Inflammatory mediators in diet-induced cardiac dysfunction

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CHAPTER 2

Gender-dependent effects of high-fat lard diet on cardiac function in C57Bl/6J mice

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Gender-dependent effects of high-fat lard diet on cardiac function in C57Bl/6J mice

Abstract

Increased availability of fatty acids released from insulin-resistant adipose tissue may lead to excess fatty acid uptake in nonadipose organs, including the heart. Accumulation of toxic fatty acid intermediates may affect cardiac function. Our aim was to identify to which extent high-fat diet feeding leads to alterations in cardiac function and whether this depends on gender and/or duration of high-fat diet feeding.

Male and female C57Bl/6J mice (n = 8 per group) of 12 to 16 weeks old were fed a low-fat (10% energy) or high-fat (45% energy) lard diet for 6 or 12 weeks. Plasma lipid levels, echocardiography, and left ventricular pressure–volume relationships were obtained at 2, 1, and 0 weeks before termination, respectively.

In both male and female mice, the high-fat diet increased body weight and plasma lipid content. At 10 weeks, significant increases were observed for plasma total cholesterol (males: +44%; females: +86%), phospholipids (+16% and +34%), and triglycerides (+27% and +53%) (all $p < 0.001$). In male mice, but not in female mice, the high-fat diet significantly affected cardiac function at 12 weeks with increased end-systolic volume (25.4 ± 6.2 vs. 17.0 ± 6.7 μL, $p < 0.05$), increased end-systolic pressure (72.1 ± 6.9 vs. 63.6 ± 6.9 mm Hg, $p < 0.01$), and decreased ejection fraction ($61.2\% \pm 4.5\%$ vs. $68.1\% \pm 3.7\%$, $p < 0.01$), indicating reduced systolic function. Multiple linear regression analysis indicated a significant diet–gender interaction for end-systolic volume and ejection fraction.

In conclusion, high-fat diet feeding increased body weight and plasma lipid levels in male and in female mice, but resulted in impairment of cardiac function only in males.

Introduction

A drastic increase in caloric intake combined with lifestyle changes and immobilizing technical innovations have caused an unprecedented epidemic of obesity in the western world. It is assumed that obesity-related heart disease, in combination with diabetes, is predominantly related to coexisting disorders such as coronary artery disease and hypertension.[1, 2] However, studies suggest that increased myocardial lipid deposition, resulting from fatty acid (FA) overload of cardiac myocytes, is directly associated with cardiac dysfunction.[3, 4]

Oxidation of long-chain FAs by mitochondrial β-oxidation supplies most of the energy for the heart under normal conditions.[5] However, the uptake of FAs by the heart in relation to FA oxidation is not always tightly controlled, which may lead to excessive storage of FAs as triglycerides in cardiac myocytes. These myocardial triglyceride stores *per se* are probably inert, but triglycerides are involved in hydrolysis-reesterification cycles, yielding FAs, fatty acyl coenzyme A esters, and diacylglycerol as intermediates. Accumulated cardiac FAs and metabolites are described in several diseases like diabetes and obesity [6-9], as well as in the ischemic and hypertrophic heart [10, 11], and can result in cardiac dysfunction and cell damage.

In humans, gender-dependent differences in myocardial function are reported in certain conditions. For instance, following ischemia, males as compared with females have decreased diastolic function, cardiac contractility, and an increased mortality risk.[12] Furthermore, evidence is accumulating that gender differences also influence metabolic changes of the heart in presence of obesity, insulin resistance, and the related lipotoxicity and cardiac steatosis. Likewise, in animal models of myocardial infarction and hypertension, males are more prone to develop heart failure [13] and detrimental effects of lipotoxicity were shown in rodent models[14].w However, the impact of gender on the effect of lipotoxicity is not fully understood. Earlier, large gender differences have been observed in mice lacking the PPARα receptor, a nuclear receptor controlling lipid utilization. A combination of PPARα-deficiency and pharmacological carnitine palmitoyltransferase (CPT1) inhibition resulted in extreme myocardial lipid accumulation leading to death in 100% of the males but only in 25% of the females.[15] Despite the fact that gender effects are relevant in studies on cardiac pathophysiology, little is known about the effect of gender on the relationship between diet-induced obesity, lipid metabolism, and cardiac function.

Therefore, the aim of our study was to investigate to what extent high-fat diet (HFD) feeding as compared with low-fat diet (LFD) feeding leads to alterations in cardiac function in a widely used C57Bl/6J mouse model, and whether this depends on gender and/or duration of HFD feeding. We used pressure-volume (PV) conductance catheters to obtain accurate assessment of left ventricular performance. This PV-loop method is the gold standard for assessing intrinsic myocardial function in humans and animals.[16] Our data indicate a gender-specific effect of HFD feeding on cardiac function: in particular we observed a more pronounced effect on systolic function in male vs. female mice, despite less pronounced effects on plasma lipids.

Materials and methods

Animals and study protocol

The experiments were performed in 12- to 16-week-old male and female C57Bl/6J mice (Charles River, Maastricht, the Netherlands). Mice were housed in a temperature and humidity-controlled room on a 12-h light / 12-h dark cycle with ad libitum access to water and food. In all animals, the specific diets were started in week 0 and continued throughout the protocol. Body weights were measured weekly. The experimental protocol to study the effects of gender, dietary fat content, and diet duration is shown schematically in figure 1. Briefly, we studied 8 groups of mice (8 animals each). Measurements of plasma lipids, echocardiography, and PV-loops were performed in weeks 4, 5, and 6 (groups 1–4) and in weeks 10, 11, and 12 (groups 5–8), respectively. At the end of the experiments the animals were sacrificed. The protocol was approved by the Animal Ethics Committee from the Leiden University Medical Center and conformed to the Guide for Care and Use of Laboratory Animals (NIH publication no. 85–23, revised 1996).

