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Cardiovascular diseases arising from atherosclerosis are the leading causes of mortality and morbidity worldwide. Lipid-lowering agents have been developed in order to treat hypercholesterolemia, a major risk factor for atherosclerosis. However, the prevalence of cardiovascular diseases is increasing, indicating a need to identify novel therapeutic targets and develop new treatment agents. Adenosine receptors (ARs) are emerging as therapeutic targets in asthma, rheumatoid arthritis, cancer, ischemia, and inflammatory diseases. This study assessed whether LJ-1888, a selective antagonist for A$_3$ AR, can inhibit the development of atherosclerosis in apolipoprotein E knock-out ($ApoE^{-/-}$) mice who are fed a western diet. Plaque formation was significantly lower in $ApoE^{-/-}$ mice administered LJ-1888 than in mice not administered LJ-1888, without any associated liver damage. LJ-1888 treatment of $ApoE^{-/-}$ mice prevented western diet-induced hypercholesterolemia by markedly reducing low-density lipoprotein cholesterol levels and significantly increasing high-density lipoprotein cholesterol concentrations. Reduced hypercholesterolemia in $ApoE^{-/-}$ mice administered LJ-1888 was associated with the enhanced expression of genes involved in bile acid biosynthesis. These findings indicate that LJ-1888, a selective antagonist for A$_3$ AR, may be a novel candidate for the treatment of atherosclerosis and hypercholesterolemia. [BMB Reports 2018; 51(10): 520-525]

INTRODUCTION

Cardiovascular diseases including atherosclerosis are the leading causes of death worldwide (1-3). Hypercholesterolemia is considered a major risk factor for atherosclerosis, which has resulted in the development of medications aimed at lowering plasma cholesterol levels (4, 5). Because atherosclerosis is characterized by increased plasma levels of low-density lipoprotein cholesterol (LDL-chol) and reduced plasma concentrations of high-density lipoprotein cholesterol (HDL-chol) (6), agents that modify LDL-chol and HDL-chol levels are needed to prevent and improve the outcomes of atherosclerotic vascular diseases. Statins, which act as 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase inhibitors, are representative lipid-lowering medications that have shown protective effects in cardiovascular diseases by inhibiting cholesterol synthesis, enhancing the expression of LDL receptors on the surface of hepatocytes, and increasing anti-inflammatory responses (7). Other medications used to lower LDL-chol levels include cholesterol absorption inhibitors, bile acid sequestrants, and protein convertase subtilisin/kexin type-9 (PCSK-9) inhibitors (8, 9). Moreover, elevated plasma levels of HDL-chol have been closely associated with clinical benefits in patients with cardiovascular diseases (10). For example, cholesteryl ester transfer protein (CETP) inhibitors and HDL mimetics have been shown to regulate HDL-chol levels, suggesting that these agents may be therapeutically applicable in the prevention and/or treatment of cardiovascular diseases (10, 11). However, the worldwide morbidity and mortality rates of cardiovascular diseases remain high (12), indicating a need to identify new therapeutic targets and develop corresponding medications.

Adenosine, an endogenous nucleoside, regulates various physiological processes, including neurotransmission, vasodilation, energy transfer, and signal transduction (13). Extracellular adenosine levels are low under normal physiological conditions, but increase in response to cellular damage resulting from inflammation, ischemia, hypoxia, and

trauma (14). Four adenosine receptor (AR) subtypes, A_1, A_{2A}, A_{2B}, and A_3, mediate cellular signaling by adenosine and modulate the biological effects of adenosine in organs (15). Although the physiological roles of these four AR subtypes are partially redundant, they are encoded by separate genes and have unique functions (15). Therefore, selective agonists and/or antagonists have been developed for each AR subtype in order to treat various diseases (16). Investigations of A_3 AR agonists and/or antagonists have provided the identification of pharmacological targets to treat myocardiac and cerebral ischemia, cancer, asthma, glaucoma, and renal fibrosis (17, 18). LJ-1888 [(2R,3R,4S)-2-[2-chloro-6-(3-iodobenzylamino)-9H-purine-9-yl]-tetrahydrothiophene-3,4-diol] is a novel, selective, species-independent A_3 AR antagonist (19) that has shown protective effects in renal fibrosis induced by unilateral ureteral obstruction (UUO) and transforming growth factor-β1 (TGF-β1) (20). However, its ability to protect against the development of atherosclerosis has not yet been determined. This study therefore investigated the effects of LJ-1888 on the development of atherosclerosis in apolipoprotein E knock-out ($ApoE^{-/-}$) mice who were fed a western diet (WD). Atherosclerotic plaque formation was significantly lower in LJ-1888-treated $ApoE^{-/-}$ mice than in untreated $ApoE^{-/-}$ mice. Moreover, LJ-1888 supplementation suppressed WD-induced hypercholesterolemia in $ApoE^{-/-}$ mice through

