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Prevention of Cardiac Hypertrophy by the Use of a Glycosphingolipid Synthesis Inhibitor in ApoE^{−/−} Mice

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Abstract

ApoE^{−/−} mice fed a high fat and high cholesterol (HFHC) diet (20% fat and 1.25% cholesterol) from 12 weeks of age to 36 weeks revealed an age-dependent increase in the left ventricular mass (LV mass) and decline in fractional shortening (FS%), which worsened with HFHC diet. These traits are indicative of maladaptive pathological cardiac hypertrophy and dysfunction. This was accompanied by loading of glycosphingolipids and increased gene expression of ANP, BNP in myocardial tissue. Masson's trichrome staining revealed a significant increase in cardiomyocyte size and fibrosis. In contrast, treatment with 5 and 10 μ M D-threo-1-phenyl-2-decanoylamino-3-morpholino-1-propanol (D-PDMP), an inhibitor of glucosylceramide synthase and lactosylceramide synthase, dose-dependently decreased the load of glycosphingolipids and preserved fractional shortening and maintained left ventricular mass to normal 12-week-old control levels over a 6 month treatment period. Our mechanistic studies showed that D-PDMP inhibited cardiac hypertrophy by inhibiting the phosphorylation of mitogen-activated protein kinase (MAPK). We propose that associating increased glycosphingolipid synthesis with cardiac hypertrophy could serve as a novel approach to prevent this phenotype in experimental animal models of diet-induced atherosclerotic heart disease.

Keywords

Glycosphingolipids; Lactosylceramide; Cardiac hypertrophy; Atherosclerotic heart disease

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Disclosures

None.

Introduction

Cardiac hypertrophy is characterized by an increase in the size of cardiomyocytes due to increased protein synthesis and pressure or volume overload [1]. This is a compensatory and protective mechanism to offset pressure overload due to hyperlipidemia-induced oxidative stress and/or other factors [2], [3]. Prolonged hypertrophy, when hypertrophy can no longer be adaptive for the increased afterload [4], contributes to irreversible myocardial damage characterized by myocardial wall thinning, ventricular dilatation, decline in myocardial contractility and may lead to heart failure [4]. This is defined by the term maladaptive and/or pathological hypertrophy. Although left ventricular hypertrophy (LVH) is known to contribute to mortality, its root cause remains unknown. Several factors contribute to LVH such as obesity, hyperlipidemia, hypertension, male sex and age [5],[6].

Increasing evidence suggest that hyperlipidemia induces vascular wall oxidative stress injury [7] which worsens with age [8] and leads to vascular growth, stiffening and calcification [9], [10]. Increased shear stress due to increased blood pressure may exert oxidative stress on the arterial wall, thus accelerating atherosclerosis [9],[10],[11]. Shear stress (i.e. "stretch") is a natural response of the vasculature to hypertension, mediated by eNOS. Thus, endothelial eNOS production leads to vasodilation and cardioprotection. The oxidative stress, or ROS production, that contributes to atherogenesis is the result of diminished shear stress [9],[12]

Epidemiological studies also show that hypercholesterolemia is associated with increased blood pressure [13], maladaptive hypertrophy, and increased LV mass, an index used in the quantification of LVH and that dyslipidemia is independent of LVmass [14]. In response to increase in blood volume, oxidative stress and pressure, the cardiomyocytes hypertrophy in the left ventricle of the heart [15]. Both angiotensin II and endothelin-1 are proteins that lead to oxidative stress and pathological hypertrophy [9],[16]. Many studies point to oxidative stress and p44 mitogen activated protein kinase/extracellular signal-related kinase-1 (MAPK/ERK-1) pathway in the activation of hypertrophy [17]. Although some glycosphingolipids such as lactosylceramide(LacCer) can induce oxidative stress by generating superoxides upon interacting with vascular cells and cardiomyocytes [18],[19], nothing is known about the role of glycosphingolipid glycosyltransferases and glycosphingolipids (GSLs) in maladaptive cardiac hypertrophy in experimental animal models of hyperlipidemia.

Glycosphingolipids (GSLs) are small molecular weight compounds mostly localized on the cell surface. They are composed of a sphingosine base to which a fatty acid is attached to form ceramide. The sequential addition of sugars from nucleotide sugars to ceramide via the action of glycosyltransferases synthesizes various GSLs [20].

