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Group 1B Phospholipase A2 Inactivation Suppresses Atherosclerosis and Metabolic Diseases in LDL Receptor-deficient Mice

Norris I. Hollie, **Eddy S. Konaniah**, **Colleen Goodin**, and **David Y. Hui**
Department of Pathology and Laboratory Medicine, University of Cincinnati College of Medicine, Cincinnati, Ohio 45237. USA

Abstract

Objective—Previous studies have shown that inactivation of the group 1B phospholipase A_2 (Pla2g1b) suppresses diet-induced obesity, hyperglycemia, insulin resistance, and hyperlipidemia in C57BL/6 mice. A possible influence of Pla2g1b inactivation on atherosclerosis has not been addressed previously. The current study utilized LDL receptor-deficient ($Ldlr^{-/-}$) mice with plasma lipid levels and distribution similar to hyperlipidemic human subjects as a preclinical animal model to test the effectiveness of Pla2g1b inactivation on atherosclerosis.

Methods and Results—The $Pla2g1b^{+/+}Ldlr^{-/-}$ and $Pla2g1b^{-/-}Ldlr^{-/-}$ mice were fed a low fat chow diet or a hypercaloric diet with 58.5 kcal% fat and 25 kcal% sucrose for 10 weeks. Minimal differences were observed between $Pla2g1b^{+/+}Ldlr^{-/-}$ and $Pla2g1b^{-/-}Ldlr^{-/-}$ mice when the animals were maintained on the low fat chow diet. However, when the animals were maintained on the hypercaloric diet, the $Pla2g1^{+/+}Ldlr^{-/-}$ mice showed the expected body weight gain but the $Pla2g1b^{-/-}Ldlr^{-/-}$ mice were resistant to diet-induced body weight gain. The $Pla2g1b^{-/-}Ldlr^{-/-}$ mice also displayed lower fasting glucose, insulin, and plasma lipid levels compared to the $Pla2g1b^{+/+}Ldlr^{-/-}$ mice, which displayed robust hyperglycemia, hyperinsulinemia, and hyperlipidemia in response to the hypercaloric diet. Importantly, atherosclerotic lesions in the aortic roots were also reduced 7-fold in the $Pla2g1b^{-/-}Ldlr^{-/-}$ mice.

Conclusion—The effectiveness of Pla2g1b inactivation to suppress diet-induced body weight gain and reduces diabetes and atherosclerosis in LDL receptor-deficient mice suggest that pharmacological inhibition of Pla2g1b may be a viable strategy to decrease diet-induced obesity and the risk of diabetes and atherosclerosis in humans.

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Corresponding Author: David Y. Hui, PhD, Department of Pathology and Laboratory Medicine, Metabolic Diseases Institute, University of Cincinnati College of Medicine, 2120 E. Galbraith Road, Cincinnati, Ohio 45237-0507. Tel: 1-513-558-9152; Fax: 1-513-558-1312; huidy@ucmail.uc.edu.

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Conflict of interest

No conflicts of interest to disclose.

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Keywords

Phospholipase; Atherosclerosis; Glucose tolerance; Lipid and lipoprotein metabolism

1. Introduction

According to the most recent data collected by the World Health Organization, ischemic heart disease and stroke remain the leading cause of death worldwide. The increasing prevalence of obesity and diabetes is also rapidly becoming a major health threat. The risk of these cardiometabolic diseases is dependent on both genetic and lifestyle factors, including the increasing consumption of meals rich in fat and carbohydrates. Reducing consumption of these caloric-rich nutrients or blocking their absorption and endogenous synthesis with drugs such as orlistat, ezetimibe and statins are effective strategies to lower the risk of these cardiometabolic diseases [1]. The development of orlistat and ezetimibe is based on the discovery that lipolysis of the ingested fat by pancreatic lipases and the intestinal cholesterol transporter NPC1L1 are required for optimal fat and cholesterol absorption, respectively [2, 3].

