

## A Systematic Review of Apolipoprotein A-I Mimetic Peptides for Atherosclerosis Therapy via Activation of the Reverse Cholesterol Transport Pathway

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### Review Article

#### Abstract

**BACKGROUND:** HDL has been identified as a potential new treatment for atherosclerosis. Targeting lipid metabolism via the Reverse Cholesterol Transport (RCT) pathway can improve HDL metabolism. Apolipoprotein A-I mimetic peptides (ApoA-I MPs) are able to increase HDL metabolism. Thus, this systematic review aimed to examine the potential effect of ApoA-I MPs against atherosclerosis in mice models through the RCT mechanism.

**METHODS:** This systematic review was conducted using previous in vivo studies published in four scientific databases over the last ten years (PubMed, SCOPUS, ProQuest, and Science Direct) and was based on the Systematic Review Protocol for Animal Intervention Studies (SYRCLE) protocol.

**RESULTS:** This study's primary outcome was a reduction in atherosclerotic plaque, where 16 articles were qualified for this study. Based on the risk of bias analysis, these articles had a low risk of bias. Most in vivo studies (13 of 16) showed that ApoA-I MPs significantly reduced atherosclerotic plaque formation. Generally, ApoA-I MPs played an important role in regulating HDL metabolism (HDL remodeling process, increased cholesterol efflux, and stimulated RCT pathway) and anti-inflammatory agent. ApoA-I MPs may differ in their ability to reduce atherosclerotic plaque depending on the peptide sequence and administration route.

**CONCLUSIONS:** ApoA-I MPs can reduce atherosclerotic plaque formation in mice by increasing cholesterol efflux via the RCT pathway. Further investigation is required to support the development of ApoA-I MPs as a new therapy for atherosclerosis in humans.

**Keywords:** Apolipoprotein A-I mimetic peptides, Atherosclerosis, HDL Metabolism, Reverse Cholesterol Transport

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#### Introduction

Atherosclerosis is a chronic plaque formation in the inner arterial wall that increases global cardiovascular disease morbidity and mortality rates.<sup>1</sup> This disorder caused 31% of global deaths in 2015.<sup>1</sup> Furthermore, atherosclerosis is negatively correlated with high-density lipoprotein (HDL) cholesterol levels, making HDL metabolism an important potential target for

the development of atherosclerosis therapy.<sup>2</sup> RCT is an alternative pathway for boosting HDL metabolism.

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HDL cholesterol is a complex molecule composed of spherical microemulsion particles, free cholesterol, and protein, with Apolipoprotein A-I (ApoA-I) contributing approximately 70%.<sup>3</sup> ApoA-I is a polypeptide with a 243-amino acid sequence and repeating amphipathic helices as its primary structural motif. It is required for cholesterol removal from the cytoplasm of a cell via the Adenosine Triphosphate Binding Cassette A1 (ABCA1) transporter.<sup>3</sup> Genetic modified ApoA-I expression in animal models indicates a protective effect against atherogenesis due to reduced HDL levels.<sup>3</sup> Apolipoprotein A-I mimetic peptides (ApoA-I MPs) are one of the potential therapies for improving HDL function. In recent years, ApoA-I MPs with various sequences of peptides have been developed, but there is no growing evidence that reviews the potential benefits of ApoA-I MPs against atherosclerosis. These peptides can affect plasma HDL remodeling, which results in the migration of ApoA-I from larger nanoparticles to smaller ones and increase the production of pre- $\beta$  HDL.<sup>4</sup> Multiple studies have demonstrated that ApoA-I MPs reduce atherosclerotic lesions, enhance the anti-inflammatory properties of HDL, and improve vascular function.<sup>5</sup>

In addition to HDL remodeling, ApoA-I MPs boost cholesterol efflux, HDL reception, and cholesterol bile excretion.<sup>2</sup> Previous *in vitro* and *in vivo* studies have suggested that ApoA-I MPs have comparable effects to 30 mg/kg BW. ApoA-I intraperitoneal infusion

administered three times per week increases the RCT pathway and reduces atherosclerosis plaque.<sup>2</sup> Consequently, this systematic review aimed to determine the potential therapy of ApoA-I MPs against RCT in atherosclerosis.

