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Systemic MCP1P1 deficiency in mice impairs lipid homeostasis

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ABSTRACT

Atherosclerosis involves interactions between inflammation system and dyslipidemia. MCP1P1 (Monocyte Chemotactic Protein induced Protein-1) is induced by proinflammatory molecules and serves as a negative feedback loop in regulating inflammatory responses. Our current study was designed to test the role of MCP1P1 in maintaining lipid homeostasis, the latter a pivotal factor that contributes to the pathogenesis of atherosclerosis. We found that MCP1P1 knockout mice displayed a decrease in levels of serum HDL-cholesterol and total triglycerides but an increase in serum LDL/VLDL-cholesterol levels when compared to wild-type mice. Additionally, ApoA-1 expression was reduced but LPL expression was upregulated in plasma from MCP1P1 knockout mice. The livers from the MCP1P1 knockout mice revealed a decrease in hepatocyte number and an increase in collagen deposition when compared to wild-type mice. These findings suggest that MCP1P1 deficiency can induce liver fibrosis, alter the expression of lipoproteins, and affect transportation and metabolism of lipids, indicating that MCP1P1 is involved in maintaining lipid homeostasis, possibly via negatively regulating inflammatory responses.

1. Introduction

Atherosclerosis is a chronic inflammatory disease that is characterized as a process of the interplay between hyperlipidemia and the inflammatory response. It begins with accumulation of oxidized LDL loaded foam cells in the intima of the artery followed by chronic inflammation which progresses to form atheromas (Alexander & Dzau, 2000; Collins et al., 2003; Pedrigo et al., 2014). Recruitment of circulating monocytes induced by monocyte chemotactic protein-1 (MCP-1) is one of the earliest cellular responses in the pathogenesis of atherosclerosis (Hansson, 2005; Libby, 2002). Differentiation of the monocytes into macrophages at the site followed by an uptake of oxidized LDL is the characteristic of atherosclerotic lesions.

Monocyte chemotactic protein induced protein-1 (MCP1P1) is a novel zinc-finger family (ZC3H12A) protein that is induced under inflammatory status (Monin et al., 2017) as well as by stimulation with pro-inflammatory molecules such as TNF- α , IL-1 β , and MCP-1 (Liang et al., 2008a; Liang et al., 2008b; Matsushita et al., 2009; Skalniak et al., 2009; Zhou et al., 2006). The function of MCP1P1 is to maintain

hemostasis of immune system function by serving as a negative regulator of immune cell activation and subsequent inflammatory responses. It does so by deubiquitinating inflammatory cytokine-associated proteins (Liang et al., 2008a, b), affecting the stability of inflammatory genes, or modulating the function (Suzuki et al., 2011), maturation and stability of microRNA of a set of inflammatory genes including IL-6 (Li et al., 2012; Matsushita et al., 2009; Mizgalska et al., 2009). The role of MCP1P1 in the pathogenesis of atherosclerosis is uncertain. It has been reported that MCP1P1 expression is increased in the atherosclerotic plaques in both human and mouse models and overexpression of MCP1P1 suppresses the interaction between monocytes and the activated endothelial cells, a crucial step in initiating the atherosclerotic process (Qi et al., 2010). Because of the anti-inflammatory nature of MCP1P1 and the role of inflammation in the pathogenesis of atherosclerosis, it was suggested that MCP1P1 is anti-atherogenic. However, recent studies revealed that MCP1P1 deficiency in mouse bone marrow is associated with systemic inflammation, but reduced plasma cholesterol levels and risk of atherosclerosis (Yu et al., 2013) and MCP1P1 is involved in angiotensin II-caused formation of unstable atherosclerotic plaques (Shu et al., 2019),

Abbreviations: MCP1P1, Monocyte Chemotactic Protein induced Protein-1; ApoB-100, apoprotein B100; ApoA-1, apoprotein A1; TNF- α , Tissue necrosis factor alpha; IL-1 β , interleukin 1 beta; MCP-1, monocyte chemoattractant protein 1; IL-6, interleukin 6.

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suggesting that MCP1P1 may be pro-atherogenic.

Because elevation of plasma cholesterol is considered a standalone risk factor that is crucial and may be sufficient enough in driving atherogenesis (Glass & Witztum, 2001), our current study was designed to study the role of MCP1P1 in maintaining lipid homeostasis by using MCP1P1 knockout mice. The purpose of this study was to better understand the role of MCP1P1 in lipid homeostasis and atherogenesis, and to provide insight for future mechanistic studies.

2. Materials and methods

2.1. Materials

Primers used for genotyping were purchased from IDT (Integrated DNA Technologies, Coralville, IA). AccuPrime™ SuperMix II was acquired from Invitrogen (through ThermoFischer Scientific). All other chemicals were obtained from Sigma–Aldrich except for chloroform (Fisher Scientific) and methanol (J.T. Baker Chemical Company).

2.2. Animals

C57/BL6 mice heterozygous for a mutated MCP1P1 gene were originally provided by Dr Pappachan Kolattukudy at University of Central Florida, 2010. We have been breeding offspring from those mice since. The mice were housed and cared for at ATSU Animal Care Facility with 12-h light and dark cycles, and a controlled room temperature of 25 °C. All mice had *ad libitum* access to food and water. The heterozygous mice were cross-bred to produce homozygous MCP1P1 knockout and homozygous/heterozygous non-mutated MCP1P1 mice (wild-type). Wild-type and litter-matched MCP1P1 knockout mice at the age of 6 weeks were used for this project to minimize litter effect. All procedures were approved by A.T. Still University-Kirkville College of Osteopathic Medicine Institution Animal Care and Use Committee (IACUC).

2.3. Genotyping

Wild-type, heterozygous, and MCP1P1 knockout mice were verified by PCR using primers pLA-up 5' ATG CCT TCC TGA TCC TAT TGG 3', pLA-low 5' CTT TCA TGA GCA ATG GTC GCA 3', and pLAZ 5' GTG CGG GCC TCT TCG CTA TTAC 3'.