Genome-wide association studies have identified *PLA2G1B* genetic polymorphism as another risk factor for central obesity in humans [4]. This gene encodes group 1B phospholipase A_2 (Pla2g1b), which is predominantly expressed in pancreatic acinar cells with trace amounts of its mRNA found in lung tissues [5]. Unlike other phospholipase A_s enzymes, Pla2g1b is not found in plasma circulation except under conditions of acute pancreatitis [6, 7]. The Pla2g1b protein synthesized in pancreatic acinar cells is stored in zymogen granules and secreted into the intestinal lumen in response to fatty meal to catalyze phospholipid digestion and lipid nutrient uptake [8]. Interestingly, inactivation of the *Pla2g1b* gene only has a minor influence on the total amount of lipids absorbed from a single meal, with only tracer amounts of ingested radiolabeled triglyceride found in the feces of $Pla2g1b^{-/-}$ mice [8, 9]. In fact, no significant difference in total fat absorption was observed between $Pla2g1b^{+/+}$ and $Pla2g1b^{-/-}$ mice measured over a 3-day period [10]. Nevertheless, the absorption of dietary and biliary phospholipids as lysophospholipids was significantly reduced in $Pla2g1b^{-/-}$ mice [11]. Our previous studies showed that *Pla2g1b* gene inactivation or pharmacological inhibition of Pla2g1b activity in wild type mice protect against diet-induced obesity and hyperglycemia [9], whereas over-expression of Pla2g1b exacerbates diet-induced obesity and insulin resistance [12]. Inactivation of the *Pla2g1b* gene also suppresses VLDL synthesis and reduces diet-induced hyperlipidemia [13], suggesting that reducing Pla2g1b activity may be a viable option to reduce atherosclerosis. This study was undertaken to test this hypothesis.

2. Methods

The $Pla2g1b^{-/-}$ mice in a congenic C57BL/6 background [11, 13] were mated with $Ldlr^{-/-}$ mice on the same background (Jackson laboratories) to obtain $Pla2g1b^{+/-}Ldlr^{+/-}$ offspring, which were then backcrossed and mated to obtain $Pla2g1b^{-/-}Ldlr^{-/-}$ mice for comparison with $Pla2g1b^{+/+}Ldlr^{-/-}$ mice. Age-matched male mice on congenic C57BL/6 background

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were used for all experiments. Mice were maintained in a specific pathogen-free environment on a 12 hr light/dark cycle and fed either a 5% fat (3.75 kcal/g) standard rodent chow diet (LM485, Harlan Teklad, Madison, WI) or a high caloric diet with 58.5 kcal% fat and 25 kcal% sucrose (D12331, Harlan Teklad) beginning at 10 to 12 weeks of age. Blood samples were collected from fasting mice into EDTA containing tubes. Fasting blood glucose levels were determined with an AccuChek Active Glucometer (Roche Applied Science). Plasma was isolated by centrifugation. Triglyceride and cholesterol levels were measured using colorimetric assay kits (ThermoFisher Scientific). Lipid distribution among various lipoproteins was determined by fast performance liquid chromatography (FPLC) gel filtration on two Superose 6 columns [13]. Plasma insulin levels were measured using an Ultra Sensitive Rat Insulin ELISA kit (Crystal Chem, Chicago). Insulin resistance were estimated by homeostasis model assessment index calculated from fasting glucose and insulin levels [12].

Atherosclerosis lesions were analyzed after 10 weeks of feeding the hypercaloric diets as described previously [14]. Briefly, mice were anesthetized with isofluorane inhalation and the heart and aorta were perfusion fixed with 4% neutral paraformaldehyde solution. Cryosections of 5-μm thickness through the aortic root were prepared for staining with Oil Red O to measure neutral lipid accumulation and counter-stained with hematoxylin. Mean lesion area and total valve area were measured from digitalized images obtained from 5 sections per mouse. Atherosclerotic lesions were analyzed as ratios of lesion area to total valve area from 9 mice in each group. All procedures and animal care were reviewed and approved by the University of Cincinnati Institutional Animal Care and Use Committee.

