Scavenger Receptor Class B Type 1 Deletion Led to Coronary Atherosclerosis and Ischemic Heart Disease in Low-density Lipoprotein Receptor Knockout Mice on Modified Western-type Diet

Jiawei Liao¹, Xin Guo¹, Mengyu Wang¹, Chengyan Dong²,³, Mingming Gao¹, Huan Wang¹, Abudurexiti Kayoumu¹, Qiang Shen¹, Yuhui Wang¹, Fan Wang²,³,⁴, Wei Huang¹ and George Liu¹,⁴

¹Institute of Cardiovascular Sciences and Key Laboratory of Molecular Cardiovascular Sciences, Ministry of Education, Peking University Health Science Center, Beijing, China
²Interdisciplinary Laboratory, Institute of Biophysics, Chinese Academy of Sciences, Beijing, China
³Medical Isotopes Research Center, Peking University Health Science Center, Beijing, China
⁴Center for Molecular and Translational Medicine, Peking University Health Science Center, Beijing, China

Aim: Atherosclerosis-prone apolipoprotein E (apoE) or low-density lipoprotein receptor (LDL-R) knockout (KO) mice are generally resistant to developing coronary atherosclerosis (CA) and ischemic heart disease (IHD). However, studies have demonstrated the occurrence of spontaneous CA and IHD in scavenger receptor class B type 1 (SR-BI)/apoE double KO (dKO) mice, which suggests that SR-BI could be a potential target for the prevention and therapy of CA and IHD. This possibility was later investigated in SR-BI/LDL-R dKO mice, but no signs of CA or IHD was identified when mice were fed a normal western-type diet. Here we explored whether SR-BI deletion could result in CA and IHD in LDL-R KO mice when fed a modified western-type diet containing higher (0.5%) cholesterol. Methods: Cardiac functions were detected by electrocardiography, single photon emission computed tomography (SPECT), echocardiography (Echo) and 2,3,5-triphenyltetrazolium chloride staining. CA was visualized by hematoxylin-eosin staining. Results: After 12 weeks on the modified diet, SR-BI/LDL-R dKO mice developed cardiac ischemia/infarction, together with systolic dysfunction and left ventricular dilatation. CA was most severe at the aortic sinus level to an extent that no dKO mice survived to 20 weeks on the modified diet. None of control mice, however, developed CA or IHD. Conclusions: SR-BI deletion led to CA and IHD in LDL-R KO mice when fed the modified western-type diet. We established SR-BI/LDL-R dKO mice as a diet-induced murine model of human IHD and developed detection methods, using a combination of SPECT and Echo, for effective in vivo evaluation of cardiac functions.

Key words: Scavenger receptor class B type 1, Coronary atherosclerosis, Ischemic heart disease

Address for correspondence: Wei Huang, Institute of Cardiovascular Sciences and Key Laboratory of Molecular Cardiovascular Sciences, Ministry of Education, Peking University Health Science Center, Beijing, China
E-mail: huangwei@bjmu.edu.cn
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have been the most widely used animal models for studying atherosclerotic diseases. However, atherosclerosis in mice was mainly distributed in the aorta, whereas coronary atherosclerosis (CA) and IHD were rather rare\(^6\).\(^7\). Scavenger receptor class B type 1 (SR-BI) is a cell-surface high-density lipoprotein (HDL) receptor that is associated with reverse cholesterol transport (RCT) and plays a vital role in the systemic homeostasis of cholesterol. The deletion of SR-BI in mice disrupts the process of RCT and leads to HDL abnormalities, including the accumulation of free cholesterol, enlargement of HDL particles, and enrichment or loss of bio-active proteins carried on the surface of HDL particles\(^6\).\(^9\). Hence, the deletion of SR-BI leads to accelerated atherosclerosis\(^10\). In addition, even the loss of SR-BI resulted in spontaneous obstructive CA and myocardial infarction in apoE KO mice\(^11\), suggesting that SR-BI could be a potential target for the prevention and therapy of CA and IHD. However, in another study, atherosclerotic LDL-R KO mice with SR-BI deletion failed to develop CA and IHD when fed a western-type diet\(^12\). As such, whether the modulation of susceptibility to the development of CA and IHD by SR-BI is apoE-dependent or -independent remains unknown. Here we explored if SR-BI deficiency could lead to CA and IHD in LDL-R KO mice when fed a modified western-type diet with higher cholesterol content than that used in normal western-type diet.

