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Basic Research

MD1 Deficiency Promotes Inflammatory Atrial Remodelling Induced by High-Fat Diets

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ABSTRACT

Background: Myeloid differentiation protein 1 (MD1) is expressed in various tissues, including the heart. However, the role of MD1 in obesity-related atrial remodelling remains incompletely understood. Here, this study intends to determine the regulatory role and underlying mechanisms of MD1 in obesity-related atrial remodelling.

Methods: A high-fat diet (HFD) feeding was performed in 6-week-old MD1-knockout (MD1-KO) mice and wild-type (WT) littermates for 20 weeks. Morphological, biochemical, functional, histological, and electrophysiological studies were conducted at the age of 26 weeks.

Results: Our results revealed that the MD1 expression levels were downregulated in the atrium of the HFD-fed induced obesity mice. An increase in body weight, glucose intolerance, hyperlipidemia, and

RÉSUMÉ

Contexte : La protéine de différenciation myéloïde 1 (MD1) est exprimée dans différents tissus, y compris celui du cœur. Toutefois, le rôle de la MD1 dans le remodelage auriculaire lié à l'obésité n'a pas été complètement élucidé. Cette étude a pour but de déterminer le rôle régulateur de la protéine MD1 et les mécanismes sous-jacents dans le remodelage auriculaire lié à l'obésité.

Méthodologie : Une alimentation riche en graisses (ARG) a été donnée pendant 20 semaines à des souris âgées de 6 semaines dont le gène de la MD1 avait été inactivé (MD1-KO) et à des souris de type sauvage (TS) de la même portée. Des analyses morphologiques, biochimiques, fonctionnelles, histologiques et électrophysiologiques ont été réalisées à l'âge de 26 semaines.

Obesity is an independent risk factor for atrial fibrillation (AF) initiation, progression, and cardiovascular outcomes.[1,2] Extensive evidence indicated that consumption of a high-fat diet (HFD) can lead to metabolic syndrome, increasing the susceptibility to myocardial damage via fibrosis and inflammation.[3,4] However, the key molecules involved in AF caused by HFD-induced obesity are yet to be determined.

The toll-like receptor 4 (TLR4)-mediated signal-transduction pathway plays an important role in cardiac remodelling caused by obesity.[5] TLR4 is a type of receptor for free fatty acids, which can mediate a series of intercellular signal-transduction processes including inflammation, oxidative stress, autophagy, and cell death.[6-8] It was found that the TLR4-signalling pathway in the heart tissues of obese animals was significantly activated, which could mediate pathological and physiological responses such as inflammation, cardiac hypertrophy, oxidative stress, autophagy, and fibrosis.[9-11]

Myeloid differentiation protein 1 (MD1) is a type of secreted glycoprotein, and it can form a complex with radioprotective protein 105 (RP105), called MD1-RP105.[12] The MD1-RP105 complex can directly interact with the MD2 (myeloid differentiation protein 2)-TLR4 complex by a lateral binding, acting as a physiological negative regulator of the TLR4 signalling pathway.[13] Our previous studies showed that MD1 was widely expressed in cardiac tissues, and MD-1 deficiency aggravated cardiac hypertrophy, cardiac dysfunction, and left ventricular (LV) electrical remodelling in response to chronic pressure overload, resulting in overt heart failure (HF) and increased electrophysiological instability.[14,15] However, the important regulatory factors and potential molecular mechanisms of MD1 in obesity-related atrial remodelling have not yet been studied.

To further investigate that, we conducted a study and found that MD1 expression is downregulated in HFD-induced obese mice atrium. Using loss-of-function approach, we observed that HFD-induced atrial fibrosis and inflammation were aggravated in MD1-knockout (MD1-KO) mice. We identified that the MD1-KO deterioration effect on atrial fibrosis and inflammation was associated with the activation of TLR4/NF-κB pathways. Our findings demonstrate that MD1 deficiency aggravates the development of atrial remodelling caused by HFD-induced obesity through the enhanced activation of TLR4/NF-κB signalling pathways.

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adverse atrial remodelling, such as atrial inflammation and fibrosis, were induced by HFD feeding in WT mice. Vulnerability to atrial fibrillation (AF) was also significantly increased by HFD feeding in WT mice. In addition, these adverse effects caused by HFD-fed induced obesity were further exaggerated in MD1-KO mice compared with WT mice. Mechanistically, MD1-KO activated TLR4/NF-κB signaling pathways, which led to atrial remodelling in mice fed by HFD by increasing the phosphorylation of p65 and IκBα.

Conclusions: Our data suggested that MD1 deficiency played an important role in accelerating the development of inflammatory atrial fibrosis and increasing vulnerability to AF in mice with HFD-fed induced obesity, providing an essential target for improving HFD-induced atrial remodelling.