lowering LDL-chol and raising HDL-chol levels. These effects of LJ-1888 were mechanistically associated with alterations in the expression of genes catalyzing bile acid biosynthesis. Taken together, these results suggest that LJ-1888 may be useful in the prevention and/or treatment of atherosclerosis and hypercholesterolemia.

RESULTS

LJ-1888 ameliorates the formation of atherosclerotic plaques in apolipoprotein E knock-out mice

To explore the effects of the selective A_3 AR antagonist LJ-1888 on the development of atherosclerosis, six-week-old $ApoE^{-/-}$ mice were fed normal chow (ND) or a western diet (WD), with or without two dosages of LJ-1888 (0.025% and 0.05%), for 12 weeks. Body weight (BW) gains were higher in WD fed than in ND fed $ApoE^{-/-}$ mice, but there were no differences among WD fed groups in BW gain (Supplementary Fig. 1A) or food intake (Supplementary Fig. 1B). After 12 weeks, the mice were sacrificed. The ratios of liver weight to BW were similar in all groups (Supplementary Fig. 1C), but the ratios of epididymal fat to BW were significantly higher in WD-fed than in ND-fed mice, with LJ-1888 supplementation having no effect on the ratio of epididymal fat to BW (Supplementary Fig. 1D).

Isolated whole aortas of these mice, from the ascending to the femoral region, were dissected longitudinally and stained with Oil red O to analyze atherosclerotic plaque formation. Plaque formation was significantly greater in WD fed (3.79%) than in ND fed (1.58%) $ApoE^{-/-}$ mice. However, supplementation with 0.025% (2.02%) and 0.05% (2.30%) LJ-1888 significantly inhibited WD-induced atherosclerotic plaque formation on the aortas of WD fed mice (Fig. 1A and B). To further validate the effects of LJ-1888 on atherosclerotic plaque formation, the aortic sinuses of hearts from the four groups of mice were dissected. As expected, atherosclerotic plaque formation on aortic sinuses was lowest in the ND fed group and highest in the WD fed group. Consistent with the plaque formation on the aortas, the plaque formation on the aortic sinuses was markedly lower in WD fed mice receiving 0.025% and 0.05% LJ-1888 than in mice fed WD alone (Fig. 1C and D). These results suggested that the selective A_3 AR antagonist LJ-1888 has anti-atherosclerotic effects in $ApoE^{-/-}$ mice fed WD.

LJ-1888 ameliorates hypercholesterolemia in western diet fed apolipoprotein E knock-out mice

Hypercholesterolemia is a major risk factor for the development of cardiovascular diseases including atherosclerosis (1). Elevated levels of LDL-chol in the circulation increase endothelial permeability, leading to the accumulation of lipids in the arterial walls and subsequently triggering atherosclerosis (3). In contrast to LDL-chol, HDL-chol contributes to the prevention of atherosclerosis development by enhancing reverse cholesterol transport (21). Therefore, we tested whether LJ-1888-mediated
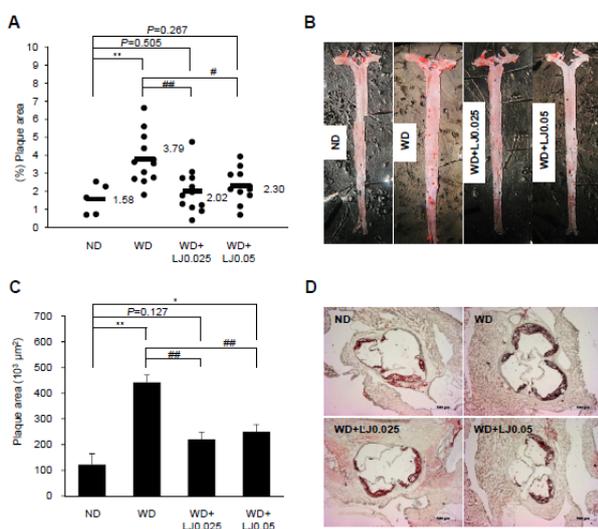