## Materials and Methods

### Data source

The systematic review was conducted per the Systematic Review Protocol for Animal Intervention Studies (SYRCLE) and was registered with PROSPERO (ID: CRD42021231543). Electronic databases, including PubMed, SCOPUS, ProQuest, and Science Direct, were used to search for relevant articles between January 2011 to July 2020. This study's search strategy was based on a systematic review titled "A step-by-step guide to identifying all relevant animal studies" "thorough analysis of previously performed experiments is essential from a scientific as well as from an ethical point of view. The method that is most suitable to carry out such a thorough analysis of the literature is a systematic review (SR. Several Medical Subject Headings (MeSH) keywords were used to search for published articles (Supplemental Material 1).

### Search strategy

Two authors conducted and evaluated the initial search, screening, and eligibility of published articles, and any disagreements were resolved by consensus. After the initial search, duplicate records, reviews, and articles without full text were eliminated.

**Table 1.** Eligibility Criteria

No.	Criteria	Description
1.	Study Design	Inclusion: <i>In vivo</i> study Exclusion: A review article, clinical study, case reports
2.	Population	Inclusion: Atherosclerotic model mice or rat Exclusion: Cellular or tissue, human, other animals
3.	Intervention	Inclusion: ApoA-I mimetic peptides (oral or injection) Exclusion: N/A*
4.	Outcome	Inclusion: Atherosclerotic plaque formation Exclusion: N/A*
5.	Language	Inclusion: English Exclusion: N/A*
6.	Time of Publication	Inclusion: January 2011- June 2020 Exclusion: N/A*

\*N/A; Not Applicable

Then, the article records were screened and excluded based on their titles and abstracts for lack of relevance to the study's keywords. After that, eligibility criteria were applied to the full-text articles (Table 1). Eligible studies were included in this systematic review.

### *Quality Assessment and Data Extraction*

In order to minimize the risk of bias (RoB) analysis, two independent reviewers analyzed the selected articles using the RoBSYRCLE protocol<sup>7</sup> but awareness of the merits of conducting such SRs is steadily increasing. As animal intervention studies differ from randomized clinical trials (RCT. The data extraction for each article comprised article information (author and publication year), study characteristics, experimental animal characteristics, intervention and comparator, and outcomes (atherosclerotic plaque formation).