2.4. Sample collection for lipid measurements

2.4.1. Serum collection

Mice were in a fasted state for 12 h prior to the experiment and were subsequently anesthetized with intraperitoneal injection of sodium pentobarbital (50 mg/kg). Blood was collected using the retrobulbar venous plexus puncture, centrifuged at 1400 g for 20 min at 4 °C and serum lipid levels were measured immediately after preparation by using commercially available kits. In order to minimizing the effect of acute heparin on lipid parameters, these mice were not injected with heparin (Katopodis et al., 2007).

2.4.2. Liver lipid extraction

Liver lipids were extracted by using Folch extraction method (Folch & Lees, 1957). After mice were sacrificed, livers were removed, snap frozen in liquid nitrogen, and stored at –80 °C for later experimentation. 50 mg of tissue was homogenized (using a Brinkmann Homogenizer, model PT 10/35) in a 1.5 mL chloroform/methanol (2:1) solution, mixed for 20 min on a Thermomixer (Eppendorf AG, 22331) at room temperature, and centrifuged (Effendorf, 541R) at 13000 rpm for 30 min at 20 °C. The lipid-containing supernatant was then extracted and washed with 200 µl of 150 mM sodium chloride (NaCl) and centrifuged at 2000 rpm for 5 min. A chloroform/lipid bilayer was formed and 200 µl of the lower organic lipid-containing layer was carefully removed from solution and mixed with a 40 µl of Triton-X Chloroform solution (1:1). The chloroform

and methanol were then removed by using a Laboport pump (KNF Neuberger, Inc, NJ), indicated by no weight loss after several measurements following prolonged vacuuming. We then re-suspended the solution in 200 µl of deionized water (final volume is 225 µl, and 1.125 dilution factor). This method extracts only total triglyceride and total cholesterol.

2.5. Sample collection for protein measurements

2.5.1. Plasma collection

Mice were in a fasted state for 12 h prior to the experiment. A bolus of 1 IU of sodium heparinate/g body weight was injected intraperitoneally to induce the release of lipoprotein lipase (LPL) from tissues (Vilella & Joven, 1998). LPL is normally anchored to the vascular endothelium. Binding of LPL to the surface of endothelial cells is through an interaction with heparin sulfate proteoglycans (Wang & Eckel, 2009). Heparin has higher binding affinity towards LPL than heparin sulfate proteoglycans. It binds to LPL and promotes the release of the LPL from its proteoglycan anchor (Brown et al., 2003; Vilella & Joven, 1998; Wang & Eckel, 2009). Thus, the total LPL levels measured in plasma represent the LPLs from endothelial cell surface of capillaries in pancreas, adipose tissue, skeletal muscle, and cardiac muscle. Mice were anesthetized by intraperitoneal injection of sodium pentobarbital (50 mg/kg). Blood was collected using the retrobulbar venous plexus puncture 20 min after the heparin injection, centrifuged at 1400 g for 20 min at 4 °C and stored at –20 °C for apoprotein measurements (Levak-Frank et al., 1995).

2.5.2. Protein extraction from liver

Following the blood collection, liver tissue was harvested from each animal, weighed, snap frozen in liquid nitrogen, and stored at –80 °C until processing. Liver protein extraction was conducted by following the modified published protocol (Ozcan et al., 2004). Briefly, the tissue (~0.3 g) was homogenized in 8 mL of lysis buffer containing 25 mM Tris–HCl (pH7.4), 2 mM Na₃VO₄, 100 mM NaF, 10 mM EDTA, 1% Igepal CA-630, 5 µg/mL leupeptin, 5 µg/mL aprotinin, and 2 mM phenylmethylsulfonyl fluoride and centrifuged at 5,000 rpm for 15 min at 4 °C. The supernatant was collected, further centrifuged at 32,000 rpm for 120 min at 4 °C, and stored at –20 °C. Protein concentration was measured using BCA protein assay reagents (Pierce, Rockford, IL) and apoprotein levels were measured by Western blot analysis.

2.6. Lipoprotein assay

Serum HDL and LDL-VLDL-cholesterol levels and liver total cholesterol were measured using Sigma–Aldrich HDL and LDL/VLDL Quantitation kit (Catalog Numbers MAK045) by following the manufacturer's instructions and quantified with a spectrophotometer (BioTek Instruments, Epoch model) at 560 nm. For serum HDL-cholesterol and LDL/VLDL-cholesterol quantitation, HDL and LDL/VLDL were separated during the process. The cholesterol concentration of each is determined using cholesterol esterase in a coupled enzyme assay which produces a color proportional to the cholesterol present. Measurements were calculated by comparing to a standard curve and expressed as µg per µl of serum. Liver total cholesterol was measured by following the similar procedure except no separation of HDL and LDL/VLDL was performed. Both serum total triglyceride and liver total triglyceride were determined by using the Sigma–Aldrich Serum Triglyceride Determination Kit (Catalog Number TR0100) following the manufacturer's instructions and quantified with a spectrophotometer (BioTek Instruments, Epoch model) at 540 nm.

2.7. Western blot analysis

Western blot analysis was used to detect protein expression of LDL receptor in liver lysates (Kim et al., 2012; Ozcan et al., 2004) and ApoA-1, ApoB-100, and LPL in plasma. Proteins were separated on SDS

polyacrylamide gels, probed with primary antibodies against LDL receptor, ApoB-100, ApoA-1, and LPL (Abcam, Cambridge, MA) respectively, and labeled with horseradish peroxidase (HRP)-conjugated secondary antibodies. Signals were detected by reacting with chemiluminescence and exposing to X-Ray film (Midsci, St. Louis, MO). Band densities were quantified by using Image J software. The membranes were stripped and re-probed with β -tubulin for liver samples or transferrin for plasma samples (Cell Signaling Technology, Beverly, MA and Proteintech Group Inc., Chicago, IL respectively) as internal controls (Johnson, 2012).

2.8. Liver histology

Mice were anesthetized by intraperitoneal injection of overdose sodium pentobarbital (120 mg/kg) and fixed by *in vivo* perfusion with 4% of formaldehyde through left ventricle. Liver tissues were dissected, further fixed in 4% of formaldehyde for 24 h, processed using a Leica TP1020 semi-enclosed benchtop tissue processor (Leica Microsystems, Nussloch, Germany), and embedded in paraffin using a Leico embedding station. Specimens were sectioned using a Leica RM2255 microtome. Cross-sections of 8 μ m were stained with hematoxylin & eosin and Masson's trichrome. Images were captured with a Leica DFC400 digital microscope camera mounted on a Leica DM4000B microscope using Surveyor Software (Objective Imaging Ltd., Cambridge, United Kingdom).