Statistical analysis was performed with SigmaPlot Version 11. Values were expressed as mean ± SD. Multiple comparisons were tested by Student's t test or ANOVA, with Student Newman-Keuls post-hoc analysis. A difference of $P<0.05$ was considered statistically significant.

3. Results

Previous studies have shown that Pla2g1b inactivation suppressed diet-induced obesity, hyperglycemia, and hyperlipidemia in wild type C57BL/6 mice [9, 11, 13]. The current study showed that *Pla2g1b* gene inactivation had minimal effect on plasma lipid levels in chow-fed LDL receptor-deficient mice but both plasma triglyceride and cholesterol levels were significantly lower in $Pla2g1b^{-/-}Ldlr^{-/-}$ mice compared to $Pla2g1b^{+/+}Ldlr^{-/-}$ mice when the animals were fed a hypercaloric diet (Fig. 1). Lipoprotein profile analysis by FPLC revealed the reduced plasma lipid levels in $Pla2g1b^{-/-}Ldlr^{-/-}$ mice were due to reduction of both VLDL and LDL (Fig. 1).

Fasting glucose levels were statistically lower in chow-fed $Pla2g1b^{-/-}Ldlr^{-/-}$ mice compared to $Pla2g1b^{+/+}Ldlr^{-/-}$ mice but their fasting insulin levels were similar. Homeostasis model assessment of insulin resistance (HOMA-IR) revealed no difference in insulin resistance between the two groups of animals under chow-fed conditions (Fig. 2). In contrast, robust elevation of fasting plasma glucose and insulin levels, leading to ~8-fold increase in HOMA-IR was observed in $Pla2g1b^{+/+}Ldlr^{-/-}$ mice when the animals were fed

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a hypercaloric diet for 10 weeks. Inactivation of the *Pla2g1b* gene protected against the hypercaloric diet-induced hyperglycemia, hyperinsulinemia, and insulin resistance in *Ldlr*$^{-/-}$ mice (Fig. 2). Additionally, consistent with previous results observed in wild type C57BL/6 mice [9], *Pla2g1b* inactivation also suppressed diet-induced body weight gain in hypercaloric diet-fed *Ldlr* knockout mice (Fig. 2D).

The influence of Pla2g1b on atherosclerosis was assessed by examining the aortic roots of *Pla2g1b*$^{+/+}$*Ldlr*$^{-/-}$ and *Pla2g1b*$^{-/-}$*Ldlr*$^{-/-}$ mice after 10 weeks of feeding the hypercaloric diet. The data revealed an approximate 7-fold reduction in atherosclerotic lesion area in mice without Pla2g1b expression ($3.72 \pm 0.5\%$ vs. $0.525 \pm 0.16\%$ of valve area, $P < 0.001$) (Fig. 3). These data documented that inactivation of Pla2g1b is effective in suppression of diet-induced atherosclerosis.

4. Discussion

Previous studies have shown that genetic inactivation or pharmacologic inhibition of Pla2g1b is effective in suppressing high fat diet-induced obesity, hyperglycemia, insulin resistance, and hyperlipidemia in wild type C57BL/6 mice [9, 11, 13, 15]. The underlying mechanism is related to the reduced absorption of lysophospholipids and the consequential effects of the absorbed lysophospholipids on hepatic fatty acid oxidation and mitochondrial activity [10, 11, 16]. Results of the current study showed that Pla2g1b inactivation is also effective in reducing diet-induced obesity, hyperglycemia, insulin resistance and hyperlipidemia in an animal model with plasma lipid distribution similar to that in human subjects, i.e., the hypercaloric diet-fed *Ldlr*$^{-/-}$ mice. Importantly, the current study also documented that Pla2g1b inactivation also suppressed atherosclerosis in LDL receptor-deficient mice fed the hypercaloric diet. Since Pla2g1b is present only in the digestive tract [5], where it generates and transport lysophospholipids to the liver resulting in reduced fatty acid oxidation and increased VLDL synthesis [10, 13], the reduced atherosclerosis observed in *Pla2g1b*$^{-/-}$*Ldlr*$^{-/-}$ mice is likely mediated through reduced VLDL production and the consequential benefit of reduced plasma lipid levels on atherosclerosis. Taken together, our data obtained from studies in LDL receptordeficient mice demonstrated proof of principle in a preclinical animal model of human hyperlipidemia and atherosclerosis.