Diets

Starting in week 0, all animals received either a lard-based LFD or HFD for 6, 10, or 12 weeks. The diets were obtained from Research Diets Inc. (Wijk bij Duurstede, the Netherlands) and supplied ad libitum. As a percentage of the total energy, the LFD contained 10% fat (4.5% lard and 5.5% soy bean oil) and 70% carbohydrate (34.5% energy from sucrose, 32% from corn starch, and 3.5% from maltodextrin). The HFD contained 45% energy from fat (39.5% lard and 5.5% soy bean oil) and 35% carbohydrate (17% energy from sucrose, 8% from corn starch and 10% from maltodextrin).

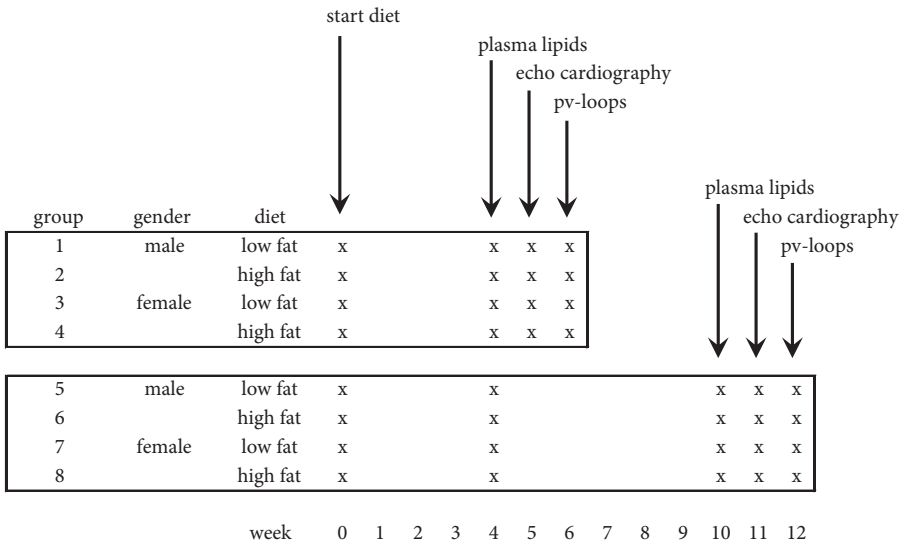

Figure 1 **Schematic study protocol.** See materials and methods for explanation.

Plasma analysis

Blood was sampled after a 4-h fast (09.00 to 13.00 h) via tail vein bleeding and was collected in potassium EDTA coated plastic tubes (Starstedt, Nümbrecht, Germany). Total plasma levels of cholesterol, triglyceride, phospholipid, and glucose were measured by using commercially available kits and standards according to the instructions of the manufacturer (kit no. 1489232, 11488872, and 101140; Roche Diagnostics, Mannheim, Germany; and 2942 Instruchemie, Delfzijl, the Netherlands). Plasma insulin concentrations were determined by ELISA (Crystal Chem Inc., Downers Grove, Ill., USA).

Echocardiography

Transthoracic echocardiography was performed using a VisualSonics Vevo 770 with a 30 MHz ultrasound transducer (VisualSonics, Toronto, Ont., Canada). During examination, mice were anesthetized with 2% inhaled isoflurane and placed on a temperature-controlled platform. Electrocardiogram, heart rate (HR), and respiratory rate were monitored continuously and recorded during the imaging process. Parasternal long axis and short axis images were recorded in all animals. Analysis of the data was performed with software provided by VisualSonics. Briefly, left ventricle (LV) internal diameters at end-diastole and end-systole (D_{ED}, D_{ES}) were obtained from short-axis M-mode images as average of 3 consecutive cardiac cycles. M-mode derived end-diastolic volume (EDV) and end-systolic volume (ESV) were estimated as [17]:

$$EDV = ((7.0 / (2.4 + D_{ED})) \times D_{ED}^3$$

$$ESV = ((7.0 / (2.4 + D_{ES})) \times D_{ES}^3$$

Ejection fraction (EF) and cardiac output (CO) were calculated as:

$$EF = 100\% \times (EDV - ESV) / EDV$$

$$CO = HR \times (EDV - ESV)$$

Left ventricular PV-loops

Hemodynamics and LV function indices were assessed by invasive PV-loops. Mice were anesthetized with an intraperitoneally injected mix of 6.25 $mg \cdot kg^{-1}$ body weight Ventranquil (Ceva Sante Animale, Naaldwijk, the Netherlands), 6.25 $mg \cdot kg^{-1}$ body weight Midazolam (Actavis, Hafnarfjordur, Iceland), and 0.3125 $mg \cdot kg^{-1}$ body weight Fentanyl (Hameln Pharmaceuticals, Hameln, Germany) diluted in sterile water. Thereafter the mice were placed supine under a surgical microscope on a temperature-controlled warming pad to maintain a normal body temperature. Via the right carotid artery a 1.2F PV catheter (FTS-1212B-4518, Scisense Inc., London, Ont., Canada) was placed into the LV. The catheter was connected to a Scisense ADV signal processor (Scisense Inc.) to generate high-fidelity pressure and volume signals. Positioning of the catheter was guided by online pressure and volume signals. On-line display and acquisition of the signals (2000 $samples \cdot s^{-1}$) was performed with a PowerLab 8/30 data acquisition system and LabChart Pro software (AD Instruments GmbH, Spechbach, Germany). Off-line data analysis was performed with custom-made software (CircLab, P. Steendijk). Raw LV volume signals obtained by conductance were calibrated by matching EF and CO with corresponding echocardiographic values. PV signals were acquired in steady state to obtain general hemodynamics via HR, stroke volume (SV), CO, and stroke work (SW). Effective arterial elastance (E_A) was calculated as the

