CHAPTER 6

Toll-like receptor 2 and Toll-like receptor 4 deficiency modestly influence cardiac function in mice on a high-fat diet

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Toll-like receptor 2 and Toll-like receptor 4 deficiency modestly influence cardiac function in mice on a high-fat diet

Abstract

High-fat diet (HFD) feeding is a potent trigger for the development of obesity and cardiovascular disease. In addition, we previously reported that HFD feeding modestly impairs cardiac function in mice. Toll-like receptors (TLR) 2 and TLR4, receptors of the innate immune system, are hypothesized to be activated by ligands associated with HFD exposure such as (modified) fatty acids. Although TLR2 and 4 are involved in inflammation in cardiac disease, a direct relationship between HFD, TLR activation and cardiac dysfunction has not been reported. We therefore investigated the role of TLR2 and TLR4 in the impairment of cardiac function after exposure to HFD.

Male TLR2-deficient ($TLR2^{-/-}$) mice, TLR4-deficient ($TLR4^{-/-}$) mice and their corresponding wild-type (WT) littermates were fed a HFD for 12 weeks. Body weights were measured weekly, plasma lipid levels were obtained at week 10, and left ventricular pressure-volume relationships were measured at week 12.

As compared to their respective WT littermates, $TLR2^{-/-}$ and $TLR4^{-/-}$ mice showed no difference in body weight, plasma phospholipid, total cholesterol and triglyceride levels. In $TLR2^{-/-}$ mice, an increased end-diastolic volume (51±12 versus 73±18 μL; p=0.03) was observed, whereas other parameters of cardiac function were not different from WT littermates. In $TLR4^{-/-}$ mice, we observed trends of a decreased pressure half time (5.3±1.0 versus 4.3±0.6 ms; p=0.06) and an increased end-systolic elastance (1.6± 0.6 versus 3.3±2.0 mm Hg/μL; p=0.07).

This study shows that deficiency for TLR2 or TLR4 only mildly impairs cardiac function in HFD fed mice, suggesting that TLR2 and TLR4 may play a modest protective role in HFD-induced cardiac dysfunction.

Introduction

Overweight and obesity are reaching epidemic proportions worldwide, due to reduced physical activity in combination with a calorie rich diet. Since adipose tissue has a limited capacity to store fat, excess fat may be stored in non-adipose tissue such as liver and muscle, contributing to the pathogenesis of type 2 diabetes and cardiovascular diseases (CVD).

Saturated fatty acids such as palmitate and stearate which are abundantly available in most HFDs induce the accumulation of lipids in non-adipose tissues which is thought to give rise to chronic activation of inflammatory signalling pathways.[1] The onset of the inflammatory response is marked by the release of pro-inflammatory cytokines[2] resulting in the infiltration of inflammatory cells, e.g. macrophages, in the tissue.[3]

The exact molecular mechanisms responsible for the activation of inflammatory pathways in obesity are still poorly understood. One mechanism may be related to the finding that fatty acids and fatty acid derivative are able to activate TLRs.[4] TLRs play a key role in the innate immune response and are not only expressed on immune cells, but also by other cell types including cardiomyocytes.[4, 5] Thus far thirteen different TLRs have been discovered, and particularly the role of TLR2 and TLR4 in CVD and atherosclerosis have been studied extensively.[6] It has been observed that the absence of TLR2 and TLR4 has favourable effects on post-infarction healing during cardiac ischemia[7-10], and in atherosclerosis results in a reduced plaque formation.[11-13] Furthermore, there is evidence that TLRs also have adverse effects on the development of acute coronary syndrome[14] and aggravate heart failure.[15]

As HFD is clearly related to the development of coronary heart diseases, deleterious effects of HFD on the heart may be, at least partially, mediated through TLRs, which to our knowledge has not been studied before. We previously demonstrated that HFD feeding impairs cardiac function, preferentially in male mice.[16] In the current study, we show that a HFD intervention in TLR2 and TLR4 deficient mice results in very mild changes in cardiac function, thereby suggesting that TLR2 and TLR4, if anything, play a moderately protective role in HFD-induced cardiac dysfunction.