The clinical implication of our observations is that Pla2g1b inactivation may be a viable strategy to reduce atherosclerosis and metabolic diseases associated with chronic consumption of hypercaloric diets. Currently, the most effective strategy to reduce hyperlipidemia and atherosclerosis is the use of statins to inhibit endogenous cholesterol biosynthesis and to promote plasma LDL catabolism through LDL receptor induction. However, a recent survey estimated that 1/200 of the general population is heterozygous for familial hypercholesterolemia due to LDL receptor mutation, and a large proportion of these individuals failed to achieve recommended cholesterol levels upon statin therapy [17]. The advent of ezetimibe to block intestinal cholesterol absorption has provided an alternative strategy to reduce plasma cholesterol levels and decrease atherosclerosis risk [18]. The current study showing the effectiveness of Pla2g1b inactivation to reduce plasma cholesterol levels and suppress atherosclerosis in animals with LDL receptor deficiency suggests another novel strategy through Pla2g1b inhibition to enhance hepatic fatty acid oxidation for

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treatment of atherosclerosis. Since cholesterol absorption is not impaired in *Pla2g1b*$^{-/-}$ mice [8], this strategy is independent of cholesterol absorption efficiency and may be used in combination with ezetimibe for treatment of a large population of individuals that are resistant to statin therapy. Furthermore, in normal hyperlipidemic and diabetic individuals, inhibition of Pla2g1b may also complement statin and metformin therapies, respectively. Since the predominant mechanism by which Pla2g1b promotes hyperlipidemia and cardiometabolic diseases is through the production and absorption of lysophospholipids in the intestinal lumen [10, 11, 13, 15], and the advantage of minimal side effects of non-systemic drugs that act primarily in the gastrointestinal tract [19], non-absorbable Pla2g1b inhibitors should be considered for development as novel therapeutics for the treatment of hyperlipidemia and cardiometabolic diseases.

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Non-standard Abbreviations

Pla2g1b	Group 1B phospholipase A_2
FPLC	fast performance liquid chromatrography
VLDL	Very low density lipoproteins
LDL	Low density lipoproteins
LDLR	LDL receptor

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Highlights

- Inactivation of the group 1b phospholipase A_2 ameliorates diet-induced atherosclerosis.
- Group 1b phospholipase A_2 inactivation improves insulin sensitivity.
- Group 1b phospholipase A_2 inactivation suppresses diet-induced body weight gain.