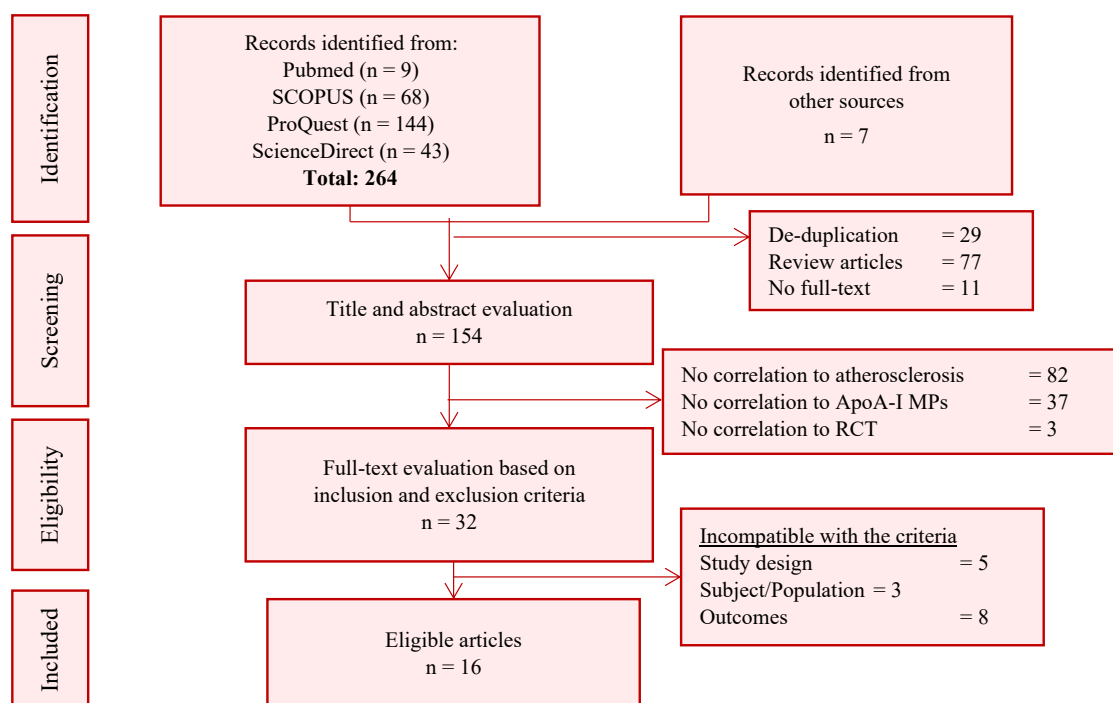
## Results

There were 264 articles from the four

databases and seven more from the bibliography of eligible articles found through additional research. The titles and abstracts of 154 of the 271 articles were evaluated for relevance to the systematic review topic. In addition, 32 articles were screened for eligibility, and 16 were ultimately included in this study (Figure 1).

The risk analysis results revealed that most eligible studies had a low risk of bias. An unclear risk of bias existed in random housing and random outcome assessment domains. A high risk of bias mainly existed in the blinding of the housing domain. However, the other domains had a low risk of bias (Figure 2).

The characteristic data of 16 eligible studies were extracted in Table 2, which were author, publication year, animal model, age, gender, diet, diet duration, peptide type, peptide dose, route of administration, duration of the treatment, and treatment groups and samples (detailed results can be found in Supplemental Material 2). Table 2 displays the heterogeneity of the eligible articles extracted from all available data.



**Figure 1.** Systematic Review Flowchart Diagram

Risk of bias	Risk of bias									
	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10
Navab et al (2011)	+	+	+	-	-	+	-	X	+	+
Ou et al (2012)	+	+	+	-	-	-	-	X	+	+
Qin et al (2012)	+	+	+	-	-	-	+	+	+	+
Chattopadhyay et al (2013)	+	X	+	-	-	-	-	+	+	+
Ditiatkovski et al (2013)	+	+	+	-	X	-	+	+	+	+
Li et al (2013)	+	+	+	-	-	+	X	+	+	+
Uehara et al (2013)	+	+	+	-	-	-	+	X	+	+
Ying et al (2013)	+	+	+	+	X	+	+	+	+	+
Averill et al (2014)	+	+	X	-	X	-	-	+	+	+
Zhao et al (2014)	+	+	+	-	-	-	+	+	+	+
Schwendeman et al (2015)	+	+	+	+	-	-	+	+	-	+
Ditiatkovski et al (2017)	+	+	+	-	-	-	+	-	+	+
Tian et al (2017)	+	+	+	-	+	-	+	+	+	+
Edmunds et al (2019)	+	+	+	+	+	-	+	+	+	+
Suematsu et al (2019)	+	+	+	-	-	-	+	+	+	+
Wu et al (2020)	+	+	+	+	-	-	+	+	+	+

D1: Random sequence generation  
D2: Baseline characteristics  
D3: Allocation concealment  
D4: Random housing  
D5: Blinding of Housing  
D6: Random outcome assessment  
D7: Blinding of outcome assessment  
D8: Incomplete outcome data  
D9: Selective reporting  
D10: Other sources of bias

Judgement  
X High  
- Unclear  
+ Low

Figure 2. Risk of bias for the eligible articles

The total types of ApoA-I MPs used in all these studies were 16 types, namely D-4F,<sup>8-11</sup> Reverse-D-4F (Rev-D-4F),<sup>12,13</sup> reverse-D4F (Rev-D4F L-4F),<sup>12,14,15</sup> reverse-D4F (Rev-D4F 6F,<sup>16</sup> Transgenic-6F (Tg6F),<sup>16</sup> ELK-2A2L2E,<sup>17,18</sup> ELKA-CH2,<sup>18</sup> ELK-2A,<sup>18</sup> Fukuoka University ApoA-I MPs (FAMP),<sup>19,20</sup> R)-(+)-1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) nanoparticle,<sup>21</sup> 5A,<sup>18</sup> 5A-palmitoyl-oleoyl-phosphatidyl-choline (POPC),<sup>22</sup> 5A-sphingomyelins (SM),<sup>22</sup> 5A-C1,<sup>18</sup> 5A-CH1,<sup>18</sup> and RG54.<sup>23</sup>

A total of 13 types of peptides (D-4F, Rev-

D-4F, 6F, Tg6F, ELKA-CH2, ELK-2A, FAMP, DMPC nanoparticle, 5A, 5A-SM, 5A-C1, 5A-CH1, and RG54) could significantly decrease atherosclerotic lesion, while the remaining (L-4F, ELK-2A2K2E, and 5A-POPC) were insignificant.

