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Severe hypertriglyceridemia caused by *Gpihbp1* deficiency facilitates vascular remodeling through increasing endothelial activation and oxidative stress

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ABSTRACT

Hypertriglyceridemia (HTG) is an independent risk factor for atherosclerosis. However, its impact on non-atherosclerotic cardiovascular diseases remains largely unknown. Glycosylphosphatidylinositol anchored high-density lipoprotein binding protein 1 (GPIHBP1) is essential for the hydrolysis of circulating triglycerides and loss of functional GPIHBP1 causes severe HTG. In this study, we used *Gpihbp1* knockout (GKO) mice to investigate the potential effects of HTG on non-atherosclerotic vascular remodeling. We compared the aortic morphology and gene expressions between three-month-old and ten-month-old GKO mice and their age-matched wild-type controls. We also conducted similar comparisons between GKO mice and wild-type controls in an angiotensin II (AngII)-induced vascular remodeling model. Our data showed that the intima-media wall of ten-month-old GKO mice but not three-month-olds was significantly thickened compared to wild-type controls. Moreover, ten-month-old GKO mice but not three-month-olds had increased aortic macrophage infiltration and perivascular fibrosis, along with increased endothelial activation and oxidative stress. Similarly, the AngII-induced vascular remodeling, as well as endothelial activation and oxidative stress, were also exacerbated in the GKO mice compared to wild-type controls. In conclusion, we demonstrated that severe HTG caused by *Gpihbp1* deficiency could facilitate the onset and progression of non-atherosclerotic vascular remodeling through endothelial activation and oxidative stress in mice.

1. Introduction

Hypertriglyceridemia (HTG) is commonly defined as a fasting serum triglyceride level ≥ 150 mg/dL (≥1.7 mmol/L) and a non-fasting triglyceride level ≥ 200 mg/dL by the American Heart Association [1] or ≥ 175 mg/dL by the European Atherosclerosis Society [2]. It is usually not an isolated condition, but is often accompanied by other metabolic disorders, such as hypercholesterolemia, obesity, and diabetes mellitus; thus, its impacts on the cardiovascular system have been underestimated for almost half a century. In the past two decades, increasing large-scale clinical studies have supported a close association between HTG and atherosclerotic cardiovascular diseases [3–8]. The causal effects of HTG on atherosclerosis have also been demonstrated in genetically-manipulated small animals (such as mice and hamsters) as well as in medium-to-large animals (such as rabbits and pigs) [9]. However, most clinical and experimental studies involving HTG have focused on atherosclerotic cardiovascular diseases, and little is known about the pathogenic effects of HTG on non-atherosclerotic cardiovascular

Abbreviations: HTG, hypertriglyceridemia; TG, triglycerides; TRL, TG-enriched lipoproteins; LPL, lipoprotein lipase; GPIHBP1, glycosylphosphatidylinositol-anchored high-density lipoprotein-binding protein 1; APO, apolipoprotein; AngII, angiotensin II; KO, knockout; TC, total cholesterol; GLU, glucose; DHE, dihydroethidium; NOX, NADPH oxidases; ROS, reactive oxygen species.

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pathology.

Triglycerides (TGs) are insoluble in an aqueous phase and thus are transported in APOB-containing lipoproteins, such as chylomicrons, very-low-density lipoproteins, and their remnants, referred to as TG-enriched lipoproteins (TRLs) [9]. HTG is caused by either increased synthesis of TG or decreased lipolysis of TG in TRLs, or both [9]. Lipoprotein lipase (LPL), mainly secreted by adipocytes and myocytes, is the key enzyme for TRL lipolysis, which converts TG into fatty acids for tissue utilization as energy substrates [10]. Glycosylphosphatidylinositol-anchored high-density lipoprotein-binding protein 1 (GPIHBP1), exclusively expressed in capillary endothelial cells, is an essential partner for LPL: it transports LPL from the subendothelial spaces to the capillary lumen, where it serves as the platform for LPL-mediated TRL lipolysis by binding LPL and TRLs to capillary endothelial cells [11,12]. Additionally, LPL-mediated TRL lipolysis is regulated by other co-factors, such as apolipoprotein (APO) C2, APOC3, APOA5, and angiopoietin-like proteins (ANGPTL3 and ANGPTL4) [13,14]. Genetic defects involving LPL, GPIHBP1, and these co-factors all disrupt TG homeostasis and cause severe HTG [13,14].

In this study, we aimed to explore the association between HTG and the onset and progression of non-atherosclerotic vascular remodeling, using *Gpihbp1* knockout (KO) mice as HTG models. Our result demonstrated that severe HTG caused by *Gpihbp1* deficiency led to spontaneous vascular remodeling in mice, and further exacerbated hypertensive vascular remodeling induced by angiotensin II (AngII), highlighting a potential causal role of HTG in non-atherosclerotic vasculopathy. Moreover, we showed that severe HTG caused by *Gpihbp1* deficiency stimulated endothelial activation and oxidative stress, which might at least partially contribute to the vascular remodeling in mice.

2. Materials and methods

2.1. Animals

Male *Gpihbp1* knockout (GKO) mice (C57BL/6 J background) and their sex-matched control wild-type littermates (Ctrls) were generated by heterozygous crosses as previously described [15]. Genotyping was performed by PCR analysis of the genomic DNA extracted from the tails.

All mice were housed in individually ventilated cages and maintained on a 12-h light/12-h dark cycle with free access to a rodent chow diet and sterilized water. All experimental procedures were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals and were approved by the Animal Care and Use Committee of Dalian Medical University.

2.2. Hypertension induction and measurement of blood pressure

Hypertension in mice was induced by subcutaneous AngII infusion at a dosage of 1000 ng/kg/min using osmotic mini-pumps (Alzet MODEL 1007D; Durect Corp, Cupertino, CA, USA) [16], while normotension controls were administered with the same dosage of saline infusion. Systolic blood pressure (SBP) was measured with a tail-cuff instrument (BP-98A; Softron, Japan) and averaged from 10 records as previously described [16]. Mice were sacrificed after two weeks on AngII or saline infusion.

2.3. Serum lipid and glucose assay

Blood samples were collected by retro-orbital plexus puncture after an overnight fast, and serum was separated by centrifugation at 4000 rpm for 10 min at 4 °C. Serum total cholesterol (TC), triglycerides (TG), and glucose (GLU) concentrations were measured with commercial enzymatic kits (BioSino, Beijing, China).

2.4. Histomorphological analysis

Mice were euthanized by a lethal dose of anesthesia and flushed with PBS through the left ventricular. Whole aortas from the base ascending aorta to the iliac bifurcation were collected and quickly rid of the perivascular adhering tissues in ice-cold PBS. The upper one-third of the thoracic aortas (approximately 1 mm long) were fixed in 4 % paraformaldehyde (Life-iLab, Shanghai, China) overnight, embedded in O.C.T. compound (Sakura Finetek, Torrance, USA), and cyto-sectioned into 5 μm thick slices for histological analysis, while the remaining parts of the aortas were pooled and stored in −80 °C for RNA extraction. Hematoxylin and eosin (H&E) staining (G1120; Solarbio, Beijing, China) and Masson's trichrome staining (G1340; Solarbio, Beijing, China) were performed using commercial kits according to the manufacturer's instructions. Intima-media thickness was defined as the distance from the endothelial surface to the adventitia and averaged from five different fields of the aortic ring for each mouse. Dihydroethidium (DHE, 1 μM; Sigma-Aldrich, St. Louis, MO, USA) staining was performed as previously described [17]. Immunofluorescent staining was performed using antibodies against Mac-2 (diluted at 1:200; ARG66239, Arigo), MCP-1 (diluted at 1:100, AF7437, Beyotime), VCAM-1 (diluted at 1:100; ARG42059, Arigo) and ICAM-1 (diluted at 1: 200; ab222736, Abcam). All quantifications were performed with Image J software.

