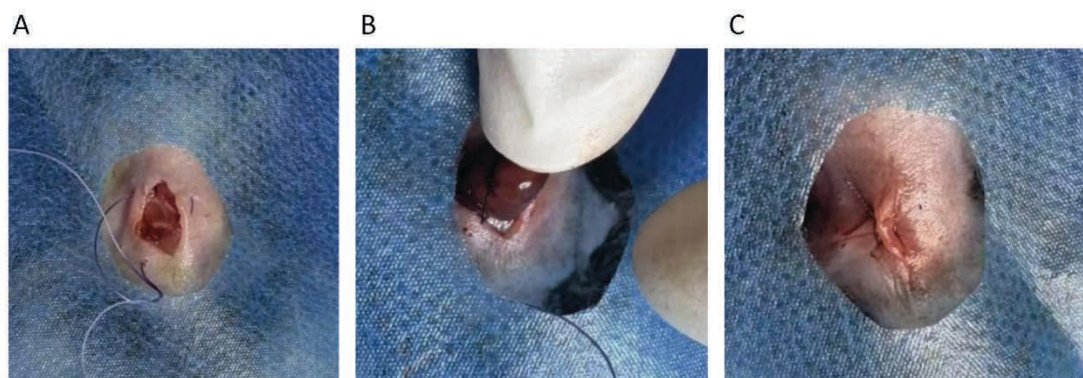


Figure 1. The operation method for the mouse model of MI caused by ligation of the left anterior descending branch of the coronary artery. (A) Expose the third and fourth intercostal spaces. (B) Cut out the heart and ligate the left anterior descending artery. (C) Closure of the cardiac exhaust incision.

2.3. Survival rate analysis

After the modeling process is completed, record the survival rates of the mice in each group:

$$(\text{Number of surviving mice} / \text{Initial number of mice}) \times 100\%$$

2.4. Heart rate (HR) and blood pressure monitoring analysis

First, power on the non-invasive blood pressure monitor for small animals, connect the sensor and heating device to adjust the temperature, and check whether the instrument is operating normally. Open the KdBpTerm software to verify the connection status and input basic animal information, then set the pressure and sensor signals. Subsequently, place the mouse into a restrainer for thermal insulation, insert its tail into the sensor, wait for the mouse to stabilize, and begin measuring heart rate and blood pressure.

2.5. Measurement and analysis of electrocardiogram

After connecting the equipment, open the Biological Information Medical Signal Acquisition and Processing System. Anesthetize the mouse with isoflurane inhalation and position it supine on a temperature-controlled operating board. Disinfect the limbs with alcohol and insert three-lead needle electrodes subcutaneously into the four limbs: right forelimb electrode (red, negative), right hindlimb electrode (black, ground), and left hindlimb electrode (green, positive). Activate the electrocardiogram measurement function and click Start to display the sampled waveform, thereby completing ECG acquisition.

2.6. 2,3,5-triphenyltetrazolium chloride (TTC) staining analysis

After removal, the hearts were immediately placed in pre-chilled physiological saline. Forceps were used to press the heart until all blood was expelled gently. The hearts were then frozen at -20°C for 30 minutes, subsequently sliced into 5 sections, and immersed in TTC solution. The TTC solution was protected from light and incubated in a 37°C water bath, with the heart sections turned over every 5 minutes. After staining for 15 minutes, the sections were transferred to fixative solution for fixation.

2.7. Statistical analysis

Data statistics and analysis were conducted using GraphPad Prism 8.0 software. The data were expressed

as mean $\pm$ standard deviation (SD). The one-way analysis of variance (ANOVA) was used for comparison between the two groups. A $p < 0.05$ indicated a significant difference between the two groups.

3. Result

3.1. Survival rate analysis

After MI modeling, the 24-hour survival rate of mice was 70%. The surviving model mice exhibited symptoms such as listlessness, increased respiratory rate, arched back and huddling posture, decreased food and water intake, ruffled fur, decreased body temperature, and abnormal heart rhythm. In contrast, no abnormalities were observed in the sham group.

3.2. Heart rate and blood pressure monitoring analysis

The results showed that there was no significant difference in heart rate between the model group and the sham group, while the diastolic and systolic blood pressures were higher in the model group than in the sham group (**Figure 2**).

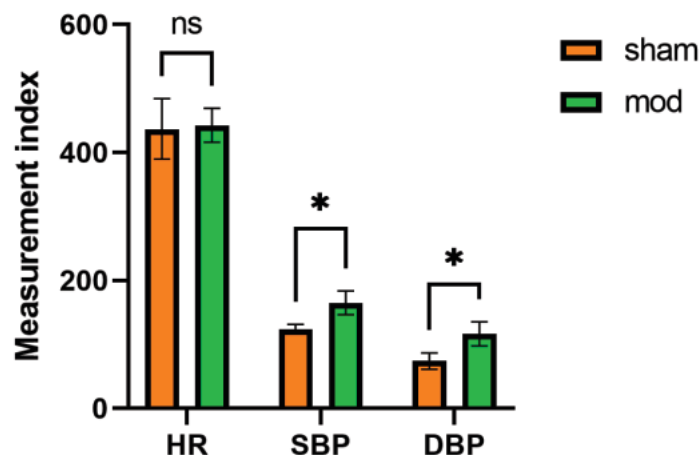


Figure 2. Comparison and analysis of the measurement results of HR, SBP and DBP in the sham group and the model group. $n = 10$. ns indicates that there is no significant difference between the two comparison groups; $*p < 0.05$.

3.3. Electrocardiogram analysis

Compared with the sham group, the model group exhibited ST segment elevation and T wave inversion on the electrocardiogram (**Figure 3**).