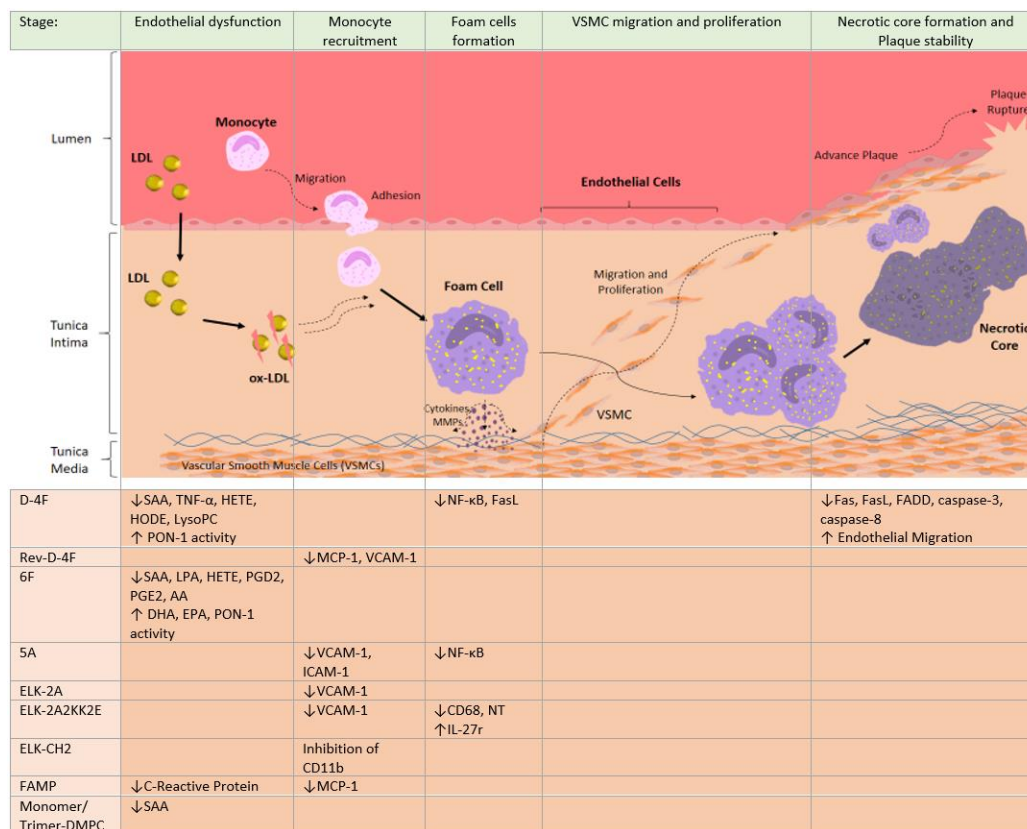
The different administration routes of peptides showed different results in reducing atherosclerosis plaque. For example, D-4F peptide administration either by oral, subcutaneous, or intraperitoneal routes significantly reduced atherosclerosis plaque.<sup>8-11</sup>