Materials and methods

Animals and experimental design

We used male B6.129-Tlr2tm1Kir/J (TLR2$^{-/-}$) mice, purchased from The Jackson Laboratory (Indianapolis, USA), TLR4 knock out (TLR4$^{-/-}$) mice, kindly provided by Prof. S. Akira (Department of Biochemistry, Hyogo College of Medicine, Hyogo, Japan) and T. van der Poll (Center for Experimental and Molecular Medicine, Academic Medical Center, Amsterdam, the Netherlands), and their respective wild-type (WT) littermates (all C57Bl/6J background). All mice were housed under standard conditions with a 12-hour light-dark cycle and had free access to food and water.

At the age of 13-15 weeks (TLR2$^{-/-}$ mice and WT littermates) or 7-12 weeks (TLR4$^{-/-}$ mice and WT littermates) mice were assigned to a lard based low-fat diet (LFD) or HFD which provided 45% energy from lipids (D12451, Research Diets Services, Wijk bij Duurstede, the Netherlands). Body weight was measured weekly and after 10 weeks of diet feeding, blood was drawn to measure plasma lipids. Heart function assessment with echocardiography and pressure volume loops (PV-loops) were performed in week 11 and 12, respectively. At the end of the experiment the mice were sacrificed and hearts were isolated for further examination. The protocol was approved by the Animal Ethics Committee from the Leiden University Medical Center and was conform to the Guide for Care and Use of Laboratory Animals (NIH publication No.85-23, Revised 1996).

Plasma lipids

After a 4 hour fast (9.00-13.00 h), blood was collected via tail vein bleeding in potassium EDTA-coated plastic tubes (Sarstedt, Germany). Plasma total cholesterol, triglycerides and phospholipids were determined individually using commercially available kits (kit no. 1489232; 11488872 and 101140, Roche Diagnostics, Mannheim, Germany) in 96-wells plates (Greiner Bio-One), according to the manufacturer's protocols.

Hemodynamic measurements

At week 12, left ventricular (LV) function was assessed by invasive PV-loops as described earlier.[16] Briefly, mice were anesthetized with 4% isoflurane, intubated, ventilated and the jugular vein was cannulated for infusion of hypertonic saline to determine parallel conductance. Via the right carotid artery a 1.2F PV catheter (FTS-1212B-4518, Scisense Inc., London, Ontario, 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/s) 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).

Immunohistochemistry

After isolation, hearts were fixed in formalin and cut into five 1-2mm thick slices perpendicular to the long axis of the heart. These slices were flat embedded in paraffin and cross-sectioned (5 μm-thick) throughout the entire heart for immunohistological analysis. To determine the amount of macrophages in the myocardium an F4/80 staining was performed (F4/80; 1:10. The F4/80 monoclonal antibody was a gift from Dr. P.J. Nijweide, (Dept. of Molecular Cell Biology, Leiden University Medical Centre, the Netherlands). Five images were acquired throughout the ventricles of the heart with a 400x magnification. The number of macrophages per image was counted by two observers blinded for the study results. A macrophage was defined as brown/red staining which touched a blue nucleus and the average number of macrophages in the heart was calculated.

Statistical analysis

Significance of differences between the groups was calculated non-parametrically using a Kruskal-Wallis test for independent samples, followed by a Mann-Whitney U-test for independent samples. P-values ≤0.05 were considered statistically significant. SPSS 17.0 for Windows (SPSS, Chicago, IL, USA) was used for statistical analysis. Values are presented as means ± SD.

Results

HFD feeding similarly increases body weight gain in TLR2$^{-/-}$, TLR4$^{-/-}$ and WT mice

To determine the effect of HFD feeding on induction of obesity in TLR2$^{-/-}$ and TLR4$^{-/-}$ mice, body weight was measured weekly. At the start of the experiment, body weight did not significantly differ between groups. After HFD feeding for 12 weeks no differences in body weight between HFD fed TLR2$^{-/-}$ and TLR4$^{-/-}$ mice and corresponding WT mice were observed, although there was a tendency towards a diminished body weight gain for the TLR2$^{-/-}$ group (figure 1). However, 12 weeks of HFD feeding significantly increased body weights in all strains as compared to their LFD controls. LFD feeding in TLR2$^{-/-}$ resulted in a lower body weight gain compared to WT mice, whereas TLR4$^{-/-}$ mice did not differ in body weight gain compared to WT mice (supplemental figure A).