2.5. Quantitative real-time PCR analysis

Aortic total RNA was extracted using TriQuick Reagent (R1100; Solarbio, Beijing, China) and reverse-transcribed to cDNA with the PrimeScript RT Reagent Kit (11141ES60; Yeasen, Shanghai, China). Quantitative real-time PCR was performed with SYBR Green qPCR reagents (11184ES03; Yeasen, Shanghai, China), using primers listed in Table 1. All samples were quantitated using the comparative CT method and normalized to β-actin levels.

2.6. Statistical analysis

All data were presented as mean ± standard deviation. The Shapiro–Wilk test was used to assess data normality. Statistical comparisons were conducted using two-way ANOVA followed by Tukey's test or the Mann-Whitney *U* test for nonparametric data with GraphPad Prism software. A p value <0.05 was considered statistically significant.

Table 1
Primer sequences used in the study.

Name	Type	Sequence (5′ - 3′)
Cd68	Forward	TGTCTGATCTTGCTAGGACCG
	Reverse	GAGAGTAACGGCCTTTTTGTGA
Il-1β	Forward	TGCCACCTTTTGACAGTGATG
	Reverse	TGATGTGCTGCTGCGAGATT
Il-6	Forward	TGATGGATGCTACCAAACTGGA
	Reverse	TGTGACTCCAGCTTATCTCTTGG
α-sma	Forward	TCCTGACGCTGAAGTATCCGATA
	Reverse	GGCCACACGAAGCTCGTTAT
Col1	Forward	GAGAGGTGAACAAGGTCCCG
	Reverse	AAACCTCTCTCGCCTCTTGC
Col3	Forward	TCCCCTGGAATCTGTGAATC
	Reverse	TGAGTCGAATTGGGGAGAAT
Mcp-1	Forward	TAAAAACCTGGATCGGAACCAAA
	Reverse	GCATTAGCTTCAGATTTACGGGT
Vcam-1	Forward	TTGGGAGCCTCAACGGTACT
	Reverse	GCAATCGTTTTGTATTCAGGGGA
Icam-1	Forward	GCCTGGCATTTCAGAGTCTGCT
	Reverse	AAACCAGACCCTGGAACTGCAC
Nox2	Forward	CTTCTTGGGTCAGCACTGGC
	Reverse	GCAGCAAGATCAGCATGCAG
Nox4	Forward	CTTGGTGAATGCCCTCAACT
	Reverse	TTCTGGGATCCTCATTCTGG
β-actin	Forward	GGCTGTATTCCCCTCCATCG
	Reverse	CCAGTTGGTAACAATGCCATGT

3. Results

3.1. Severe HTG in the Gpihbp1 KO mice

The *Gpihbp1* KO mice are born with familial chylomicronemia due to the loss of functional GPIHBP1 protein and disruption of LPL-mediated TRL lipolysis. As expected, the serum of three-month-old *Gpihbp1* KO mice exhibited a milky appearance (Fig. 1A), and the serum TG levels were remarkably elevated, approximately 50-fold higher than age-matched wild-type controls (Fig. 1B). The *Gpihbp1* KO mice also developed hypercholesterolemia, with serum total cholesterol (TC) levels two-fold higher than wild-type controls (Fig. 1C). The severe HTG observed in adult *Gpihbp1* KO mice did not progress with age, as the serum TG levels of ten-month-old *Gpihbp1* KO mice were comparable to those of three-month-olds (Fig. 1B). However, the serum TC levels in ten-month-old *Gpihbp1* KO mice were slightly higher than those of three-month-olds (Fig. 1C). Notably, the serum glucose levels of *Gpihbp1* KO mice did not differ significantly from those of wild-type controls, regardless of age (three months or ten months) (Fig. 1D).

3.2. Severe HTG caused by Gpihbp1 deficiency led to spontaneous vascular remodeling in mice

Wild-type mice, with their high-density lipoprotein-dominant lipid profile, are naturally resistant to developing atherosclerosis. However, severe hypertriglyceridemic *Gpihbp1* KO mice begin to present mild early atherosclerosis at the aortic root when they reach eleven-to-twelve months of age [18]. To investigate the effects of HTG on non-atherosclerotic vasculopathy, we therefore evaluated the histomorphology of the thoracic aorta ring and gene expression in the aorta of severe hypertriglyceridemic *Gpihbp1* KO mice and normotriglyceridemic wild-type control mice at three and ten months of age. H&E staining revealed that the intima-media wall thickness of three-month-old *Gpihbp1* KO mice did not differ significantly from that of age-matched controls. However, when the mice reached ten months of age, the intima-media wall of *Gpihbp1* KO mice was significantly thickened, compared with that of control mice (Fig. 2A). Mac-2 immunofluorescent staining showed that ten-month-old *Gpihbp1* KO mice had more macrophages accumulated in their thoracic aortas than age-matched wild-type controls, whereas three-month-old *Gpihbp1* KO mice did not exhibit this phenomenon (Fig. 2B). This observation was further confirmed by increased expression of the aortic macrophage marker gene (*Cd68*) and macrophage-derived pro-inflammatory cytokines (*Il-1β* and *Il-6*) in ten-month-old *Gpihbp1* KO mice, as determined by quantitative real-time PCR (Fig. 2C). Similarly, Masson staining in combination with higher aortic *α-sma* and *Collagen (Col) 1/3* expression revealed increased perivascular fibrosis in ten-month-old *Gpihbp1* KO mice, but not in three-month-olds (Fig. 2D and E). Taken together, these