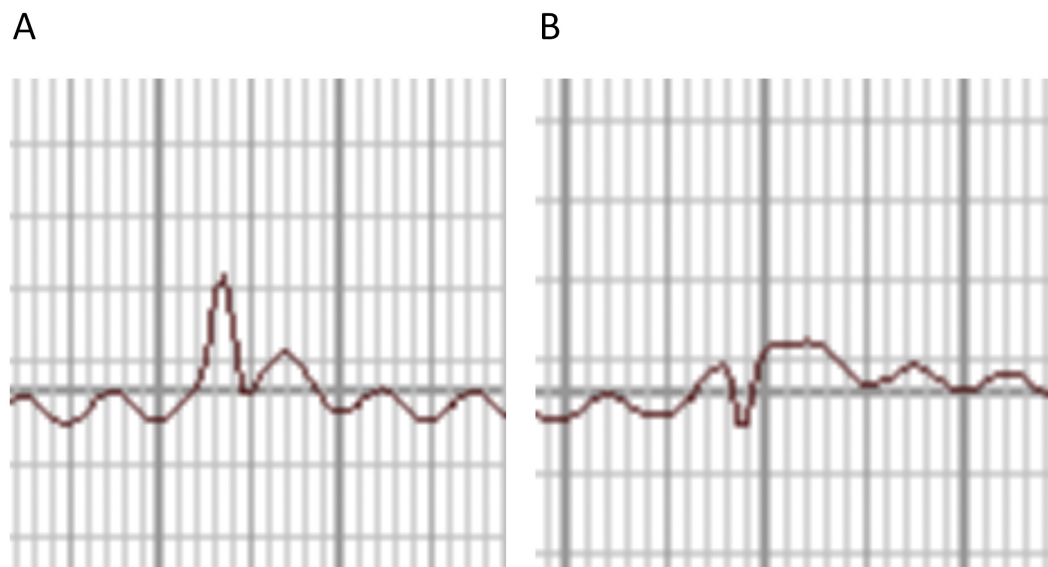


Figure 3. Electrocardiogram test results. (A) sham group, (B) model group.

3.4. TTC staining analysis

TTC staining is regarded as the gold standard method for assessing myocardial infarct size. In this technique, normal myocardium stains brick red, while infarcted myocardium appears pale. TTC staining results of heart sections from the two groups showed no infarcted areas in the sham group, whereas distinct pale infarcted regions were clearly observed in the model group (**Figure 4**).

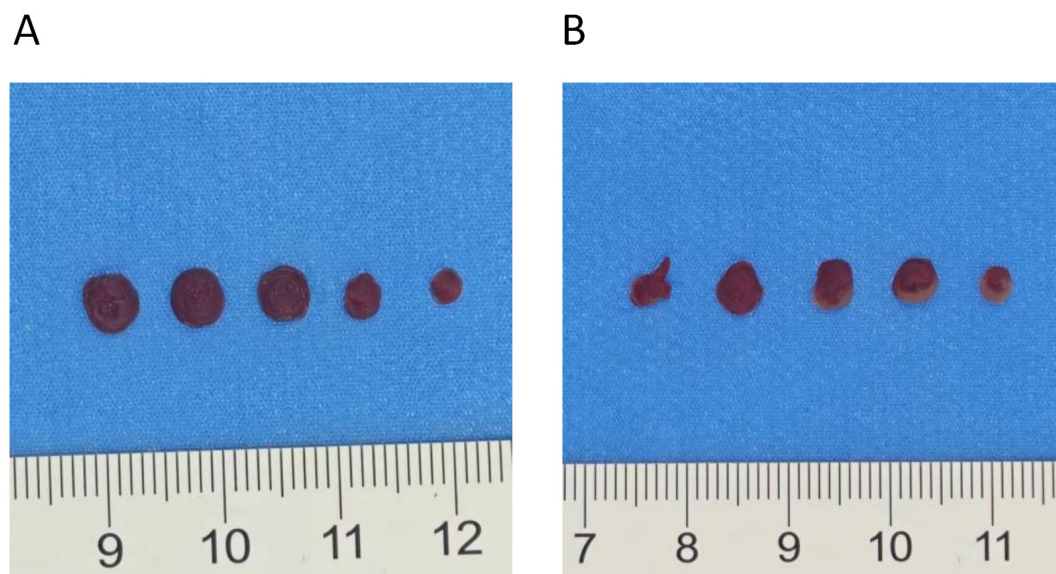


Figure 4. TTC staining result. (A) sham group, (B) model group.

4. Discussion and conclusion

In recent years, the incidence of MI has been increasing annually in China, making it a research hotspot for many scientists. Consequently, the establishment of MI models has become particularly crucial in scientific research, as it plays a significant role in elucidating and exploring disease mechanisms. In this study, an MI model was established by ligating the left anterior descending coronary artery in mice. The results showed a survival rate of 70% in the model group. Blood pressure and heart rate measurements revealed higher diastolic and systolic blood pressures in the model group, which represents an acute compensatory response. Electrocardiogram findings demonstrated ST segment elevation and T wave inversion in the model group, indicative of post-ischemic changes. TTC staining revealed distinct pale infarcted areas in the hearts of the model group, indicating myocardial necrosis, loss of mitochondrial function, and inactivation of dehydrogenases, which prevented reaction with the TTC dye. These findings collectively demonstrate that this modeling method successfully induces myocardial ischemia and necrosis in mice and can be reliably used for studying MI in mouse models.

The left anterior descending coronary artery ligation method is the most widely used experimental approach for establishing mouse models of myocardial infarction. This technique closely recapitulates the pathophysiological process of acute myocardial infarction in humans, avoiding the potential metabolic variability associated with pharmacological induction methods while more accurately reflecting the clinical reality of coronary occlusion, and preserving the option for either ischemia-reperfusion or permanent occlusion studies ^[5-7]. At the cellular mechanism level, following LAD ligation, local myocardial tissue experiences a sharp decline in oxygen supply, leading to impaired mitochondrial oxidative phosphorylation and ATP synthesis failure, which subsequently triggers a cascade of events including reactive oxygen species burst and opening of the mitochondrial permeability transition pore ^[8,9]. These processes ultimately result in irreversible myocardial cell damage, with the pale areas revealed by TTC staining corresponding precisely to cellular changes such as dehydrogenase inactivation and mitochondrial dysfunction. At the functional systemic level, the elevated blood pressure observed in the model group indicates activation of the sympathetic-adrenal system and the renin-angiotensin-aldosterone system ^[10,11]; the ST segment elevation and T wave inversion on electrocardiograms reflect electrophysiological remodeling following transmural ischemia ^[12,13]. Both aspects collectively validate the successful establishment of the model in this study.