**Methods**

**Animals and Diet**

SR-BI KO and LDL-R KO mice were supplied by Peking University Experimental Animal Center and crossbred to generate SR-BI/LDL-R dKO mice. Modified western-type diet (see **Supplemental Table 1**) containing 0.5% cholesterol (AMRESCO, USA) and 20% fat were fed to 10- to 12-week-old SR-BI/LDL-R dKO mice and age-matched control LDL-R KO mice. All mice included in the experiment were females. The housing, care, and all the experimental procedures were conducted following the regulations of the National Institute of Health and approved by the Animal Care Committee at Peking University.

**Plasma Lipids Analysis**

Blood samples were collected by retro-orbital venous plexus puncture after mice were fasted for 4 h. Plasma total cholesterol (TC) and triglycerides (TG) were measured using commercial kits (BioSino, China). Plasma HDL-cholesterol (HDL-C) was measured with the same kit for TC assay after plasma samples precipitated with 20% polyethylene glycol solution to remove apoB-containing lipoproteins. For the analysis of lipoprotein distribution, pooled plasma samples from 4–5 mice of the same group were fractioned by fast protein liquid chromatography (FPLC) as previously reported\(^13\).

**Electrocardiography (ECG), Single Photon Emission Computed Tomography (SPECT), and Echocardiography (Echo) Analysis**

ECG, SPECT, and Echo were obtained under anesthetization with 1.5% isoflurane inhalation. ECGs were obtained using four needle electrodes in each of the limbs with a multiple physiological signal recording system (Chengdu Instrument Factory, China). SPECT images were acquired at 40 min after the intravenous administration of 37.0 MBq (1.0 mCi) of \(^{99m}\)Tc-MIBI with nanoScan (Mediso, Hungary). Echo images were obtained with a high-resolution Vevo 770 imaging system (VisualSonics Inc., Canada). Left ventricle (LV) dimensions and wall thicknesses were determined using parasternal short axis M-mode images at the level of the papillary muscle and averaged from three cardiac cycles. Ejection fraction (EF), fractional shortening (FS), and LV volume were calculated using the Vevo770 software.

**Myocardial 2,3,5-triphenyltetrazolium Chloride (TTC) Staining**

Mice were sacrificed and their hearts were quickly removed, stored at \(-20^\circ\)C for 30 min and sliced into 5–6 slices (2 mm/slice) from the apex towards the auricle. The slices were then incubated in 1% TTC (Sigma, USA) at 37\(^\circ\)C for 15 min. Images were taken by a digital camera (Sony, Japan).

**CA Analysis**

Mice were sacrificed and flushed with 20 ml 0.01 M phosphate buffer solution through the LV. The hearts were harvested, fixed in 4% paraformaldehyde solution for 4 h and stored in 20% sucrose solution overnight. After these preparations, the hearts were embedded in paraffin and cross-sectioned (5 µm/slice) from the level of the aortic sinus to the papillary muscle at an interval of 300 µm. For each heart, six levels were obtained and analyzed individually. Atherosclerotic plaques were visualized by hematoxylin–eosin (HE) staining. The severity of CA was divided into four degrees defined as: none (<5% stenosis), <50% stenosis (5%–50% stenosis), >50% stenosis (50%–95% stenosis), and occlusion (>95% stenosis). Data were presented as the percentage of the number of coronary arteries with the same degree of CA severity to the total number of coronary arteries.
Myocardial Histological Analysis

Once SR-BI/LDL-R dKO mice on the modified western-type diet died, their hearts were collected, fixed in 4% paraformaldehyde solution for 6 h, and stored in 20% sucrose solution overnight. The hearts were then embedded in paraffin and cross-sectioned as described above. Sirius red staining was applied to visualize potential fibrotic scars in the myocardium. Control hearts from LDL-R KO mice on the modified western-type diet for 20 weeks were prepared in the same way.