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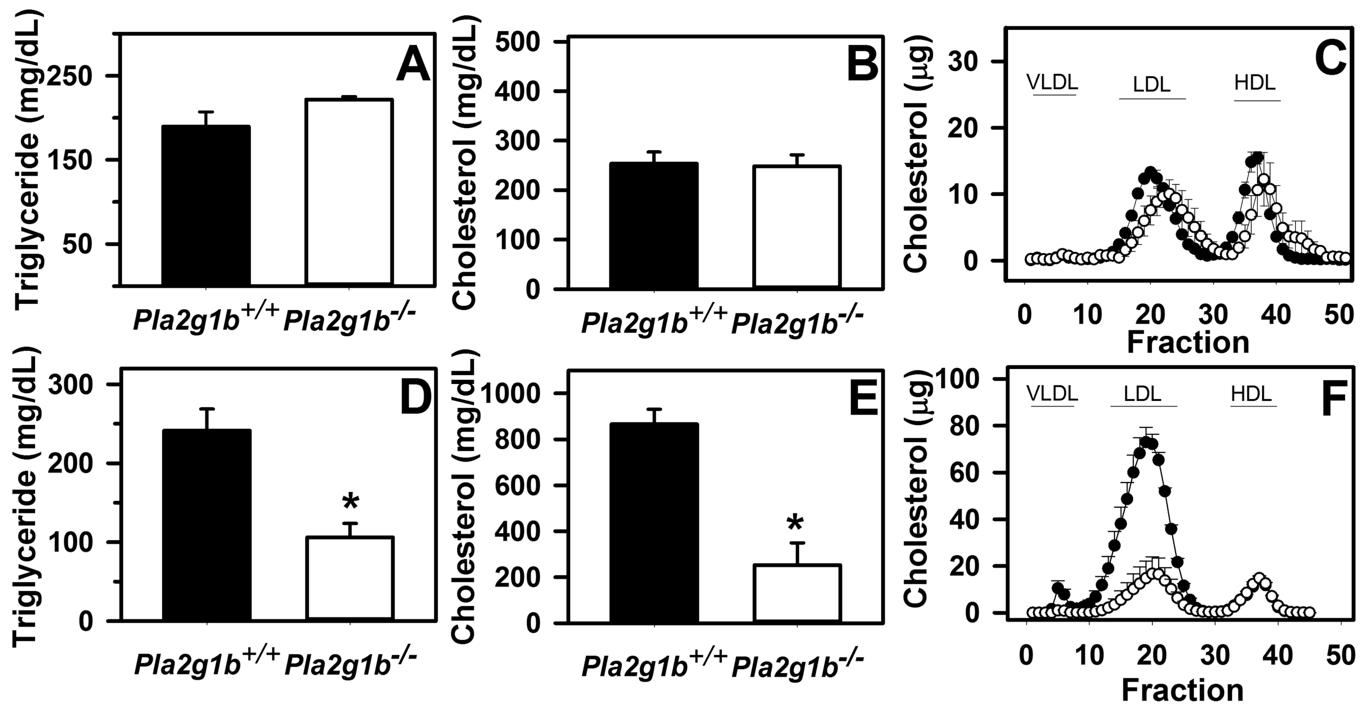

Figure 1. Fasting plasma lipid levels in *Pla2g1b*$^{+/+}$*Ldlr*$^{-/-}$ and *Pla2g1b*$^{-/-}$*Ldlr*$^{-/-}$ mice

Male *Pla2g1b*$^{+/+}$*Ldlr*$^{-/-}$ (filled bars and symbols) and *Pla2g1b*$^{-/-}$*Ldlr*$^{-/-}$ mice were fed low fat chow diet (A–C) or hypercaloric diet (D–F) for 10 weeks. Animals were fasted overnight prior to obtaining blood samples to measure plasma triglyceride (A,D) and cholesterol (B,E) levels. An 100 μL of the samples were fractionated on FPLC to determine lipid distribution among the various lipoprotein fractions compared to standards as indicated (C,F). Data represent mean ± SEM from 4–6 mice in each group. * denotes differences from *Pla2g1b*$^{+/+}$*Ldlr*$^{-/-}$ mice at $P < 0.01$.