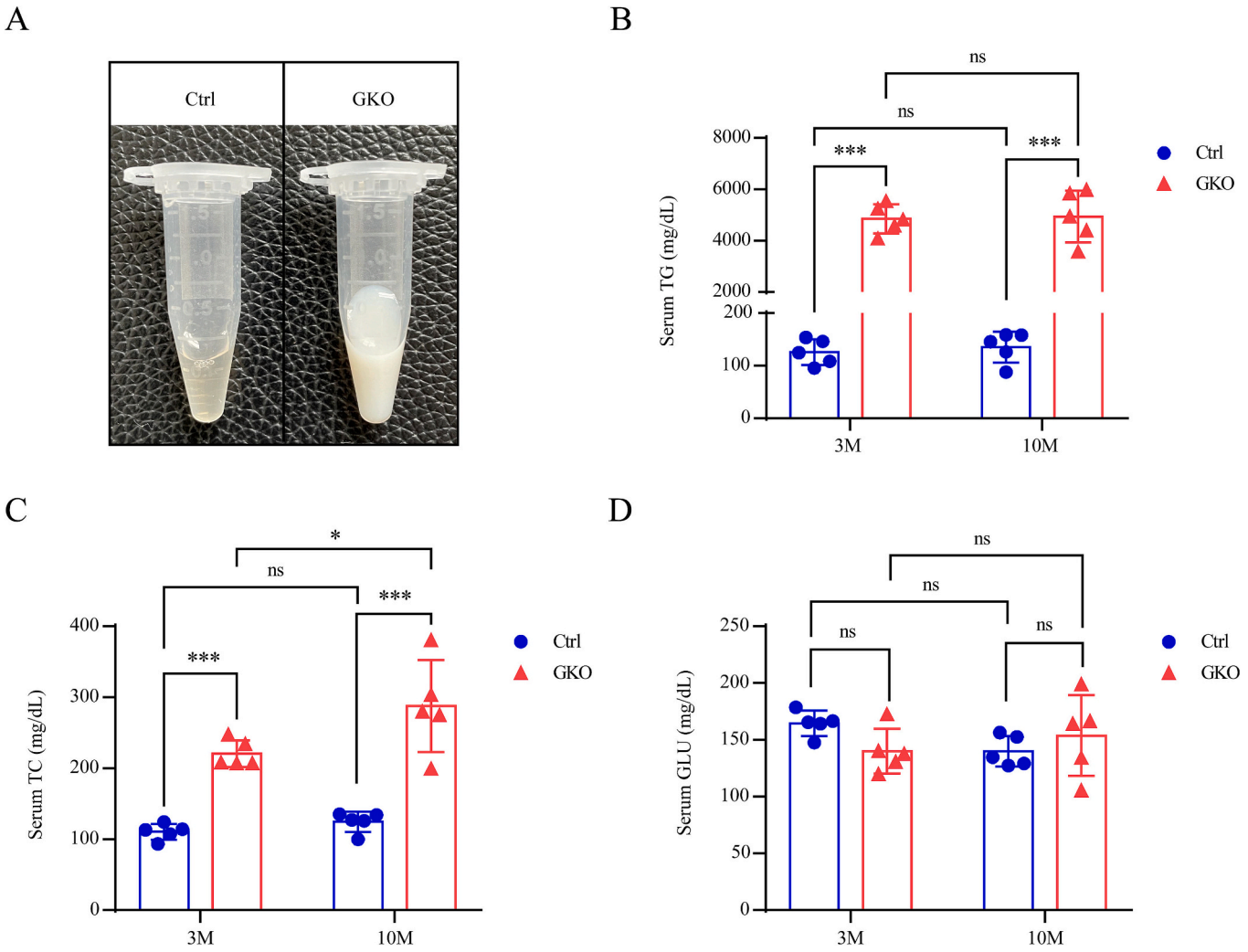

Fig. 1. *Gpihbp1* deficiency induced severe HTG. (A) Serum appearance of three-month-old *Gpihbp1* KO mice (right) and their matched littermates (left); (B) Fasting serum triglycerides levels; (C) Fasting serum total cholesterol levels; (D) Fasting serum glucose levels.
n = 5 per group. *: $p < 0.05$, ***: $p < 0.001$, ns: no significance.

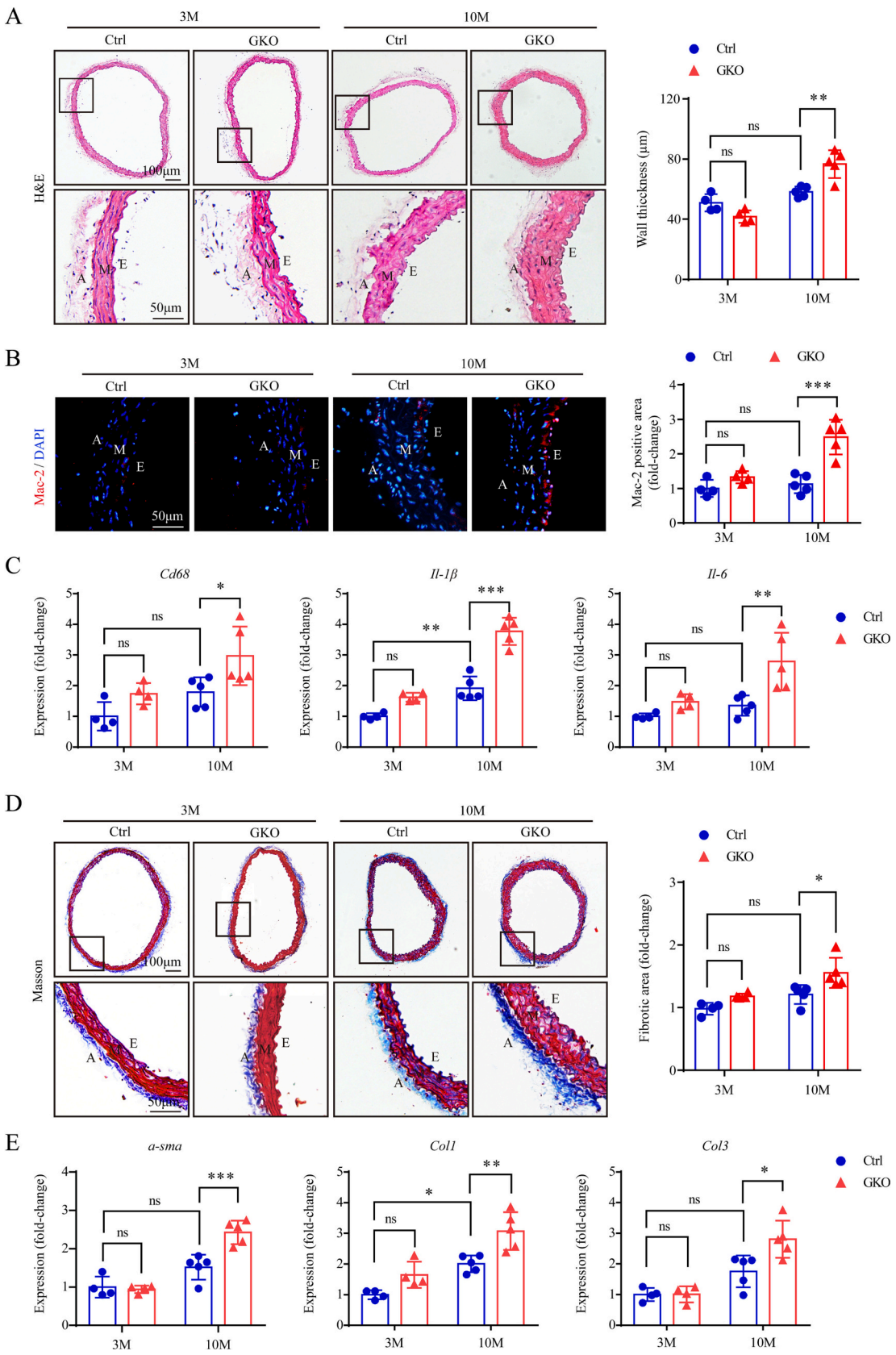

Fig. 2. Severe HTG caused by *Gpihbp1* deficiency led to spontaneous vascular remodeling in mice. (A) H&E staining of the thoracic aortic rings and quantification of the intima-media wall thickness; (B) Mac-2 immunofluorescent staining of the thoracic aortic rings and quantification of the Mac-2 positive area; (C) RT-qPCR analysis showing aortic *Cd68*, *Il-1β*, and *Il-6* gene expressions; (D) Masson staining of the thoracic aortic rings and quantification of the fibrotic area; (E) RT-qPCR analysis showing aortic *α-sma*, *Col1*, and *Col3* gene expressions.
n = 4–5 per group. *: $p < 0.05$, **: $p<0.01$, ***: $p < 0.001$, ns: no significance.

results suggest that middle-aged *Gpihbp1* KO mice have already developed vascular remodeling with signs of increased aortic stiffness.