**Table 2.** Characteristics of eligible studies

No	Authors (Year)	Animal Models	Age	Gender	Diet	Diet Duration	Peptide Type	Dose	Route	Duration	Groups and Samples
1	Navab et al. (2011)	Mice ApoE <sup>-/-</sup> C57BL/6	8-9 month	female	WD	8 wk	D-4F, Sc-D-4F	900 µg/day	SQ and PO	8 wk	I: Sc-D-4F SQ; II: D-4F SQ; III: Sc-D-4F PO; IV: D-4F PO (n=12/ group)
2	Ou et al. (2012)	Mice LDLR <sup>-/-</sup> C57BL/6	8 wk	female	WD	10 wk	D-4F	1 mg/ kg/day	IP	4,7, 10 wk	I: Standard Feed; II: WD 10 wk; III: WD 10 wk + D-4F 4 wk; IV: WD 10 wk + D-4F 7 wk; V : WD 10 wk + D-4F 10 wk (n=6 / group)
3	Qin et al. (2012)	Mice ApoE <sup>-/-</sup> C57BL/6	4 wk	female	Standard Feed	6 wk	L-4F and Rev-D-4F	1.6 mg/day	PO	6 wk	I: Control (n=15); II: L-4F (n=12); III: Rev-D-4F (n=12)
4	Chattopadhyay et al. (2013)	Mice ApoE <sup>-/-</sup> C57BL/6	4-6 month	female	WD	6-7 wk	L-6F	60 mg/kg/day	PO	6-7 wk	IA: WD (n=30) ; IIA: WD + 6F (n=30)
		Mice LDLR <sup>-/-</sup> C57BL/6	10 wk	female	WD	2 wk	EV and Tg6F Tomatoes	40 mg/kg/day	PO	2 wk	IB: WD (n=20); IIB: WD + WT Tomatoes (n=8); IIIB: WD + Tg6F (n=8)
		Mice LDLR <sup>-/-</sup> C57BL/6	4-5 month	female	WD	13 wk	EV and Tg6F Tomatoes	45 mg/kg/day	PO	13 wk	IC: WD (n=28); IIC: WD + EV Tomatoes (n=8); IIIC: WD + Tg6F (n=20)
5	Ditiatkovski et al. (2013)	Mice ApoE <sup>-/-</sup> C57BL/6	10 wk	male	HFD	4 wk	ELK-2A2K2E peptide	30mg/kg/wk	IP	16 wk	I: Placebo; II: ELK-2A2K2E (n=8/group)
6	Li et al. (2013)	Mice LDLR <sup>-/-</sup> C57BL/6	3 month	male	HFD, UFP	10 wk	D-4F	0.2 mg/ml/Mice	SQ	10 wk	I: FA; II: FA + D-4F; III: UTP; IV: UTP + D4F (n=9/group)
7	Uehara et al. (2013)	Mice LDLR <sup>-/-</sup> and C57BL/6	6-7 wk	male	HFD	3x / wk for 16 wk	ScFAMP, LD FAMP5, HD FAMP5	10 and 50 mg/kg	IP	3x /wk for 16 wk	I: ScFAMP (n=7); II: LD FAMP5 (n=5); III: HD FAMP5 (n=7)
8	Ying et al. (2013)	Mice ApoE <sup>-/-</sup> C57BL/6 and WT	8 wk	male	HFD	16 wk	L-4F	1 mg/ kg/day	IP	16 wk	I: Control; II: HFD; III: Simva; IV: L-4F; V: Simva + L-4F (n=6/group)
9	Averill et al. (2014)	Mice LDLR <sup>-/-</sup> C57BL/6	10 wk	male	HFH SC	12 wk	L-4F	100 µg/day	SQ	12 wk	I: Control; II: L-4F (n=5)
10	Zhao et al. (2014)	Mice LDLR <sup>-/-</sup> C57BL/6	10 wk	female	HFD	10 wk	DMPC nanoparticle	7.5, 40, 75 mg/kg	IP and PO	10 wk	IP I: Control (n=10); IP II: DMPC ULV (n=10); IP III: monomer / DMPC (n=12); IP IV: trimer / DMPC (n=15); PO I: kontrol (n=18); PO II: DMPC MLV (n=8); PO III: monomer / DMPC (n=10); PO IV: trimer / DMPC 75 mg/kg (n=10); PO V: trimer / DMPC 7.5 mg/kg (n=8)
11	Schwendeman et al. (2015)	Mice LDLR <sup>-/-</sup> C57BL/6	8 wk	male	HCD	14 wk	5A-POPC and 5A-SM rHDL	50 mg/kg	IP	3x/ wk for 6 wk	I: Baseline; II: PBS; III: 5A-POPC; IV: 5A-SM rHDL (n=7-8/group)
12	Ditiatkovski et al. (2017)	Mice ApoE <sup>-/-</sup> C57BL/6	6-7 wk	male	HFD	12 wk	5A, ELK-2A2K2E, 5A-C1, ELK-2A2K2E+5A-C1, ELKA-CH2, ELK-2A, 5A-CH1	30 mg/kg	IP	4 wk	I: Control; II: 5A; III: ELK-2A2K2E; IV:5A-C1; V: ELK-2A2K2E+5A-C1; VI: ELKA-CH1; VII: ELK-2A; VIII: 5A-CH1
13	Tian et al. (2017)	Mice LDLR <sup>-/-</sup> C57BL/6	7 wk	male	HFD	8 wk	Sc-D-4F and D-4F	1 mg/ kg/day	IP	6 wk	I: Control; II: Sc-D-4F; III: D-4F (n=8/group)