2.9. Statistical analysis

Data were analyzed using GraphPad Prism 5 (GraphPad Software Inc.). An unpaired *t*-test was performed and data are expressed as mean \pm standard error. *p* value of less than 0.05 was considered statistically significant.

3. Results

3.1. MCPIP1 knockout mice displayed reduced serum HDL-cholesterol and total triglyceride levels but increased LDL/VLDL-cholesterol levels

Although the previous study found that organ-specific (bone marrow) MCPIP1 deficiency mice displays drastic reduction of atherosclerotic lesions when compared to wild-type mice, the minor reduction of plasma total cholesterol observed in these mice is not sufficient to explain the eradication of atherosclerotic lesions (Yu et al., 2013). The two major limitations of their experiment are: firstly, HDL-cholesterol was not separated from LDL- and VLDL-cholesterol in the abovementioned study. Total plasma cholesterol mainly reflects the LDL- and HDL-cholesterol levels. Since elevation of plasma LDL-cholesterol is a major contributor to the pathogenesis of atherosclerosis and HDL-cholesterol may be anti-atherogenic by reverse transporting cholesterol from peripheral tissues to the liver to be metabolized, it is not clear if the anti-atherogenic effect observed in bone marrow MCPIP1 deficient mice is mainly due to reduced LDL-cholesterol alone or a combination of reduced LDL-cholesterol and increased HDL-cholesterol (since only a slight reduction of total cholesterol was found). Secondly, the model used in the abovementioned study was organ-specific MCPIP1 deficient mice. It does not reflect the systemic effects of MCPIP1, especially the effects of MCPIP1 as an inflammatory modulator on organs and tissues that are involved in maintaining lipid homeostasis. Based on these results, it appears that the anti-atherogenic effect of MCPIP1 observed in these mice is dissociated from the severe systemic inflammation. Hence, our current experiment was designed to study the effect of MCPIP1 on lipid homeostasis by separating HDL-cholesterol from LDL/VLDL-cholesterol in MCPIP1 knockout mice. In addition, we also measured the changes of total triglyceride in wild-type and MCPIP1 knockout mice. We first compared the differences among MCPIP1 knockout mice at ages of 4, 6, and 8 weeks and we found no significant differences in plasma lipid

levels (data not shown). Therefore, mice at the age of 6 weeks were used in the following experiments. We used a commercially available cholesterol kit that separates HDL-cholesterol from LDL/VLDL-cholesterol but not LDL-cholesterol and VLDL-cholesterol. Inconsistent with the findings from the previous study, we found that compared to wild-type mice, MCPIP1 knockout mice showed an average of 40% reduction of serum HDL-cholesterol (0.90 ± 0.06 versus 0.54 ± 0.04 μ g/ μ l, *p* < 0.05, Fig. 1A) but an 87% increase in serum LDL/VLDL-cholesterol (0.17 ± 0.01 versus 0.31 ± 0.03 μ g/ μ l, *p* < 0.05, Fig. 1B). In addition, MCPIP1 knockout mice also showed a significant reduction of serum total triglyceride when compared to wild-type mice. The average reduction of total triglyceride in MCPIP1 knockout mice was 37% when compared to wild-type mice (1.54 ± 0.13 versus 0.97 ± 0.09 mg/mL, *p* < 0.05, Fig. 1C).

3.2. MCPIP1 knockout modified the expression of plasma apoproteins and LPL

To understand the potential mechanisms behind the changes of serum HDL-cholesterol, LDL-cholesterol, and total triglyceride levels observed in our experiments, our next experiments were designed to quantify the levels of several major structural apoproteins in these lipoproteins, including plasma ApoA-1 protein levels (a major structural protein in HDL) and ApoB-100 (a major structural protein in LDL). We found that consistent with the reduction of HDL-cholesterol observed in serum, MCPIP1 knockout mice showed a significant decrease in plasma ApoA-1 expression when compared to wild-type mice (*p* < 0.05, Fig. 2A). The protein level of plasma ApoA-1 in MCPIP1 knockout mice was about 10% of that in wild-type mice. ApoA-1 is predominantly synthesized in the liver. Biosynthesis of ApoA-1 is the first step in HDL formation. The reduced ApoA-1 in MCPIP1 knockout mice indicates that MCPIP1 deficiency reduces HDL-cholesterol by decreasing its synthesis. In contrast to the increase in serum LDL/VLDL-cholesterol, our data revealed that MCPIP1 knockout mice displayed a significant reduction in plasma ApoB-100 expression when compared to wild-type mice (*p* < 0.05, Fig. 2B). Since ApoB-100 is the major apoprotein in LDL and acts as a ligand for the LDL receptor and thus plays an important role in the metabolism of lipoprotein particles, the reduction of ApoB-100 suggests that the increase in serum LDL/VLDL-cholesterol observed in MCPIP1 knockout mice may not occur as the result of increased synthesis of LDL/VLDL-cholesterol but possibly decreased metabolism. Consistent with the reduction of total triglyceride observed in plasma, the protein levels of total plasma LPL in MCPIP1 knockout mice increased by about 4-fold when compared to wild-type mice (*p* < 0.05, Fig. 2C). Since LPL is responsible for hydrolysis of triglycerides to fatty acids in chylomicrons and VLDL, the increased activity of LPL indicates that the reduction of serum triglyceride may be the result of increased metabolism by LPL.

3.3. MCPIP1 knockout caused a slight but not significant decrease in the expression of LDL receptors in liver tissue

Cholesterol is mainly synthesized and also metabolized in the liver. Liver is responsible for removal of about 70% of LDL-cholesterol from plasma. It does so by LDL receptor-mediated pathways. LDL receptors on hepatocytes recognizes the ApoB-100 in LDL-cholesterol particles and promotes endocytosis and degradation of LDL-cholesterol in endosomes (Brown & Goldstein, 1986). Impairment of LDL receptor pathways is associated with familial hypercholesterolemia, while on the other hand, prolonging the life span of LDL receptors has become an important target of antihypercholesterolemic and antiatherosclerotic therapy (Leren, 2014).