3.3. Endothelial activation and oxidative stress contributed to spontaneous vascular remodeling in the severe hypertriglyceridemic Gpihbp1 KO mice

The release of monocyte chemoattractive and adhesive molecules, such as MCP-1, VCAM-1, and ICAM-1, are commonly used as an indicator of endothelial activation. Here we demonstrated that ten-month-old *Gpihbp1* KO mice, but not three-month-old mice, expressed higher levels of MCP-1, VCAM-1, and ICAM-1, as evidenced by immunofluorescent staining and gene expression analysis, compared to their age-matched control mice (Fig. 3A–D). Additionally, we evaluated aortic oxidative stress levels using DHE staining and expression of NADPH oxidases (NOXs). We found that the DHE immunofluorescent intensity in ten-month-old *Gpihbp1* KO mice, but not three-month-old, was significantly higher than that of age-matched wild-type controls (Fig. 3E and F). Moreover, the expression of *Nox2* and *Nox4*, two major subunits of aortic *Noxs*, were also increased in ten-month-old *Gpihbp1* KO mice, but not three-month-old mice, compared to their age-matched wild-type controls (Fig. 3G). These findings indicate that endothelial activation and oxidative stress may contribute to the spontaneous vascular remodeling observed in middle-aged *Gpihbp1* KO mice.

3.4. Severe HTG caused by Gpihbp1 deficiency aggravated AngII-induced vascular remodeling

We next explored whether severe HTG in the *Gpihbp1* KO mice could contribute to stress-induced vascular remodeling. We infused eight-week-old *Gpihbp1* KO mice and age-matched wild-type controls with AngII (1000 ng/kg/min) for two weeks to induce hypertension and hypertensive vascular remodeling. We found no significant difference in blood pressure between severe hypertriglyceridemic *Gpihbp1* KO mice and wild-type controls during AngII infusion (Supplemental Fig. 1). However, H&E staining revealed that AngII infusion induced significant intima-media thickening, which was further exacerbated in severe hypertriglyceridemic *Gpihbp1* KO mice compared to wild-type controls (Fig. 4A). Additionally, AngII-induced macrophage infiltration and perivascular fibrosis, as indicated by Mac-2 immunofluorescent staining (Fig. 3B) and Masson staining (Fig. 3D), were also more pronounced in severe hypertriglyceridemic *Gpihbp1* KO mice. Consistently with these findings, the aortic expression of the macrophage marker gene (*Cd68*) and pro-inflammatory cytokines (*Il-1β* and *Il-6*) (Fig. 4C), as well as collagen-associated genes (*α-sma*, *Col1*, and *Col3*) (Fig. 4E), were all increased in severe hypertriglyceridemic *Gpihbp1* KO mice.

3.5. Severe HTG caused by Gpihbp1 deficiency aggravated AngII-induced endothelial activation and oxidative stress

Endothelial activation and oxidative stress are two pivotal factors that contribute to AngII-induced vascular remodeling. Here, we demonstrated that the infusion of AngII led to a significant increase in the expression of MCP-1, VCAM-1, and ICAM-1 in the aorta, as evidenced by immunofluorescent stainings and gene expression analysis, which was more pronounced in severe hypertriglyceridemic *Gpihbp1* KO mice (Fig. 5A–D). Similarly, AngII-induced oxidative stress in the aorta was also exacerbated in severe hypertriglyceridemic *Gpihbp1* KO mice, as indicated by increased DHE intensity (Fig. 5E and F) and aortic gene expression of *Nox2* and *Nox4* (Fig. 5G). These findings suggested that severe HTG may enhance AngII-induced endothelial activation and oxidative stress during stress-induced vascular remodeling.

4. Discussion

In this study, we demonstrated that (1) severe HTG caused by *Gpihbp1* deficiency led to spontaneous vascular remodeling, including intima-media thickening, increased pro-inflammatory macrophage infiltration, and perivascular fibrosis; (2) severe HTG caused by *Gpihbp1* deficiency exacerbated AngII-induced hypertensive vascular remodeling without affecting blood pressure; (3) severe HTG caused by *Gpihbp1* deficiency induced endothelial activation and oxidative stress to facilitate vascular remodeling.

The vessel wall is a dynamic system composed of endothelial cells, vascular smooth muscle cells, and fibroblast cells [19]. It is capable of sensing mechanical and metabolic stimulations (such as hypertension, hyperlipidemia, and diabetes mellitus) within its milieu and integrating these signals into the production of mediators that consequently alter its structure and function [20]. This process, known as vascular remodeling, is a well-established pathological basis of vascular diseases, including atherosclerosis, hypertension, aortic aneurysms, and diabetic vascular complications [20]. Both clinical and experimental evidence has indicated that HTG plays a critical role in atherosclerotic vascular remodeling [21]. In carotid stenosis patients underwent carotid endarterectomy or carotid artery stenting, HTG is identified as a residual risk for the progression of atherosclerotic carotid stenosis and precedes carotid restenosis after successful revascularization [22–25]. In patients with type 2 diabetes mellitus, postprandial HTG is closely associated with carotid intima-media thickness, an early sign predicting atherosclerosis development [26,27]. In diabetic mice, severe HTG caused by *Gpihbp*1 deficiency accelerates atherosclerosis and leads to vascular dilated remodeling [15]. Furthermore, the reduction of HTG in hypertriglyceridemic mouse models by interventional APOC3 lowering using an APOC3 antisense oligonucleotide reduces atherosclerotic lesion progression and improves plaque stability by reducing necrotic core area and increasing fibrous cap thickness [28]. In contrast, the effects of HTG on non-atherosclerotic vascular remodeling are still not well understood. Here in this study, we found that severe HTG caused by *Gpihbp*1 deficiency led to spontaneous vascular remodeling, and promoted stress-induced vascular remodeling in mice. Our findings provide preliminary evidence that increased circulating triglyceride levels might be a potential risk factor for vascular remodeling. Further studies, however, are needed to confirm the pathogenic and causative effects of HTG on the onset and progression of vascular remodeling.

Although all types of vascular cells are potential active players in the vascular remodeling process, the endothelial cells, which form a monolayer on the interior walls of vasculatures, are the first-line sensor to alterations in the vascular milieu due to their unique localization [29]. Upon exposure to mechanical and metabolic stresses, these cells not only adjust the release of relaxing and contracting factors (such as nitric oxide, prostacyclin, and endothelin) to control vascular tone and blood flow, but also secret multiple pro-inflammatory cytokines, such as MCP-1, VCAM-1 and ICAM-1, to facilitate the recruitment and adhesion of inflammatory cells for defense or repair reactions, a process known as endothelial activation [29]. Mounting evidence has indicated endothelial activation as the initial event in almost all types of vascular pathologies including atherosclerosis, aging-related and stress-induced vascular remodeling. Previously, endothelial activation is demonstrated to contribute to HTG-aggravated atherosclerosis [15,30–33]. Here in this study, we showed signs of endothelium activation involving in the spontaneous and AngII-induced hypertensive vascular remodeling in the severe hypertriglyceridemic *Gpihbp1* KO mice, indicating a contribution of endothelium activation to the onset and progression of HTG-associated vascular remodeling. Further studies, however, are needed to elucidate how HTG activates endothelium and therefore promotes non-atherosclerotic vascular pathologies.