Recently a study of various GSL revealed that only Lactosylceramide could exert a concentration and time-dependent increase in hypertrophy in freshly cultured neonatal rat ventricular myocytes (NRVM) and H9C2 cells, a transformed mouse cardiomyocyte cell line. This required the generation of superoxides and ERK-1/p44 MAPK activation [19]. These observations led us to hypothesize that in vivo GSL loading in mice heart tissue (by way of increased biosynthesis) may lead to maladaptive cardiac hypertrophy. Conversely,

inhibiting glycosyltransferase activity and decreasing GSL load may well prevent maladaptive cardiac hypertrophy. In this report we have used apoE^{-/-} male mice fed a high fat and high cholesterol (HFHC) diet over a period of 6 months to expedite atherosclerosis and pathological cardiac hypertrophy. Treatment with an inhibitor of GSL glycosyltransferases, D-PDMP by oral gavage for the duration of this study was also conducted. We show that maladaptive cardiac hypertrophy increased and decreased in tandem with GSL loading and unloading in these apoE^{-/-} mice.

Our studies identify GSL loading and consequently increased oxidative stress as a bona fide and independent factor contributing to maladaptive cardiac hypertrophy. This study also offers a novel approach to mitigating maladaptive cardiac hypertrophy by way of inhibiting GSL synthesis.

Materials and Methods

Treatment of apolipoproteinE^{-/-} mice fed a western diet with a glycosyltransferase inhibitor

ApoE^{-/-} male mice aged 11 weeks were purchased from the Jackson Laboratory (Bar Harbor, ME). At 12 weeks old, the apo E^{-/-} mice were started on a HFHC diet consisting of 20% fat, and 1.25% cholesterol (D12108C, Research Diet Inc., New Brunswick, NJ) until 36 weeks of age and treated daily with 5 mg/kg or 10 mg/kg D-PDMP. Treated mice were compared to control mice fed only chow diet and placebo mice fed HFHC and vehicle (5% Tween-80 in phosphate buffered saline). Diet was rationed once a week to estimate the weekly growth rate and food intake. Physiological studies were performed around the age of 12, 20 and 36 weeks.

Animals were subjected to physiological measurements such as body weight (BW), heart weight to body ratio (HW/BW), mean arterial pressure (MAP), percent fractional shortening (FS), percent ejection fraction (EF), relative wall thickness (RWT), left ventricular mass (LV mass), left ventricle end diastolic and systolic dimension (LVEDD and LVESD), inter-ventricular septal and posterior wall thickness at end diastolic (IVSED and PWTED) phase, and heart rate (HR) (Table S1).

A group of mice (n=5) was euthanized to obtain baseline values and blood samples were collected. The rest of the mice were divided into several groups. These were: Placebo (HFHC and vehicle), 5 mg/kg D-PDMP, and 10 mg/kg D-PDMP. Vehicle and D-PDMP were delivered daily by oral gavage. The physiological measurements were repeated at 16, 20 weeks and 36 weeks. Mice were then euthanized under sedation and tissues were harvested for further studies. The Johns Hopkins University Animal Care and Use Committee approved all animal protocol and experiments.

Trans-thoracic echocardiography

Trans-thoracic echocardiography was performed in conscious mice using the 2100 Visualsonic ultrasound system (Toronto, Ontario, Canada), equipped with an ultra-high frequency linear array micro-scan transducer of 30–40MHz [21].

The two-dimensional (2-D) and M-mode echocardiogram were obtained in the parasternal short and long axis view of the left ventricle (LV) at the level of the papillary muscles and sweep speed of 200 mm/sec. Using the M-mode echocardiogram image, the following four parameters were derived: (i) left ventricular posterior wall thickness at end of diastole (PWTEd), (ii) inter-ventricular septal thickness at end of diastole (IVSEd), (iii) left ventricle chamber diameter at end of diastole (LVEDD), and (iv) left ventricle chamber diameter at end of systole (LVESD). The fractional shortening (FS) represents the percent change in left ventricular chamber dimension and systolic contraction. We used fractional shortening in the estimation of the LV wall contractility or the systolic function based on the following equation:

$$fs(\%) = [lvedd - lvesd] / lvedd \times 100$$

The left ventricular mass (LV mass) was derived and used in the assessment of left ventricular hypertrophy and enlargement, using the following equation²²:

$$lvmass(mg) = 1.055[(ivsd + lvedd + pwted)^3 - (lvedd)^3]$$

where 1.055 is the specific gravity of the myocardium, IVSEd is the inter-ventricular septal thickness at end diastole, and PWTEd is the posterior wall thickness at end of diastole.