To assess if the increased serum LDL/VLDL-cholesterol is caused by reduced hepatic metabolism of cholesterol via decreased expression of LDL receptors, our next experiment was designed to measure the LDL receptor expression in liver tissue in both wild-type and MCPIP1

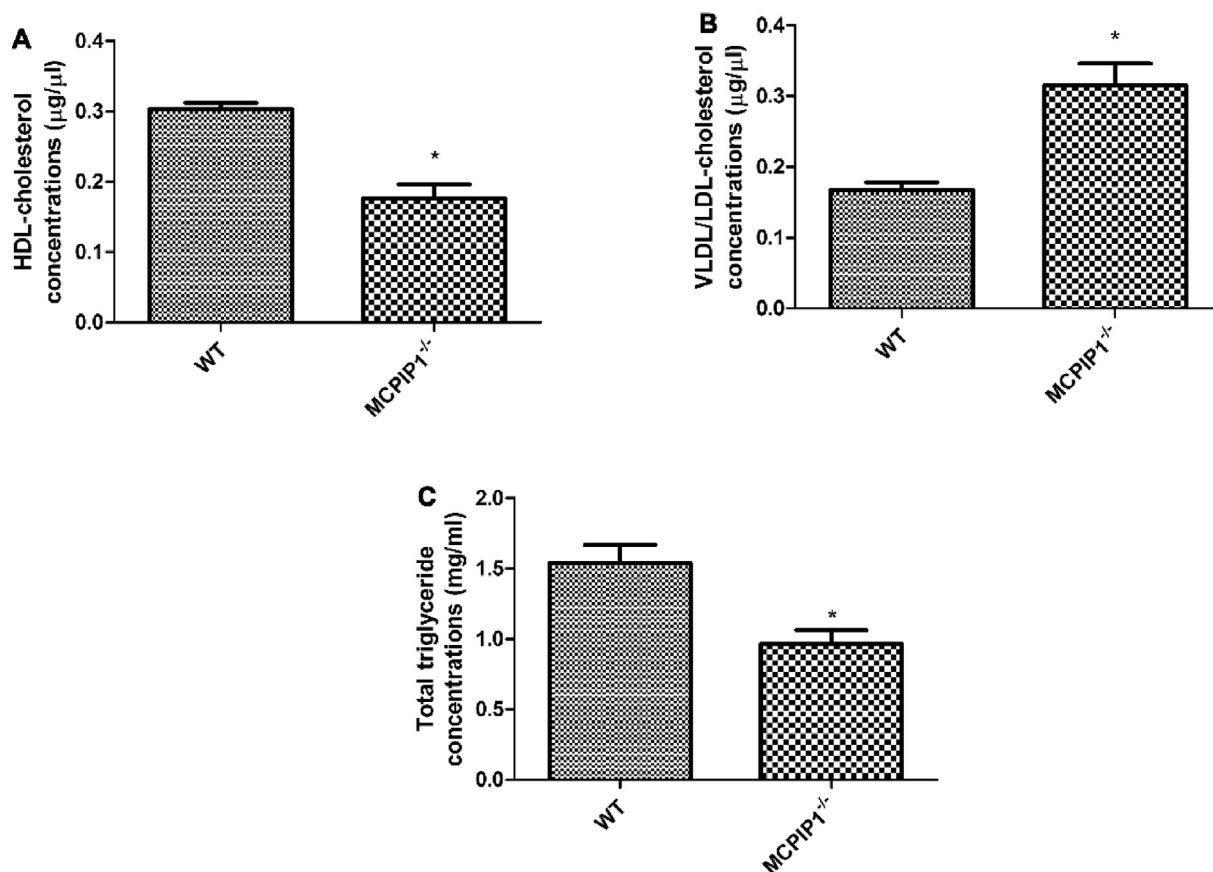


Fig. 1. Effect of MCPIP1 knockout (MCPIP1^{-/-}) on mouse serum HDL-cholesterol (1A), LDL/VLDL-cholesterol (1B), total triglyceride (1C) at the age of 6 weeks. Data are expressed as mean ± SE (n = 8 for each group). * denotes p < 0.05 compared to wild-type mice (WT).

knockout mice. We found that although there was a trend of reduction in LDL receptor protein levels in MCPIP1 knockout mice (about 75% of that in wild-type mice), no statistically significant difference was observed (p > 0.05, Fig. 2D).

3.4. MCPIP1 knockout reduced white adipose tissue weight along with total body weight

White adipose tissue is one of the major storage sites of triglyceride. The main function of white adipose tissue is to synthesize and store triglycerides and to release free fatty acids into the blood stream when needed. The size of adipocytes affects the weight of adipose tissue and is positively correlated to blood triglyceride level (Angel & Farkas, 1974; Kovanen et al., 1975; Schreiber & Dell, 1975; Krause & Hartman, 1976). Our current study was designed to test if the reduced serum total triglyceride levels observed in MCPIP1 knockout mice affects adipose tissue weight. A pool of the fat tissues from inguinal, epididymal, abdominal, and intrascapular white fat pads of WT and MCPIP1 knockout mice were dissected, weighed, and compared. Consistent with the reduction of serum total triglyceride levels, we found that MCPIP1 knockout mice showed about 92% reduction of total ratio of white fat/body weight compared to wild-type mice (p < 0.05, Fig. 3D), indicating that MCPIP1 is essential for maintaining the size of white adipocytes and adipose tissue weight, possibly by regulating the triglyceride levels. Consistent with the findings from the other studies (Matsushita et al., 2009), our data also revealed that the average body weight and length of MCPIP1 knockout mice was about 47% and 40% less than wild-type mice, respectively (p < 0.05, Fig. 3A, B, and C), suggesting that MCPIP1 is not only involved in maintaining adipose tissue weight but also essential in maintaining normal growth.