Fig. 1. LJ-1888 ameliorates the formation of atherosclerotic plaque in apolipoprotein E knock-out mice. (A) Quantification of Oil red O-stained areas on whole aortas from indicated groups (n = 5-12). (B) Representative *en face* images of whole aortas from indicated groups. (C) Quantification of plaque areas on aortic sinuses of indicated groups (n = 5-12). (D) Representative images of Oil red O-stained areas of frozen sections of aortic sinuses from indicated groups. Data are shown as mean ± SEM. *P < 0.05, **P < 0.01 versus ND group; #P < 0.05, ##P < 0.01 versus WD group.

anti-atherogenic effects are associated with reduced hyper-cholesterolemia in $ApoE^{-/-}$ mice. Total cholesterol levels were found to be about two-fold higher in WD fed (1218 ± 40 mg/dl) than in ND fed (679 ± 41 mg/dl) $ApoE^{-/-}$ mice. Supplementation with 0.025% (719 ± 53 mg/dl) and 0.05% (964 ± 74 mg/dl) LJ-1888 significantly reduced WD-mediated hypercholesterolemia compared to the WD group (Fig. 2A). Plasma LDL-chol levels showed a similar trend as total cholesterol, with WD fed mice having the highest level (279 ± 13 mg/dl), ND fed mice having the lowest level (97 ± 6 mg/dl), and mice fed WD plus 0.025% (151 ± 14 mg/dl) and 0.05% (216 ± 20 mg/dl) LJ-1888 having significantly lower plasma LDL-chol concentrations than $ApoE^{-/-}$ mice fed WD alone (Fig. 2B). Supplementation with LJ-1888 also prevented the WD-mediated reduction in HDL-chol levels in $ApoE^{-/-}$ mice. HDL-chol levels were lower in WD fed (18 ± 1.2 mg/dl) than in ND fed (23 ± 3.5 mg/dl) mice, whereas mice fed WD plus 0.025% (30 ± 1.9 mg/dl) and 0.05% (30 ± 3.2 mg/dl) LJ-1888 had significantly higher HDL-chol concentrations than mice fed WD alone (Fig. 2C). In contrast, although plasma triglyceride (TG) levels were lower in WD fed than in ND fed $ApoE^{-/-}$ mice, LJ-1888 supplementation had no effect on plasma TG levels (Fig. 2D), and glucose levels were slightly, but not significantly, higher in WD fed than in ND fed mice (Fig. 2E).

To test the liver toxicity of LJ-1888, we measured the serum concentrations of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and albumin (ALB) in $ApoE^{-/-}$ mice.

We found that ALT and AST levels, but not ALB levels, were higher in WD fed than in ND fed $ApoE^{-/-}$ mice (Fig. 2F-G), with LJ-1888 supplementation slightly ameliorating WD-induced liver damage.

LJ-1888 enhances the expression of bile acid biosynthesis genes in the livers of apolipoprotein E knock-out mice