No	Authors (Year)	Animal Models	Age	Gender	Diet	Diet Duration	Peptide Type	Dose	Route	Duration	Groups and Samples
14	Edmunds et al. (2019)	Mice ApoE <sup>-/-</sup> C57BL/6	12 wk	female	WD	6 wk	RG54	12 mg/kg	IP	3x/ wk for 6 mg	I: NaCl; II: Liraglutide; III: ApoA-I; IV: RG54 (n=9)
15	Suematsu et al. (2019)	Mice ApoE <sup>-/-</sup> C57BL/6 and CETP Tg	6 wk	male	HFD	2x/wk for 16 wk	FAMP and i-FAMP-D1	FAMP (50 mg/kg), i-FAMP-D1 (50 mg/kg)	IP	3x/ wk for 16 wk	I: Control; II: FAMP; III: i-FAMP-D1 (n=6/group)
16	Wu et al. (2020)	Mice LDLr <sup>-/-</sup> C57BL/6	6-8 wk	male	HCD	12 wk	Rev-D-4F	2.1 mg/kg	IV	9 wk	I: PBS; II: ST; III: MNC@M-ST; IV: AP; V: MNC@M-AP; VI: MNC@M-ST/AP (n=6/group)

AP, Apolipoprotein; ApoA-I, Apolipoprotein A-I; ApoE, Apolipoprotein E; CETP, Cholesteryl Ester Transfer Protein; DMPC, R(+)-1,2-dimyristoyl-sn-glycero-3-phosphocholine; EV, empty vector; FA, filtered air; FAMP, Fukuoka University ApoA-I MPs; HCD, high cholesterol; diet; HD, high dose; HFD, high-fat diet; HFHSC, High Fat High Sucrose diet with added Cholesterol; IP, intraperitoneal; kg, kilogram; LD, low dose; LDLr, low-density lipoprotein receptor; mg, milligram; MNC@M, Fe<sub>3</sub>O<sub>4</sub> magnetic nanoclusters coated with anchored leukocyte membrane fragments; MLV, multilamellar vesicle; NaCl, Sodium Chloride; PBS, phosphate buffer saline; PO, peroral; POPC, palmitoyl oleoyl phosphatidyl choline; Rev-D-4F, reverse-D-4F; rHDL, reconstituted-HDL; Sc, Scrambled; SM, sphingomyelins; ST, Simvastatin; SQ, subcutaneous; Tg6F, transgenic 6F; UFP, ultrafine particle; ULV, unilamellar vesicle; WD, western diet; Wk, week; WT, wild-type; µg, microgram; \* = p<0.05; \*\* = p<0.01; \*\*\* = p<0.001.



**Figure 3.** The Role of ApoA-I MPs against atherosclerosis.

AA, Arachidonic Acid; CD, Cluster of Differentiation; DHA, Docosahexaenoic Acid; EPA, Eicosapentaenoic Acid; FADD, Fas-Associated Death Domain; FAMP, Fukuoka University Apolipoprotein A-I Mimetic Peptide; FasL, Fas Ligand; HETE, Hydroxyeicosatetraenoic Acid; HOPE, Hydroxyoctadecadienoic Acid; ICAM-1, Intercellular Adhesion Molecule 1; IL, Interleukin; LDL, Low Density Lipoprotein; LPA, lysophosphatidic Acid; LysoPC, Lysophosphatidylcholine; MCP-1, Monocyte Chemoattractant Protein 1; NF-κB, Nuclear Factor Kappa B; NT, Nitrotyrosine; Ox-LDL, Oxidized Low Density Lipoprotein; PGD2, Prostaglandin D2; PGE2, Prostaglandin E2; PON-1, Paraonase-1; Rev-D-4F, Reverse-D-4F; SAA, Serum Amyloid A; VCAM-1, Vascular Cell Adhesion Molecule 1; VSMCs, Vascular Smooth Muscle Cells

Intraperitoneal administration of L-4F peptide significantly reduced plaque formation,<sup>14</sup> while oral and subcutaneous administration had no significant effect.<sup>12,15</sup>

The Rev-D-4F peptide was utilized by Wu *et al.* (2020). Rev-D-4F is an analog of the peptide D-4F. Intravenous administration of this peptide in conjunction with self-driven bioinspired nanovehicles in the form of Fe<sub>3</sub>O<sub>4</sub> magnetic nanocluster (MNCs) coated by Simvastatin (ST) and associated with leukocyte membrane fragments (MNC@M-ST-AP) could reduce plaque formation.<sup>13</sup>