Résultats : Nos résultats ont montré que les degrés d'expression de MD1 étaient régulés négativement dans l'oreillette des souris dont l'obésité avait été induite par l'ARG qu'elles avaient reçue. Chez les souris TS, une augmentation du poids corporel, l'intolérance au glucose, l'hyperlipidémie et le remodelage auriculaire, qui se manifeste notamment par l'inflammation et la fibrose auriculaires, ont été induits par l'ARG. La vulnérabilité à la fibrillation auriculaire (FA) a aussi été fortement augmentée par l'ARG chez les souris TS. De plus, ces effets indésirables causés par l'obésité induite par l'ARG étaient beaucoup plus marqués chez les souris MD1-KO que chez les souris TS. Du point de vue des mécanismes en jeu, chez les souris MD1-KO, les voies de signalisation TLR4/NF-κB ont été activées, ce qui a conduit à un remodelage auriculaire chez les souris ayant reçu une ARG en stimulant la phosphorylation de p65 et d'IκBα.

Conclusions : Nos données laissent croire que la carence en MD1 a joué un rôle important en accélérant l'apparition de la fibrose auriculaire inflammatoire et en augmentant la vulnérabilité à la FA chez les souris présentant une obésité induite par l'ARG, mettant ainsi en évidence une cible essentielle pour améliorer le remodelage auriculaire induit par l'ARG.

Methods

Experimental animals

All experiments involving animals were approved by the Animal Care and Use Committee of Renmin Hospital at Wuhan University and confirmed by the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (8th Edition, NRC 2011). All animals were housed in an environment with controlled light cycles (12 hours light/12 hours dark), temperature, and humidity; food and water were provided *ad libitum*. All animal experiments were performed by an experimenter who was blinded to the animals' genotypes.

MD1-KO mice

CL57/BL6 MD1-KO mice were generated as described by previous studies.[14,15] Male MD1-KO mice were purchased from the Japan RIKEN Bio Resource Centre Mouse (BRC) (B6.129P2-MD-1 <tm1Kmiy>). The deletion of MD1 was confirmed in the obtained MD1-KO mice by Western blot analyses of left atrium (LA) tissues. Mice were fed with a HFD (60% kcal from fat) starting at the age of 6 weeks; the normal diet (ND) contained 10% of kcal from fat. To achieve the research objectives, the experimental animals were divided into 4 groups: wild-type (WT) + ND (WT-ND), WT+ HFD (WT-HFD), MD1-KO + ND (KO-ND), and MD1-KO + HFD (KO-HFD). HFD and age-matched ND mice were weighed weekly and killed at the age of 26 weeks.

Biochemical and serum analysis

Following a 12-hour fast, 2.0 g/kg glucose was administered intraperitoneally, and blood samples were taken immediately before and 15, 30, 60, 90, and 120 minutes after glucose injection. Blood glucose levels were assessed by using an Accu-Chek glucose analyzer (Roche Diagnostics, Indianapolis, IN).[16] Blood samples were obtained by removing eyeballs from overnight-fasted mice and centrifuged for serum. Biochemical parameters were analyzed at the clinical laboratory of Renmin Hospital at Wuhan University by using Advia 2400 automatic biochemical analyzer (Siemens, Berlin and Munich, Germany) and kits for total cholesterol (TC), triglyceride (TG), and low-density lipoprotein cholesterol (LDL-c).

Echocardiography assessment of heart function

Echocardiography was performed to evaluate cardiac function at the end of the 20-week HFD feeding. In brief, echocardiography was performed under continuous anesthesia with 1.5% to 2% isoflurane, using a Mylab30CV (ESAOTE) ultrasound system with a 15Mz probe. Cardiac measurements included examination of left atrial dimension (LAD), left ventricle end-diastolic dimension (LVDd), left ventricle end-systolic diameter (LVSd), and left ventricle ejection fraction (LVEF).

Histology

Isolated LAs were fixed in 4% paraformaldehyde, then embedded in paraffin and cut into 5-μm−thick sections. Masson trichrome staining was used to evaluate interstitial fibrosis. The percentage of fibrosis was determined by calculating the ratio of fibrotic tissue area to the normal myocardial tissue area.

Quantitative real-time PCR (qRT-PCR) and Western blot analysis

Total LA RNA was purified from the LA samples using TRIzol reagent (15596-026, Invitrogen). RNA was transcribed into complementary DNA with the PrimeScript RT reagent Kit (#RR047A, TaKaRa). Then, qRT-PCR was conducted in a 20-μl reaction system containing cDNA, forward primers, reverse primers, and SYBR Premix Ex Taq (#RR420A, TaKaRa). The relative gene expression was normalized to the internal reference gene GAPDH. The sequences of the primers used for RT-PCR in this experiment are described in Supplemental Table S1.

Total proteins were extracted from the frozen LA tissues. Protein concentrations were determined and normalized using the Bicinchoninic Acid (BCA) Protein Assay Kit

(AS1086, ASPEN). Following that, proteins (40 μg) were separated by sodium dodecylsulphate (SDS)-polyacrylamide gel electrophoresis (PAGE), then transferred onto a polyvinylidene difluoride (PVDF) membrane and incubated with primary antibodies (Supplemental Table S2) overnight at 4°C (39.2°F). Finally, secondary antibodies were incubated with the membranes for 30 minutes at room temperature. Enhanced chemiluminescence was used to visualize the signals.