Blood pressure

Systolic, diastolic and mean arterial blood pressure and heart rate were measured non-invasively in conscious mice using the tail cuff plethysmograph (CODA2 blood pressure system, Kent Scientific Corporation USA) as described [22].

Chemicals and supplies

All chemicals were from Sigma-Aldrich (St. Louis, MO) unless specified otherwise. D-PDMP and glycolipid standards were purchased from Matreya, LLC (Pleasant Gap, PA).

Histopathology

Mice were euthanized under sedation. Bodyweight, heart weight, and tibia lengths were recorded. The hearts were sectioned in cross-section at mid-papillary level. The basal to mid-papillary level was fixed in 10% formalin and paraffin-embedded for the histological evaluation. Thin five micron heart sections were stained with Masson's trichrome to assess and estimate the amount of fibrosis, cardiomyocyte size and morphology. Nikon 80I Eclipse equipped with Nikon DS-EI1 camera and NIS-Elements software (Nikon, Japan) were used for image analysis.

Chromatographic analysis of glycosphingolipid levels in heart tissue in apoE^{-/-} mice

A sample of approximately 10 mg of heart tissue was homogenized in chloroform-methanol (2:1, v/v) and lipids were extracted according to the Bligh and Dyer method [23]. The total lipid extracts were dried in nitrogen and subject to deacylation using sphingosine ceramide N-deacylase, followed by o-phthalaldehyde derivatization and quantification of the levels of

glucosylceramide and lactosylceramide by reversed phase HPLC (RP-HPLC) [24]. A C18 column was used with an isocratic organic mobile phase (methanol-water, 88:12, v/v) and calibrated with standard glycosphingolipids of known chemical structure and column affinity. All samples were analyzed in triplicate and a representative quantity (n = 3) of heart tissue samples was used for each treatment from control, placebo, 5mpk and 10mpk D-PDMP-treated apoE^{-/-} mice.

Quantitative Real-Time PCR

A sample of approximately 10 mg of left ventricular tissue was homogenized from each mouse and total RNA was isolated using TRIzol reagent according to the manufacturer's instruction (Invitrogen, Carlsbad, CA). Two micrograms of RNA were reverse-transcribed with SuperScript II (Invitrogen) using random primers. Real-time PCR was performed using SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA) in an Applied Biosystems Step One Realtime PCR system with the following thermal cycling conditions: 10 min at 95 °C, followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min for denaturation, annealing and elongation. Relative mRNA levels were calculated by the method of 2^{-DDCt}. Data were normalized to GAPDH mRNA levels. To determine the specificity of amplification, melting curve analysis was applied to all final PCR products. All samples were performed in triplicate. Primers were synthesized by Integrated DNA Technologies (Coralville, IA) (Table S2). Expression suite software (Applied Biosystem) was used to analyze the data.

Western immunoblot analysis of phospho p44/42 MAP kinase in heart tissues

A sample of approximately 10 mg of left ventricular tissues was homogenized in Tris HCl buffer with SDS and centrifuged. About 70 µg of supernatant protein were subjected to SDS gel electrophoresis. Anti- phospho p44/42 MAP kinase (9101) and anti GAPDH (14C10) antibodies were purchased from Cell Signaling. Following immunoblotting the gel bands were subjected to densitometric scanning.

Statistical analysis

All values are expressed as Mean ± SEM. Comparison between groups was performed by oneway ANOVA with the Bonferroni's multiple comparison tests. Comparisons between two groups were performed using non-paired 2-tailed Student t test. A value of P<0.05 was considered significant.

Ethics Statement

This study was approved by The Johns Hopkins Medicine Institutional Animal care and Use committee, permit # MO11M492. The relevant efforts were taken to ameliorate animal suffering including anesthesia (Ketamine and Xylazine) and check on animals daily.

Results

Treatment with glycosphingolipid synthesis inhibitor prevents cardiac hypertrophy in apoE^{-/-} mice