Controlling lipoprotein and cholesterol levels is important for reducing the risk of atherosclerosis (1, 21, 22). Hypercholesterolemia may be ameliorated by lowering LDL-chol and TG concentrations and by increasing HDL-chol concentrations, using agents such as HMG-CoA reductase inhibitors, cholesterol absorption inhibitors, bile acid sequestrants, and proprotein convertase subtilisin/kexin type-9 (PCSK-9) inhibitors (7-9, 23, 24). We therefore assessed the mechanism by which LJ-1888 improves hypercholesterolemia in WD fed $ApoE^{-/-}$ mice. First, we measured the expression levels of cholesterol transport genes, including LDL receptor (*Ldlr*) and the ATP-binding cassette sub-family G member 5/8 (*Abcg5/8*), in the livers of the four groups of mice. *Ldlr* gene expression was significantly lower in the WD and WD+LJ0.025 groups than in the ND group, but this was not the case in the WD+LJ0.05 group, suggesting that *Ldlr* gene expression was not associated with the anti-atherogenic effects of LJ-1888 (Fig. 3A). In addition, *Abcg5/8* mRNA levels did not differ among the four groups (Fig. 3A), indicating that LJ-1888 does not ameliorate hypercholesterolemia in $ApoE^{-/-}$ mice by altering
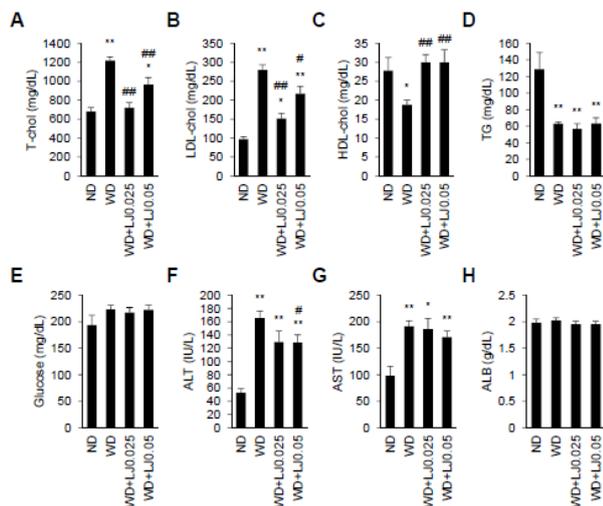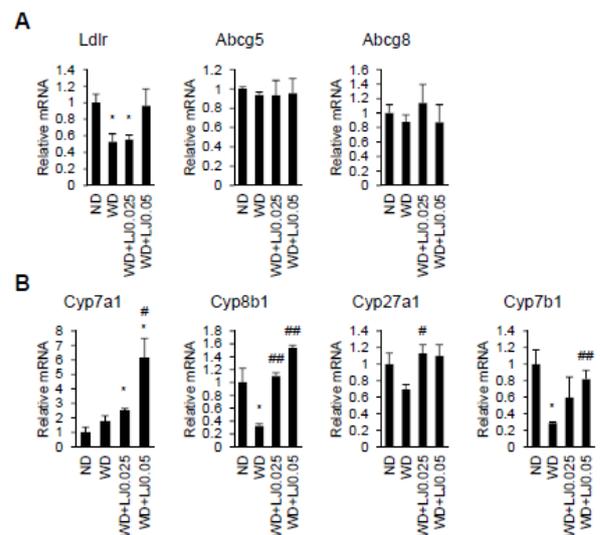

Fig. 2. LJ-1888 ameliorates hypercholesterolemia in western diet fed apolipoprotein E knock-out mice. Plasma concentrations of (A) total cholesterol (T-chol), (B) low density lipoprotein cholesterol (LDL-chol), (C) high density lipoprotein cholesterol (HDL-chol), (D) triglycerides (TG), (E) glucose, (F) ALT, (G) AST, and (H) ALB of indicated groups (n = 5-12). Data are shown as mean $\pm$ SEM. *P < 0.05, **P < 0.01 versus ND group; #P < 0.05, ##P < 0.01 versus WD group.

Fig. 3. LJ-1888 enhances the expression of bile acid synthesis related genes in the liver. Levels of expression of (A) cholesterol transport related genes and (B) bile acid biosynthesis genes in the livers of indicated groups (n = 4). Data are shown as mean $\pm$ SEM. *P < 0.05, **P < 0.01 versus ND group; #P < 0.05, ##P < 0.01 versus WD group.

the expression of cholesterol transport genes.

Next, we tested the effects of LJ-1888 on the expression of genes involved in bile acid biosynthesis (25). The classical pathway of bile acid synthesis is initiated by cholesterol 7α-hydroxylase (*Cyp7a1*), with bile acid intermediates further hydroxylated by sterol 12α-hydroxylase (*Cyp8b1*). In contrast, the major enzymes in the alternative pathway of bile acid synthesis include sterol 27-hydroxylase (*Cyp27a1*) and 25-hydroxycholesterol 7α-hydroxylase (*Cyp7b1*) (26). LJ-1888 supplementation induced the expression of bile acid biosynthetic genes. *Cyp7al* mRNA levels were significantly higher in both the WD+LJ0.025 and WD+LJ0.05 groups than in the ND group, but this was not the case in the WD group. Moreover, *Cyp7a1* mRNA levels were markedly higher in the WD+LJ0.05 group than in the WD group. *Cyp8b1, Cyp27a1,* and *Cyp7b1* mRNA levels were lower in the WD group than in the ND group, but were similar in the ND, WD+LJ0.025, and WD+LJ0.05 groups (Fig. 3B).