Electrocardiograph analysis

Mice were lightly anaesthetized using inhaled isoflurane (1.5% isoflurane in 98% O_2). The 2 subcutaneous leads were positioned approximately on electrocardiograph (ECG) limb lead II. Data were analyzed off-line using LabChart 7 Pro (ADInstruments, Sydney, Australia). ECG waveforms averaged over 4 consecutive beats were used for analyses.[17]

Electrophysiological studies using isolated perfused hearts

Langendorff-perfused hearts were prepared according to published methods.[18] Electrophysiological studies in isolated perfused hearts were conducted using the Langendorff apparatus with HEPES-buffered Tyrode's solution (130 mM NaCl; 5.4 mM KCl; 1.8 mM $CaCl_2$; 1mM $MgCl_2$; 0.3 mM $Na2HPO_4$; 10 mM HEPES; 10 mM glucose; pH adjusted to 7.4 with NaOH) bubbled with 95% O_2−5% CO^2 at 37°C (98.6°F) and at a constant pressure of 60 mm Hg to evaluate the induction of atrial fibrillation (AF), interatrial conduction time (IACT), and atrial effective refractory periods (ERPs) as previously described.[18] Langendorff-perfused hearts were stimulated with a pair of electrodes placed on the right atrium. All isolated hearts were stabilized for 20 minutes by perfusion at a constant flow before programmed electric stimulation. The hearts that did not recover to a regular spontaneous rhythm or had irreversible myocardial ischemia were discarded.

Teflon-coated (except at the tips) silver bipolar electrodes were placed on the appendages of the right atrium (RA), LA, and left ventricle (LV). The interelectrode distance between the RA and LA was set at 5 mm to measure the IACT. The ERPs of the left and right atria were measured by the S2 extrastimulus method using 8 regularly paced beats with cycle lengths of 100, 80, 60, and 40 ms. IACT was measured during RA pacing. AF inducibility was tested by burst pacing methods. The induction of AF was tested by applying 3 chains of a 2-second burst pacing using the automated stimulator. Specifically, the first 2-second burst had a cycle length (CL) of 40 ms (pulse duration = 5 ms). Following 3 minutes of stabilization, the second 2-second burst was applied with a CL of 20 ms (pulse duration = 5 ms). After 3 minutes of stabilization, the last 2-second burst with a CL of 20 ms was applied with a 10-ms pulse duration. AF was defined as a rapid irregular atrial rhythm with irregular RR intervals lasting at least 1 second. The duration of AF was measured from the end of burst pacing to the first P wave detected after the rapid irregular atrial rhythm.

Statistical analysis

Statistical analysis was performed using SPSS (IBM, Armonk, NY) or GraphPad Prism software (GraphPad Software, San Diego, CA). Continuous variables are shown as means ± SEM and were evaluated with Student's 2-tailed unpaired Students' *t*-test or 2-way analysis of variance (ANOVA) followed by Bonferroni *post hoc* test. Categorical data are expressed as percentages and were analyzed using Fisher exact test. $P < 0.05$ was considered statistically significant.

Results

Expression of MD1 was downregulated in HFD-fed mouse atria

To explore the correlation between MD1 and atrial pathology after obesity, we first examined the expression levels of MD1 and its ligand RP105 in mouse LA following an HFD.

The Western blots and RT-PCR results showed that the expression of both MD1 and RP105 were significantly downregulated at 12 and 20 weeks after HFD feeding compared with the higher expression levels before HFD feeding (Fig. 1, A and B). These results suggest that MD1 may be involved in the development of obesity-related atrial remodelling.

Deletion of MD1-intensified metabolic disorder in HFD-fed mouse

At the end of the 20-week HFD feeding, the body weights of WT and MD1-KO mice were significantly elevated compared with the corresponding ND-fed groups, with significant difference between the 2 HFD-fed groups (Fig. 2A). HFD induced abnormal glucose intolerance and hyperlipidemia in WT-HFD mice, and these effects were significantly accentuated in MD1-KO mice (Fig. 2, B and C). These results suggest that MD1-intensified HFD induced obesity-related metabolic disorder.

MD1-KO aggravated HFD-induced structural remodelling of the atrium

To confirm the obesity-related atrial remodelling effects of MD1, we then performed the HFD feeding on KO mice whose MD1 was absent in all tissues, including the heart (Fig. 3A). Echocardiographic measurements were taken, including LAD, LVDs, LVDd, LVEF. MD1-KO mice exhibited enlargement of both LA and LV after 20 weeks of HFD feeding, but there was no observed difference in LVEF (Fig. 3, B-E).