This study has certain limitations. First, the experimental evaluation was conducted only at the acute phase (24 hours post-surgery), without tracking the progression of cardiac function or long-term survival during the chronic phase following myocardial infarction. Second, although the infarct area was validated by TTC staining, histopathological and molecular analyses were not performed to investigate mechanisms such as the inflammatory response.

Nevertheless, the mouse model of myocardial infarction established by left anterior descending coronary artery ligation in this study demonstrates promising potential for future applications. This model can serve as a reliable tool for drug screening and efficacy evaluation, enabling the assessment of therapeutic effects on cardiac function protection and infarct size reduction following treatment ^[14-16]. Moreover, when combined with techniques such as gene knockout, this model can be employed for functional validation of specific molecules involved in the pathological progression of myocardial infarction ^[17-18]. Building on this foundation, integrating dynamic monitoring using echocardiography with molecular biology approaches will facilitate systematic investigation of the mechanisms underlying post-infarction myocardial injury, as well as

the temporal dynamics of key events including inflammatory signaling pathways, oxidative stress, pyroptosis, and autophagy, thereby providing a theoretical basis for the identification of novel therapeutic targets^[19–21].

Ethical approval

Experimental Animal Ethics Committee of Tianjin Xinrui Biotechnology Co., Ltd. (Project No.: 2026001)

Disclosure statement

The authors declare no conflict of interest.

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Insulin and Cardiovascular Health: New Insights in the Field of Coronary Heart Disease Complicated with Type 2 Diabetes

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Abstract: Insulin is currently a crucial treatment for treating type 2 diabetes and plays a significant role in improving the prognosis of patients. When patients with cardiovascular disease who also have type 2 diabetes, the all-cause mortality rate increases by approximately 1.5 times. Consequently, many patients choose insulin as a treatment method for type 2 diabetes. However, the effects and mechanisms of insulin treatment on cardiovascular disease are rarely discussed. This review systematically explores the role and mechanisms of insulin and its related receptors in the progression of cardiovascular disease in patients with both conditions. It focuses on the impact of insulin on coronary plaques, cardiovascular neogenesis, and myocardium, aiming to guide clinical treatment and improve patient outcomes.

Keywords: Insulin; Insulin receptor; IR; Coronary heart disease; Signal transduction pathway; Atherosclerotic plaque

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1. Background

Insulin is a polypeptide hormone synthesized and secreted by pancreatic β -cells. It binds to tyrosine kinase receptors on the target cell membrane and regulates glucose transport and gene expression by activating downstream signaling pathways. Currently, the harm caused by diabetes, resulting from insulin deficiency or resistance, is increasing year by year. In the United States, deaths due to diabetes account for 8% of the total number of deaths. Among these patients, the mortality rate due to complications related to cardiovascular

diseases is 7.9%. Numerous studies have demonstrated that individuals with type 2 diabetes combined with cardiovascular diseases exhibit significantly worse incidence rates and prognoses compared to non-diabetic individuals. According to statistics, these patients often present with high-risk factors such as obesity and insulin resistance^[1,2]. Research indicates that patients with these risk factors experience approximately a two-fold increase in mortality from cardiovascular diseases, myocardial infarction incidence, and stroke risk, as well as a 1.5-fold increase in all-cause mortality^[3]. Currently, it is understood that insulin resistance can lead to water and sodium retention in the body, peripheral vascular constriction, and promote hepatic synthesis of lipid substances, resulting in elevated triglyceride levels, increased low-density lipoprotein cholesterol, higher apolipoprotein B, and increased small dense low-density lipoprotein cholesterol. These changes ultimately contribute to atherosclerosis or heart failure^[3,4]. At present, the use of exogenous insulin to control blood glucose levels in diabetic patients is recognized as a common cause of insulin resistance. The DCCT study reported that intensive insulin therapy was associated with significant metabolic abnormalities, including hypertension, increased insulin demand, and dyslipidemia. Subsequent follow-up revealed the emergence of early signs of cardiovascular disease^[5]. Research has found that, for patients with cardiovascular disease and early-stage diabetes, insulin therapy can reduce the risk of stroke and hospitalization due to heart failure. However, this treatment also induces hyperinsulinemia, which has various adverse effects. For example, it can cause insulin resistance, obesity, lipid distribution disorders, and inflammation^[6,7]. Therefore, the specific mechanisms by which insulin affects patients with type 2 diabetes and cardiovascular diseases, as well as its clinical impact, remain incompletely understood. This review systematically compiles existing evidence and aims to explore in depth the dual roles and potential mechanisms of insulin in the progression and prognosis of cardiovascular diseases.