Similar to endothelium activation, oxidative stress is another key contributor to almost all types of vascular pathologies [34,35]. Due to an imbalance between reactive oxygen species (ROS) generation and antioxidant defense, oxidative stress leads to the accumulation of free radicals and oxygen metabolites (such as superoxide, hydrogen peroxide, hydroxyl radical as well as the nitric oxide radical and

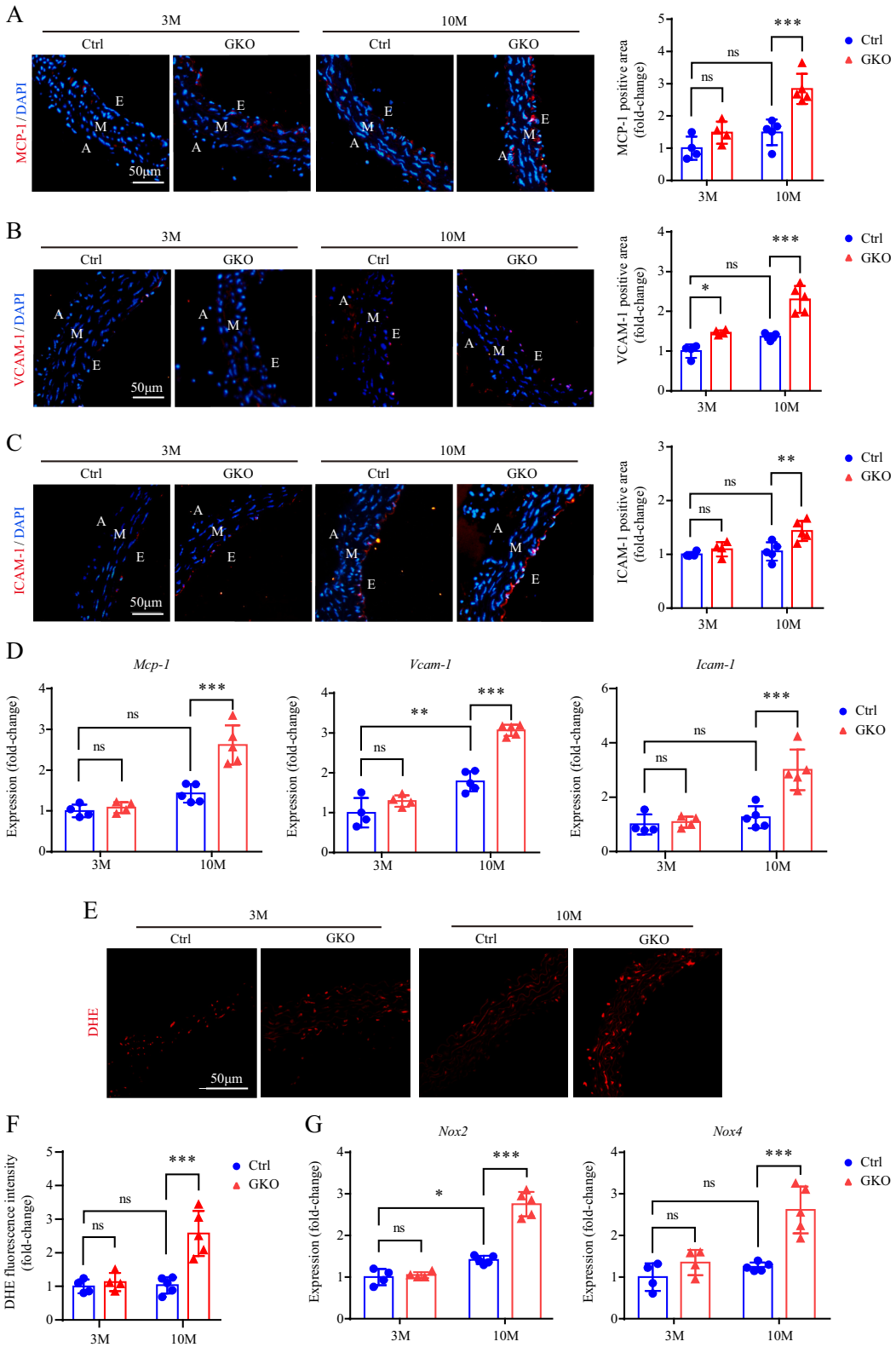

Fig. 3. Severe HTG caused by *Gpihbp1* deficiency activated endothelial cells and induced aortic oxidative stress in mice. (A) MCP-1 immunofluorescent staining of the thoracic aortic rings and quantification of MCP-1 positive area; (B) VCAM-1 immunofluorescent staining of the thoracic aortic rings and quantification of VCAM-1 positive area; (C) ICAM-1 immunofluorescent staining of the thoracic aortic rings and quantification of ICAM-1 positive area; (D) RT-qPCR analysis showing aortic *Mcp-1*, *Vcam-1*, and *Icam-1* gene expressions; (E) DHE staining of the thoracic aortic rings and quantification of the fluorescence intensity; (G) RT-qPCR analysis showing aortic *Nox2* and *Nox4* gen expressions.
n = 4–5 per group. *: $p < 0.05$, **: p<0.01, ***: $p < 0.001$, ns: no significance.