3.5. MCPIP1 knockout reduced hepatic total triglyceride but had no effect on total cholesterol levels

The liver plays a central role in lipid metabolism, including lipoprotein uptake, synthesis and export to the circulation. Chronic liver disease can impact hepatic lipid metabolism leading to alterations in circulating lipid levels thus contributing to dyslipidemia. Our next experiment was designed to test the involvement of liver in dyslipidemia observed in MCPIP1 knockout mice by comparing hepatic lipid changes with wild-type mice. Consistent with the reduced triglyceride in serum observed in our abovementioned experiments, MCPIP1 knockout mice showed a significant (71.6%) reduction of hepatic total triglyceride levels when compared to wild-type mice (6.27 ± 1.53 versus 1.78 ± 0.15 µg/g of tissue, p < 0.05, Fig. 4A), suggesting that in addition to the increased expression of LPL, the reduced synthesis of triglyceride by liver may also be responsible for the decreased serum triglyceride in MCPIP1 knockout mice. No statistically significant difference was found in liver total cholesterol levels in MCPIP1 knockout mice when compared to wild-type mice (P = 0.87, Fig. 4B). Since the liver synthesizes and metabolizes cholesterol, our result indicates that the increased serum LDL/VLDL-cholesterol is not due to increased hepatic synthesis, but possibly reduced metabolism.

3.6. MCPIP1 knockout mice displayed hepatic fibrosis

It is well established that hepatic dysfunction is associated with dyslipidemia. Previous studies revealed that MCPIP1 is a negative regulator of inflammatory responses and mice lacking MCPIP1 present with multi-organ inflammation, manifested as infiltration of inflammatory cells including liver. Our next experiment was designed to study the impact of MCPIP1 deficiency on morphological changes of liver in order

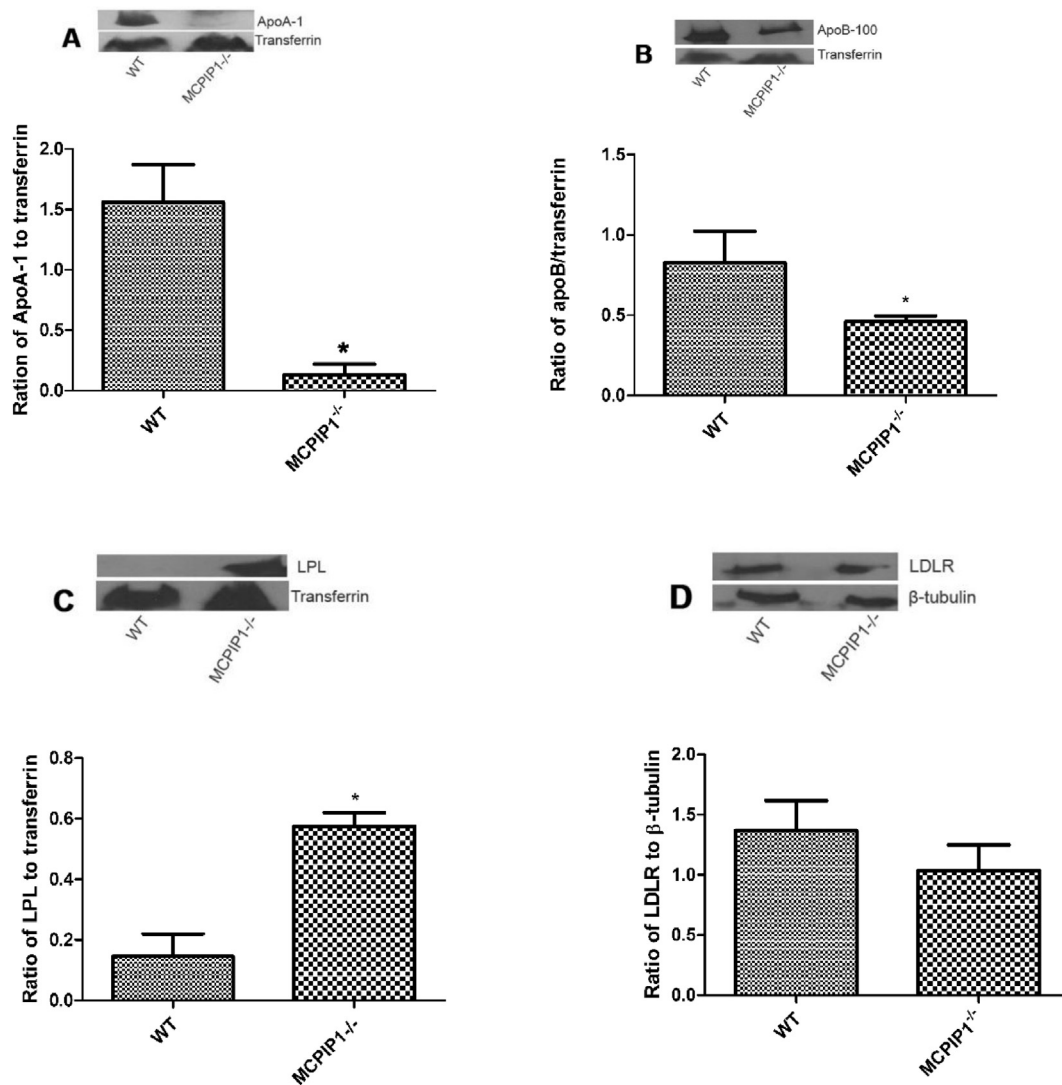


Fig. 2. Effect of MCPIP1 knockout (MCPIP1^{-/-}) on expression of plasma ApoA-1 (2A), ApoB-100 (2B), LPL (2C) and hepatic LDL receptor (2D). Upper panels indicate the representative Western blot. Lower panels depict the averaged ratio apoproteins to Transferrin or LDL receptor to β-tubulin. The results are expressed as mean ± SE n = 5. * denotes p < 0.05 when compared to WT.

to further understand the mechanisms behind the dyslipidemia in MCPIP1 knockout mice. Gross observation revealed an enlarged and pale liver with an external nodular surface in MCPIP1 knockout mice (data not shown). During dissection of the tissue, livers from MCPIP1 knockout mice appeared to be denser and fibrous compared to livers from wild-type mice, although no difference was observed in total liver weight between wild-type and MCPIP1 knockout mice. Liver hematoxylin and eosin histology staining from MCPIP1 knockout mice revealed a reduced number of hepatocytes and multiple foci that contain collagen and bile duct in the interlobular area (Fig. 5). Masson-trichrome staining showed matrix deposition in the centrilobular portal tract area in MCPIP1 knockout mice but not in wild-type mice, indicating the presence of hepatic fibrosis and remodeling in MCPIP1 deficient mice.