HFD feeding does not lead to differences in plasma lipids between TLR2$^{-/-}$ and TLR4$^{-/-}$ and WT mice

After 10 weeks of HFD feeding, blood was drawn to determine the concentration of total cholesterol, triglycerides and phospholipids in plasma (figure 2). For total cholesterol levels no differences were observed between the TLR2$^{-/-}$ and TLR4$^{-/-}$ mice and WT mice after HFD feeding, whereas HFD feeding did increase total cholesterol levels in all groups as compared to the LFD controls. LFD feeding did not influence cholesterol levels (supplemental figure B).

HFD feeding did not induce differences in triglyceride levels between TLR2$^{-/-}$ and TLR4$^{-/-}$ mice and WT mice. No differences in triglyceride levels were observed between HFD and LFD fed mice for TLR$^{-/-}$ and WT groups. Also, compared to LFD feeding, triglyceride levels were hardly affected. However, triglyceride levels tended to increase in TLR2$^{-/-}$ mice compared to WT mice, whereas TLR4$^{-/-}$ mice did not show an increase in triglyceride levels compared to WT mice. Phospholipids showed no differences between

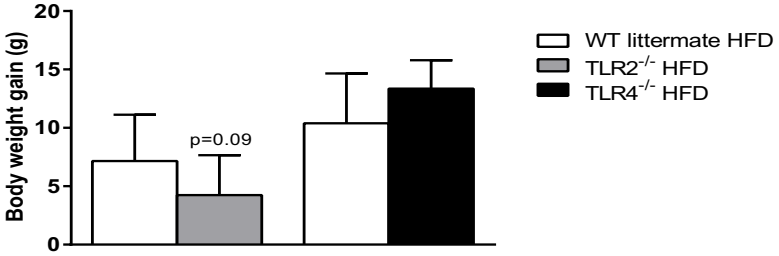

Figure 1 **Effect of TLR deficiency in HFD fed mice on body weight gain.** Male TLR2$^{-/-}$ and TLR4$^{-/-}$ mice and WT littermates were fed a high-fat diet (HFD) for 12 weeks. Body weight was measured at the beginning and end of the dietary intervention. Values represent means ± SD (n=7-8 per group), p=0.09 as compared to WT littermates.

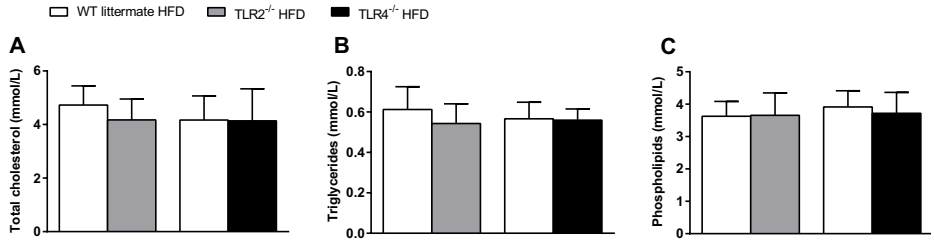

Figure 2 **Effect of TLR deficiency on HFD fed mice on plasma lipids.** Male TLR2$^{-/-}$ and TLR4$^{-/-}$ mice and corresponding WT littermates were fed a high-fat diet (HFD) for 12 weeks. After 10 weeks blood was drawn to determine total cholesterol (A), triglyceride (B) and phospholipid levels (C). Values represent means ± SD (n=5-8 per group)

both TLR$^{-/-}$ and WT mice after HFD. Levels were increase in all groups upon HFD feeding compared to LFD feeding, whereas phospholipid levels after LFD feeding alone did not differ between both TLR$^{-/-}$ and WT mice.

HFD feeding influences heart function modestly in TLR2$^{-/-}$ and TLR4$^{-/-}$ mice

To examine the effect of HFD feeding on cardiac function in TLR$^{-/-}$ mice, PV-loops were performed in all groups and the obtained results are summarized in table 1. Based on the mean values for end-diastolic and end-systolic volumes, average PV-loops of HFD groups were generated and corresponding end-diastolic and end-systolic PV-relations were calculated (figure 3).