Furthermore, Masson staining revealed that the LA of fibrosis was larger in HFD-fed MD1-KO mice than in WT mice (Fig. 3, F and I). We then quantified the extent of cardiac fibrosis by measuring collagen volume in the LA and the expression of fibrotic markers (collagen I, collagen III, and TGFβ1) (Fig. 3, G and H). Our results demonstrated that atrial fibrosis was more prominent in KO-HFD mice. Finally, as inflammation is the most important pathological response in tissue damage and repair, we also measured the expression of cytokines secreted by inflammatory cells. The mRNA levels of proinflammatory cytokines IL-1β, IL-6, and TNF-α were increased, and the levels of anti-inflammatory cytokine IL-10 were reduced in the LA tissue of KO-HFD mice (Fig. 3J).

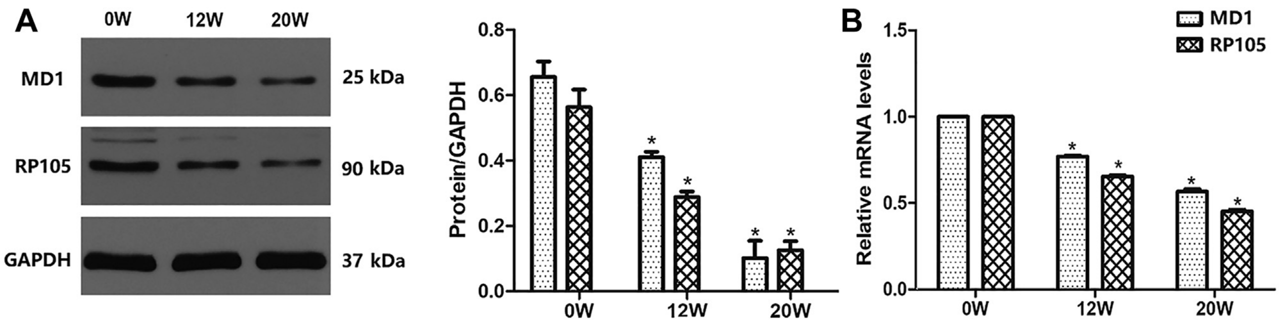

Figure 1. MD1 and RP105 expression is downregulated in high-fat diet feeding mouse atrial. (**A**) Representative Western blots and statistical analysis of the MD1 and RP105 protein levels in left atrial from wild-type mice 0, 12, and 20 weeks after high-fat diet. (**B**) Levels of the MD1 and RP105 mRNAs in left atrial from wild-type mice 0, 12, and 20 weeks after high-fat diet. Mean ± SEM; n = 4 mice per group; *$P < 0.05$ vs 0W group.

MD1 deficiency aggravated HFD-induced electrical remodelling of the atrium

Because we observed worsened structural remodelling in MD1-KO mice after HFD feeding, we hypothesized that silencing of the MD1 gene would also influence cardiac electrophysiological properties. Surface ECG (lead II) recordings were performed on mice under light anesthesia. No statistically significant differences in P waves, PR intervals, or QRS durations among the 4 groups were detected (Fig. 4, A and B).

Langendorff-perfused hearts were used to characterize changes in electrophysiological parameters (AERP, ICAT, and incidence of AF) (Fig. 5). In the ND-fed mice groups (WT-ND and KO-ND), burst stimuli was only able to induce AF in 1 mouse in each group (1/11, 9.1%). In contrast, AF was induced in 5/11 (45.5%) hearts of the WT-HFD mice and 9/10 (90 %) of the KO-HFD mice (Fig. 5C). The AF induction rate of KO-HFD group was significantly higher than that of the WT-ND group ($P < 0.05$), and the KO-HFD group was also higher than the WT-HFD group ($P < 0.1$). These results indicated that MD1 deficiency increased incidence of AF in HFD-feeding mice.

When compared with the WT-ND mice, IACT in the WT-HFD mice was prolonged in all the basic cycle lengths tested ($P < 0.05$ at the basic cycle lengths of 40, 60, 80, 100 ms [Fig. 5A]). This prolonged duration was further exaggerated in KO-HFD mice ($P < 0.01$ at the basic cycle lengths of 40, 60, 80, 100 ms [Fig. 5A]). The difference in LA ERP between ND-fed mice and HFD-fed mice was also more significant in the KO mice. There was a significant difference in the LA ERP between WT-ND and WT-HFD mice, and significantly shorter LA ERPs were observed in the KO-HFD mice (Fig. 5A). Altogether, this loss-of-function data indicated that MD1 deficiency aggravated HFD- induced electrical remodelling of the atria.

MD1-modulated NF-κB signalling pathways in the atrium

The aforementioned results suggest that MD1-KO may have facilitated obesity-related atrial remodelling. However, the underlying mechanism by which MD1-KO exerts its proinflammatory response is unclear. The TLR4/NF-κB signalling pathway is reported to be important in regulating inflammation.[19] In addition, according to a previous study, MD1 had an effect on NF-κB suppression.[14] Thus, the TLR4/NF-κB signalling pathway was investigated to confirm the function of MD1. As shown in Figure 6, the levels of phosphorylated p65 and IκBα were substantially increased in the KO-HFD mice, indicating that the TLR4/NF-κB pathway was strongly activated by MD1 deletion. This data suggest that the MD1 modulated atrial remodelling by affecting the TLR4/NF-κB pathway to some extent.