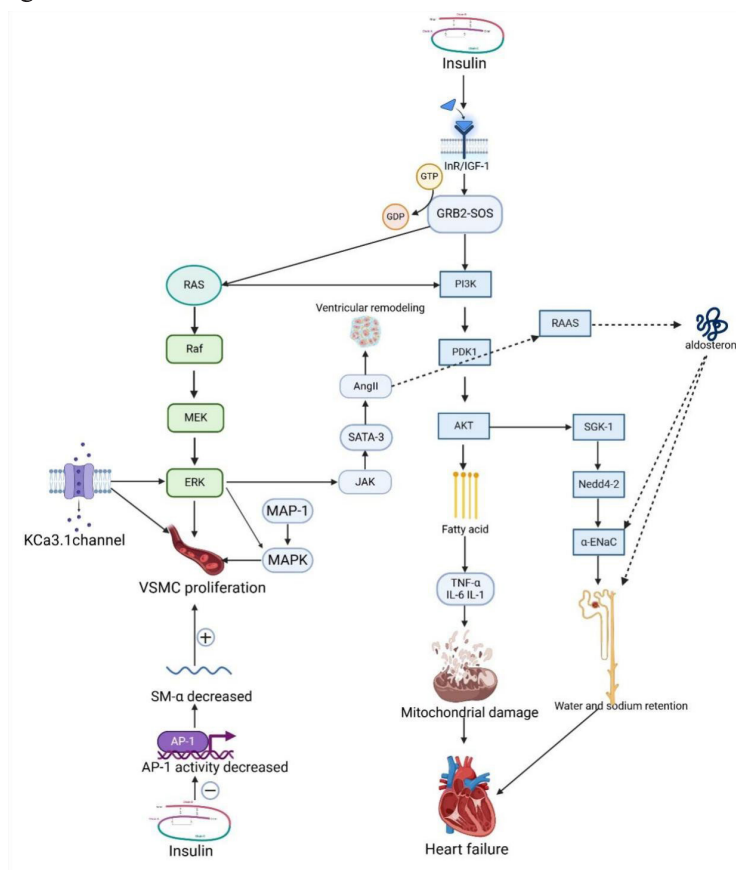


Figure 1. The specific mechanism by which insulin affects disease prognosis. InR/IGF-1: Insulin receptor/Insulin-like Growth Factor 1; VSMC: Vascular smooth muscle cells; AP-1:Activator protein-1; SM-α:Smooth Muscle Protein-α

2. Accelerate the progression of arteriosclerosis

The impact of insulin on the progression of cardiovascular diseases mainly involves influencing the formation of atherosclerotic plaques, regulating the heart muscle, promoting angiogenesis, and water and sodium retention, etc. Insulin affects the prognosis of cardiovascular diseases by influencing the activation of various signaling pathways and gene transcription (**Figure 1**).

2.1. Increased expression of adhesion molecules on endothelial cells

Coronary atherosclerotic plaques are a key feature of coronary heart disease and the primary cause of ischemic changes in the heart. Previous studies have demonstrated that the formation of these plaques is not merely due to lipid accumulation but involves ongoing inflammatory stimulation, including smooth muscle cell migration and proliferation, as well as the release of various cytokines^[8]. This inflammatory response is associated with the expression of adhesion molecules, such as intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1), on the surface of vascular endothelial cells.

Studies have shown that smooth muscle cells treated with high concentrations of insulin (10^{-9} to 10^{-7} mol/L) for 24 hours and up to 3 days exhibit increased expression of vascular cell adhesion molecule-1 (VCAM-1). Moreover, VCAM-1 expression increases further as the insulin concentration rises, reaching approximately a two-fold increase at the highest concentration^[9]. Another study demonstrated that mice with defective VCAM-1 expression exhibited significantly reduced early foam cell lesions in the aorta. This finding suggests that VCAM-1 plays a crucial role in the early stages of coronary atherosclerosis, possibly by promoting monocyte aggregation in the arterial intima and thereby facilitating the formation of atherosclerotic plaques^[10]. However, VCAM-1 is currently believed to be involved only in early coronary artery lesions. High levels of VCAM-1 are thought to indicate the severity of a patient's lesion or a heavy plaque burden, but there is no significant correlation with the overall risk of coronary heart disease^[11].

Unlike VCAM-1, plasma levels of intercellular adhesion molecule-1 (ICAM-1) in patients with peripheral vascular disease and ischemic heart disease are significantly higher than those in healthy individuals. Moreover, plasma ICAM-1 levels in patients with acute myocardial infarction are significantly elevated compared to those in patients with chronic stable angina pectoris and healthy control subjects^[12,13]. Subsequent studies have also confirmed that insulin resistance directly affects the levels of soluble cell adhesion molecules (sICAM). The authors found that the peak insulin value and the area under the insulin curve were significantly correlated with sICAM, with the strongest correlation observed for ICAM-1 ($r = 0.7$, $p < 0.001$). Additionally, there was a modest correlation with elevated postprandial triglycerides ($r = 0.29$), which is also an important risk factor for cardiovascular disease.

E-selectin, a member of the selectin family, is a cell adhesion molecule primarily involved in regulating its relatively low expression on normal vascular cells. However, its expression can be upregulated in response to various stimuli, including cytokines and oxidants, facilitating the adhesion of monocytes to the vascular wall^[14]. Existing studies have shown that type 2 diabetes, an inflammatory disease, is characterized by elevated levels of E-selectin in patients. The level of E-selectin correlates with glycosylated hemoglobin levels ($r = 0.25$, $P = 0.0351$). Previous research has demonstrated that E-selectin-mediated cell adhesion plays a significant role in the early stages of coronary artery disease. E-selectin promotes the adhesion of white blood cells and vascular inflammation by binding to ligands such as sLeX. Abnormal insulin signaling can activate pathways like NF- κ B, thereby enhancing E-selectin expression and increasing the production

of inflammatory factors within atherosclerotic plaques ^[15,16]. Furthermore, some studies have reported that although E-selectin expression increases in early-stage coronary heart disease patients, its levels are lower in unstable plaques and those with extensive calcified deposits. This suggests that during plaque progression, calcification and inflammation may reach a state of equilibrium.

2.2. Increased cell transendothelial migration

In a study ^[17], neutrophil migration was quantified by measuring myeloperoxidase activity, and the surface expression of endothelial adhesion molecules was assessed using enzyme immunoassay. The results demonstrated a significant enhancement in neutrophil migration across the endothelium. Furthermore, compared to healthy subjects, neutrophil migration across the endothelium was also increased in diabetic patients with hyperinsulinemia. This enhanced neutrophil migration induced by high insulin levels may contribute to the elevated incidence of atherosclerotic diseases, such as acute myocardial infarction (AMI) and stroke, in diabetic patients. Neutrophil migration appears to play a role in both the formation and destabilization of atherosclerotic plaques; for example, increased secretion of matrix metalloproteinase-9 by monocytes can weaken the fibrous cap of plaques, making them more susceptible to rupture ^[18].