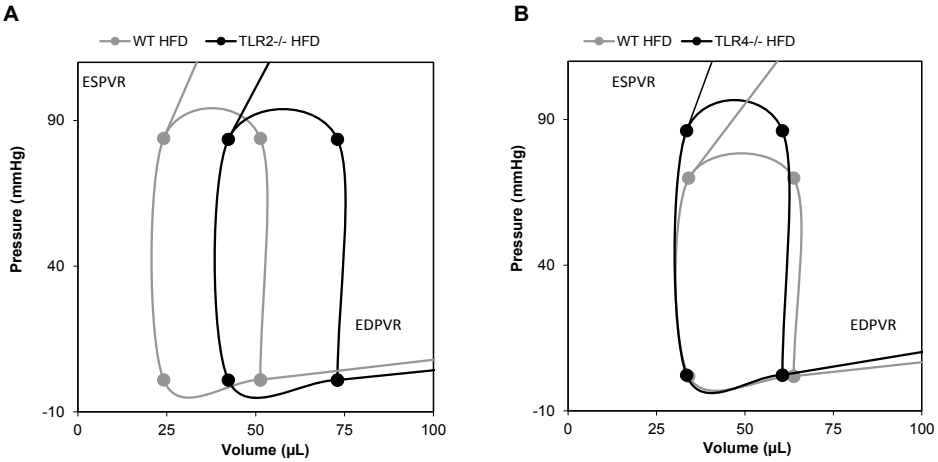

Figure 3 **Effect of TLR deficiency on HFD fed mice on pressure-volume loops and pressure-volume relations.** Male TLR2$^{-/-}$ (A) and TLR4$^{-/-}$ (B) mice and corresponding WT littermates were fed a high-fat diet (HFD). After 12 weeks pressure-volume loops (PV-loops) were recorded in each mouse, and average PV-loops are shown per group (n= 3-8 per group).

After HFD feeding differences were observed between the $TLR^{-/-}$ and WT mice. $TLR2^{-/-}$ mice increased ESV (+76%, p<0.009) and EDV (+42%, p<0.03) compared to their respective littermates. $TLR4^{-/-}$ mice tended to decrease pressure half time (-18%, P<0.06) and tended to increase end-systolic elastance (E_{ES}) (+200%, p<0.07) under HFD conditions compared to their respective littermates.

Both studies showed differences between LFD and HFD fed $TLR^{-/-}$ and WT mice, although not on similar parameters and not in a similar direction. $TLR2^{-/-}$ mice fed a HFD had a tendency towards an increased ESV (+46%, p<0.09) and an EDP (+360%, p<0.06) compared to LFD fed mice. WT mice in this study increased arterial elastance