The effects of feeding a HFHC diet and the effects of treatment with a glycosyltransferase inhibitor on the thickening of the heart wall and heart function were measured by echocardiography (Table S1 and Fig 1A). As shown in Figure 1A, at 36 weeks of age, there was significant cardiac hypertrophy in the placebo group (Fig 1A, B, top middle panel) compared to control (Fig 1A, A, top left panel). In contrast, in mice fed D-PDMP (Fig 1A, C, top right panel), the wall thickness was comparable to the control. Masson's-Trichrome staining (Fig 1G) of thin sections of the left ventricle revealed extensive collagen deposition (white arrow) in mice fed with HFHC diet (Fig 1G, B) compared to control and this was reversed and/or prevented upon treatment. The echocardiographic (Fig 1A) assessment above was substantiated by quantitative measurement of fractional shortening (Fig 1C) an index of cardiac contractility. The results show that treatment for 6 months (36 week old mice) not only mitigated cardiac hypertrophy due to HFHC feeding, but also maintained cardiac contractility/heart function to the same level as 12 weeks old mice, thus mitigating the ageing effect in these mice. Hypertrophy in placebo mice was accompanied by an age-dependent increase in LV mass (Fig 1D), an index of a significant increase in wall thickening and size of the heart. In particular, mice fed chow only progressively increased their LV mass due to ageing. While, the HFHC diet substantially increased LV mass further. In contrast, treatment with varied D-PDMP doses dependently decreased LV mass. Additional details of our echocardiography study parameters are presented in supplement (Table S1). Feeding a HFHC diet significantly increased Mean arterial pressure (MAP) (Fig. E), which was improved upon treatment with D-PDMP. The heart weight to body weight ratio (Fig. 1F) and cardiomyocyte size (Fig 1. G) were also increased in placebo mice fed HFHC compared to control and were dose-dependently decreased upon treatment.

Treatment with inhibitor decreases the glycosphingolipid mass

As shown in Figure 2, feeding 5 mg/kg and 10 mg/kg of D-PDMP 36 weeks old mice dose-dependently decreased the mass of lactosylceramide and glucosylceramide compared to placebo mice heart tissues.

Treatment with D-PDMP decreases the expression of genes implicated in cardiac hypertrophy and induces anti-oxidant defense in hypertrophic heart tissue

The genes regulating the expression of ANP and BNP serve as biomarkers for cardiac hypertrophy. Quantitative RT-PCR measurements of these genes (Fig. 3A) showed that treatment with D-PDMP reversed the expression of ANP and BNP mRNAs. This observation is compatible with the cardiovascular and functional studies above (Fig. 1). The primer sequence used in this study is presented in supplement (Table S2). Quantitative RT-PCR analysis revealed that several genes encoding the proteins implicated in anti-oxidant defense were induced by 10 mg/kg D-PDMP (Fig 3B). For example, the mRNA levels of superoxide dismutase I and II (SOD1, SOD2), catalase (CAT) were increased by treatment. In contrast, mRNA levels of hypoxia inducible factor-A was markedly decreased in these

tissues. Collectively, treatment with D-PDMP accelerated the anti-oxidant defense in mice heart.

D-PDMP abrogates the expression of phospho p44MAPK/ERK-1 protein

Western immunoblot of phospho p44MAPK in left ventricle tissue extracts showed that 10 mg/kg D-PDMP decreased the expression of p44MAPK compared to the placebo mice heart tissues (Fig. 4).

Discussion

This study demonstrates the following: (i) Feeding a western diet HFHC to transgenic mice e.g. apo E^{-/-} mice exerts a time- and dose-dependent increase in cardiac hypertrophy and decline in cardiac contractility indicative of maladaptive (decompensated) or pathological cardiac hypertrophy and remodeling. (ii) Hyperlipidemia in these mice (Placebo) was improved upon treatment. (iii) Treatment with D-PDMP maintained baseline left ventricular mass and thus overcame the adverse effect of HCHF diet in these transgenic mice. (iv) Treatment reduced the gene and protein expression of BNP and ANP and p44MAPK, established biomarkers of cardiac hypertrophy. (v) In addition, treatment also increased the expression of genes known to improve anti-oxidant defense. (vi) Most importantly, treatment markedly reduced the load of glycosphingolipids by way of inhibiting the activity of various glycosyltransferases. These findings are summarized in a hypothetical model (Fig S1).

Cardiac hypertrophy involves the enlargement of the myocardial cells due to pressure and volume overload and contributes to the pathophysiology in various cardiovascular diseases. While several factors may contribute to pathological hypertrophy and dysfunction, this study suggests that increased GSL's in heart tissues may well be additional factor contributing to cardiac hypertrophy. We used a transgenic mouse model, the apoE^{-/-} mouse, which spontaneously develops atherosclerosis upon aging. However, atherosclerosis in these mice can be expedited by feeding a western diet consisting of high fat and cholesterol (HFHC). Herein, marked hyperlipidemia and hyperlipoproteinemia raises blood viscosity, pressure and volume thus contributing to cardiac hypertrophy.