4. Discussion

Atherosclerosis is a multifactorial inflammatory disease involving the interaction among plasma lipoproteins, genetics, and environmental factors. It is well established that MCPIP1 is an important regulator of inflammation and immune homeostasis. MCPIP1 deficient mice not only show fatal immune-related lesions but also increased production of inflammatory cytokines and systemic inflammation (Miao et al., 2013).

Over-expression of MCPIP1 results in a decrease in the transcription of inflammatory cytokines (Mizgalska et al., 2009) by promoting the degradation of inflammatory cytokine mRNA and inhibiting the signaling pathway of NF-κB (Iwasaki et al., 2011; Liang et al., 2008a, b; Matsushita et al., 2009; Mizgalska et al., 2009). A previous study revealed that MCPIP1 expression is significantly upregulated in the atherosclerotic plaques in both human and mouse models and that overexpression of MCPIP1 inhibits monocyte adhesion to the activated endothelial cells, a crucial step in initiating atherosclerotic process (Qi et al., 2010), suggesting that MCPIP1 may serve as a negative feedback regulator in the atherogenic interaction between macrophages and endothelial cells. However, a recent study revealed that although bone marrow MCPIP1 deficient mice present with severe inflammation, there is only slight but significant reduction of plasma total cholesterol levels, and no atherosclerotic lesions in spite of being fed a western diet (Yu et al., 2013). Others found that MCPIP1 is a mediator in angiotensin II-induced formation of unstable atherosclerotic plaques (Shu et al., 2019). These results contradict the anti-inflammatory nature of MCPIP1 and the role of inflammation in the pathogenesis of atherosclerosis, suggesting that the role of MCPIP1 in pathogenesis of atherosclerosis is uncertain.

Because elevation and retention of blood cholesterol are considered unique and crucial, and may be sufficient in driving the development of

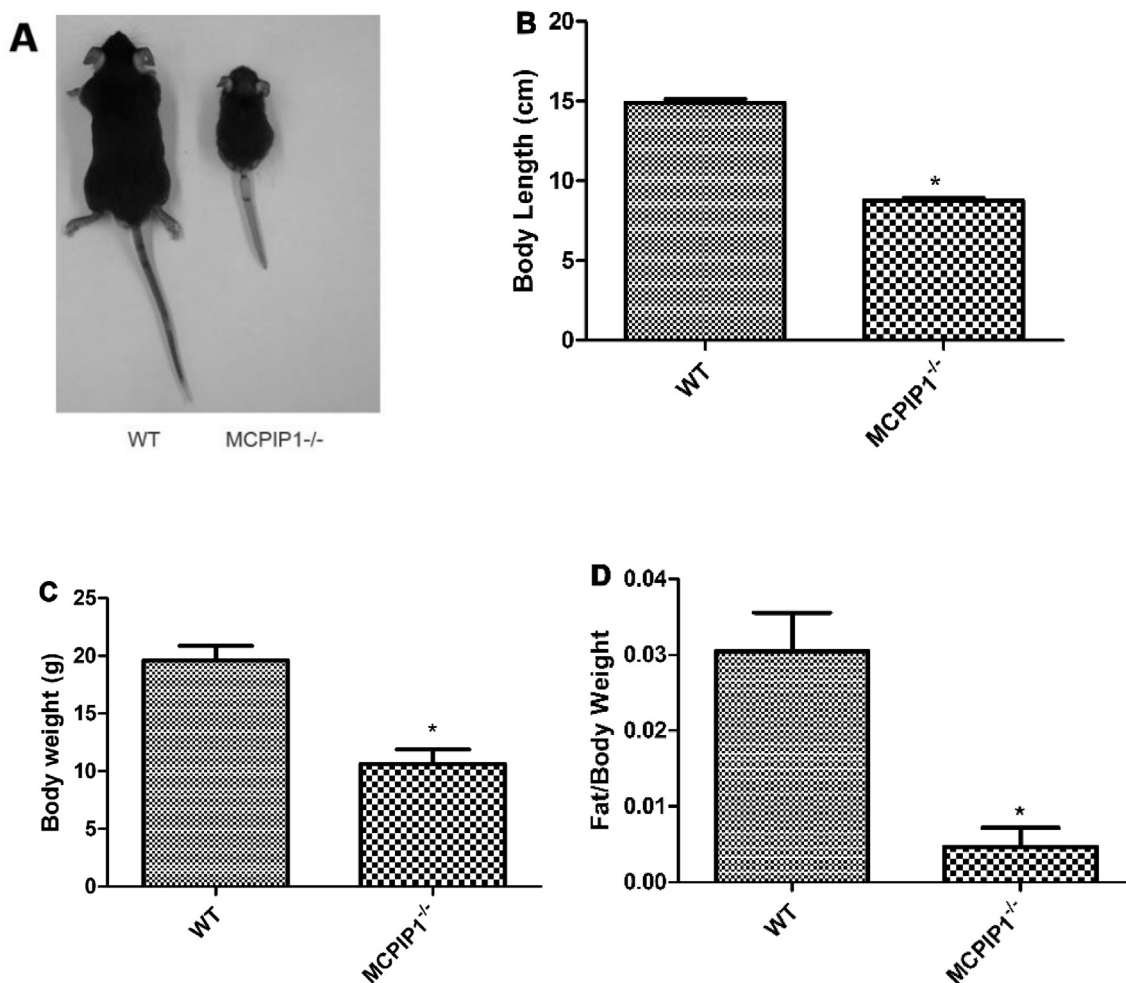


Fig. 3. Effect of MCPIP1 knockout (MCPIP1^{-/-}) on mouse body size (3A and 3B), weight (3C), ratio of fat tissue to body weight (3D) at the age of 6 weeks. Data are expressed as mean ± SE (n = 8 for each group). * denotes p < 0.05 compared to wild-type mice (WT).