In contrast to the previous study, Chattopadhyay *et al.* (2013) demonstrated oral administration of L-6F and 6F peptides from transgenic tomatoes using transgenic tomatoes constructed with Empty Vector (EV) and a vector expressing 6F peptide could significantly reduce plaque formation.<sup>16</sup>

Other studies utilized intraperitoneal administration of various peptides, including 5A, ELK-2A2K2E, 5A-C1, ELK-2A2K2E and 5A-C1 combination, ELKA-CH2, ELK-2A, and also 5A-CH.<sup>18</sup> Ditiatkovski *et al.* (2017) evidenced a significant reduction of plaque formation, particularly in the aortic arch than other locations of histological lesions.<sup>18</sup> Regarding treatment duration, ELK-2A2K2E peptide did not affect thoracic and abdominal aortic plaque during week 16.<sup>18</sup> On the other hand, Schwendeman *et al.* (2015) used a combination of 5A peptide with SM and POPC with HDL reconstruction (5A-SM and 5A-POPC rHDL). When compared to 5A-SM, 5A-POPC rHDL significantly reduced atherosclerotic plaque formation.<sup>22</sup>

## Discussion

In this systematic review, 16 studies that developed ApoA-I MPs for the treatment of atherosclerosis in mice were examined. Depending on peptide type, administration route and time, and location of histological lesions, the majority of ApoA-I MPs could reduce atherosclerotic plaque. In addition, this systematic review describes the potential mechanism of ApoA-I MPs against the

formation of atherosclerotic plaques. As the HDL metabolism regulator (HDL remodeling, cholesterol efflux, and RCT) and anti-inflammatory agent, it decreases the formation of atherosclerosis plaques by reducing plaque formation.

### *Apolipoprotein A-I as HDL Metabolism Regulator*

ApoA-I MPs help to increase the pre- $\beta$ 1-HDL formation and improve the RCT mechanism. In the early stages of RCT, these nanoparticles play an important role as acceptors of free cholesterol from ABCA1.<sup>4</sup> The lipid-poor pre- $\beta$ 1-HDL is gradually enlarged due to cholesterol uptake and esterification catalyzed by the LCAT. The enlarged HDL contributes to the lipid core, which can receive phospholipids and free cholesterol from peripheral tissue via Adenosine Triphosphate Binding Cassette G1 (ABCG1). These HDL molecules release cholesterol ester into the hepatocytes via scavenger receptor class B type I (SR-BI) or transfer them to LDL via a CETP-mediated mechanism involving transfer protein (PLTP) and diverse lipases for HDL remodeling.<sup>4</sup>

A previous study showed that D-4F could increase cholesterol removal from foam cells and plasma pre- $\beta$ 1-HDL formation and regulate cholesterol levels.<sup>11</sup> Subcutaneous administration of L-4F also could reduce atherosclerosis formation by upregulation of ABCA1 and ABCG1 expression in the macrophages, liver, and aortic walls, as well as SR-BI expression in the liver and aortic walls.<sup>14</sup> Moreover, the L-4F peptide significantly increased cholesterol efflux,<sup>14</sup> but this peptide became less unstable by oral administration due to digestion by intestinal proteases compared to the D-4F.<sup>14,15</sup> Previous studies have shown that oral administration of D-4F is safe and well-tolerated.<sup>24</sup>

On the other hand, the 5A peptide is a bi-helix amphipathic peptide with high specificity for ABCA1-mediated cholesterol efflux and low cytotoxicity.<sup>25</sup> This mimetic peptide could stimulate a 3.5-fold increase in ABCA1-mediated cell efflux and a 2.5-fold increase when combined with phospholipid.<sup>25</sup> The 5A-C1 peptide significantly increased cholesterol

efflux.<sup>18</sup> and 5A-POPC was also found to increase cholesterol efflux by ABCG1. To this end, 5A-POPC binds to HDL and LDL and increases the transfer of cholesterol from LDL to HDL.<sup>25</sup>