ratio of end-systolic pressure (ESP) and SV.[18] Systolic LV function was quantified by ESP, ESV, EF, and maximal rate of pressure increase (dP/dt_{MAX}). In addition, we determined end-systolic elastance (E_{ES}) as a load-independent index of intrinsic LV function, using a validated single-beat approach.[19] Ventricular-arterial coupling was quantified as E_{ES}/E_A.[20] Diastolic LV function was assessed by end-diastolic pressure (EDP), EDV, relaxation time constant τ, and the maximal rate of pressure decline ($-dP/dt_{MIN}$). Intrinsic LV diastolic function was quantified by end-diastolic stiffness (E_{ED}) (1/compliance) and the diastolic stiffness constant (K_{ED}). Thus, all presented hemodynamic data were obtained from invasive PV loops and echocardiographic volumes were used to calibrate the conductance-derived volumes.

Statistical analyses

Results for body weight, plasma lipids, insulin and glucose levels, and cardiac function are shown as means ± SD and were compared using unpaired t-tests and DEXA scan data followed by a Tukey's multiple comparison test (SPSS for windows 17.0, SPSS Inc., Chicago, IL, USA).

To investigate the effects of gender, and type and duration (time) of the diet intervention, and in particular the interaction between these factors, the data were submitted to a multiple linear regression model:

$$Y = A_O + A_G \times G + A_D \times D + A_T \times T + A_{GD} \times G \times D + A_{GT} \times G \times T + A_{TD} \times T \times D$$

In this model, Y represents the independent measurement variable, G codes the gender (female: G = –1, male: G = 1), D the type of diet (LFD: D = –1, HFD: D = 1), and T codes the diet intervention duration (6 weeks: T = –1, 12 weeks: T = 1). By using effects coding, coefficient A_O provides the overall mean value of Y; A_G, A_D, and A_T are the magnitude of the main effects; and A_{GD}, A_{GT}, and A_{DT} are the magnitude of the corresponding interactive effects.[21] The p values of the various coefficients indicate whether the corresponding effects are statistically significant. Grubb's test for detecting outliers was used to identify and exclude outliers. $p < 0.05$ was considered significant. All data are presented as means ± SD.

Results

HFD feeding increases body weight in males and females

To determine the effect of duration of HFD feeding on cardiac function, male and female mice underwent diet intervention for 6 or 12 weeks. All male and all female groups had similar body weights at the start of the study (figure 2). Weekly monitoring of body weight showed that HFD feeding gradually increased body weight as compared with LFD feeding, the difference reached statistical significance from 5 weeks onwards for males and from 4 weeks onwards for females (not shown) and remained significant until the end of the study. After 6 weeks, body weight was increased for males by 19% ± 8% in the HFD group vs. 5% ± 2% in the LFD group ($p < 0.01$), which was similar to the females with increases of 18% ± 12% vs. 2% ± 5%, respectively ($p < 0.05$). After 12 weeks the difference between the HFD and LFD groups was more pronounced with 32% ±

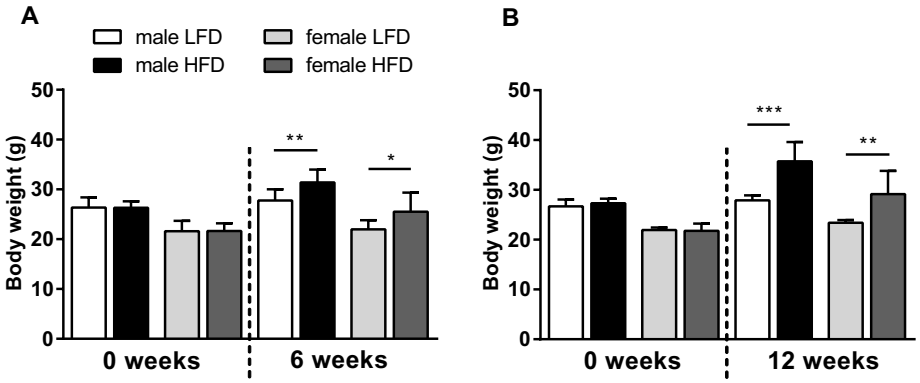

Figure 2 **Effect of diet intervention on body weight.** Male and female C57Bl/6J mice were fed a low-fat diet (LFD) or high-fat diet (HFD) for 6 weeks (A) or 12 weeks (B). Body weight was measured at baseline and at the end of dietary intervention. Values represent means ± SD (n=8 per group). *p<0.05, **p<0.01, ***p<0.001, compared to the corresponding LFD group.

10% vs. 5% ± 3% for the males (p< 0.001) and 32% ± 15% vs. 7% ± 2% for the females, respectively (p< 0.01). HFD-induced body weight gain was not significantly different between males and females after 6 and 12 weeks of diet intervention.