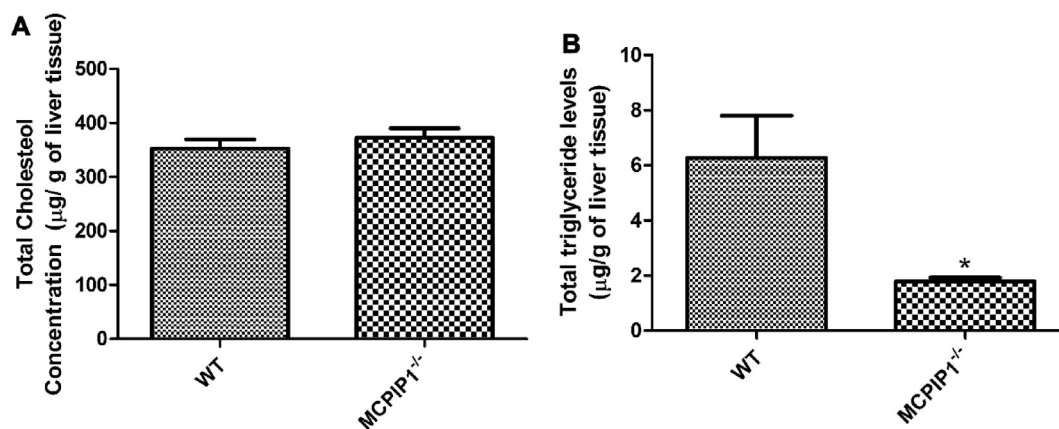


Fig. 4. Effect of MCPIP1 knockout (MCPIP1^{-/-}) on hepatic total triglyceride (4A) and cholesterol (4B) levels at the age of 6 weeks. Data are expressed as mean ± SE (n = 8 for each group). * denotes p < 0.05 compared to wild-type mice (WT).

atherosclerosis, even without the presence of other risk factors (Glass & Witztum, 2001), our current study was designed to test the possible role of MCPIP1 in atherogenesis by studying the effect of systemic MCPIP1 deficiency on lipid homeostasis. It should be noted that instead of testing total serum cholesterol as other studies did (Yu et al., 2013), we separated HDL-cholesterol from LDL- and VLDL-cholesterol. We found that mice with systemic MCPIP1 deficiency displayed a significant elevation

in serum LDL/VLDL-cholesterol levels. Since blood concentration of cholesterol is maintained by the balance between synthesis and metabolism, our next experiment was designed to determine if LDL- and VLDL-cholesterol elevation is caused by increased synthesis by measuring the plasma level of ApoB-100, a major structural apoprotein in LDL and VLDL. ApoB-100 is synthesized in the liver and serves as a ligand for the LDL receptor and therefore plays a critical role in the metabolism

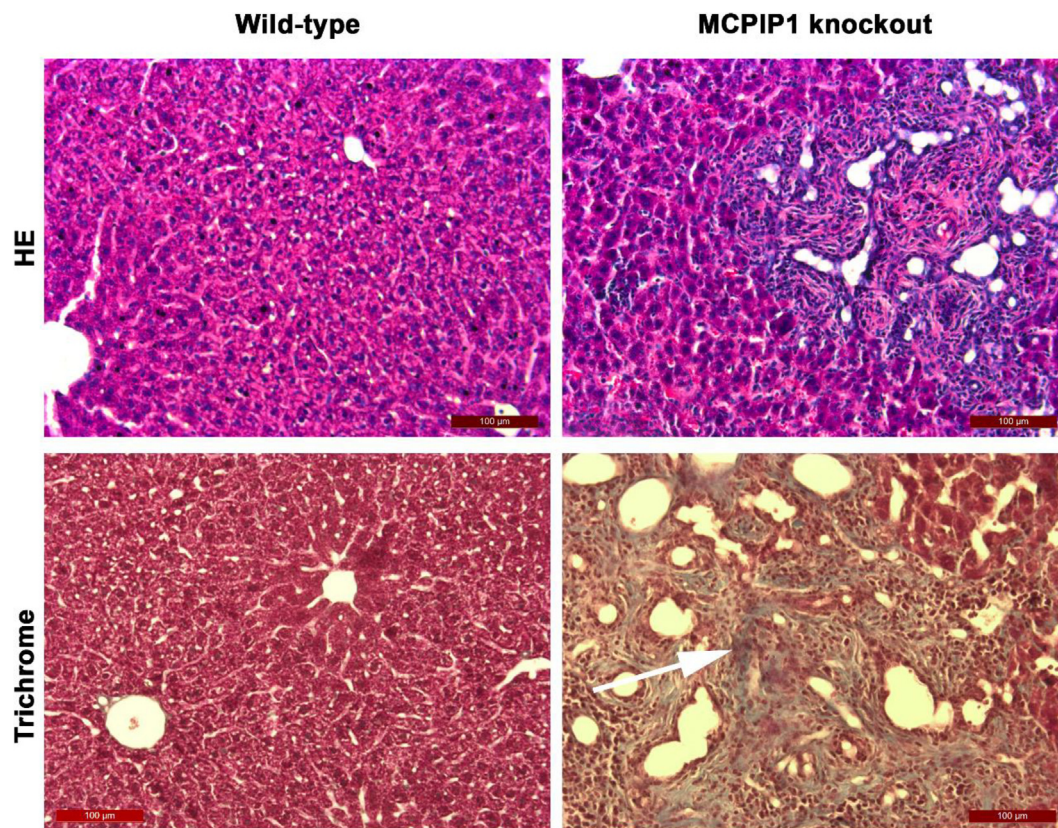


Fig. 5. Morphological changes of liver tissue in WT and MCPIP1 knockout mice. Arrows indicate collagen staining.