To analyze the mechanism by which LJ-1888 induces the expression of these genes, including those encoding bile acid biosynthesis proteins, we analyzed the mRNA levels of the transcription factors involved in regulating enzymes for bile acid biosynthesis. Liver X receptors (LXRs) are a family of pivotal transcription factors involved in regulating lipid and cholesterol metabolism. LXRα and LXRβ form heterodimers with the retinoic acid receptor (RXR), leading to transcription of various genes such as *Cyp7a1* and sterol regulatory element
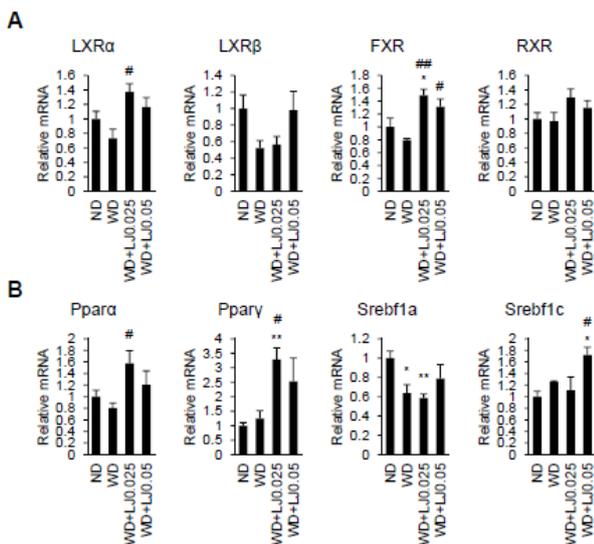

Fig. 4. Effects of LJ-1888 on the expression of transcription factors for bile acid synthesis. Levels of expression of mRNAs encoding transcription factors regulating the synthesis of (A) bile acids and (B) lipids in the livers of indicated groups (n = 4). Data are shown as mean ± SEM. *P < 0.05, **P < 0.01 versus ND group; #P < 0.05, ##P < 0.01 versus WD group.

binding factor 1c (*Srebf1c*) (26). We found that LXRα (*Nr1h3*) mRNA levels were significantly higher in the WD+LJ0.025 group than in the WD group (Fig. 4A) and tended to be higher in the WD+LJ0.05 group than in the WD group. In contrast, the levels of LXRβ (*Nr1h2*) and Rxra mRNAs did not differ among the four groups.

Farnesoid X receptor (Fxr) suppresses *Cyp7a1* transcription by controlling the expression of small heterodimer partner (Shp) (27). Paradoxically, *Fxr* mRNA levels were higher in the WD+LJ0.025 and WD+LJ0.05 groups than in the WD group. In addition, LJ-1888 supplementation altered the expression of genes encoding peroxisome proliferator-activated receptors (*Ppars*) and *Srebf1c* (Fig. 4B). These findings indicate that LJ-1888 may inhibit hypercholesterolemia in *ApoE*$^{-/-}$ mice by altering the expression of genes involved in bile acid biosynthesis.

DISCUSSION

Hypercholesterolemia, or high levels of cholesterol in the blood, enhances the risks of cardiovascular diseases, such as heart attack and stroke, induced by atherosclerosis (5, 6). High levels of circulating cholesterol generate sticky deposits on the walls of arteries, narrowing or blocking blood flow to organs and resulting in heart attack or stroke (22). Lifestyle modifications and various medications can lower blood cholesterol (6, 28). Statins are a class of HMG-CoA reductase inhibitors commonly used to reduce high cholesterol level that are effective in most individuals, reducing total cholesterol concentrations by about 50% on average (29). However, statins cannot be used in certain people, including pregnant women and patients with liver disease, and they also have side effects such as myositis, joint pain, stomach upset, and liver damage (30). Other drugs used to treat high LDL-chol, low HDL-chol, and high TG, alone or in combination with statins, include niacin, bile acid sequestrants, cholesterol absorption inhibitors, and fibric acid derivatives, but these agents also have side effects as well as low efficacy (31, 32). Therefore, it is necessary to identify novel targets and develop drugs in order to treat hypercholesterolemia and atherosclerosis.