Table 1 **Effect of TLR deficiency in HFD fed mice cardiac function**

	HFD			HFD		
	WT littermate	**$TLR2^{-/-}$**	**P-value**	**WT littermate**	**$TLR4^{-/-}$**	**P-value**
General						
HR (beats/min)	494 ± 45	514 ± 33	0.49	546 ± 77	601 ± 74	0.14
SV (µL)	27 ± 10	31 ± 8	0.30	30 ± 13	27 ± 16	0.12
CO (mL/min)	13 ± 5	16 ± 4	0.36	17 ± 9	17 ± 10	0.29
SW (mm Hg.µL)	2559 ± 866	2883 ± 803	0.42	2178 ± 1029	2468 ± 1535	0.29
E_A (mm Hg/µL)	3.6 ± 1.3	2.9 ± 0.7	0.22	2.7 ± 1.0	4.1 ± 1.8	0.19
PHT (ms)	5.3 ± 0.8	4.9 ± 0.5	0.77	5.3 ± 1.0	4.3 ± 0.6	**0.06**
Systolic						
ESP (mm Hg)	84 ± 11	84 ± 11	1.00	70 ± 18	86 ± 8	0.37
ESV (µL)	24 ± 4	42 ± 14	**0.009**	34 ± 17	33 ± 9	0.17
EF (%)	52 ± 8	43 ± 9	0.30	48 ± 23	43 ± 13	0.94
dP/dt_{MAX} (mm Hg/ms)	7120 ± 1245	7254 ± 1250	0.91	6060 ± 2499	8583 ± 1738	0.12
$SB\text{-}E_{ES}$ (mm Hg/µL)	2.8 ± 0.4	2.3 ± 0.5	0.09	1.6 ± 0.6	3.3 ± 2.0	**0.07**
Diastolic						
EDP (mm Hg)	1.0 ± 2.5	0.9 ± 2.1	0.64	1.9 ± 1.0	2.3 ± 2.8	0.94
EDV (µL)	51 ± 12	73 ± 18	**0.028**	64 ± 5	61 ± 17	0.12
Tau (ms)	10.6 ± 1.5	9.9 ± 0.8	0.91	10.3 ± 2.3	8.4 ± 1.3	0.14
$-dP/dt_{MIN}$ (mm Hg/ms)	-6096 ± 1493	-6358 ± 904	0.73	-5209 ± 1937	-7911 ± 1499	0.17
$SB\text{-}E_{ED}$ (mm Hg/µL)	0.14 ±0.03	0.12 ± 0.05	0.20	0.13 ± 0.04	0.20 ±0.09	0.12

Mice were fed a high-fat diet (HFD) for 12 weeks and cardiac function was determined. 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= 4-8 per group). HR, heart rate; SV, stroke volume; CO, cardiac output; SW, stroke work; E_A, arterial elastance (afterload); PHT, pressure half time; ESP, end-systolic pressure; ESV, end-systolic volume; EF, ejection fraction; dP/dt_{MAX}, maximal rate of pressure increase; $SB\text{-}E_{ES}$, end-systolic elastance; EDP, end-diastolic pressure; EDV, end-diastolic volume; Tau, relaxation time constant; $-dP/dt_{MIN}$, maximal rate op pressure decline; $SB\text{-}E_{ED}$, end-diastolic elastance (diastolic stiffness).

(E_A) (+55%, p=0.03) and E_{ES} (+44%, p=0.02) and tended to increase the ESP (+15%, p=0.09) on a HFD compared to WT mice on a LFD. TLR4$^{-/-}$ mice fed a HFD showed a tendency towards a decreased pressure half time (-12%, p=0.06) and an increased end-diastolic elastance (E_{ED}) (+62%, p=0.07) compared to TLR4$^{-/-}$ mice on a LFD. WT mice in this study on a HFD showed a tendency towards a decreased dP/dt_{MAX} (-32%, p=0.08) and a decreased SB-E_{ES} (-50%, P=0.07) compared with LFD feeding in WT mice.

In LFD conditions, differences in cardiac function were observed between the TLR$^{-/-}$ and WT mice (supplemental table 1 and supplemental figure C). Compared with their respective LFD WT littermates, TLR2$^{-/-}$ mice showed an increase in E_{ES} (+54%, P<0.02), whereas TLR4$^{-/-}$ mice on a LFD showed an increased stroke volume (+69%, P<0.02) and EDV (+32%, P=0.05) and a decreased heart rate (-13%, P<0.02), dP/dt_{MAX} (-19%, P=0.05), E_A (-41%, P=0.05), E_{ES} (-38%, P=0.05) and E_{ED} (-46%, P<0.03).

HFD feeding does not increase cardiac macrophage numbers in TLR2$^{-/-}$ TLR4$^{-/-}$ and WT mice

To determine if macrophages infiltrated more in cardiac tissue by HFD feeding, and their possible influence on cardiac dysfunction, the number of macrophages in the ventricles was counted. Images of representative pictures are given in figure 4. There were no differences in the average number of macrophages per image in HFD fed TLR2$^{-/-}$ mice compared to WT (1.2±1.9 vs. 2.9±1.2 macrophages per image). Likewise, no difference was found between HFD TLR4$^{-/-}$ and WT mice (11.4±16.4 vs. 14.1±13.8 macrophages per image). HFD feeding itself did not result in an increased number of macrophages neither in both TLR$^{-/-}$ groups nor WT groups as compared to the LFD control groups. Furthermore, no difference was observed for the amount of macrophages in all LFD fed groups (supplemental figure D).