Statistical Analysis

Data were presented as mean ± standard error of the mean (SEM). Statistical significance was evaluated by Student’s t-test, and a P value < 0.05 was regarded as significant.

Results

Premature Death in SR-BI/LDL-R dKO Mice on the Modified Western-type Diet

After 10 weeks on the modified western-type diet, dKO mice began to die suddenly. In fact, no dKO mice survived to week 20 on the modified western-type diet, while no deaths were recorded in the age-matched LDL-R KO controls (Fig. 1). The median time of death for dKO mice on the modified western-type diet was 98 days. Autopsies conducted on the dKO mice that died prematurely showed that their hearts were significantly enlarged (Fig. 2A) and the mass of their hearts was almost twice that of their LDL-R KO controls that were sacrificed after 20 weeks on the modified western-type diet (Fig. 2B-C). Myocardial Sirius red staining of the dKO mice that died prematurely revealed the presence of massive fibrotic scars (Fig. 2D-G). Therefore, our data suggest that dKO mice that were fed the modified western-type diet may have suffered from myocardial infarction and heart failure.

Cardiac Ischemia and Infarction in SR-BI/LDL-R dKO Mice on the Modified Western-type Diet

We used ECG, SPECT, and myocardial TTC staining to evaluate the cardiac blood supply in mice that were fed the modified western-type diet. Before the modified western-type diet was served, the ECG of the dKO mice showed no significant abnormalities in the ST segment. After 12 weeks on the modified western-type diet, approximately 50% (five in 11 mice) of the surviving dKO mice showed ST segment elevation or depression in their ECGs (Fig. 3A). ST segment changes in the ECG indicated that these dKO mice may have suffered cardiac ischemia/infarction, which was later confirmed by both SPECT scanning (Fig. 3B-C) and myocardial TTC staining (Fig. 3D). In contrast, no signs of cardiac ischemia/infarction, shown either in ECG and SPECT or by TTC staining, were identified in LDL-R KO controls (Fig. 3).

Heart Dysfunction in SR-BI/LDL-R dKO Mice on the Modified Western-type Diet

Before feeding the mice with the modified western-type diet, no significant differences of the main cardiac parameters measured by Echo were identified between dKO and LDL-R KO mice (Fig. 4 and Table 1). After 12 weeks on the modified western-type diet, the heart rates of dKO mice increased significantly when compared with those of their LDL-R KO controls (Table 1). In addition, EF and FS declined while end systolic LV internal diameter and volume increased significantly in dKO mice, indicating systolic dysfunction and LV dilation (Fig. 4 and Table 1). Data collected from Echo demonstrated that the modified western-type diet induced heart dysfunction in dKO mice.

CA in SR-BI/LDL-R dKO Mice on the Modified Western-type Diet

In dKO mice fed the modified western-type diet for 12 weeks, atherosclerotic lesions could be found in more than 50% of the coronary arteries at the aortic sinus level. Of the lesional coronary arteries, approximately 21.7% were totally occluded, 17.4% were more than half-occluded, and only 13.0% were less than half-occluded (Fig. 5B and 5D-E). At the papillary muscle level, however, CA was significantly reduced as compared with that at the aortic sinus level, with only 5.3% of the coronary arteries being more than half occluded and 7.0% being less than half occluded.
the papillary muscle level (Fig. 5F).