Previous studies have shown that adenosine/AR signaling participates in the modulation of lipid availability, including lipolysis and cholesterol efflux (33, 34). Adenosine/A$_1$ AR signaling has been shown to suppress lipolysis on adipocytes through the cyclic adenosine monophosphate (cAMP)-mediated inhibition of lipase activity (35, 36). Activation of A$_{2A}$ AR has also been shown to augment ATP-binding cassette transporter ABCA1 and sterol 27-hydroxylase (CYP27A1) expression, leading to enhancing cholesterol efflux from macrophages (37). However, the role of adenosine/A$_3$ AR signaling in hyperlipidemia and atherosclerosis is unclear. Jones *et al.* has shown that A$_3$ AR deficiency does not affect the formation of atherosclerotic plaque in *ApoE*$^{-/-}$ mice fed high-fat diet (30% fat) for five months, however, the levels of plasma cholesterol

and lipids were not determined in these mice (38). The differences in experimental design would affect any comparison of the results between the findings of Jones *et al.* and the present findings. In addition, LJ-1888 may have poly-pharmacological effects, even though LJ-1888 was developed as a selective A_3 AR antagonist. Recently, Yu *et al.* presented the poly-pharmacological effects of A_3 AR agonist (IB-MECA), which have shown the agonistic role of PPARγ and antagonistic role of PPARδ (39). Therefore, further study is needed to examine whether the role of LJ-1888 as a selective antagonist for A_3 AR is responsible for its anti-atherogenic effects or not.

Atherosclerosis is a chronic inflammatory disease induced by high levels of lipids and reactive oxygen species (3). This condition may be inhibited by targeting molecules involved in inflammatory responses and hypercholesterolemia (40, 41). LJ-1888 treatment was shown to block UUO- and TGF-β1-induced activation of c-Jun N-terminal kinase and extracellular signal- regulated kinase involved in inflammatory responses (20). While we have not explored the role of LJ-1888 in inflammation, the possibility that LJ-1888 shows anti-inflammatory properties in atherosclerosis remains. This study showed that LJ-1888 protected WD-fed $ApoE^{-/-}$ mice against the development of atherosclerosis by ameliorating hypercholesterolemia. Administration of LJ-1888 induced the expression of genes involved in bile acid biosynthesis, which may be related to the amelioration of hypercholesterolemia. Taken together, these findings show that LJ-1888, a selective antagonist for A_3 AR, might be a novel candidate for preventing and/or treating atherosclerosis and hypercholesterolemia.

MATERIALS AND METHODS

Animal experiments
All animal studies were approved by the Institutional Animal Care and Usage Committee of Ewha Womans University (IACUC 2001-01-008). $ApoE^{-/-}$ (B6.KOR/Stm-Apoe) mice were purchased from Central Laboratory Animal Inc and housed under a 12-hour day-night cycle with free access to water and food in a specific pathogen-free system. Six-week-old $ApoE^{-/-}$ mice were randomly divided into four groups. The ND group consisted of five mice fed a normal chow diet supplemented with vehicle; the WD group consisted of 12 mice fed a western diet, consisting of 20% fat and 0.15% cholesterol (D09072603) and purchased from Research Diets Inc (USA), supplemented with vehicle; and the WD+LJ0.025 and WD+LJ0.05 groups consisted of 12 mice each fed a western diet supplemented with 0.025% and 0.05% LJ-1888, respectively.

Statistical analysis
All data shown in figures are presented as mean ± SEM. Statistical significance was determined by Student's t-tests (for in vitro experiments) and Mann-Whitney U tests (for in vivo experiments).