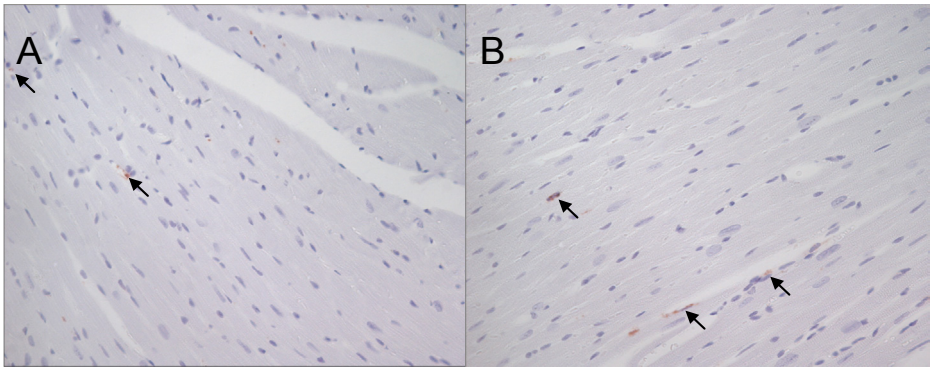

Figure 4 **Effect of TLR deficiency in HFD fed mice on cardiac macrophage infiltration.** Representative images of macrophage staining by F4/80 in cardiac tissue for of high-fat diet (HFD) fed WT mice (A) and TLR2$^{-/-}$ mice (B). The shown example was from the TLR2$^{-/-}$ experiment, no between group differences were observed. Magnification 400x.

Discussion

Since the discovery that HFD derived ligands activate TLR2- and TLR4-dependent pathways and play a role in HFD induced obesity, insulin resistance[4] and atherosclerosis[13], studies have been performed to further investigate the underlying mechanisms. We previously demonstrated that HFD feeding impairs cardiac function specifically in male and not female mice. Therefore we set out to determine the role of TLR2 and TLR4 signaling in HFD-induced cardiac function. Here, we show that TLR2 or TLR4 deficiency does not induce major differences in heart function under HFD conditions. HFD intervention for 12 weeks results in subtle differences in cardiac function in TLR2$^{-/-}$ and TLR4$^{-/-}$ mice compared to HFD fed WT littermates, even though body weight and some plasma lipid parameters are increased due to HFD feeding.

The modestly induced cardiac dysfunction between TLR deficient mice and WT littermates after HFD intervention is independent of the HFD, as high-fat feeding did induce several other previously described metabolic changes. First, HFD feeding significantly induced body weight gain in all groups. Second, as expected, HFD feeding increased total cholesterol levels in all HFD fed groups. Plasma phospholipids levels also tended to be increased upon high-fat feeding. Interestingly, possibly due to the genetic background, the mice displayed triglyceride levels just above detection level. Therefore, small variations in triglyceride levels may result in larger and thus significant differences. Third, contrary to expectations, we observed no difference in macrophages between the different groups. The HFD we used, without supplemented cholesterol, might be a too mild inflammatory trigger unable to stimulate macrophage attraction to the myocardium. Overall, these data show that HFD feeding resulted in the expected phenotypic effects, even though cardiac dysfunction was only mildly induced.

Despite evidence that mice lacking TLRs have a lower incidence of atherosclerosis and other cardiovascular pathology, in this study cardiac function in the TLR2$^{-/-}$ and TLR4$^{-/-}$ mice was slightly impaired when compared to their corresponding HFD fed WT littermates.

One of the contributing factors to the activation of TLRs, leading to the onset of inflammatory pathways, are fatty acids derived from the diet. Lard based HFD is rich in mainly C18:1 (43.3%), C16:0 (29.2%) and C18:0 (15.0%). *In vitro* studies show that in particular C12:0 and C16:0; and C14:0 and C18:0; are able to activate TLR2 and TLR4, respectively.[4, 17, 18] Therefore we assume that this particular lard diet is able to stimulate those specific TLRs, and thus does not clarify the lack of the TLR effect.