HFD feeding increases plasma lipids in males and females

After 4 and 10 weeks, blood samples were obtained and plasma levels of total cholesterol, phospholipid, and triglyceride levels were determined (figure 3). After 4 weeks of HFD feeding, total cholesterol levels were significantly increased in males (+31%, p< 0.05) and females (+55%, p< 0.001) as compared with the LFD groups. Furthermore, after 4 weeks of HFD feeding, phospholipid levels were increased in females only (+20%, p< 0.01) as compared with the LFD group, whereas HFD feeding did not affect triglyceride levels in

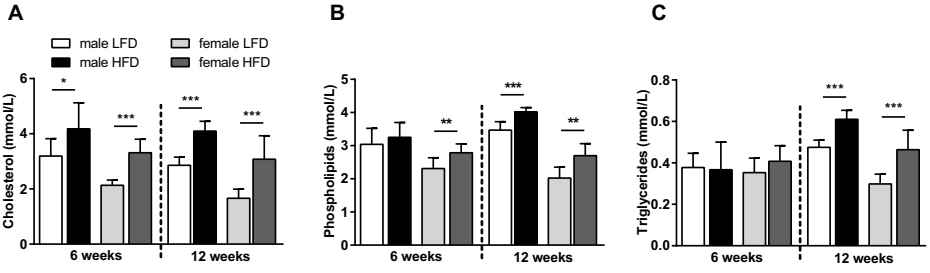

Figure 3 **Effect of diet intervention on plasma lipids.** Male and female C57Bl/6J mice were fed a low-fat diet (LFD) or high-fat diet (HFD) for 6 weeks or 12 weeks. Plasma levels of cholesterol (A), phospholipids (B) and triglycerides (C) were measured at 4 and 10 weeks, respectively. Values represent means ± SD (n=7-8 per group). *p<0.05, **p<0.01, ***p<0.001, compared to the corresponding LFD group.

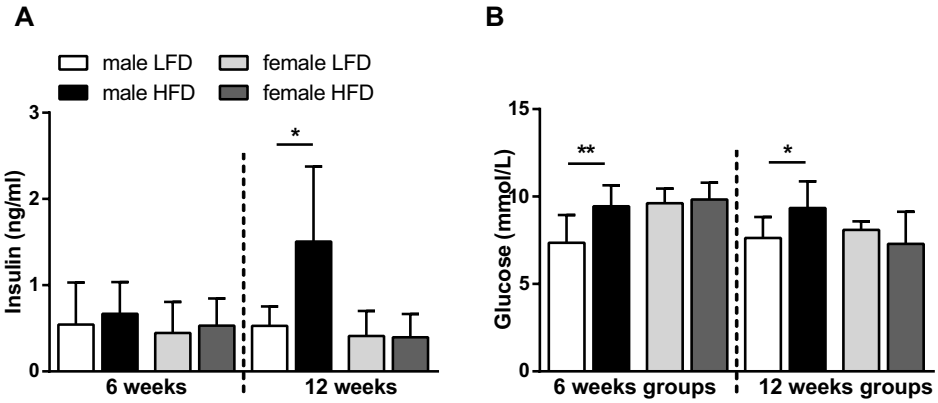

Figure 4 **Effect of diet intervention on plasma insulin and glucose levels.** Male and female C57Bl/6J mice were fed a low-fat diet (LFD) or high-fat diet (HFD) for 6 weeks or 12 weeks. Plasma levels of insulin (A) and glucose (B) were measured at 4 and 10 weeks, respectively. Values represent means ± SD (n=7-8 per group). *p<0.05, **p=0.01, compared to the corresponding LFD group.

both genders. After 10 weeks, all plasma lipid parameters for both males and females were significantly increased in the HFD group in comparison with the corresponding LFD group: total cholesterol (males: +44%, p< 0.001; females: +86%, p< 0.001), phospholipids (males: +16%, p< 0.001; females: +34%, p< 0.01) and triglycerides (males: +27%, p< 0.001; females: +53%, p< 0.001).

HFD feeding increases insulin and glucose levels in males

To determine the effect of HFD feeding on glucose metabolism, insulin and glucose levels were measured in plasma. As compared with the LFD group, 4 weeks of HFD feeding did not increase plasma insulin levels in males and females, whereas glucose levels were mildly increased in only male mice (+28%, p=0.01) (figure 4). After 10 weeks of HFD feeding, insulin (+186%, p< 0.05) as well as glucose levels (+22%, p< 0.05) were significantly increased in males, while the levels for females fed a LFD and HFD were not different.

HFD feeding selectively impairs cardiac function in males

Cardiac function measurements, obtained by combined echocardiography and PV-loops, are summarized in table 1. Based on the mean values for end-diastolic and end-systolic pressures and volumes, schematic PV-loops were created for all groups and corresponding end-systolic and end-diastolic PV-relations were added, based on mean E_{ES} and E_{ED} values (figure 5).

When compared with LFD, in male mice, but not in female mice, the HFD significantly affected cardiac function at 6 weeks with increased EDV (58.0 ± 12.2 vs. 39.9 ± 15.4 μL,