**Plasma Lipids of SR-BI/LDL-R dKO Mice on the Modified Western-type Diet**

Before being put on the modified western-type diet, SR-BI/LDL-R dKO mice displayed twice the
Fig. 3. ECG, SPECT, and myocardial TTC staining in mice before and after the modified western-type diet (mWD) feeding
A: Representative ECG images obtained from LDL-R KO and dKO mice before and 12 weeks after mWD feeding. The arrow indicated ST segment elevation/depression. B–C: Representative myocardial SPECT images obtained from LDL-R KO (B) and dKO mice (C) on the mWD for 12 weeks. The triangle indicated myocardium with low blood perfusion. E: Representative images of myocardial TTC staining obtained from LDL-R KO (D) and dKO mice (E) on the mWD for 12 weeks. Normal myocardium was stained red while infarcted myocardium white.
In LDL-R KO mice, there was only a two-fold increase of plasma TC in dKO mice (from basal 42.7 ± 23.1 mg/dl to 89.5 ± 57.5 mg/dl). These increases led to similar plasma TC levels between LDL-R KO and dKO mice on the modified western-type diet (Fig. 6A). FPLC showed that the increase of plasma TC was mainly distributed in very low-density lipoprotein (VLDL) fractions in both mice on the modified western-type diet (Fig. 6E). As for plasma HDL-C, there was a significant decrease (from 188 ± 14.4 mg/dl to 106 ± 8.34 mg/dl) in dKO mice, in contrast to an increase (from basal 87.0 ± 2.90 mg/dl to 152 ± 9.39 mg/dl) in the LDL-R KO mice (Fig. 6C). In addition to plasma TC, the modified western-type level of plasma TC (427 ± 23.1 mg/dl in dKO mice vs. 222 ± 9.58 mg/dl in LDL-R KO mice) and HDL-C (188 ± 14.4 mg/dl in dKO mice vs. 87.0 ± 2.90 mg/dl in LDL-R KO mice), compared with control LDL-R KO mice (Fig. 6A and 6C). FPLC showed that plasma cholesterol was mainly distributed in LDLs and HDLs in both dKO and LDL-R KO mice (Fig. 6D). No difference in plasma TG (129 ± 6.88 mg/dl in dKO mice vs. 126 ± 5.38 mg/dl in LDL-R KO mice) was observed between the two groups (Fig. 6B). After 12 weeks on the modified western-type diet, plasma TC in both LDL-R KO and dKO mice increased significantly. However, compared with an estimated four-fold increase of plasma TC (from basal 222 ± 9.58 mg/dl to 817 ± 43.3 mg/dl) in LDL-R KO mice, there was only a two-fold increase of plasma TC in dKO mice (from basal 427 ± 23.1 mg/dl to 895 ± 57.5 mg/dl). These increases led to similar plasma TC levels between LDL-R KO and dKO mice on the modified western-type diet (Fig. 6A). FPLC showed that the increase of plasma TC was mainly distributed in very low-density lipoprotein (VLDL) fractions in both mice on the modified western-type diet (Fig. 6E). As for plasma HDL-C, there was a significant decrease (from 188 ± 14.4 mg/dl to 106 ± 8.34 mg/dl) in dKO mice, in contrast to an increase (from basal 87.0 ± 2.90 mg/dl to 152 ± 9.39 mg/dl) in the LDL-R KO mice (Fig. 6C). In addition to plasma TC, the modified western-type

**Table 1.** Echocardiographic analysis of mice before and after the modified western-type diet (mWD) feeding

|                  | pre mWD | post mWD |
|------------------|---------|----------|
|                  | LDL-R KO | dKO | LDL-R KO | dKO |
| HR (bpm)         | 398 ± 27.4 | 400 ± 33.3 | 415 ± 7.04 | 457 ± 11.8, * |
| LVID;d (mm)      | 4.16 ± 0.11 | 4.11 ± 0.07 | 4.09 ± 0.13 | 4.22 ± 0.09 |
| LVID;s (mm)      | 2.61 ± 0.07 | 2.57 ± 0.05 | 3.03 ± 0.12 | 3.44 ± 0.13, * |
| LV Vol;d (µL)    | 76.9 ± 4.82 | 74.7 ± 3.01 | 74.5 ± 5.38 | 79.7 ± 3.88 |
| LV Vol;s (µL)    | 24.9 ± 1.64 | 23.9 ± 1.12 | 36.3 ± 3.20 | 49.3 ± 4.47, * |
| EF (%)           | 67.5 ± 1.57 | 67.8 ± 1.74 | 51.2 ± 3.00 | 38.7 ± 2.99, * |
| FS (%)           | 37.2 ± 1.23 | 37.5 ± 1.38 | 26.0 ± 1.84 | 18.7 ± 1.61, * |

Data were presented as mean ± SEM.
mWD: modified western-type diet; HR: heart rate; LV: left ventricle; ID: internal diameter; d: diastole; s: systole; Vol: volume; EF: ejection fraction; FS: fractional shortening