We observed a time –and dose-dependent increase in cardiac hypertrophy and dysfunction in apoE^{-/-} mice fed the HFHC diet. This was exhibited by a continuous increase in the left ventricular mass, decreased fractional shortening, increase in blood pressure, increased cardiomyocyte size and fibrosis (Fig 1 and Table S1). Treatment with D-PDMP in HFHC fed apoE^{-/-} mice exerted a dose and time- dependent reversal in both the left ventricular mass, fractional shortening and blood pressure to baseline levels as compared to 12 week old mice. These mice show significantly less cardiomyocyte hypertrophy and fibrosis than the placebo group (Fig 1G). These findings imply that treatment can overcome the adverse effects of hyperlipidemia on left ventricular mass, and thus preserve heart function. Our previous study also showed that LacCer can independently induce cardiomyocyte hypertrophy in dose and time dependent manner [20].

We have observed that feeding a western diet to apoE^{-/-} mice increases the serum level of oxidized-LDL about 4-fold and this was mitigated by treatment with D-PDMP [23]. This could be explained by a remarkable increase in anti-oxidant gene expression in D-PDMP treated mice heart tissue. One mechanistic explanation by which D-PDMP can prevent pathological cardiac hypertrophy and dysfunction in HFHC fed mice could involve preventing LDL oxidation, thus disallowing an increase in GSL load. In particular, an increase in the level of LacCer which has been shown to raise the level of ROS in cardiomyocyte contributes to hypertrophy [20]. Since treatment reduced LacCer mass, fewer reactive oxygen species were generated, thus reducing hypertrophy. We observed that treatment increased the expression of genes implicated in antioxidant defense, which may suggest that the acceleration of the scavenging of reactive oxygen species generated upon LacCer interaction with the vascular endothelium and cardiomyocytes (Fig S1) may well contribute to reduced hypertrophy.

Previous studies have shown that structurally diverse agonists of cell proliferation can induce the phosphorylation of p44 mitogen-activated protein kinase, via the LacCer synthase-LacCer pathway [20,21]. Phosphorylation of p44MAPK accompanies cardiac hypertrophy and that it is required to activate hypertrophic gene expression [16, 20]. Therefore, one mechanistic explanation for our observations is that since D-PDMP mitigated the phosphorylation of p44MAPK in heart tissue along with the increase in the mRNA expression of proteins involved in ROS moderation in this dietary model of hyperlipidemia, it prevented cardiac hypertrophy and dysfunction.

In sum, our in vitro studies [20] and this in vivo study in apoE^{-/-} mice fed HFHC suggest that increased level of GSL, in particular LacCer, may well be a bona fide and an independent factor contributing to cardiac hypertrophy. We also report that inhibiting glycosphingolipid synthesis by the use of D-PDMP is a novel approach to mitigate cardiac hypertrophy, and thus preventing maladaptive cardiac hypertrophy/ dysfunction in a mouse model of hyperlipidemia.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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Highlights

Western diet increases cardiac hypertrophy in Apo E^{-/-} mice.

Treatment with D-PDMP maintained left ventricular mass in placebo mice.

D-PDMP markedly reduced the load of glycosphingolipids.

D-PDMP reduced biomarkers of cardiac hypertrophy.

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Figure 1. Treatment with glycosphingolipid synthesis inhibitor prevents cardiac hypertrophy in apoE^{-/-} mice

Figure 1 represent results obtained with apo E^{-/-} mice at baseline, 16, 20 and 36 weeks of age and after 4, 8 and 24 weeks on HFHC diet (consisting of 20% fat and 1.25 cholesterol) with and without 5 and 10 mg/kg of D-PDMP.

Fig 1A. Representative M-mode echocardiogram from a control ApoE^{-/-} mouse fed chow alone (A), fed HFHC (B), fed HFHC and treated with 10 mg/kg (C). Fig 1B.

Representative images of the heart in control (A) fed chow, fed HFHC (B), fed HFHC and

treated with 10 mg/kg D-PDMP (C). Fig 1C. Graphical representation of cardiac

contractility estimated by the percent fractional shortening (FS%), and Fig 1D. cardiac

hypertrophy estimated by the left ventricular mass (LV mass) at 12weeks (Baseline), 16

weeks, 20 and 36 weeks of age. Fig 1E. Mean arterial pressure (MAP), Fig 1F. Heart weight

over the body weight ratio (HW/BW), Fig 1G. Thin five micron heart sections were stained

with Masson's trichrome to assess and estimate the morphology and amount of fibrosis/

collagen deposition (white arrow). Bar = 100 μ m. Figure shows myocyte size and fibrotic

status in control (A) fed chow, fed HFHC (B), fed HFHC and treated with 5 mg/kg D-PDMP

(C), fed HFHC and treated with 10 mg/kg D-PDMP (D). P < 0.05*, p<0.01*, p<0.001**, p<0.0001*** vs. Treatment.