Table 1 **Effect of diet intervention on cardiac function.**

	Male		Female		Male		Female	
	6 weeks LFD	6 weeks HFD	6 weeks LFD	6 weeks HFD	12 weeks LFD	12 weeks HFD	12 weeks LFD	12 weeks HFD
General								
HR (beats/min)	378 ± 38	369 ± 36	377 ± 125	421 ± 125	414 ± 63	384 ± 66	317 ± 94	328 ± 63
SV (μL)	28.1 ± 7.3	38.7 ± 9.7	35.6 ± 6.1	35.1 ± 1.8	40.2 ± 8.0	39.7 ± 6.0	27.5 ± 6.9	33.9 ± 9.8
CO (mL/min)	10.6 ± 3.6	14.4 ± 4.1	12.9 ± 2.8	13.7 ± 4.5	17.2 ± 3.7	15.3 ± 3.7	8.5 ± 2.7	10.8 ± 2.5
SW (mm Hg.μL)	1854 ± 541	2504 ± 635	2436 ± 312	2232 ± 620	2650 ± 523	2847 ± 361	1725 ± 509	2298 ± 745
E_A (mm Hg/μL)	2.60 ± 0.84	1.84 ± 0.37	2.39 ± 0.85	2.21 ± 0.60	1.69 ± 0.38	1.87 ± 0.35	2.69 ± 0.91	2.27 ± 0.59
Ees/Ea	1.37 ± 0.18	1.43 ± 0.19	1.48 ± 0.31	1.21 ± 0.35	1.19 ± 0.24	1.25 ± 0.19	1.36 ± 0.37	1.40 ± 0.21
Systolic								
ESP (mm Hg)	67.1 ± 5.7	66.0 ± 4.1	78.1 ± 14.1	69.3 ± 13.7	63.6 ± 4.2	72.1 ± 6.9**	70.8 ± 4.0	71.8 ± 5.5
ESV (μL)	11.8 ± 8.7	19.3 ± 4.8	15.0 ± 6.6	14.3 ± 5.3	17.0 ± 6.2	25.4 ± 6.2*	15.1 ± 6.9	15.4 ± 4.7
EF (%)	74.2 ± 11.7	66.5 ± 7.0	71.6 ± 9.1	70.6 ± 6.0	68.1 ± 3.1	61.2 ± 4.5**	65.6 ± 11.5	68.7 ± 6.6
dP/dt_{MAX} (mm Hg/ms)	5.7 ± 2.0	5.4 ± 0.5	6.6 ± 3.0	6.1 ± 2.7	5.9 ± 0.4	6.0 ± 1.2	4.8 ± 2.1	5.3 ± 0.8
E_{ES} (mm Hg/μL)	3.53 ± 1.11	2.63 ± 0.77	3.27 ± 0.47	2.63 ± 0.82	2.04 ± 0.71	2.29 ± 0.32	3.85 ± 2.02	3.10 ± 0.60
Diastolic								
EDP (mm Hg)	4.29 ± 1.57	3.15 ± 1.01	7.73 ± 3.11	5.49 ± 4.65	4.03 ± 1.49	5.01 ± 2.10	6.07 ± 3.24	6.11 ± 3.70
EDV (μL)	39.9 ± 15.4	58.0 ± 12.2*	50.6 ± 10.9	47.3 ± 9.2	57.2 ± 13.0	65.1 ± 11.1	42.5 ± 11.2	49.4 ± 13.0
Tau (ms)	14.4 ± 3.8	14.4 ± 1.8	14.4 ± 4.4	14.8 ± 5.3	11.1 ± 1.2	12.7 ± 1.8	18.0 ± 5.7	16.0 ± 2.4
$-dP/dt_{MIN}$ (mm Hg/ms)	3.6 ± 0.9	3.5 ± 0.3	4.7 ±2.0	4.0 ± 1.8	4.3 ± 0.3	4.2 ± 0.8	3.1 ± 1.2	3.4 ± 0.7
E_{ED} (mm Hg/μL)	0.160 ± 0.06	0.139 ± 0.05	0.200 ±0.06	0.170 ± 0.11	0.144 ± 0.06	0.183 ± 0.08	0.220 ± 0.09	0.234 ± 0.14
K_{ED} (1/μL)	0.051 ± 0.02	0.061 ± 0.03	0.068 ± 0.03	0.066 ± 0.02	0.043 ± 0.02	0.061 ± 0.02	0.051 ± 0.01	0.066 ± 0.02

Male and female C57Bl/6J mice were fed a low-fat diet (LFD) or high-fat diet (HFD) for 6 weeks or 12 weeks, and cardiac function was determined. Left ventricle (LV) volume signals obtained by conductance catheter were calibrated by matching EF and CO with corresponding echocardiographic values obtained by measurements four days earlier. Values represent means ± SD (n=8 per group). *p<0.05, **p<0.01 as compared to LFD group. HR, heart rate; SV, stroke volume; CO, cardiac output; SW, stroke work; E_A, arterial elastance (afterload); E_{ES}/E_A, ventricular arterial coupling; ESP, end-systolic pressure; ESV, end-systolic volume; EF, ejection fraction; dP/dt_{MAX}, maximal rate of pressure increase; E_{ES}, end-systolic elastance; EDP, end-diastolic pressure; EDV, end-diastolic volume; Tau, relaxation time constant; $-dP/dt_{MIN}$, maximal rate op pressure decline; E_{ED}, diastolic stiffness; K_{ED}, diastolic stiffness constant.

p< 0.05) and at 12 weeks with increased ESV (at 12 weeks: 25.4 ± 6.2 vs. 17.0 ± 6.7 μL, p< 0.05), increased ESP (72.1 ± 6.9 vs. 63.6 ± 6.9 mm Hg, p< 0.01), and decreased EF (61.2 ± 4.5 vs. 68.1 ± 3.7%, p< 0.01), indicating reduced systolic function after 12 weeks of HFD feeding (table 1).

Subsequently, we used a multiple linear regression model to test whether the gender, the type of diet, or the duration of the diet interventions had significant effects on the various hemodynamic indices. In addition to these main effects, this approach was used to statistically analyze possible interactive effects, in particular to investigate if the diet effects were statistically different for male and female mice. The statistical analyses are presented in table 2.