* P < 0.05 vs. LDL-R KO mice. n = 4–6 per group

Fig. 4. Representative M-mode short axis views of the LV in mice before and after the modified western-type diet (mWD) feeding
Fig. 5. Analysis of CA in mice on the modified western-type diet (mWD) for 12 weeks

A–D: HE staining of coronary arteries at the aortic sinus level. The boxes in A and B indicated coronary arteries. C and D: Magnified views of the box areas in A and B, respectively. E: Quantitative analysis of CA at the aortic sinus level in mice on the mWD for 12 weeks. F: Quantitative analysis of CA at the papillary muscle level in mice on the mWD for 12 weeks. $n=7$ per group
diet also led to a significant increase of plasma TG in LDL-R KO mice (from 126 ± 5.38 mg/dl to 259 ± 26.6 mg/dl), whereas it induced an unexpected decrease of plasma TG in dKO mice (from 129 ± 6.88 mg/dl to 74.1 ± 6.02 mg/dl) (Fig. 6B).

**Discussion**

In this study, we increased the cholesterol content in the western-type diet from standard 0.15% to moderate 0.5%. A higher plasma TC level (700 – 1000 mg/dl) was induced by the modified western-type diet than by the standard western-type diet (500 – 750 mg/dl), and the modified western-type diet aggravated plaque development in LDL-R KO mice. When SR-BI/LDL-R dKO mice were fed this modified western-type diet, they developed CA and cardiac ischemia/infarction as indicated by ECG, SPECT, and myocardial TTC/Sirius red staining, along with heart dysfunction as indicated by Echo, suggesting the successful induction of diet-induced CA and IHD. While we were preparing this manuscript, Fuller et al also reported diet-induced CA and myocardial infarction in SR-BI/LDL-R dKO mice that were fed various atherogenic diets with high cholesterol, including Paigen diet (1.25% cholesterol) with or without sodium cholate and pure high-cholesterol diet (2% cholesterol) 14. Altogether, our data demonstrated that SR-BI deficiency could lead to CA and IHD in LDL-R KO mice when challenged with the appropriate atherogenic diet. However, the mechanism by which SR-BI modulates the susceptibility of CA development in mice is still unknown. We found that the plaque composition in dKO mice was quite different from that in control LDL-R KO mice, for example, abnormal collagen accumulated in the atherosclerotic plaques, including coronary atherosclerotic plaques, in dKO mice (see **Supplemental Fig. 1**). It remains undefined whether SR-BI could interact with collagen metabolism and thus contribute to CA development.
In this study, we observed that in SR-BI/LDL-R dKO mice, CA at the aortic sinus level was continuous to the lesion of the aortic sinus. To explore whether it was an extension of the aortic sinus lesions, we adopted a serial-section protocol from the aortic sinus level down to the papillary muscle level at an interval of 300 µm to characterize the severity and spatial distribution of CA. We found that in the dKO mice fed the modified western-type diet for 12 weeks, CA was mainly at the aortic sinus level or in the common coronary artery as suggested by Weicheng et al. However, only atherosclerotic SR-BI deficient mice such as SR-BI/apoE dKO mice and SR-BI/LDL-R dKO mice developed CA and IHD, suggesting that these mice could be used as valuable tools to explore the relationship between HDL dysfunction and cardiovascular diseases and also to screen for potential therapeutic targets and drugs to improve HDL function. While CA and IHD in SR-BI/apoE dKO mice fed a chow diet and SR-BI/LDL-R dKO mice fed high cholesterol diets progressed too rapidly to allow heart remodeling, SR-BI/LDL-R dKO mice fed the modified western-type diet with moderate cholesterol could survive with heart dysfunction. Thus, SR-BI/LDL-R dKO mice on the modified western-type diet could be used as a preferred murine model for studies on chronic cardiac ischemia/infarction and the progression of heart dysfunction to failure.