Discussion

The present study utilized loss-of-function approaches to study the role of MD1 in obesity-related atrial remodelling.

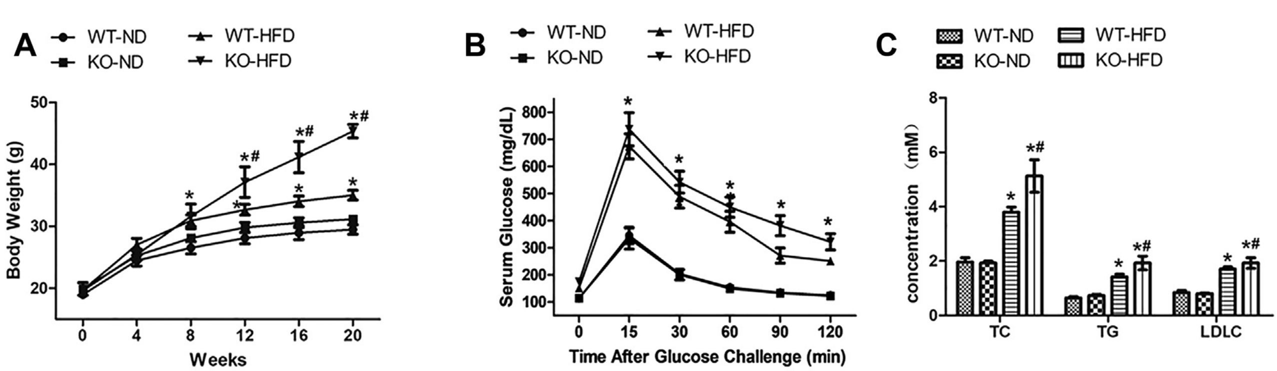

Figure 2. Effect of 20-week high-fat diet intake on (**A**) body weight; (**B**) OGTT; (**C**) serum lipid. Mean ± SEM, n = 6 to 8 mice per group; *$P < 0.05$ vs WT-ND group; # $P < 0.05$ vs WT-HFD group.