To explain the results of the multiple linear regression model, ESV is taken as an example. The coefficient A_0 (16.66) yields the mean value of ESV averaged over all mice. Since p_F, reflecting the overall significance of the model, was significant (p = 0.004), the investigated effects (gender, diet, and/or time) apparently significantly affected ESV. A significant effect was observed for gender (p = 0.032): the positive value of A_G (1.72) indicates that the average ESV in male mice (G = 1) was higher (16.66 + 1.72 mL) than in females (G = –1, thus 16.66 – 1.72 mL). The significant (p = 0.017) coefficient A_D indicates that average ESV in mice subjected to HFD (D = 1) was 1.94 mL higher than the overall mean, and for mice with a LFD (D = –1) ESV was 1.94 mL lower. Finally, the coefficient A_{GD} indicates a significant (p = 0.012) gender-diet interaction (thus, the diet effect is different between males and females) with an additional 2.03 mL increase in ESV for males with HFD (and corresponding interactive effects in the other groups).

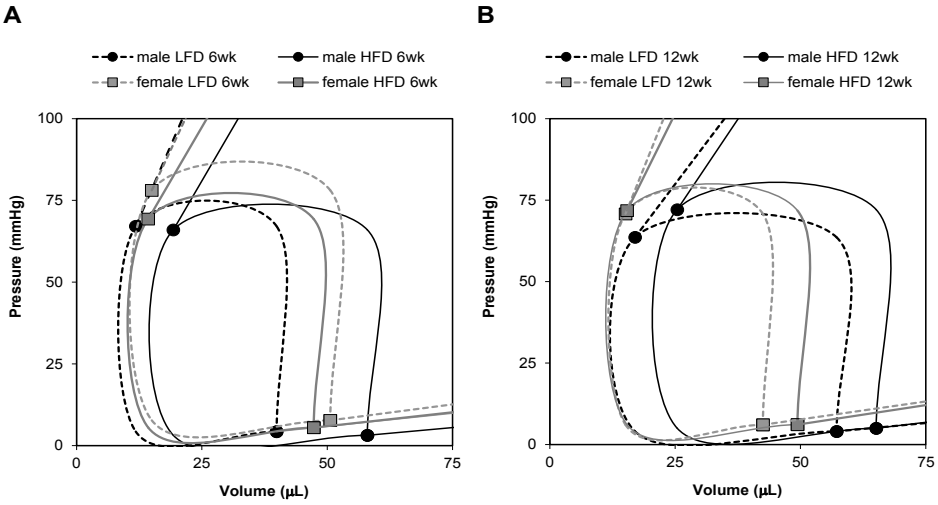

Figure 5 **Effect of diet intervention on pressure-volume loops and pressure-volume relations.** Male and female C57Bl/6J mice were fed a low-fat diet (LFD) or high-fat diet (HFD) for 6 weeks (A) or 12 weeks (B). Pressure-volume loops were recorded in each mouse, and average pressure-volume loops are shown per group.

Table 2 **Effects of diet intervention on cardiac function (multiple linear regression model)**

	Intercept		Main Effects						Interactive Effects					
	A_0	(p_F)	A_G	(p)	A_D	(p)	A_T	(p)	A_{GD}	(p)	A_{GT}	(p)	A_{DT}	(p)
General														
HR	373.54	(0.226)	12.82	(0.245)	2.12	(0.847)	-12.76	(0.247)	-11.80	(0.284)	25.37	(0.024)	-6.66	(0.544)
SV	34.85	(0.003)	1.82	(0.052)	2.00	(0.033)	0.47	(0.609)	0.53	(0.565)	2.79	(0.004)	-0.50	(0.587)
CO	12.93	(0.000)	1.44	(0.002)	0.64	(0.152)	0.04	(0.925)	-0.16	(0.722)	1.85	(0.000)	-0.53	(0.234)
SW	2318.3	(0.002)	145.6	(0.038)	152.0	(0.030)	61.7	(0.371)	59.9	(0.385)	223.0	(0.002)	40.5	(0.557)
E_A	2.19	(0.024)	-0.20	(0.019)	-0.15	(0.074)	-0.06	(0.430)	0.00	(0.983)	-0.15	(0.063)	0.09	(0.295)
E_{ES}/E_A	1.34	(0.267)	-0.03	(0.439)	-0.01	(0.677)	-0.03	(0.308)	0.04	(0.199)	-0.05	(0.104)	0.04	(0.221)
Systolic														
ESP	69.85	(0.036)	-2.66	(0.013)	-0.06	(0.957)	-0.29	(0.781)	1.89	(0.072)	0.92	(0.377)	2.45	(0.022)
ESV	16.66	(0.004)	1.72	(0.032)	1.94	(0.017)	1.55	(0.053)	2.03	(0.012)	1.26	(0.116)	0.24	(0.759)
EF	68.28	(0.069)	-0.81	(0.418)	-1.57	(0.122)	-2.42	(0.019)	-2.09	(0.041)	-0.43	(0.670)	0.61	(0.541)
dP/dt_{MAX}	5.711	(0.635)	0.021	(0.928)	-0.033	(0.886)	-0.221	(0.342)	-0.038	(0.871)	0.426	(0.070)	0.191	(0.412)
E_{ES}	2.92	(0.006)	-0.29	(0.020)	-0.26	(0.043)	-0.10	(0.423)	0.09	(0.457)	-0.36	(0.005)	0.13	(0.298)
Diastolic														
EDP	5.23	(0.068)	-1.12	(0.003)	-0.29	(0.416)	0.07	(0.845)	0.26	(0.478)	0.33	(0.361)	0.55	(0.130)
EDV	51.26	(0.002)	3.80	(0.015)	3.69	(0.018)	2.28	(0.138)	2.82	(0.068)	3.79	(0.015)	-0.01	(0.997)
Tau	14.49	(0.026)	-1.33	(0.005)	0.02	(0.964)	-0.02	(0.970)	0.41	(0.371)	-1.22	(0.010)	-0.10	(0.823)
$-dP/dt_{MIN}$	3.853	(0.124)	0.060	(0.683)	-0.068	(0.642)	-0.106	(0.471)	-0.024	(0.867)	-0.447	(0.003)	-0.140	(0.338)
E_{ED}	0.181	(0.232)	-0.025	(0.021)	0.000	(0.976)	0.014	(0.183)	0.004	(0.686)	-0.007	(0.500)	0.013	(0.216)
K_{ED}	0.058	(0.199)	-0.004	(0.110)	0.005	(0.052)	-0.003	(0.237)	0.002	(0.486)	0.001	(0.699)	0.003	(0.249)