Furthermore, this experiment demonstrated that in the high insulin group ($> 50 \mu\text{U/mL}$, for 24 hours), the migration of neutrophils across the endothelium was enhanced, which was associated with the increased expression of platelet endothelial cell adhesion molecule (PECAM-1) ^[17]. Under physiological conditions, although PECAM-1 can promote the adhesion and migration of white blood cells through the PI3K/Akt-FoxO1 pathway, it also has an inhibitory effect on inflammation. After the formation of atherosclerotic plaques, the PECAM-1-dependent immunosuppression is disrupted, which in turn promotes the recruitment of white blood cells and supports the atherosclerotic inflammatory response ^[19]. This is indeed the case. In a comparative study involving 137 patients diagnosed with coronary heart disease (with stenosis $\geq 70\%$) through coronary angiography and 110 healthy individuals, the levels of PECAM-1 in the plasma of patients diagnosed with coronary heart disease were significantly elevated ($r = 0.314$, $P = 0.006$), but there was no significant correlation between the number of affected vessels, and only a weak correlation with TG, LDL-C, and HDL-C ^[20]. Another study confirmed that ICAM-1, which is also a cell adhesion factor, plays a role in promoting the transendothelial migration of T cells. Its relationship with the migration of white blood cells is not significant. As for VCAM-1, no effect on promoting cell transendothelial migration was found ^[21].

The study found that in the experiment, high insulin-mediated neutrophil migration increase persisted for 72 hours, while the expression of endothelial PECAM-1 on the surface only increased within 24 hours. This indicates that the increase in neutrophil migration stimulated by high insulin in the latter 48 hours may be related to another mechanism ^[17]. Previous studies have noted that, in addition to PECAM-1, some intercellular junction proteins in endothelial cells, such as vascular endothelial cadherin (VE-cadherin), junctional adhesion molecule (JAM-1), CD99, and reactive oxygen species (ROS), can all affect the transendothelial migration of white blood cells ^[22–24]. During the following 48 hours, the migration of neutrophils increased, which might be related to this. At least for now, in individuals with insulin resistance or abnormal glucose tolerance, indicators such as VE-cadherin and ROS are significantly higher than those in healthy individuals ^[25].

However, in addition to the increased cell migration associated with inflammatory responses (such as neutrophils), some cells responsible for healing, such as fibroblasts and vascular endothelial cells, also

migrate to the site of myocardial injury and repair the damaged tissue. During this process, senescent fibroblasts accumulate in the myocardial infarction area, which can lead to deterioration of cardiac function. Therefore, promoting the differentiation of undifferentiated mesenchymal stem cells into new fibroblasts is an important part of restoring cardiac function. Previous studies have shown that rat mesenchymal stem cells treated with insulin-like growth factor-1 (IGF-1) can promote their differentiation into cardiomyocyte-like cells, and the presence of growth factor HGF in the cells can further enhance this effect ^[26,27]. Insulin and insulin-like growth factor (IGF) belong to the same insulin family and share homology in structure. Both of them are involved in cell proliferation, apoptosis, protein synthesis and metabolism, indicating that the insulin family also plays an important role in the recovery of cardiac function.

2.3. Pro-inflammatory effect

Studies have shown that in patients with insulin resistance, insulin resistance prompts macrophages to differentiate into the pro-inflammatory M1 phenotype, thereby secreting a large amount of inflammatory factors (such as TNF- α , IL-1 β , IL-6, etc.) and reactive oxygen species (ROS), inhibiting the transduction of insulin-related signaling pathways and promoting the apoptosis of pancreatic β cells, forming a vicious cycle of “insulin resistance - inflammation - more severe insulin resistance” ^[28]. Although insulin-induced endothelial-derived Nitric oxide (NO) is an important anti-inflammatory mediator in the human body under normal conditions, in individuals with insulin resistance, the signal transduction of the PI3K/Akt pathway is impaired, resulting in reduced activation of endothelial nitric oxide synthase (eNOS) and decreased NO production. Consequently, its anti-inflammatory effect is significantly weakened ^[29,30]. Previous studies have shown that other inflammatory factors (such as IL-1, TNF- α) are effective inducers of VSMC proliferation. In the mouse arterial endothelial cells activated by IL-1, it was observed that VSMCs proliferated significantly and the collagen and fibrous cap decreased, indicating an increase in plaque instability and even a clear correlation with in-stent restenosis ^[31,32].

Studies have shown that TNF- α synthesized by macrophages in insulin-resistant patients activates the NF- κ B/MAPK inflammatory signaling pathway and induces the production of matrix metalloproteinases (MMP), vascular cell adhesion molecule-1 (VCAM-1), and heparin-binding epidermal growth factor-like growth factor (HB-EGF) by endothelial cells and smooth muscle cells. These factors promote inflammatory responses and vascular dysfunction, thereby accelerating the progression of atherosclerosis (see Figure 2) ^[33]. In addition, the TNF- α secreted by fat cells can also phosphorylate the serine residue of IRS-1 and reduce the expression of GLUT-4, resulting in the disruption of glucose metabolism regulated by insulin and the occurrence of obesity. This study also revealed that TNF- α can trigger the inflammatory response in the body through the NF- κ B/MAPK signaling pathway (a signaling pathway related to the inflammatory response) (Figure 2).

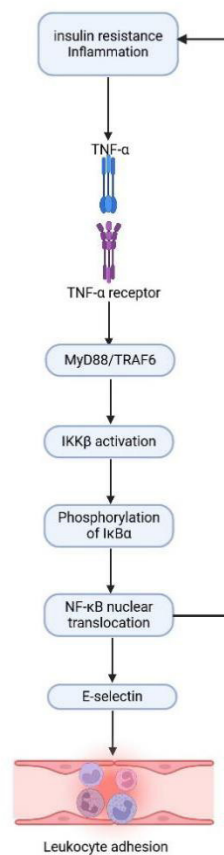


Figure 2. Relationship between TNF- α -mediated NF- κ B signaling pathway and e-selectin.