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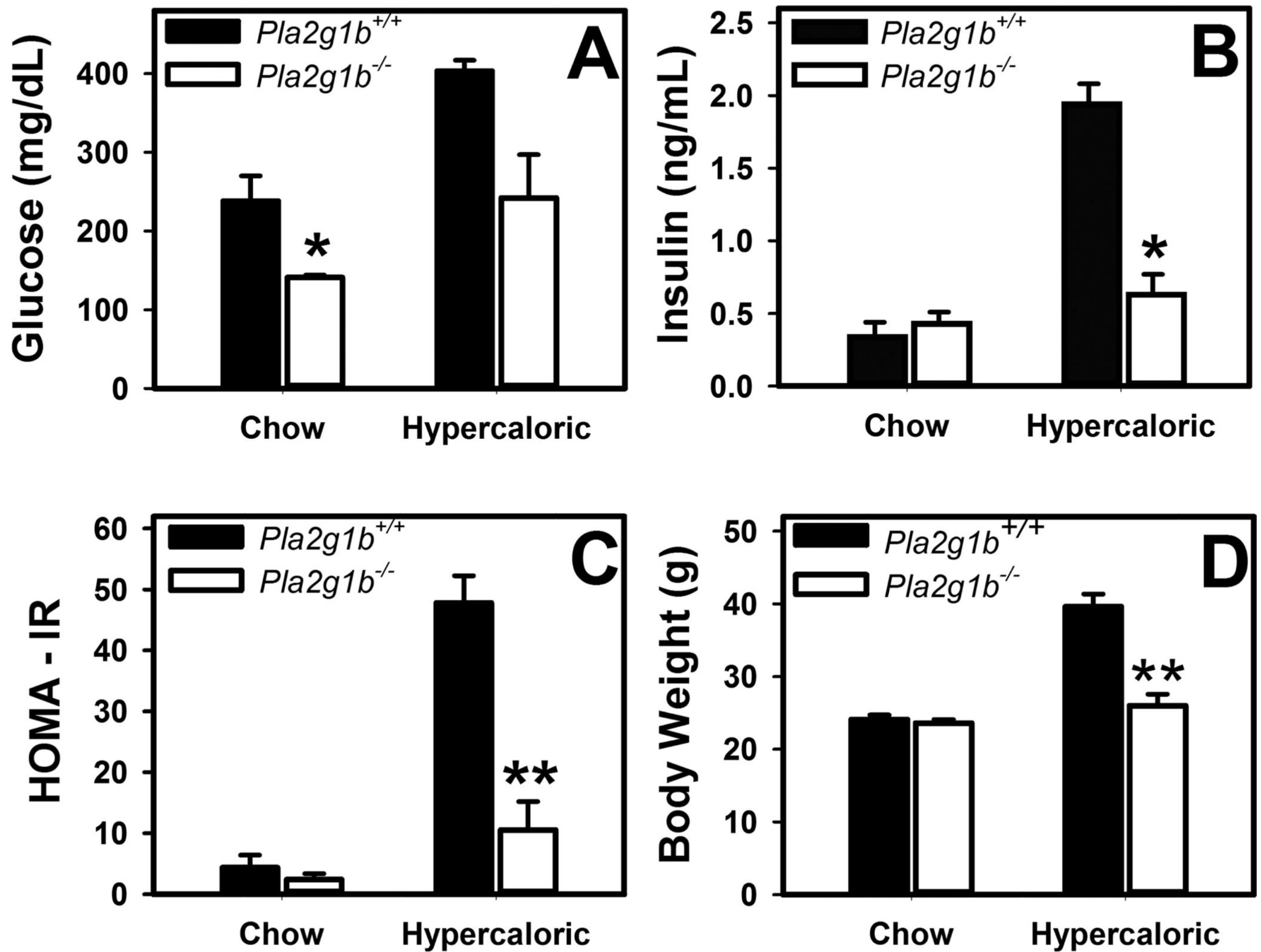

Figure 2. Blood chemistry and body weight of *Pla2g1b*$^{+/+}$*Ldlr*$^{-/-}$ and *Pla2g1b*$^{-/-}$*Ldlr*$^{-/-}$ mice

Male *Pla2g1b*$^{+/+}$*Ldlr*$^{-/-}$ (filled bars) and *Pla2g1b*$^{-/-}$*Ldlr*$^{-/-}$ mice (open bars) were fed either a low fat regular mouse chow or a hypercaloric diet containing 58.5 kcal% fat and 25 kcal% sucrose for 10 weeks. The mice were fasted overnight. (A) Blood glucose levels were measured by glucometer. (B) Plasma insulin levels were determined by ELISA. (C) Insulin resistance was estimated based on homeostasis model assessment index of insulin resistance (HOMA-IR) calculated from the glucose and insulin data. (D) Body weight was measured using a Denver 300K scale. The data represent mean ± SEM from 5 mice in each group. * and ** denote differences from *Pla2g1b*$^{+/+}$*Ldlr*$^{-/-}$ mice on the same diet at $P = 0.02$ and $P < 0.001$, respectively.