Detailed materials and methods are available in the supplementary file.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Nelson RH (2013) Hyperlipidemia as a risk factor for cardiovascular disease. Prim Care 40, 195-211
2. Jeong SJ, Lee MN and Oh GT (2017) The Role of Macrophage Lipophagy in Reverse Cholesterol Transport. Endocrinol Metab 32, 41-46
3. Weber C and Noels H (2011) Atherosclerosis: current pathogenesis and therapeutic options. Nat Med 17, 1410-1422
4. Hebert PR, Gaziano JM, Chan KS and Hennekens CH (1997) Cholesterol lowering with statin drugs, risk of stroke, and total mortality. An overview of randomized trials. Jama 278, 313-321
5. Pignone M, Phillips C and Mulrow C (2000) Use of lipid lowering drugs for primary prevention of coronary heart disease: meta-analysis of randomised trials. BMJ 321, 983-986
6. Cheung BM and Lam KS (2010) Is intensive LDL-cholesterol lowering beneficial and safe? Lancet 376, 1622-1624
7. Nissen SE, Tuzcu EM, Schoenhagen P et al (2005) Statin therapy, LDL cholesterol, C-reactive protein, and coronary artery disease. N Engl J Med 352, 29-38
8. Davis HR Jr, Compton DS, Hoos L and Tetzloff G (2001) Ezetimibe, a potent cholesterol absorption inhibitor, inhibits the development of atherosclerosis in ApoE knockout mice. Arterioscler Thromb Vasc Biol 21, 2032-2038
9. Smith L, Mosley J, Yates J and Caswell L (2016) The New Face of Hyperlipidemia Management: Proprotein Convertase Subtilisin/Kexin Inhibitors (PCSK-9) and Their Emergent Role As An Alternative To Statin Therapy. J Pharm Pharm Sci : a publication of the Canadian Society for Pharmaceutical Sciences, Societe canadienne des sciences pharmaceutiques 19, 137-146
10. Brousseau ME, Schaefer EJ, Wolfe ML et al (2004) Effects of an inhibitor of cholesteryl ester transfer protein on HDL cholesterol. N Engl J Med 350, 1505-1515
11. White CR, Datta G, Zhang Z et al (2008) HDL therapy for cardiovascular diseases: the road to HDL mimetics. Curr Atheroscler Rep 10, 405-412
12. Writing Group M, Mozaffarian D, Benjamin EJ et al (2016)

Heart Disease and Stroke Statistics-2016 Update: A Report From the American Heart Association. Circulation 133, e38-360

13. Layland J, Carrick D, Lee M, Oldroyd K and Berry C (2014) Adenosine: physiology, pharmacology, and clinical applications. JACC Cardiovasc Interv 7, 581-591

14. Bowser JL, Lee JW, Yuan X and Eltzschig HK (2017) The hypoxia-adenosine link during inflammation. J Appl Physiol 123, 1303-1320

15. Sheth S, Brito R, Mukherjea D, Rybak LP and Ramkumar V (2014) Adenosine receptors: expression, function and regulation.Int J Mol Sci 15, 2024-2052

16. Chen JF, Eltzschig HK and Fredholm BB (2013) Adenosine receptors as drug targets--what are the challenges? Nat Rev Drug Discov 12, 265-286

17. Jacobson KA (1998) Adenosine A3 receptors: novel ligands and paradoxical effects. Trends Pharmacol Sci 19, 184-191

18. Borea PA, Varani K, Vincenzi F et al (2015) The A3 adenosine receptor: history and perspectives. Pharmacol Rev 67, 74-102

19. Jeong LS, Choe SA, Gunaga P et al (2007) Discovery of a new nucleoside template for human A3 adenosine receptor ligands: D-4'-thioadenosine derivatives without 4'-hydroxymethyl group as highly potent and selective antagonists. J Med Chem 50, 3159-3162

20. Lee J, Hwang I, Lee JH, Lee HW, Jeong LS and Ha H (2013) The selective A3AR antagonist LJ-1888 ameliorates UUO-induced tubulointerstitial fibrosis. Am J Pathol 183, 1488-1497

21. Libby P, Ridker PM and Hansson GK (2011) Progress and challenges in translating the biology of atherosclerosis. Nature 473, 317-325

22. Libby P, Schoenbeck U, Mach F, Selwyn AP and Ganz P (1998) Current concepts in cardiovascular pathology: the role of LDL cholesterol in plaque rupture and stabilization. Am J Med 104, 14S-18S