2.4. The impact of reduced insulin receptors on plaques

The research has found that the reduction or absence of insulin receptor (IR) expression caused by a high-fat diet (HFD) can prompt cells to express more inflammatory factors, leading to instability of the plaques and thereby increasing the incidence of adverse events ^[34]. Recently, it has been discovered that the reduction in insulin receptor expression leads to the pro-atherosclerotic effect, which is associated with multiple genes. For instance, in VSMC cells with high expression of ICAM-1, genes such as Myh11, Dendritic, and Cnn1, which control myocardial contractility, were observed to be decreased, while genes related to inflammation, such as Mmp2, Mmp9, C1ra, Vcam1, Thbs1, Cc119, Tnfsf1b, and C4b, were found to be increased ^[35]. Other studies have also confirmed that, compared with the arteries of non-diabetic patients, the expression activity of metalloproteinase-related genes (MMP-2 and MMP-9) in the arterial endothelium of diabetic patients has increased by approximately 40% and 110%, respectively. Moreover, the degree of arteriosclerosis in diabetic patients is highly correlated with the activity of MMP-2 and MMP-9 ($r = 0.738$, $P = 0.0002$) ^[36]. In the analysis of the obese and metabolic syndrome population, it was found that the expression of platelet reaction protein 1 (Thbs1, a fat-derived stromal cell protein, which is considered a potential factor for obesity, insulin resistance, and the occurrence of fat inflammation) in visceral and adipose tissues was significantly increased and was correlated with body mass index ($P = 0.033$), abdominal obesity ($P < 0.001$), hyperglycemia ($P = 0.02$), and hypertension ($P = 0.04$) ^[37]. Compared with individuals with normal blood sugar levels, the expression of secreted frizzled-related protein 3 (Sfrp 3) in the serum of patients

with type 2 diabetes was significantly lower (0.156 ± 0.04 vs. 0.376 ± 0.07 , $P < 0.05$), and it was positively correlated with the serum Sfrp3 level ($r = 0.505$, $P < 0.05$). Previous studies have demonstrated that the Sfrp family plays an important role in promoting the proliferation and differentiation of cardiac cells and maintaining cardiac structure. Sklepkiwicz et al. reported that long-term absence of the Sfrp1 gene could even lead to changes in heart structure^[38]. However, the specific pathogenic mechanisms of some genes remain unclear, and further research is needed at present.

Additional experiments revealed that in mice with insulin receptors knocked out, the degree of aortic lipid deposition on the surface of vascular smooth muscle was approximately 90% higher than that of the control group mice ($P = 0.02$), the necrotic area of the plaque increased by 41% ($p = 0.045$), and the collagen content in the plaque decreased by 29% ($P = 0.01$). Moreover, in the rat group with insulin resistance (where insulin receptor expression was reduced), thickening of the aortic wall and disordered aortic media structure were observed (108.00 ± 20.0 mm VS 83.28 ± 12.15 mm), but no statistical differences were found in this experiment^[39]. This was also observed in other studies, and it was believed that after insulin receptor activation, it might promote VSMC proliferation and migration by down-regulating the expression of p21 WAF1 through microRNA-208 and inducing dysfunction of dopamine D1 receptors, thereby thickening the aortic wall. Past studies have shown that changes in the structure of the arterial wall can serve as an independent predictor of the incidence and mortality of cardiovascular diseases (CVD). The thickness of the aortic wall is closely related to the short-term and long-term incidence and mortality of CVD. This provides further evidence that the reduced expression of insulin receptors is an important factor contributing to the increased risk of cardiovascular diseases^[34].

3. Effects on the myocardium

3.1. The lipid toxicity of insulin on cardiomyocytes

Research has found that insulin has a dual effect on Cardiomyocytes: under ischemic conditions, insulin plays a crucial role in the cardiac muscle's adaptation to stress. It is particularly important in enhancing glucose metabolism, reducing the oxygen consumption of Cardiomyocytes, and inhibiting the apoptosis of Cardiomyocytes^[40]. However, under long-term high insulin levels in the body, insulin continuously increases the expression of angiotensin II (Ang II) through the MEK/STAT 3 pathway, continuously activating the renin-angiotensin-aldosterone system, thereby exacerbating ventricular remodeling and myocardial lipid toxicity damage^[40].

As mentioned earlier, the use of exogenous insulin leads to an increase in insulin concentration in the body. This long-term high insulin environment reduces the sensitivity of fat cells and skeletal muscle cells to insulin, thereby causing insulin resistance. However, studies have shown that cardiomyocytes remain sensitive to insulin in this high insulin environment. In a clinical trial, it was observed that the expression of the PI 3-Akt pathway in cardiomyocytes increased in patients with insulin-dependent diabetes mellitus (NIDDM), and as the expression of this signaling pathway increased, the uptake of glucose by cardiomyocytes decreased, while the uptake of fatty acids increased and gradually accumulated. This change is known as myocardial lipotoxicity^[41,42]. The pathogenic mechanism includes an increase in reactive oxygen species (ROS) due to lipid overload, which leads to damage of the mitochondrial respiratory chain and a reduction in ATP production; an increase in the release of inflammatory factors (such as TNF- α)

and the induction of apoptosis in Cardiomyocytes; endoplasmic reticulum stress interferes with protein folding and calcium homeostasis, further exacerbating cell damage and insulin resistance ^[43–45]. Research has found that long-term exposure of Cardiomyocytes to lipid toxicity leads to cellular damage, which results in decreased contractility of Cardiomyocytes, cardiac hypertrophy, and fibrosis ^[46]. Furthermore, since individuals with insulin resistance often have impaired mitochondrial function, the mitochondria in Cardiomyocytes have a reduced ability to oxidize fatty acids. This undoubtedly leads to an increase in fatty acid accumulation, exacerbates mitochondrial damage, and forms a vicious cycle. This manifestation is particularly evident in patients with advanced heart failure ^[47]. Another survey indicates that in patients with insulin resistance, due to the increase in glucose and lipid metabolites, as well as the elevated expression of oxidants and inflammatory cytokines, the combined effect of these factors leads to an increase in de novo synthesis of diacylglycerol (DAG); DAG is an activator of protein kinase C(PKC)subtypes ^[48]. The activated PKC subtypes PKC β and PKC δ enhance the activity of the IRS/1/2/PI 3 K/Akt signaling pathway and simultaneously increase the expression of mitogen-activated protein kinase (MAPK). This indicates that lipid accumulation not only affects the structure and function of the heart but also accelerates the progression of atherosclerotic plaques.

3.2. The impact of insulin on the risk of heart failure

The heart, as an insulin-dependent energy-consuming organ, insulin not only plays a crucial role in the absorption and utilization of glucose, but also supports the growth of Cardiomyocytes and the recovery of cardiac function ^[49].