As stated in the introduction, the establishment of appropriate disease models serves the urgent needs to explore not only pathologic mechanisms of the disease onset and progression but also potential therapeutic solutions. For the latter, the development of highly effective evaluating systems is no less important than the establishment of the disease models itself. To date, several murine models of human IHD, including apoE/LDL-R dKO mice, SR-BI/apoE dKO mice, SR-BI/LDL-R dKO mice, SR-BI KO/apoE<sup>mut/−</sup> mouse, PDZK1/apoE dKO mice, eNOS/apoE dKO mice, SR-uPA<sup>mut</sup>/apoE KO mice, Akt1/apoE dKO mice, and Fbn1<sup>mut/−</sup>/apoE KO mice, have been established. Of these models, cardiac ischemia/infarction was mainly evaluated by histological staining such as myocardial TTC staining and Masson staining. No sensitive and specific in vivo diagnostic approach has been developed in these models. Although SPECT has been widely used in clinical practice, in this study, we are the first to apply in vivo SPECT scanning to detect cardiac ischemia in a murine model of human IHD. Compared with coronary angiography, which cannot achieve a resolution high enough to obtain clear images in a fast-beating living mouse heart, SPECT is sensitive to not only the diagnosis of cardiac ischemia but also the in vivo evaluation of cardiac reperfusion. The combined use of SPECT and Echo, as demonstrated in this study, could provide not only in vivo evidence of the disease progression but also the effects of drug interventions on cardiac reperfusion and function in murine models of human IHD.

**Conclusion**

We demonstrated that SR-BI deletion led to CA and IHD in LDL-R KO mice that were fed the modified western-type diet. We have not only established SR-BI/LDL-R dKO mice as a diet-induced murine model of human IHD but have also provided an option of an effective in vivo evaluating system by the combined use of SPECT and Echo for cardio-protective drug discovery in established murine models of IHD.
human IHD.

**Abbreviations**

ABCA1: ATP-binding cassette transporter 1; apo: apolipoprotein; CA: coronary atherosclerosis; dKO: double knockout; ECG: electrocardiography; Echo: echocardiography; EF: ejection fraction; FPLC: fast protein liquid chromatography; FS: fractional shortening; HDL: high-density lipoprotein; HDL-C: high-density lipoprotein cholesterol; IHD: ischemic heart disease; KO: knockout; LCAT: lecithin cholesterol acyl transferase; LDL-C: low-density lipoprotein cholesterol; LDL-R: low-density lipoprotein receptor; LV: left ventricular; mWD: modified western-type diet; RCT: reverse cholesterol transport; SPECT: single photon emission computed tomography; SR-BI: scavenger receptor class B type 1; TC: total cholesterol; TTC: 2,3,5-triphenyltetrazolium chloride; VLDL: very low-density lipoprotein

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**Conflict of Interest**

None declared.

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Supplemental Table 1. Ingredients of the modified western-type diet.

|                          | Modified western-type diet | Murine chow diet                  |
|--------------------------|----------------------------|-----------------------------------|
| Cholesterol (%)          | 0.5                        |                                   |
| Lard (%)                 | 20                         |                                   |
| Powdered murine chow (%) | 79.5                       |                                   |
| Raw protein (g/100 g)    | 23.8                       |                                   |
| Raw fat (g/100 g)        | 5.4                        |                                   |
| Raw fiber (g/100 g)      | 3.5                        |                                   |
| Raw ash (g/100 g)        | 6.8                        |                                   |
| Moisture (%)             | 9.0                        |                                   |
| Calcium (%)              | 1.24                       |                                   |
| Phosphorus (%)           | 1.08                       |                                   |
Supplemental Fig. 1. Sirius red staining of the atherosclerotic plaques at the aortic sinus level
A–B: Representative images obtained from LDL-R KO (A) and dKO mice (B) that were fed the modified western-type diet (mWD) for 12 weeks. C–D: Magnified views of the black box areas in A and B, respectively, showing collagen in the atherosclerotic plaques of the aortic sinus. E–F: Magnified views of the blue box areas in A and B, respectively, showing collagen in the coronary atherosclerotic plaques. The arrow indicated an obstructive coronary artery. Collagen was stained red.
Supplemental Fig. 2. Masson-trichrome staining of the coronary atherosclerotic plaques at non-aortic sinus level.

A: A representative image obtained from dKO mice that died prematurely from the modified western-type diet (mWD) feeding. B: Magnified views of the box areas in A. The arrow indicated an obstructive coronary artery. Normal myocardium was stained red while fibrotic tissues stained blue.