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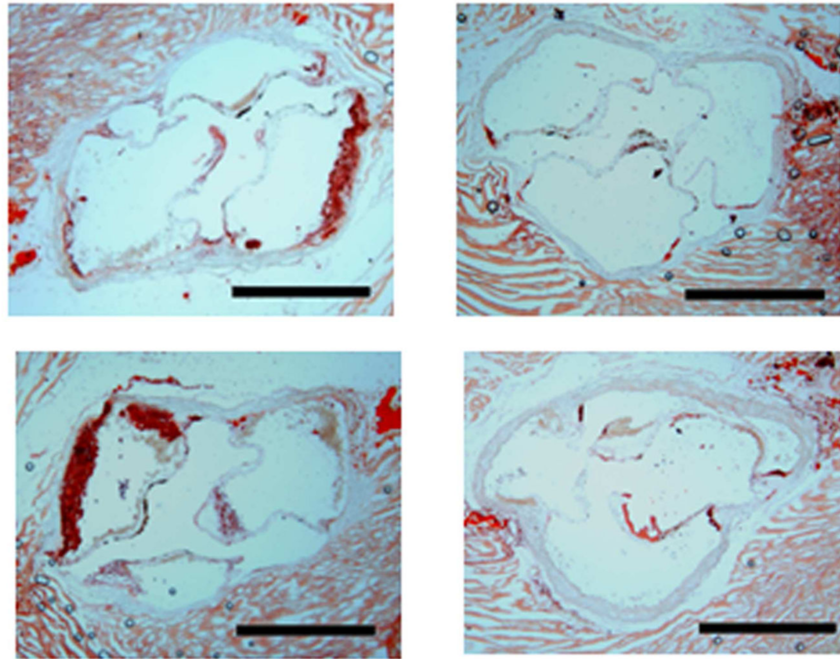

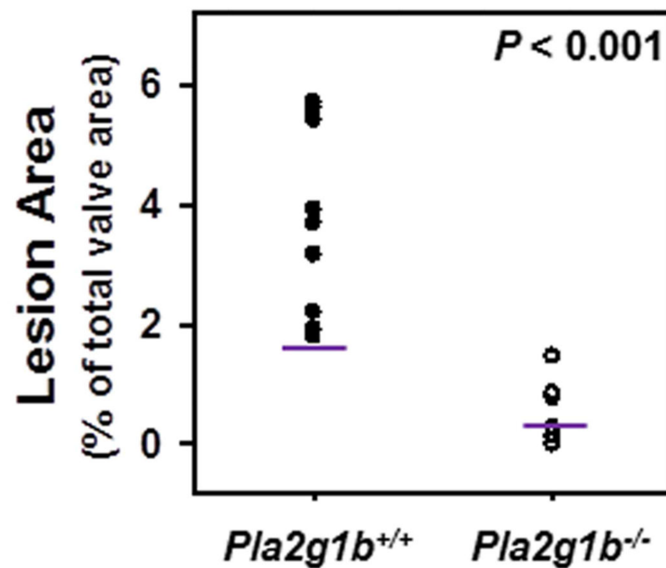

Figure 3. Atherosclerotic lesions in the aortic roots of *Ldlr*$^{-/-}$ mice with or without Pla2g1b expression

Male *Pla2g1b*$^{+/+}$ and *Pla2g1b*$^{-/-}$*Ldlr*$^{-/-}$ mice were fed a hypercaloric diet for 10 weeks. Atherosclerotic lesions were assessed in the aortic roots. The top panels show photomicrographs of aortic roots from 2 different mice in each group stained with oil red O. Scale bar = 500 μm. The bottom panel shows morphometric analysis of lesion areas expressed as ratios of lesion area to total valve area from 9 mice in each group. Each data point represents lesion area in one mouse with the geometric mean indicated with a line.