of lipoprotein particles. We found a significant reduction in plasma ApoB-100 expression in MCPIP1 knockout mice, indicating that the upregulation of LDL/VLDL-cholesterol is not due to increased synthesis but possibly reduced metabolism. Therefore, we assessed possible changes of the LDL receptor, a facilitator of cholesterol intake and metabolism. The LDL receptors are present on the surface of hepatocytes and mediate the uptake and subsequent metabolism of LDL-cholesterol by recognizing ApoB-100. Hence, LDL receptors play an important role in determining blood concentration of LDL-cholesterol. Our result revealed that although there is a downward trajectory of LDL receptor expression in MCPIP1 knockout mice when compared to wild-type mice, no statistically significant difference was observed. There are two possible explanations for our findings: 1) although not statistically significant, the level of reduction of LDL receptor expression is sufficient enough to reduce the metabolism of cholesterol, or 2) other factors may be involved in metabolism of LDL/VLDL-cholesterol, such as lipolysis-stimulated lipoprotein (LSR), a receptor that is believed to be involved in ApoB-containing lipoprotein metabolism in liver, may be affected in this case (Jun et al., 2011). We also found that MCPIP1 knockout mice developed severe hepatic fibrosis, a possible result of systemic inflammation. These results, together with the significant reduction of ApoB-100 expression, slight but insignificant reduction of LDL receptors, and marked hepatic fibrosis, suggest that it is possible that the elevation of serum LDL/VLDL-cholesterol may occur through a reduction in metabolism but not an increase in synthesis (Meisenberg & Simmons, 2017e).

We also found a significant decrease in serum HDL-cholesterol levels. HDL is a reverse transporter of cholesterol that is considered anti-atherogenic. Since elevation of HDL-cholesterol is associated with reduced risk of cardiovascular events (Holven et al., 2013), the combination of increased LDL/VLDL-cholesterol and reduced HDL-cholesterol suggests that systemic MCPIP1 deficiency may be associated with increased risk of atherosclerosis. Our findings are opposite to the ones

revealed by the others using MCPIP1 deficient bone marrow cells (Yu et al., 2013) but consistent with the ones showing the inhibitory role of MCPIP1 in interaction between monocyte and endothelial cells, a key step in initiating atherosclerotic process (Qi et al., 2010). Together, dyslipidemia observed in MCPIP1 knockout mice and the anti-inflammatory nature of MCPIP1 shown by others suggest that MCPIP1 is anti-atherogenic. Consistent with the reduction of serum HDL-cholesterol observed in MCPIP1 knockout mice, we found that plasma ApoA-1, a structural apoprotein in HDL, was drastically decreased in MCPIP1 knockout mice. As a major structural and functional apoprotein in HDL, ApoA-1 is essential for cholesterol reverse transport (55). It is synthesized primarily in hepatocytes and plays a crucial role in the transportation of cholesterol from the peripheral tissues to the liver to be metabolized (Kuyil & Mendelsohn, 1992; Perez-Martinez, Lopez-Miranda, Perez-Jimenez, & Ordovas, 2008; Sorci-Thomas et al., 1989). The decreased ApoA-1 protein levels in MCPIP1 knockout mice indicates that the reduction of HDL-cholesterol may be the result of decreased synthesis, possibly due to the pathological changes in the liver.

We also discovered some unique findings associated with our MCPIP1 deficient mouse model - a significant reduction of both serum and liver total triglyceride levels and an increase in plasma total lipoprotein lipase (LPL) expression, indicating the reduction of serum triglycerides is due to enhanced metabolism. This result is in line with the decreased white adipose tissue weight observed in the MCPIP1 knockout mice. The exact function of the upregulated LPL in atherogenesis is not fully understood. Some studies indicate LPL is pro-atherogenic but others suggest that it is anti-atherogenic (Alaupovic, Mack, Knight-Gibson, & Hodis, 1997; Gaziano, Hennekens, O'Donnell, Breslow, & Buring, 1997; Mack, Krauss, & Hodis, 1996; McGill Jr. et al., 1997). Synthesized in muscle, heart, and adipose tissue, LPL hydrolyzes triglycerides in chylomicrons and VLDL to fatty acids, which can be taken up and used by cells. LPL expression is regulated by a variety of physiological and pathophysiological stimulations. It has been discovered that LPL mRNA levels are increased in

macrophage-derived foam cells in atherosclerotic lesions (Yla-Herttuala et al., 1991) and LPL protein expression in arterial atherosclerotic plaques is associated with progression of atherosclerotic lesions (Clee et al., 2000; Takahashi et al., 2008; Wilson, Fry, Chappell, Sigmund, & Medh, 2001), suggesting that LPL may be pro-atherogenic. Our results from systemic MCP1P1 knockout mice, including the reduced HDL-cholesterol and increased LDL/VLDL-cholesterol, support the hypothesis that the upregulation of LPL may be associated with increased risk of atherosclerosis.

We also found a reduction of hepatocyte number and multiple collagen-containing foci in MCP1P1 knockout mice, providing the evidence of hepatic remodeling, a process that may occur through increased inflammatory cell infiltration (Miao et al., 2013). These changes may contribute to the alterations of functional properties of the liver, such as lipid synthesis and metabolism.

5. Conclusions

In conclusion, we are the first to show that the systemic absence of MCP1P1 is associated with increased LDL/VLDL-cholesterol, reduced HDL-cholesterol, upregulation of LPL, and liver remodeling, suggesting that MCP1P1 is involved in maintaining lipid homeostasis. These findings suggest that systemic MCP1P1 deficiency may be associated with increased risk of atherosclerosis. Our results are in line with the anti-inflammatory nature of MCP1P1 and consistent with the concept that sustained inflammation (occurred in MCP1P1 deficiency mice) potentially alters lipid metabolic homeostasis, leading to dyslipidemia and increased risk of atherosclerosis (Hansson, Libby, & Tabas, 2015). Modulation of MCP1P1 could be a potential therapeutic target for prevention or treatment of atherosclerotic cardiovascular disease.

One of the major limitations of this study is that MCP1P1 knockout mice have shorter lifespan (maximal of 10 weeks) and premature death prevents us from directly testing if these mice have increased or decreased risk of atherosclerosis when fed with western diet since it usually takes several months to develop atherosclerotic lesions, even with genetically modified mice.

Author contributions

Joshua Moody, Methodology, Validation, Formal analysis, Investigation, Data Curation, Funding acquisition, Writing - Review & Editing.

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Declaration of Competing Interest

None declared.

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