A possible explanation for the mild effects of the HFD in TLR deficient mice on cardiac function might be that we have chosen for male mice in this experiment. A sexual dimorphism has been reported for TLR deficient mice, showing that TLR2$^{-/-}$ and TLR4$^{-/-}$ female mice fed a HFD are more evidently protected against metabolic disturbances compared to males, who showed no difference in insulin response between WT and TLR deficient mice.[4, 19] In addition, as more often is seen with receptors belonging to the innate immune system, it is possible that the absence of one TLR causes compensatory up regulation of other TLRs, resulting in a similar inflammatory status which thus not lead to cardiac dysfunction.

Contradictory to previous findings[16], in the current study HFD-induced cardiac dysfunction in WT mice did not reach statistical significance. The fact that these WT mice have a less pronounced cardiac impairment could explain why only subtle differences were found in cardiac function between $TLR^{-/-}$ and WT mice. We cannot explain why the WT mice react differently to the HFD feeding compared to an earlier study, however it might be that small variations in study setup, e.g. the average age of the mice or environmental factors in the animal facilities, are causing this effect. Clearly, more research is needed to fully determine the exact role of TLR2 and TLR4 signaling in HFD-induced cardiac function.

To summarize, in this study we show that HFD feeding in $TLR2^{-/-}$ mice and $TLR4^{-/-}$ mice, compared to WT mice, neither results in differences in body weight nor in plasma lipids. Cardiac dysfunction is observed HFD fed $TLR2^{-/-}$ mice for the EDV parameter, whereas HFD fed $TLR4^{-/-}$ mice showed a tendency towards a decreased pressure half time and an increased SB-E_{ES}, leading to the conclusion that TLR2 and TLR4, if anything play a moderately protective role in HFD-induced cardiac dysfunction.

Acknowledgements

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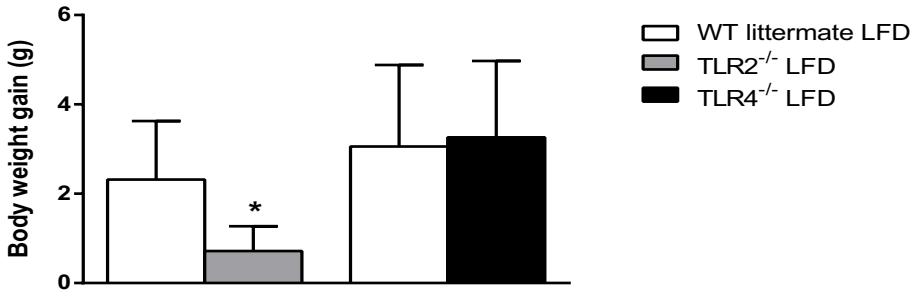
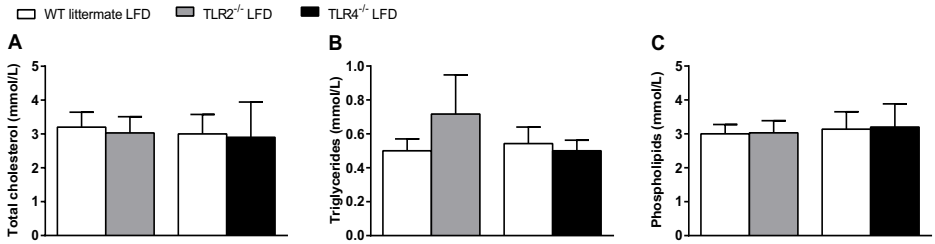
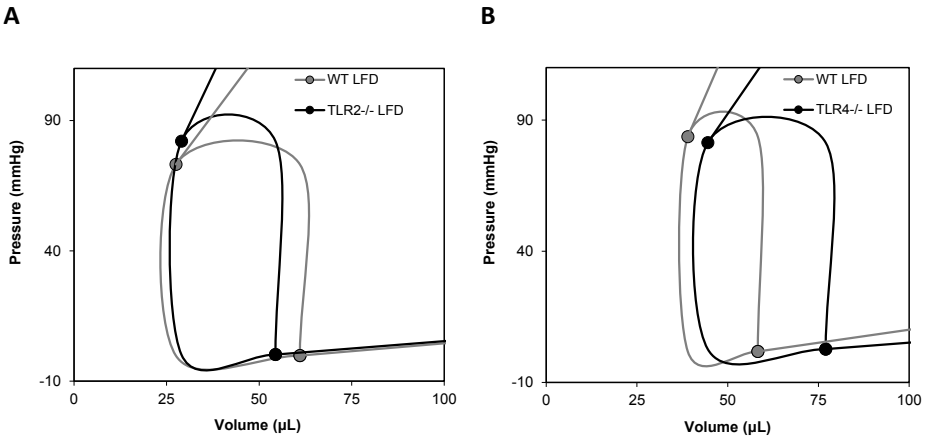
Supplemental data