All hemodynamic indices from table 1 were fitted to the multiple linear regression model: $Y = A_O + A_G \times G + A_D \times D + A_T \times T + A_{GD} \times G \times D + A_{GT} \times G \times T + A_{DT} \times D \times T$. In this model, Y represents the independent measurement variable, G codes the gender (female: G=-1, male: G=1), D codes the type of diet (low-fat: D=-1, high-fat: D=1), and T codes the diet intervention duration (6-week: T=-1, 12-week: T=1). With this coding, the coefficient A_0 gives the overall mean value of each index; A_G, A_D, and A_T indicate the magnitudes of the main effects; A_{GD}, A_{GT}, and A_{DT} the interactive effects. The significance of the model is given by p_F, the significance of each coefficient by the corresponding p-value shown in the same column. For abbreviations see legends of table 1.

First, calculations were made for the entire model fit for each hemodynamic index. The p values are presented in the Intercept block in table 2, reflecting the significance of this F test. The model showed significant effects for general hemodynamic function (SV, CO, SW, and E_A), for systolic function (ESP, ESV and E_{ES}), and diastolic function (EDV and τ).

Then, the effects of the separate factors, gender, type of diet, and duration of diet intervention were calculated for the affected indices. These calculations are presented in the main effects block in table 2. All these indices showed a significant gender effect (except SV, which just fell short of statistical significance at p = 0.052), with higher CO, SW, ESV, and EDV (indicated by the positive AG) and lower E_A, ESP, E_{ES}, and τ (negative A_G) in males (G = 1) compared with females (G = –1). The type of diet (coefficient A_D) had a significant main effect on SV, SW, E_A, (marginal, p=0.074) ESV, E_{ES}, and EDV. HFD feeding (D = 1) resulted in higher values for SV, SW, ESV, and EDV, and lower values for E_A and E_{ES}. For none of the indices, diet duration (reflected by coefficient A_T) had a significant main effect, indicating that for the full cohort (thus males and females on both LFD and HFD) there was no significant difference between 6 weeks and 12 weeks diet intervention.

Furthermore, the interaction of the different factors on the hemodynamic indices was calculated (interactive effects block, table 2). For several indices there was a significant interaction between gender and diet duration, indicating that either the effect of diet duration was different between males and females or the effect of gender was significantly different between the 6 and 12 weeks groups. This significant interaction was found for SV, CO, SW, E_{ES}, EDV, and τ. Furthermore, a significant interactive diet duration–diet type effect was found for ESP, whereas interaction between gender and diet type was present for ESV, EF, ESP, and EDV, the latter being only marginally significant.

Discussion

The present study was designed to investigate to what extent high-fat feeding leads to alterations in cardiac function in C57Bl/6J mice and whether this depends on gender and/or durations of high-fat feeding. The data obtained from both echocardiography and conductance catheter indicates that in male mice cardiac function was significantly altered by prolonged HFD feeding. HFD feeding also increased the insulin and glucose levels in males, implying an impaired glucose tolerance. These effects were absent in female mice despite their more pronounced elevation of plasma lipid levels, indicating the relevance of gender in this mouse model of obesity-related cardiac disease.

Previous studies have provided strong evidence that excessive accumulation of triglycerides in cardiac myocytes of obese animals results in impaired cardiac function, as characterized by an increase in LV end-diastolic diameter and a significant reduction in cardiac contraction.[4,22] In addition, increased triglyceride stores are found in cardiac myocytes of streptozotocin-induced diabetic rats [23,24] and cholesterol-fed hyperlipidemic

rats [25]. However, in these experiments it has never been investigated whether there is a difference in cardiac function between male and female mice, and thus whether gender choice of the animal model is of importance in future research of obesity-induced cardiac disease.

We have now showed that 12 weeks of intervention with HFD induced obesity and increased plasma lipid levels in both male and female mice. However, insulin and glucose levels, as well as the effects on cardiac function, were only affected in males. The difference in heart function was observed by comparing the HFD fed animals to the corresponding LFD group, revealing that only in males systolic function was attenuated by feeding HFD for 12 weeks. This was evidenced by increased ESP and ESV and a reduced EF. The most pronounced effect of the diet is seen in males after 12 weeks as compared with 6 weeks of HFD feeding, which explains that a longer exposure results in a stronger effect. In females the HFD did not affect any cardiac function parameter after 6 or 12 weeks.

We further quantified the contributions of the separate factors and their interactions in a multiple linear regression model. This analysis (table 2) showed that gender had the most prominent effects on cardiac function. Also, these gender effects were not confined to a sole parameter, but were observed in most parameters for general, systolic, and diastolic indices. Fully consistent with previous studies in humans [26, 27], the males showed higher ventricular volumes and cardiac output and lower pressures, whereas E_{ES} and E_A, representing intrinsic systolic function and afterload, respectively, were also both lower in males. Thus, lower systolic function is more prominent in males and is compensated by lower afterload resulting in maintained, or even higher, cardiac output compared with females.