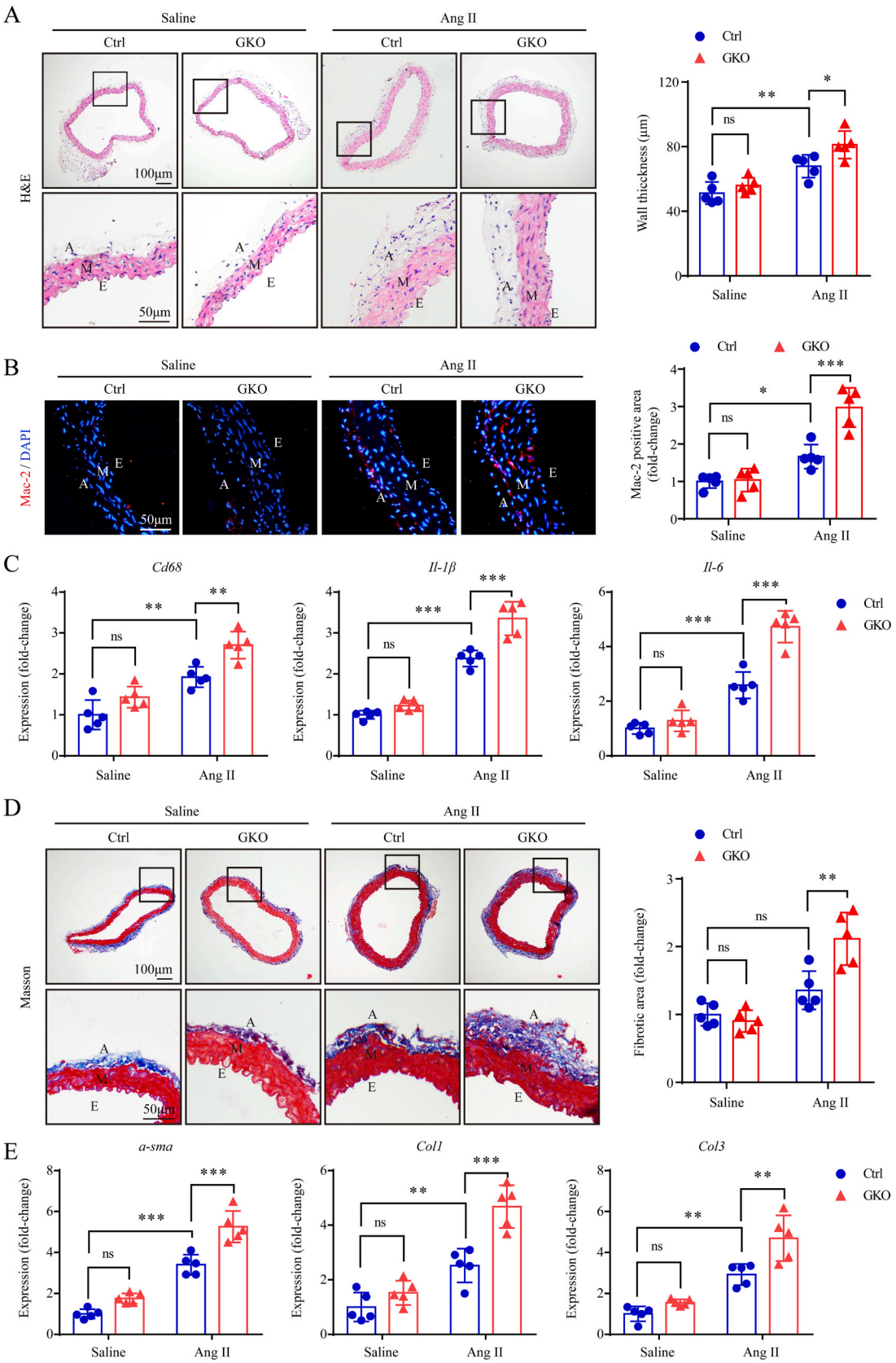

Fig. 4. Severe HTG caused by *Gpihbp1* deficiency promoted AngII-induced vascular remodeling in mice. (A) H&E staining of the thoracic aortic rings and quantification of the intima-media wall thickness; (B) Mac-2 immunofluorescent staining of the thoracic aortic rings and quantification of the Mac-2 positive area; (C) RT-qPCR analysis showing aortic *Cd68*, *Il-1β*, and *Il-6* gene expressions; (D) Masson staining of the thoracic aortic rings and quantification of the fibrotic area; (E) RT-qPCR analysis showing aortic *α-sma*, *Col1*, and *Col3* gene expressions.
n = 5 per group. *: $p < 0.05$, **: p<0.01, ***: $p < 0.001$, ns: no significance.

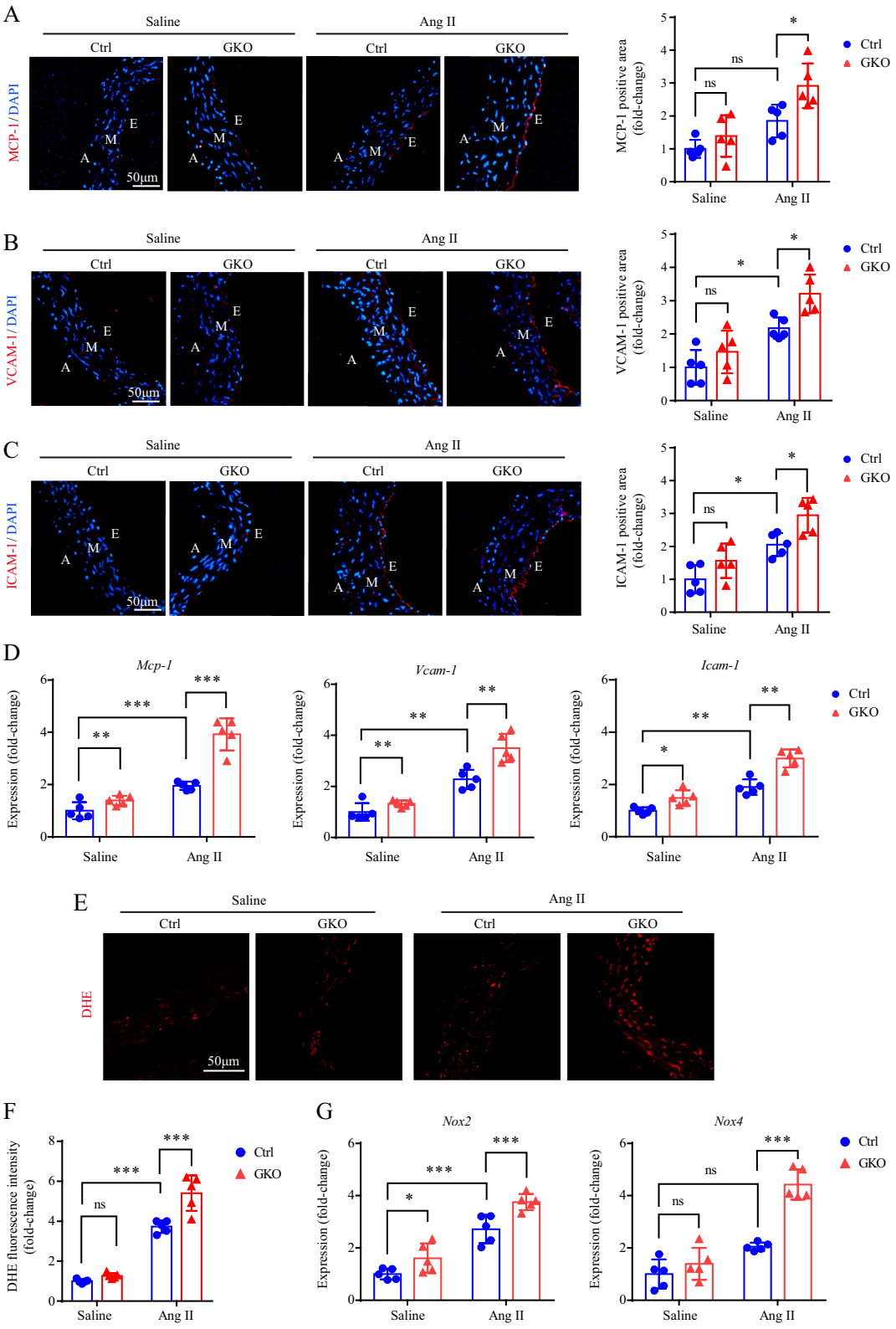

Fig. 5. Severe HTG caused by *Gpihbp1* deficiency promoted AngII-induced endothelial activation and oxidative stress in mice. (A) MCP-1 immunofluorescent staining of the thoracic aortic rings and quantification of MCP-1 positive area; (B) VCAM-1 immunofluorescent staining of the thoracic aortic rings and quantification of VCAM-1 positive area; (C) ICAM-1 immunofluorescent staining of the thoracic aortic rings and quantification of ICAM-1 positive area; (D) RT-qPCR analysis showing aortic *Mcp-1*, *Vcam-1*, and *Icam-1* gene expressions; (E) DHE staining of the thoracic aortic rings and quantification of the fluorescence intensity; (G) RT-qPCR analysis showing aortic *Nox2* and *Nox4* gen expressions.
n = 5 per group. *: $p < 0.05$, **: p<0.01, ***: $p < 0.001$, ns: no significance.

peroxynitrite), finally disrupting redox signaling and causing cellular damages [36]. NADPH oxidases (NOXs) are one of the major ROS-generating systems in living cells [37] and indeed have been known as the primary source of oxidative stress in vascular diseases, such as atherosclerosis [38,39], hypertension [40,41], aortic aneurysms [42,43] and diabetic vascular complications [38,39]. Activation of NOXs has been shown to activate other ROS-generating systems, such as the uncoupled endothelial nitric oxide synthase, dysfunctional mitochondria, and xanthine oxidase, inducing secondary sources of ROS production [37]. In addition, the over-activation of NOXs also stimulates the endothelium to start pro-inflammatory cascades [34,35]. Here we demonstrated the involvement of oxidative stress as shown by DHE staining and increased *Noxs* gene expression in the spontaneous and stress-induced vascular remodeling in the severe hypertriglyceridemic *Gpihbp1* KO mice; however, how HTG causes oxidative stress and whether oxidative stress further activates the endothelium to promote the non-atherosclerotic vasculopathy are not explained in the current study and need further explorations.