Recent clinical studies have shown that a significant proportion of patients with heart failure have insulin resistance. Patients with insulin resistance or type 2 diabetes have approximately 1.5 to 2 times higher risk of heart failure. ^[50,51]. It can be seen that there is a close connection between insulin resistance and heart failure. Combined with previous studies, the possible mechanisms leading to the occurrence of heart failure are as follows: We found that insulin needs to continuously bind to IRs to inhibit the expression of the angiotensinogen gene mediated by Foxo1 and the production of Ang II, in order to lower blood pressure and maintain normal cardiac function. Therefore, in patients with insulin resistance or type 2 diabetes, the lack of utilization of insulin leads to an increase in the expression of the angiotensinogen gene mediated by Foxo1, thereby increasing the incidence of heart failure or ventricular remodeling ^[49,52].

Insulin can also affect the contractile function of the heart muscle and hemodynamics by activating Gi-biased beta 2-adrenergic signaling. After mouse cardiomyocytes are pre-treated with insulin, the Gi-beta 2 signal can effectively weaken the cAMP/PKA signal transduction in cardiomyocytes after beta-adrenergic nerve stimulation, thereby inhibiting PKA phosphorylation and the contraction response of cardiomyocytes, and ultimately leading to cardiovascular dysfunction. In the study, they believe that heart failure is a manifestation of insulin resistance or hyperinsulinemia ^[53,54].

Therefore, some researchers believe that for patients with heart failure and insulin resistance, compared to the sole use of insulin, the combined application of GLP-1 receptor agonists, SGLT2 inhibitors, ACEI/ARB drugs, etc., can reduce the incidence of adverse cardiovascular events in these patients ^[55,56].

3.3. Water-sodium retention

The occurrence of cardiovascular adverse events in patients receiving insulin treatment is related to its

characteristic of causing water and sodium retention in the patient's body. Currently, it is believed that insulin activates corresponding signaling pathways (such as PI3K/Akt), which leads to the expression and phosphorylation of α -ENaC (one of the subunits of epithelial sodium channels), enhancing the reabsorption capacity of sodium in renal tubules and resulting in water and sodium retention. Additionally, insulin can enhance the effect of aldosterone on α -ENaC, reducing the excretion of water and sodium. This is particularly significant in patients with hyperinsulinemia and type 2 diabetes^[57]. In the study by Vicent et al., the rehospitalization rate due to heart failure within one year for patients with heart failure who received treatment for type 2 diabetes was evaluated. The results showed that the risk of rehospitalization due to HF within one year for patients receiving treatment for type 2 diabetes was significantly higher than that for diabetic patients who did not receive insulin treatment (risk ratio 1.28; $P < 0.022$)^[58]. Another European clinical trial demonstrated that patients with HF who received insulin treatment had significantly increased risks of all-cause mortality (OR: 2.02) and re-hospitalization due to HF (OR: 1.42), and their usage rate of loop diuretics was also significantly higher than that of the control group^[59]. Loop diuretics are also an important factor influencing the prognosis of heart failure^[60]. It is worth noting that patients using SGLT-2 inhibitors had a significantly lower mortality rate from heart failure compared to the control group (5.7% vs 8.5%, HR: 0.66, $P < 0.001$), and the usage rate of loop diuretics was also relatively lower. This suggests that, on the basis of ensuring good glycemic control, SGLT-2 inhibitors may be a more preferable choice^[61].

4. The impact of angiogenesis

4.1. Stimulate the proliferation of vascular smooth muscle cells

The proliferation of human arterial smooth muscle cells can be stimulated by insulin in the plasma, indicating that insulin is a serum growth factor that can stimulate the proliferation of smooth muscle cells in the vascular wall^[62]. In a study conducted on spontaneously hypertensive rats (SHR), it was found that insulin, under conditions of high insulinemia or hypertension in the rats, causes an impairment in MKP-1-induced signaling, leading to an increase in signal transduction of the MAPK signaling pathway. This results in differential stimulation of the mitosis of vascular smooth muscle cells (VSMCs), thereby promoting the proliferation of vascular smooth muscle cells in the rats^[63]. However, insulin not only promotes the proliferation of VSMCs through the MAPK pathway, but also the ERK 1/2 pathway and the KCa 3.1 pathway can all promote the proliferation of VSMCs under the influence of insulin^[64]. The ERK 1/2 pathway is located downstream of IGF-1 in the signal cascade that affects cell proliferation. Since insulin and IGF-1, as well as insulin receptors and IGF-1R, all have considerable homology and structural similarities, high concentrations of insulin can additionally bind to IGF-1R, activating IGF-1 and subsequently activating ERK1/2, thereby promoting the proliferation of VSMCs^[65]. In this study, it was also confirmed that the phosphorylation level of ERK 1/2 increased after insulin treatment (compared with the control group, the phosphorylation degree of the ERK1/2 pathway increased by 35%, $n = 5$, $P < 0.01$)^[64]. Recent studies have shown that in addition to the MAPK signaling pathway, insulin also promotes the proliferation of vascular smooth muscle cells through the transcriptional activator protein (activator protein-1, AP-1) and smooth muscle-microfilament alpha (SM- α) signaling pathways. In this study, different concentrations of insulin were added to the culture of rat VSMCs, and the results showed that the expression of AP-1 and SM- α in rat VSMCs stimulated by insulin significantly decreased (decreased by more than 50% compared to the blank control)^[66]. Previous studies have shown that rats without SM- α expression died within 1-9 days after birth due to abnormal proliferation

function of VSMCs. Another study indicated that in patients with high-risk factors for coronary heart disease (such as high cholesterol levels, vascular endothelial damage, atherosclerotic plaque formation, etc.), the expression of SM22- α in VSMCs was also significantly reduced ^[67].