The multiple linear regression model further showed significant main effects of the diet intervention on cardiac function as indicated by increased volumes and a decreased E_{ES}. This is in line with studies in humans, which show an association between obesity and impaired cardiac function.[1, 28] However, to our knowledge no studies investigated possible differences between men and women on this association. In our study, the overall effects of diet did not reach significance in all individual groups (table 1). In particular, the females showed limited or no changes in volume, whereas the changes were much more pronounced in males. This differential effect was indeed reflected by (nearly) significant interactive gender–diet effects on ESV ($p= 0.012$) and EVD ($p= 0.068$). With regard to E_{ES}, the overall significant decrease with HFD feeding was reflected by clear trends for a decrease in all individual groups, except for males after 12 weeks on HFD (table 1). This is interesting since in this particular group ESP and ESV both increased significantly, but E_{ES} remained unchanged. However, given the E_{ES} of approximately 2 mm Hg·μL^{-1}, the observed increase in ESV (8.4 μL) could not be explained merely by the higher ESP (8.5 mm Hg). Thus, in this group the end-systolic pressure-volume relation apparently showed a more or less parallel rightward shift (as shown in figure 5B), which is also associated with a decreased contractile state.[29, 30]

Furthermore, while in our study HFD feeding resulted in a reduced systolic function, it had only minor effects on diastolic function. This is in contrast with earlier findings in

literature, which showed that in obese patients mainly the diastolic function seemed to be affected.[31,32] Although this discrepancy could be due to species differences, additional factors are likely to play a role. In particular, we studied the effect of a single isolated factor, the lard diet, whereas previous studies in humans are clearly multifactorial.

A possible explanation for the different response in cardiac function between males and females may be a difference in susceptibility to obesity. Unpublished data from our group (S.A.A. van den Berg 2008, unpublished data) showed that sex hormones are the main contributing factors to obesity. We observed that HFD fed males compared with HFD fed females have a similar lean body mass, while males have an excessive increase in fat mass, which is not observed in females. Furthermore, ovariectomized females fed a HFD showed similar weight gain as males fed a HFD, which is explained by an increase in body fat. Conversely, male mice treated with estrogen fed a HFD showed a largely decreased weight gain, similar to HFD fed females, which is explained by a largely reduced body fat (supplementary figure S1). Collectively, these data indicate that sex hormones greatly influence HFD-induced increase in fat mass.

Besides the effect of estrogen on obesity, differences in sex hormones in males and females themselves can also influence heart function. Cardiac protection by estrogen is reported in several studies in different species. Studies with gene-targeted mouse strains revealed protection against hypertrophy in females.[33, 34] Also in male rats an accelerated progression to heart failure is reported after hypertension.[35] Additionally, comparable results are observed in humans: in postmenopausal women estrogen replacement reduced the risk of cardiovascular events.[36] Similarly, the survival rate after ischemic heart failure is higher in females compared with males.[37] Thus, it can be speculated that gender differences in sex hormones and fat content may contribute to the alterations in cardiac function we observed in our study.

Several limitation of our study should be mentioned. The number of animals investigated was relatively small (8 mice per group). Thus, potential group differences that currently showed clear trends could have reached significance with larger sample sizes. Assessment of PV relations was based on a single-beat estimates rather than load interventions. This approach has been validated, but its accuracy remains debated. We quantified afterload by effective E_A based on ventricular parameters, rather than a more detailed but complex aortic impedance analysis based on aortic pressure and flow data. However, the present study was mainly focused on ventricular function and E_A has been shown useful parameter to study ventricular-arterial coupling.[38] Calibration of the conductance catheter was based on echocardiographic data obtained 4 days earlier, thus using a non-simultaneous reference method. However, the same protocol was followed in all animals so this approach is unlikely to have influenced the observed effects or comparisons between groups.

In conclusion, the present study indicates that high-fat feeding gradually increases body weights and plasma lipids levels in C57Bl/6J mice independent of gender. However, at 12 weeks cardiac function is impaired in male mice, but not in female mice. These results indicate a gender-specific effect of high-fat feeding on cardiac function in mice, independent of increased plasma lipids. This was confirmed by additional statistical

analyses, calculating the impact of the separate factors and their interactions. We thus propose that male mice are the preferred model to investigate effects of ischemia and drug treatments on cardiac function in a setting of diet induced obesity.

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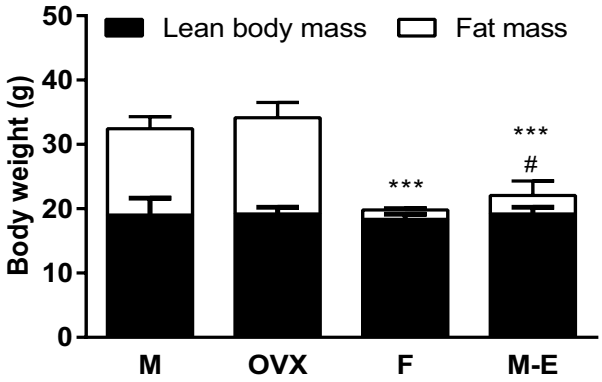
Supplemental data



Supplemental figure S1 **Effect of sex hormones on lean body mass and fat mass.** Male and female C57Bl/6J mice were fed a high-fat diet (HFD) for 10 weeks. M= male, OVX= ovariectomy, F= female, M-E= male treated with estrogen. Values represent means ± SD (n=5-6 per group). ***p<0.001, compared both with males and OVX females for fat mass; #p<0.05, compared with females for lean body mass.