In conclusion, our study demonstrated that severe HTG caused by *Gpihbp1* deficiency led to spontaneous vascular remodeling and exacerbated AngII-induced hypertensive vascular remodeling in mice. Moreover, we showed that severe HTG caused by *Gpihbp1* deficiency stimulated endothelial activation and oxidative stress, which might at least partially contribute to vascular remodeling in mice. These findings indicate that HTG might facilitate the onset and progression of non-atherosclerotic vascular remodeling through endothelial activation and oxidative stress. Further studies, however, are needed to confirm the pathogenic effects and elucidate the underlying mechanisms of HTG on vascular remodeling in other animal models and patients.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbalip.2023.159330.

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Institutional review board statement

The animal study was approved by the Animal Care and Use Committee of Dalian Medical University and performed under the guidelines for the care and use of laboratory animals of the National Institute of Health.

CRediT authorship contribution statement

Conceptualization, Y.X. and J.L.; investigation, R.F., X.A., Y.W., J.Z., S.L., J.B. and J.L.; methodology, Q.L. and Y.X.; writing—original draft preparation, R.F. and X.A.; writing—review and editing, Y.X. and J.L.; supervision, Y.X. and J.L.; project administration, R.F. and X.A.; funding acquisition, Y.X. and J.L.. All authors have read and agreed to the published version of the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability statement

The data used to support the findings of this study are available from the corresponding authors upon reasonable request.

References

[1] M. Miller, N.J. Stone, C. Ballantyne, V. Bittner, M.H. Criqui, H.N. Ginsberg, A. C. Goldberg, W.J. Howard, M.S. Jacobson, P.M. Kris-Etherton, T.A. Lennie, M. Levi, T. Mazzone, S. Pennathur, Triglycerides and cardiovascular disease: a scientific statement from the American Heart Association, Circulation 123 (2011) 2292–2333.

[2] B.G. Nordestgaard, A. Langsted, S. Mora, G. Kolovou, H. Baum, E. Bruckert, G. F. Watts, G. Sypniewska, O. Wiklund, J. Boren, M.J. Chapman, C. Cobbaert, O. S. Descamps, A. von Eckardstein, P.R. Kamstrup, K. Pulkki, F. Kronenberg, A. T. Remaley, N. Rifai, E. Ros, M. Langlois, S. European Atherosclerosis, C. the European Federation of Clinical, i, Laboratory medicine joint consensus, fasting is not routinely required for determination of a lipid profile: clinical and laboratory implications including flagging at desirable concentration cut-points-a joint consensus statement from the European Atherosclerosis Society and European Federation of Clinical Chemistry and Laboratory Medicine, Eur. Heart J. 37 (2016) 1944–1958.

[3] A. Patel, F. Barzi, K. Jamrozik, T.H. Lam, H. Ueshima, G. Whitlock, M. Woodward, Serum triglycerides as a risk factor for cardiovascular diseases in the Asia-Pacific region, Circulation 110 (2004) 2678–2686.

[4] B.G. Nordestgaard, M. Benn, P. Schnohr, A. Tybjaerg-Hansen, Nonfasting triglycerides and risk of myocardial infarction, ischemic heart disease, and death in men and women, Jama 298 (2007) 299–308.

[5] N. Sarwar, J. Danesh, G. Eiriksdottir, G. Sigurdsson, N. Wareham, S. Bingham, S. M. Boekholdt, K.T. Khaw, V. Gudnason, Triglycerides and the risk of coronary heart disease: 10,158 incident cases among 262,525 participants in 29 Western prospective studies, Circulation 115 (2007) 450–458.

[6] M. Miller, C.P. Cannon, S.A. Murphy, J. Qin, K.K. Ray, E. Braunwald, Impact of triglyceride levels beyond low-density lipoprotein cholesterol after acute coronary syndrome in the PROVE IT-TIMI 22 trial, J. Am. Coll. Cardiol. 51 (2008) 724–730.

[7] B.G. Nordestgaard, A. Varbo, Triglycerides and cardiovascular disease, Lancet 384 (2014) 626–635.

[8] R. Klempfner, A. Erez, B.Z. Sagit, I. Goldenberg, E. Fisman, E. Kopel, N. Shlomo, A. Israel, A. Tenenbaum, Elevated triglyceride level is independently associated with increased all-cause mortality in patients with established coronary heart disease: twenty-two-year follow-up of the Bezafibrate infarction prevention study and registry, Circ. Cardiovasc. Qual. Outcomes 9 (2016) 100–108.

[9] D. Basu, K.E. Bornfeldt, Hypertriglyceridemia and atherosclerosis: using human research to guide mechanistic studies in animal models, Front. Endocrinol. (Lausanne) 11 (2020) 504.

[10] C.L. Chang, Lipoprotein lipase: new roles for an 'old' enzyme, Curr. Opin. Clin. Nutr. Metab. Care 22 (2019) 111–115.

[11] S.G. Young, L.G. Fong, A.P. Beigneux, C.M. Allan, C. He, H. Jiang, K. Nakajima, M. Meiyappan, G. Birrane, M. Ploug, GPIHBP1 and lipoprotein lipase, partners in plasma triglyceride metabolism, Cell Metab. 30 (2019) 51–65.

[12] C. Liu, L. Li, D. Guo, Y. Lv, X. Zheng, Z. Mo, W. Xie, Lipoprotein lipase transporter GPIHBP1 and triglyceride-rich lipoprotein metabolism, Clin. Chim. Acta 487 (2018) 33–40.

[13] D. Basu, I.J. Goldberg, Regulation of lipoprotein lipase-mediated lipolysis of triglycerides, Curr. Opin. Lipidol. 31 (2020) 154–160.

[14] S.A. Wu, S. Kersten, L. Qi, Lipoprotein lipase and its regulators: an unfolding story, Trends Endocrinol Metab 32 (2021) 48–61.

[15] X. Liu, J. Li, J. Liao, H. Wang, X. Huang, Z. Dong, Q. Shen, L. Zhang, Y. Wang, W. Kong, G. Liu, W. Huang, Gpihbp1 deficiency accelerates atherosclerosis and plaque instability in diabetic Ldlr(−/−) mice, Atherosclerosis 282 (2019) 100–109.

[16] Y. Deng, Z. Li, X. An, R. Fan, Y. Wang, J. Li, X. Yang, J. Liao, Y. Xia, Hyperhomocysteinemia promotes cardiac hypertrophy in hypertension, Oxidative Med. Cell. Longev. 2022 (2022) 1486157.

[17] Y.P. Xie, S. Lai, Q.Y. Lin, X. Xie, J.W. Liao, H.X. Wang, C. Tian, H.H. Li, CDC20 regulates cardiac hypertrophy via targeting LC3-dependent autophagy, Theranostics 8 (2018) 5995–6007.