Figure 2. D-PDMP inhibits the mass of glycosphingolipids in the heart tissue in apoE^{-/-} mice
At 36 weeks, the mass of lactosylceramide (A) and glucosylceramide (B) was also decreased, as measured by RP-HPLC.

Figure 3. Treatment with D-PDMP decreases the expression of genes implicated in cardiac hypertrophy and induces the expression of genes encoding antioxidant defense

Treatment with 10 mg/kg D-PDMP blunts cardiac hypertrophic marker genes expression in left ventricular tissues from Apo E^{-/-} mice. RNA was extracted from ventricular tissues of mice from different experimental groups. Real Time quantitative PCR was performed. For RT-PCR reactions (20 μ l), equal amount (500 ng) of total RNA was used. Expression levels of 18S RNA, beta actin and GAPDH were used to normalize for variations in the amount of RNA. RNA expression levels were quantified by using Expression suite software. 10 mg/kg drug minimized the ANP and BNP mRNA expression levels induced in Apo E^{-/-} mice fed with high fat and cholesterol diet (HFHC) (A). 10mg/kg drug also induces the SOD1, SOD2 and catalase mRNA expression levels in Apo E^{-/-} mice (fed with high fat and cholesterol diet). HIF-1 α expression was increased in hearts from Apo E^{-/-} placebo animals compared with 10 mg/kg drug treated group (B).

Figure 4. Expression of phospho p44MAPK in hypertrophic mouse heart is mitigated by the use of D-PDMP

Left ventricular heart homogenates were prepared from placebo, 5 mg/kg and 10 mg/kg treated mice hearts. Western blot analysis was done using anti phospho p44MAPK antibody. Expression level of GAPDH was used to normalize the protein expression. Expression levels were quantified by densitometric scanning using Image J v.1.45s (NIH, USA) software. (A) Western immunoblot of phosphorylated form of p44MAPK in placebo heart tissue as compared to control, (B) corresponding densitometric scan.

Table 1
Structural and functional changes of the left ventricle in treated and untreated Apo E^{-/-} mice.

Week	Baseline (12wk)	Control (n=4)	Placebo (n=7)	5 mg/kg (n=8)	10 mg/kg (n=9)	Anova
Cardiac parameters	(n=10)					p<0.05
LVEDD (mm)	3.04±0.08	3.19±0.01	3.23±0.04	3±0.09	2.95±0.03	NS
LVESD (mm)	1.21±0.03	1.47±0.03	1.96±0.1	1.39±0.06	1.15±0.03	0.0001
IVSD (mm)	1.01±0.01	1.23±0.02	1.34±0.03	1.23±0.03	1±0.02	0.0001
PW/TED (mm)	0.95±0.02	1.19±0.008	1.3±0.03	1.17±0.03	0.96±0.02	0.0001
FS (%)	60.07±0.34	53.88±1.17	39.34±2.82	53.37±0.52	61.13±0.61	0.0001
EF (%)	84.04±0.23	78.68±1.09	62.73±3.47	78.24±0.49	84.86±0.47	0.0001
LV mass (mg)	102.86±5.09	151.9±1.54	178.04±4.49	136.9±5.32	97.84±3.28	0.0001
RWT	0.63±0.02	0.74±0.02	0.81±0.03	0.79±0.04	0.65±0.01	0.0001
HR (b/min)	703.34±7.64	694.44±27.86	678.89±18.99	706.3±11.1	707.08±6.27	NS

One way ANOVA analysis with p<0.01*,

** p<0.001,

*** p<0.0001,

Mean±SD, not significant (NS)

Echocardiography parameters of placebo in HFHC diet and D-PDMP treated with 5 and 10 mg/kg mice. Left ventricle end diastolic and end systolic diameter (LVEDD and LVESD), interventricular septal and posterior wall thickness at end diastolic and end systolic phase (IVSED and PW/TED), fractional shortening (FS), ejection fraction (EF), left ventricular mass (LV mass), relative wall thickness (RWT), and heart rate (HR).