The other type of peptide is ELK/ELKA. ELK-2A2K2E significantly increases cholesterol efflux.<sup>17, 18</sup> Moreover, Fukuoka University ApoA-I MPs (FAMP) were reported to demonstrate two roles in HDL metabolism, especially in the production of pre- $\beta$ -HDL metabolism. FAMP could increase cholesterol efflux through ABCA1-dependent or -independent mechanism to produce new pre- $\beta$  HDL particles.<sup>26</sup> Furthermore, incubation of FAMP with human HDL or plasma could produce both small HDL particles and ApoA-I-rich particles. These particles migrated as pre-HDL on agarose electrophoresis.<sup>27</sup>

#### *Apolipoprotein A-I as Anti-Inflammatory Agent*

Most ApoA-I MPs play a role as an anti-inflammatory agent. ApoA-I functions as an anti-inflammatory agent through the uptake of oxidized lipids, which is facilitated by the high affinity of active peptides for oxidized fatty acids, sterols, and phospholipids.<sup>4</sup> ApoA-I MPs, as anti-inflammatory agents in monocyte chemotactic activity (MCA) and atherosclerosis, have a high affinity against oxidized fatty acids, cholesterol, and phospholipids. ApoA-I MPs can also inhibit MCA stimulation mediated by LDL. ApoA-I MPs also play a role in stimulating endothelial nitric oxidase synthase (eNOS), thus leading to vasodilated blood vessels.<sup>28</sup>

Studies of ApoA-I MPs had been developed from a physicochemical and biological aspect (in vitro) into animal models (in vivo). Recent studies have examined various ApoA-I MPs as anti-inflammatory agents (Figure 3).<sup>11,12,17,18</sup> an apolipoprotein A-I mimetic peptide, on nuclear factor- $\kappa$ B (NF- $\kappa$ B D-4F and L-4F peptides have been proven to regulate various plasma and tissue biomarkers to prevent or reduce atherosclerosis. D-4F had a role as an atheroprotective agent through oxidative stress and inflammation inhibition.<sup>11</sup> Rev-D-4F was also developed as ApoA-I MP against

atherosclerosis. This peptide significantly inhibited VCAM-1 and MCP-1, which play a role in monocyte adhesion and chemotaxis.<sup>12</sup>

Another type of ApoA-I MPs, 6F, was proven to reduce the total plasma cholesterol, triglycerides, SAA, LPA, 5-HETE, 15-HETE, PGD2, PGE2, and AA. LPA has been shown to alter the secretion of apoB-containing lipoproteins from hepatocytes, accelerating atherosclerosis in a mouse model.<sup>29</sup>

Oxidation of LDL generates lysophosphatidylcholine, which is the main substrate for the lysophosphatidic acid (LPA). The Tg6F also could decrease systemic inflammation and dyslipidemia in WD-induced mice by preventing the increase of SAA and LPA levels in the small intestine.<sup>16</sup>

In addition, ELK-2A2K2E could decrease CD68, VCAM-1, and NT expression and raise RCT value via cholesterol and fecal bile acid. ELKA-CH2 was observed to be a selective CD11b inhibitor in monocytes. Furthermore, ELK-2A has been demonstrated to be a selective inhibitor of VCAM-1 expression in endothelial cells.<sup>17, 18</sup> Monocyte CD11b expression can be suppressed by ABCA1-dependent or -independent mechanisms.<sup>25</sup> Moreover, ELK-2A and ELKA-CH2 could increase IL-27 expression.<sup>18</sup>

However, no research has been conducted on the safety and tolerability of other ApoA-I MPs. Concerning its applicability, the primary issue with ApoA-I MPs is their high production costs.<sup>30</sup>

### Conclusions

ApoA-I MPs can inhibit atherosclerosis through RCT and anti-inflammatory pathways. Nonetheless, the characteristics of eligible items are extremely diverse. Administration of ApoA-I MPs could reduce the formation of atherosclerotic plaques in mice models, depending on the peptide type and method of administration. Some ApoA-I MPs also affect the lipid profile and other biomarkers in the plasma and tissue. Several ApoA-I MPs (4F, 5A, ELKs, and FAMP) inhibit atherosclerosis by increased of cholesterol efflux in the RCT



pathway.

A recent study revealed no results regarding the safety and toxicity of ApoA-I MPs except for the D-4F peptide. Several types of ApoA-I MPs, however, have not been evaluated for cholesterol efflux or RCT value; therefore, additional research is required. Moreover, research on ApoA-I MPs safety, toxicity, and human clinical trials is required to support the development of ApoA-I MPs as a new therapy for atherosclerosis in humans.

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