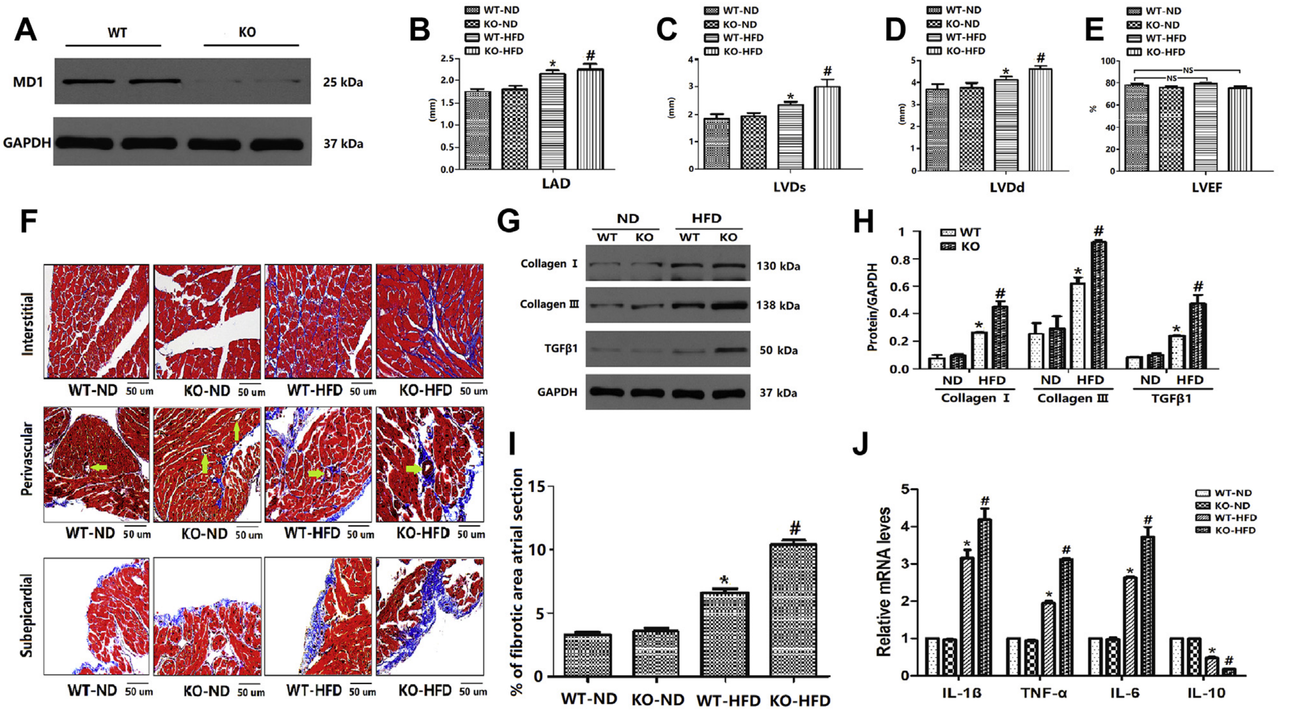

Figure 3. MD1-KO exaggerates high-fat diet feeding-induced atrial structure modelling. (**A**) MD-1 protein levels in samples obtained from WT and KO mice. The cardiac functions of LAD (**B**), LVDs (**C**), LVDd (**D**), and LVEF (**E**) were measured in mice after normal or high fat diet feeding in the absence or presence of MD1 expression (Raw echocardiography in Supplemental Fig. S1) (n = 6 to 8). (**F, I**) Representative images of Masson trichrome staining and quantitative analysis of fibrosis of the interstitial, subepicardial, perivascular of left atrium (n = 6). The **green arrow** points to where the vessels are. (**G, H**) Representative Western blots and statistical analysis of the fibrosis-related proteins levels in samples obtained from WT and KO mice left atrial tissues 20 weeks after the normal or high-fat diet feeding (n = 4). (**J**) Relative levels of IL-1b, TNF-a, IL-6, and IL-10 mRNA in samples obtained from WT and KO mice left atrial tissues 20 weeks after the normal or high-fat diet feeding (n = 4). Mean ± SEM; * $P < 0.05$ vs WT-ND group; # $P < 0.05$ vs WT-HFD group.

The major novel findings of this study were as follows: (1) MD1 expression was significantly reduced in HFD-induced obesity; (2) the structural and electrical remodeling of the atrium caused by HFD-induced obesity was exacerbated in MD1-KO mice compared with WT mice, because of inflammation and fibrosis in the atrium; (3) the MD1-KO—mediated proinflammatory effect was at least partially dependent on the regulation of the TLR4/NF-κB signalling pathway. These findings suggest that MD1 may play a vital role in atrial remodelling after HFD feeding, and it may be a novel therapeutic target for the treatment of obesity-related structural and electrical remodelling of the atrium.

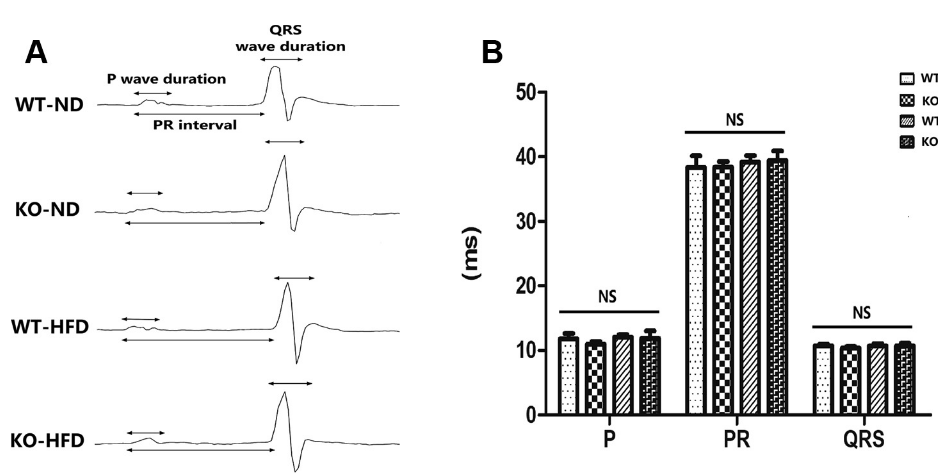

Figure 4. Analysis of surface electrocardiograph in 4 groups. (**A**) A representative trace of surface electrocardiograph in 4 groups, respectively. P wave duration, PR interval, and QRS duration were indicated with **arrow**. (**B**) Surface electrocardiograph parameters in 4 groups. Mean ± SEM, n = 7 to 8 mice per group.