Studies have shown that insulin receptor (IR)-negative microvessels consistently display clear expression of type IV collagen. Generally, the expression of type IV collagen indicates the maturation of vascular endothelium. This suggests that the presence of IR may stimulate the formation of new blood vessels within coronary plaques ^[68,69]. OTUD 6B, as a member of the functional deubiquitinating protease subfamily that encodes ovarian tumor domains, plays a significant role in regulating cell proliferation, migration, and other cellular processes. OTUD6B can arrest cells in the G1 phase to inhibit cell proliferation. In the experiment, the author used a mouse model of diabetes-induced atherosclerosis. The results showed that the expression of OTUD6B in diabetic atherosclerosis patients was significantly decreased, thereby promoting the growth of new blood vessels in the coronary artery and reducing the stability of atherosclerotic plaques ^[70]. A7r5 cells are fibroblasts derived from the smooth muscle of the thoracic aorta of rat embryos. In a recent study, it was discovered that at a concentration of 10 μ M (the optimal concentration that can induce proliferation of rat aortic smooth muscle cells) ^[71]. After 24, 48, and 72 hours of insulin treatment, the proliferation rates of A7r5 cells were 223.38%, 235.71%, and 192.01% of the control group (without insulin treatment), respectively. It can be seen that the proliferation rate of A7r5 cells significantly increased after insulin treatment. This process involved changes in the expression of multiple genes, such as Myc, Jun, Ccnd1, etc., which are involved in the positive regulation of cell proliferation and the regulation of the cell cycle ^[72].

4.2. Promoting angiogenesis effect

Insulin and insulin-like growth factor (IGF) belong to the same insulin family and share homology in structure. Therefore, both of them are involved in cell proliferation, apoptosis, protein synthesis, and metabolism. The angiogenic effect of IGF-1 plays a crucial role in preventing CVD from progressing to MI. The rapid formation of microvessels has the potential to rescue ischemic myocardium in the early stage of MI, thereby avoiding impairment of cardiac function ^[73]. Studies have shown that in vascular endothelial cells treated with IGF-1, the formation of microvessels is accelerated compared to before, and the expression of angiogenesis-related genes and proteins in endothelial cells is upregulated. According to the research, the activation of the PI 3 K/Akt signaling pathway plays an important role in this process ^[74]. Although no studies have yet demonstrated that insulin has a direct role in promoting angiogenesis, insulin shares homology with IGF and can bind to the receptors of IGF to a certain extent. Moreover, some research has indicated that in a normal physiological concentration of insulin environment, insulin can activate the relevant signaling pathways of vascular endothelial cells and vascular smooth muscle cells, thereby upregulating the expression of angiogenic factors (VEGF). And VEGF has been proven to play an important role in the formation of collateral circulation in coronary arteries ^[75]. Therefore, this article suggests that insulin can, to some extent, promote the regeneration of coronary blood vessels. Moreover, in patients with non-ST-segment elevation myocardial infarction (NSTEMI), the vasodilatory effect of insulin can reduce ischemia by either increasing the blood flow in partially blocked culprit vessels or by enhancing collateral blood flow, which also facilitates the establishment of subsequent collateral circulation ^[76].

However, the angiogenic effect of insulin only takes effect at normal physiological concentrations. In patients with type 2 diabetes or IR, due to the high insulin environment in the body, the formation of

coronary collateral vessels is observed to be reduced instead. The main reasons for this may be the decreased bioavailability of nitric oxide, increased expression of endothelin-1 and adhesion molecules, intensified oxidative stress, and increased vascular permeability. Furthermore, cystatin C, as an endogenous anti-angiogenic factor, a large cohort study involving 866 patients with coronary heart disease showed that 57% (n = 498) of type 2 diabetic patients had a significant correlation between the inhibition of coronary artery collateral formation and elevated cystatin C levels. Therefore, when evaluating the prognosis of diabetic patients with cardiovascular diseases, the increase in cystatin C is an important predictor of poor prognosis for the patients ^[77].

4.3. Vascular proliferation within the plaque

Studies have shown that after being treated with insulin, the capillary-like vessels within the microvascular endothelial cells of the human body significantly increase in formation, thereby leading to the formation of new microvessels within the plaque. An increase in vascular density within the plaque significantly increases the incidence of perioperative adverse events (7.1% vs 3.8%, 95% CI), and compared with healthy patients, the risk of adverse events within 3 years is significantly higher (30% vs 23.8%, HR = 1.4) ^[78]. The mechanism may be that after the microvascular density within the plaque increases, it becomes easier for the blood vessels to dilate. After dilation, the white blood cells in the blood vessels are more likely to be released into the plaque, triggering an inflammatory response within the plaque (such as the release of proteolytic enzymes and an increase in plaque matrix breakdown), thereby making the plaque no longer stable ^[79]. Furthermore, insulin itself can also cause adverse changes such as angiogenesis through its binding to its own receptors.

5. Conclusion

On the one hand, insulin exacerbates the progression of atherosclerosis by activating various signaling pathways, thereby increasing the risk of cardiovascular events. On the other hand, at physiological concentrations, insulin has a protective effect on cardiomyocytes. Therefore, in clinical practice, when formulating treatment strategies for type 2 diabetes combined with cardiovascular diseases, the impact of insulin on metabolism and cardiovascular function should be comprehensively considered. It is not advisable to rely solely on insulin treatment; instead, drugs with cardiovascular protective effects should be combined to achieve more comprehensive control of metabolic and cardiovascular risks. Further research is needed to understand how insulin affects the expression of related genes through signaling pathways, in order to provide new targets and strategies for precise treatment of diabetes-related cardiovascular diseases.

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Disclosure statement

The authors declare no conflict of interest.

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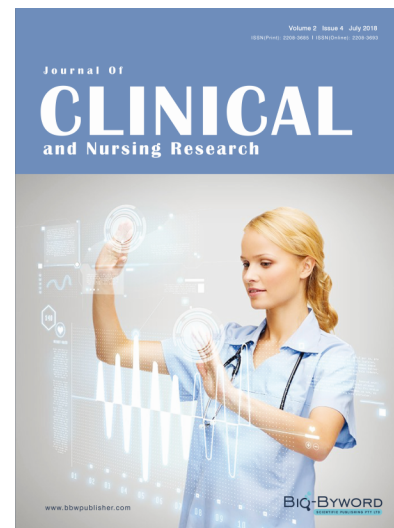
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