[18] M.M. Weinstein, L. Yin, Y. Tu, X. Wang, X. Wu, L.W. Castellani, R.L. Walzem, A. J. Lusis, L.G. Fong, A.P. Beigneux, S.G. Young, Chylomicronemia elicits atherosclerosis in mice–brief report, Arterioscler. Thromb. Vasc. Biol. 30 (2010) 20–23.

[19] B.R. Evans, A. Yerly, E.P.C. van der Vorst, I. Baumgartner, S.M. Bernhard, M. Schindewolf, Y. Doring, Inflammatory mediators in atherosclerotic vascular remodeling, Front. Cardiovasc. Med. 9 (2022), 868934.

[20] G.H. Gibbons, V.J. Dzau, The emerging concept of vascular remodeling, N. Engl. J. Med. 330 (1994) 1431–1438.

[21] Y. Miura, H. Suzuki, Hypertriglyceridemia and atherosclerotic carotid artery stenosis, Int. J. Mol. Sci. 23 (2022).

[22] Y. Miura, Y. Suzuki, H. Kanamaru, M. Shiba, R. Yasuda, N. Toma, H. Suzuki, Higher non-fasting serum triglyceride preceding the carotid stenosis progression, Neurol. Med. Chir. 61 (2021) 422–432.

[23] Y. Miura, H. Kanamaru, R. Yasuda, N. Toma, H. Suzuki, Nonfasting triglyceride as an independent predictor of carotid restenosis after carotid endarterectomy or carotid artery stenting, World Neurosurg. 156 (2021) e415–e425.

[24] Y. Miura, R. Yasuda, N. Toma, H. Suzuki, Non-fasting hypertriglyceridemia burden as a residual risk of the progression of carotid artery stenosis, Int. J. Mol. Sci. 23 (2022).

[25] M. Kitagami, R. Yasuda, N. Toma, M. Shiba, M. Nampei, Y. Yamamoto, Y. Nakatsuka, H. Sakaida, H. Suzuki, Impact of hypertriglyceridemia on carotid stenosis progression under normal low-density lipoprotein cholesterol levels, J. Stroke Cerebrovasc. Dis. 26 (2017) 1793–1800.

[26] Y. Mori, Y. Itoh, H. Komiya, N. Tajima, Association between postprandial remnant-like particle triglyceride (RLP-TG) levels and carotid intima-media thickness (IMT) in Japanese patients with type 2 diabetes: assessment by meal tolerance tests (MTT), Endocrine 28 (2005) 157–163.
[27] S. Teno, Y. Uto, H. Nagashima, Y. Endoh, Y. Iwamoto, Y. Omori, T. Takizawa, Association of postprandial hypertriglyceridemia and carotid intima-media thickness in patients with type 2 diabetes, Diabetes Care 23 (2000) 1401–1406.
[28] B. Ramms, S. Patel, X. Sun, A.R. Pessentheiner, G.M. Ducasa, A.E. Mullick, R. G. Lee, R.M. Crooke, S. Tsimikas, J.L. Witztum, P.L. Gordts, Interventional hepatic apoC-III knockdown improves atherosclerotic plaque stability and remodeling by triglyceride lowering, JCI Insight 7 (2022).
[29] A. Krüger-Genge, A. Blocki, R.P. Franke, F. Jung, Vascular endothelial cell biology: an update, Int. J. Mol. Sci. 20 (2019).
[30] X. Zhang, R. Qi, X. Xian, F. Yang, M. Blackstein, X. Deng, J. Fan, C. Ross, J. Karasinska, M.R. Hayden, G. Liu, Spontaneous atherosclerosis in aged lipoprotein lipase-deficient mice with severe hypertriglyceridemia on a normal chow diet, Circ. Res. 102 (2008) 250–256.
[31] H. Li, Y. Han, R. Qi, Y. Wang, X. Zhang, M. Yu, Y. Tang, M. Wang, Y.N. Shu, W. Huang, Aggravated restenosis and atherogenesis in ApoCIII transgenic mice but lack of protection in ApoCIII knockouts: the effect of authentic triglyceride-rich lipoproteins with and without ApoCIII, Cardiovasc. Res. 107 (2015) 579–589.
[32] H. Yingchun, M. Yahong, W. Jiangping, H. Xiaokui, Z. Xiaohong, Increased inflammation, endoplasmic reticulum stress and oxidative stress in endothelial and macrophage cells exacerbate atherosclerosis in ApoCIII transgenic mice, Lipids Health Dis. 17 (2018) 220.
[33] X. Lin, P. Ma, C. Yang, J. Wang, K. He, G. Chen, W. Huang, J. Fan, X. Xian, Y. Wang, G. Liu, Dietary-induced elevations of triglyceride-rich lipoproteins promote atherosclerosis in the low-density lipoprotein receptor knockout Syrian Golden Hamster, Front. Cardiovasc. Med. 8 (2021), 738060.
[34] M.A. Incalza, R. D'Oria, A. Natalicchio, S. Perrini, L. Laviola, F. Giorgino, Oxidative stress and reactive oxygen species in endothelial dysfunction associated with cardiovascular and metabolic diseases, Vasc. Pharmacol. 100 (2018) 1–19.
[35] S. Steven, K. Frenis, M. Oelze, S. Kalinovic, M. Kuntic, M.T. Bayo Jimenez, K. Vujacic-Mirski, J. Helmstädter, S. Kröller-Schön, T. Münzel, A. Daiber, Vascular inflammation and oxidative stress: major triggers for cardiovascular disease, Oxidative Med. Cell. Longev. 2019 (2019) 7092151.
[36] H. Sies, Oxidative stress: a concept in redox biology and medicine, Redox Biol. 4 (2015) 180–183.
[37] Y. Zhang, P. Murugesan, K. Huang, H. Cai, NADPH oxidases and oxidase crosstalk in cardiovascular diseases: novel therapeutic targets, Nat. Rev. Cardiol. 17 (2020) 170–194.
[38] A.M. Amanso, K.K. Griendling, Differential roles of NADPH oxidases in vascular physiology and pathophysiology, Front. Biosci. (Scholar edition) 4 (2012) 1044–1064.
[39] A. Konior, A. Schramm, M. Czesnikiewicz-Guzik, T.J. Guzik, NADPH oxidases in vascular pathology, Antioxid. Redox Signal. 20 (2014) 2794–2814.
[40] R. Loperena, D.G. Harrison, Oxidative stress and hypertensive diseases, Med. Clin. North Am. 101 (2017) 169–193.
[41] K.K. Griendling, L.L. Camargo, F.J. Rios, R. Alves-Lopes, A.C. Montezano, R. M. Touyz, Oxidative stress and hypertension, Circ. Res. 128 (2021) 993–1020.
[42] Y. Kigawa, T. Miyazaki, X.F. Lei, T. Nakamachi, T. Oguchi, J.R. Kim-Kaneyama, M. Taniyama, S. Tsunawaki, S. Shioda, A. Miyazaki, NADPH oxidase deficiency exacerbates angiotensin II-induced abdominal aortic aneurysms in mice, Arterioscler. Thromb. Vasc. Biol. 34 (2014) 2413–2420.
[43] K.L. Siu, Q. Li, Y. Zhang, J. Guo, J.Y. Youn, J. Du, H. Cai, NOX isoforms in the development of abdominal aortic aneurysm, Redox Biol. 11 (2017) 118–125.