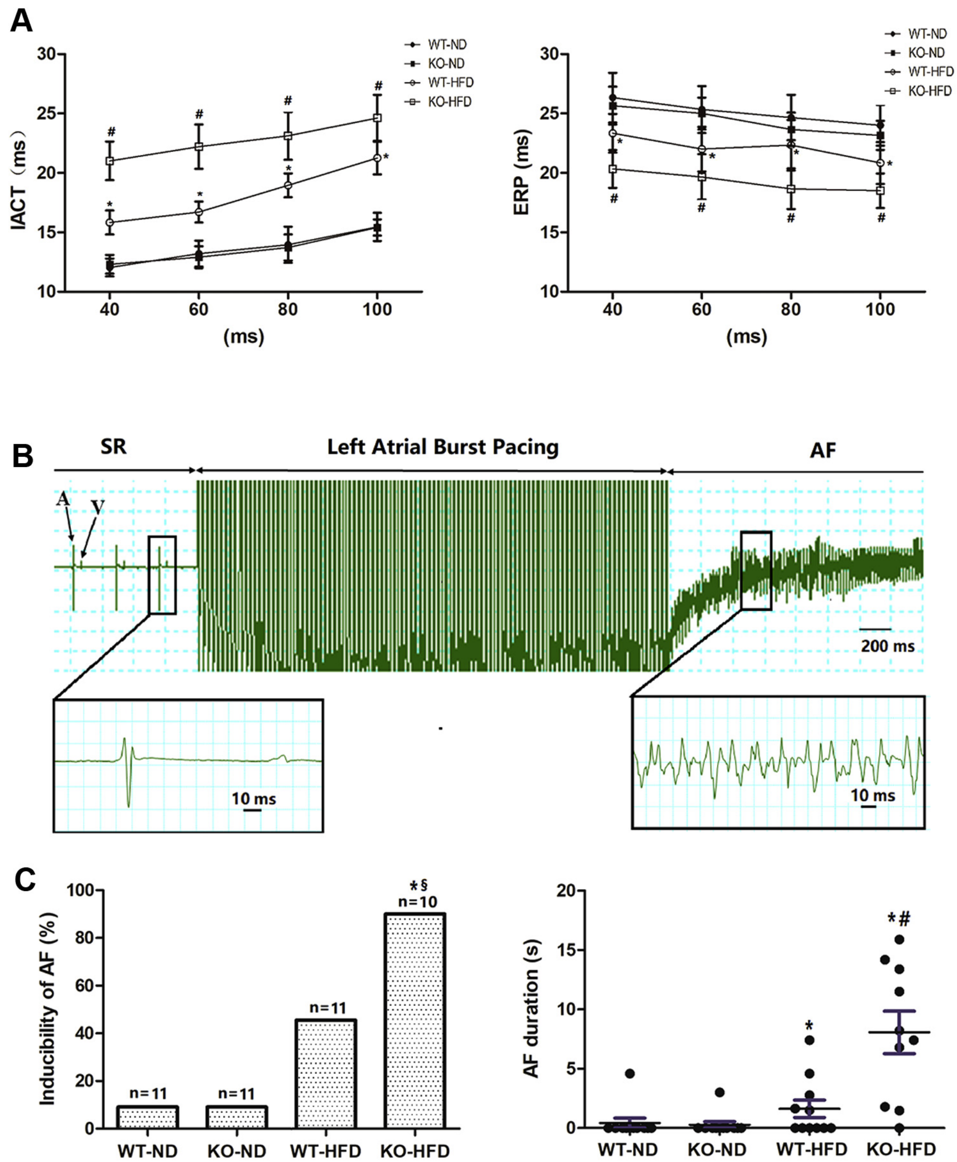

Figure 5. Deletion of MD1 alters electrophysiological properties of the atrial in high-fat diet feeding mice. (**A**) Intra-atrial conduction time (IACT), effective refractory period (ERP) of left atrium (LA) evaluated in the study using isolated perfused heart mice (n = 8). (**B**) Representative electrograms (EGMs) of AF induction after atrial burst pacing in isolated perfused heart using Langendorff apparatus in a KO-HFD mouse. (**C**) Inducibility and duration of AF in the study using isolated perfused heart (n = 10 to 11). Mean ± SEM; * $P < 0.05$ vs WT-ND group; $^{\#}$ $P < 0.05$ vs WT-HFD group; § $P < 0.1$ vs WT-HFD group.

High fat-induced metabolic syndrome is a significant risk factor in the development of cardiac disease.[20] An HFD containing 60% lard-based lipid chow is an established experimental model mimicking obesity, which is used to study the pathogenesis of obesity-related cardiovascular disease in vivo.[17,21] Obesity is closely related to atrial fibrosis. Recent studies found that, during obesity, adipose tissue expansion may have favoured fibrosis of neighbouring myocardium by secreting Activin A or triggering an immune response.[22,23] In addition, there is substantial evidence demonstrating that atrial fibrosis is important in the pathogenesis of AF.[24,25] Similar results were revealed in our study. It is found in our study that MD1-KO enhanced protein expression related to fibrosis, including the expression of Collagen I, Collagen III, and TGF-β1, inducing cardiac injury in HFD-fed mice. In addition, measurement values of heart function (LAD, LVDs, and LVDd) also suggested that MD1 deficiency significantly accelerated cardiac dysfunction. The data here illustrate that MD1 removal contributed to the deterioration in cardiac injury induced by HFD feeding.

In addition, the study confirmed that vulnerability to AF is exacerbated in HFD-induced obesity. The electrophysiological results of isolated perfused hearts showed ERP was significantly shortened in HFD-fed mice in this study. A report demonstrated that obese patients with AF had shorter atrial and pulmonary vein effective refractory periods than their normal weight counterparts.[26] According to previous research, the ERP shortening may be explained by an increase