23. Istvan ES and Deisenhofer J (2001) Structural mechanism for statin inhibition of HMG-CoA reductase. Science 292, 1160-1164

24. Hou R and Goldberg AC (2009) Lowering low-density lipoprotein cholesterol: statins, ezetimibe, bile acid sequestrants, and combinations: comparative efficacy and safety. Endocrinol Metab Clin North Am 38, 79-97

25. Lee MR, Lim CJ, Lee YH et al (2014) The adipokine Retnla modulates cholesterol homeostasis in hyperlipidemic mice. Nat Commun 5, 4410

26. Chiang JY (2009) Bile acids: regulation of synthesis. J Lipid Res 50, 1955-1966

27. Chiang JY, Kimmel R, Weinberger C and Stroup D (2000) Farnesoid X receptor responds to bile acids and represses cholesterol 7alpha-hydroxylase gene (CYP7A1) transcription. J Biol Chem 275, 10918-10924

28. Bonovas S, Nikolopoulos G and Sitaras NM (2011) Efficacy and safety of more intensive lowering of LDL cholesterol. Lancet 377, 715; author reply 715-716

29. Tobert JA (2003) Lovastatin and beyond: the history of the HMG-CoA reductase inhibitors. Nat Rev Drug Discov 2, 517-526

30. Pedersen TR and Tobert JA (1996) Benefits and risks of HMG-CoA reductase inhibitors in the prevention of coronary heart disease: a reappraisal. Drug Saf 14, 11-24

31. Dembowski E and Davidson MH (2009) Statin and ezetimibe combination therapy in cardiovascular disease. Curr Opin Endocrinol Diabetes Obes 16, 183-188

32. Michos ED, Sibley CT, Baer JT, Blaha MJ and Blumenthal RS (2012) Niacin and statin combination therapy for atherosclerosis regression and prevention of cardio-vascular disease events: reconciling the AIM-HIGH (Atherothrombosis Intervention in Metabolic Syndrome With Low HDL/High Triglycerides: Impact on Global Health Outcomes) trial with previous surrogate endpoint trials. J Am Coll Cardiol 59, 2058-2064

33. Leiva A, Guzman-Gutierrez E, Contreras-Duarte S et al (2017) Adenosine receptors: Modulators of lipid availability that are controlled by lipid levels. Mol Aspects Med 55, 26-44

34. Reiss AB and Cronstein BN (2012) Regulation of foam cells by adenosine. Arterioscler Thromb Vasc Biol 32, 879-886

35. Ohisalo JJ (1981) Effects of adenosine on lipolysis in human subcutaneous fat cells. J Clin Endocrinol Metab 52, 359-363

36. Heseltine L, Webster JM and Taylor R (1995) Adenosine effects upon insulin action on lipolysis and glucose transport in human adipocytes. Mol Cell Biochem 144, 147-151

37. Bingham TC, Fisher EA, Parathath S, Reiss AB, Chan ES and Cronstein BN (2010) A2A adenosine receptor stimulation decreases foam cell formation by enhancing ABCA1-dependent cholesterol efflux.J Leukoc Biol 87, 683-690

38. Jones MR, Zhao Z, Sullivan CP et al (2004) A(3) adenosine receptor deficiency does not influence atherogenesis. J Cell Biochem 92, 1034-1043

39. Yu J, Ahn S, Kim HJ et al (2017) Polypharmacology of N(6)-(3-Iodobenzyl)adenosine-5'-N-methyluronamide (IB-MECA) and Related A3 Adenosine Receptor Ligands: Peroxisome Proliferator Activated Receptor (PPAR) gamma Partial Agonist and PPARdelta Antagonist Activity Suggests Their Antidiabetic Potential. J Med Chem 60, 7459-7475

40. Park HJ, Kim MK, Kim Y et al (2017) Gastrin-releasing peptide promotes the migration of vascular smooth muscle cells through upregulation of matrix metalloproteinase-2 and -9. BMB Rep 50, 628-633

41. Jung HJ, Im SS, Song DK, Bae JH (2017) ffects of chlorogenic acid on intracellular calcium regulation in lysophosphatidylcholine-treated endothelial cells. BMB Rep 50, 323-328