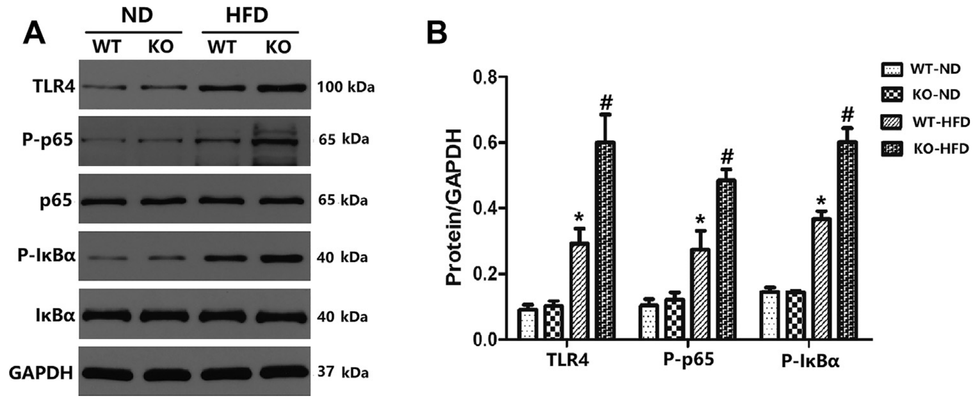

Figure 6. MD1 deficiency promotes atrial remodeling through the TLR4/NF-κB signalling pathway activation. Representative Western blots (**A**) and statistical analysis (**B**) of TLR4, P-p65 and P- IκBa in WT and KO mouse atrial tissues 20 weeks after the normal or high fat diet feeding. Mean ± SEM, n = 4 mice per group; * $P < 0.05$ vs WT-ND group; $^{\#}$ $P<0.05$ vs WT-HFD group.

in fibroblasts which shortened the duration of the action potential.[27] It is widely agreed upon the importance of atrial fibrosis in the initiation and maintenance of AF, especially in the matter of conduction disturbances.[24,25] According to the current study, HFD feeding evoked interstitial LA fibrosis and prolonged IACT in WT-HFD compared with the WT-ND group. This finding was supported by a previous study conducted by Abed et al. According to that study, 8 months of a high-calorie diet caused increased atrial fibrosis and inflammation, which was associated with decreased atrial conduction, leading to increased AF inducibility.[28]

As described previously, MD1 is a potent anti-inflammatory cytokine that may attenuate adverse cardiac effects. We previously reported that MD1 played a significant role in the pathogenesis of pressure overload-induced heart failure in mice.[14,15] In the current study, we also found that MD1 may be a key molecule in obesity-induced atrial remodelling because the protein levels of MD1 in WT-HFD mice after 20 weeks were significantly reduced compared with those in the ND-fed mice. According to findings of this study, deficiency of MD1 may have exacerbated the inflammation, fibrosis, and vulnerability to AF caused by HFD-induced obesity.

The possible mechanisms by which MD1 regulates atrial remodelling may be associated with its downstream targets in the TLR4/NF-κB signalling pathway. TLR4 can be activated by TLR ligands such as LPS, interferons, and viral antigens.[13] Similarly, the current study found that the TLR4/NF-κB signalling pathway was activated after HFD feeding, and this effect was enhanced in MD1-KO mice. TLR4 signalling pathway activation was reported to be of great importance in regulating inflammatory responses through upregulating NF-κB phosphorylation.[29] Thus, the inhibition of the TLR4/NF-κB pathway may have a beneficial effect on anti-inflammation.

In addition, HFD feeding may induce an inflammatory response, which can injure various organs through TLR4/NF-κB activation, causing large secretions of IL-1b, TNF-a, and IL-6.[30] In line with these results, the current study showed that proinflammatory cytokines were extremely upregulated in mice fed by the HFD. MD1 deficiency significantly enhanced this inflammatory cytokine secretion. The studies above indicate that MD1 played an important role in suppressing the NF-κB pathway after HFD feeding.

Based on previous studies, lifestyle-related diseases, such as hypertension, obesity, diabetes, and dyslipidemia, were associated with the development of AF,[1,31] and a Mediterranean diet could decrease the vulnerability to AF.[32] However, molecular mechanisms leading to these electrophysiological changes remain unclear. Structural remodelling characterized by atrial fibrosis and dilatation increased the vulnerability to AF by promoting multiple re-entries in the atrium. Recent studies demonstrated that inflammation might be a critical mediating factor of electrophysiological remodelling and arrhythmia.[33-35] Indeed, various large clinical and preclinical cohort studies showed that several markers of systemic inflammation were significantly associated with development of AF.[36,37] Although the mechanisms of arrhythmia caused by increased inflammation are not fully understood, alterations in ion channel properties, calcium handling, and homeostasis and atrial fibrosis are likely to be involved.[38,39] Therefore, we propose that inhibition of inflammation can have a positive effect in preventing the development of arrhythmia.

Conclusions

We have revealed that knockout MD1 enhanced activation of the TLR4/NF-κB signalling pathway, by aggravating the development of inflammatory atrial fibrosis and vulnerability to AF in mice with HFD-induced obesity. MD1 may be a novel therapeutic target to prevent AF in the obese human population.

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Disclosures

The authors have no conflicts of interest to disclose.

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Supplementary Material

To access the supplementary material accompanying this article, visit the online version of the *Canadian Journal of Cardiology* at www.onlinecjc.ca and at http://doi.org/10.1016/j.cjca.2018.11.020.