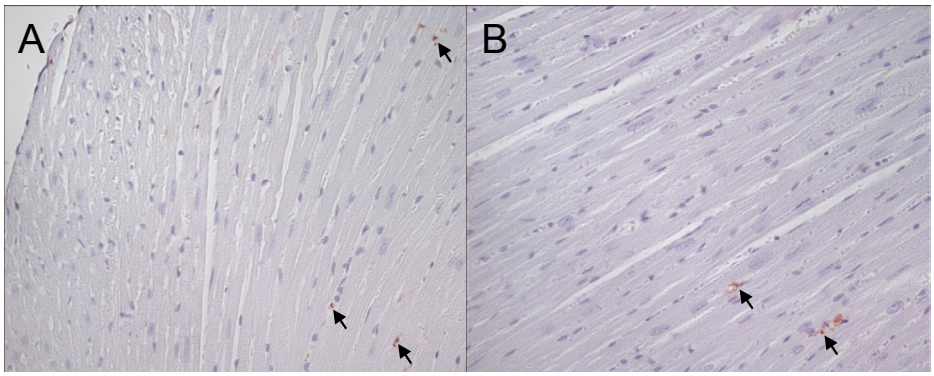
Supplemental figure A **Effect of LFD feeding on body weight gain.** Male TLR2$^{-/-}$ and TLR4$^{-/-}$ mice and WT littermates were fed a low-fat diet (LFD) for 12 weeks. Body weight was measured at the beginning and end of the dietary intervention. Values represent means ± SD (n=6-8 per group), *p<0.05 as compared to WT littermates.



Supplemental figure B **Effect of LFD feeding on plasma lipids.** Male TLR2$^{-/-}$ and TLR4$^{-/-}$ mice and corresponding WT littermates were fed a low fat diet (LFD) for 12 weeks. After 10 weeks blood was drawn to determine total cholesterol (A), triglyceride (B) and phospholipid (C) levels. Values represent means ± SD (n=5-6 per group).



Supplemental figure C **Effect of LFD feeding on pressure-volume loops and pressure-volume relations.** Male TLR2$^{-/-}$ (A) and TLR4$^{-/-}$ (B) mice and corresponding WT littermates were fed a low-fat diet (LFD). After 12 weeks pressure-volume loops (PV-loops)were recorded in each mouse, and average PV-loops are shown per group (n= 4-5 per group).



Supplemental figure D **Effect of LFD feeding on cardiac macrophage infiltration.** Representative images of macrophages stained cardiac tissue for A) low-fat diet (LFD) WT littermate group and B) LFD TLR$^{-/-}$ group. The shown example was from the TLR2$^{-/-}$ experiment, no between group differences was observed. Magnification 400x.

Supplemental Table A **Effect of diet intervention in TLR2$^{-/-}$, TLR4$^{-/-}$ and littermates on cardiac function**

	LFD			LFD		
	WT littermate	TLR2$^{-/-}$	P-value	WT littermate	TLR4$^{-/-}$	P-value
General						
HR (beats/min)	499 ± 59	526 ± 51	0.46	640 ± 18	558 ± 46	**0.014**
SV (µL)	33 ± 8	25 ± 14	0.33	19 ± 6	33 ± 12	**0.014**
CO (mL/min)	17 ± 5	13 ± 7	0.33	12 ± 4	18 ± 8	0.22
SW (mm Hg.µL)	2331 ± 612	2192 ± 1372	0.62	2105 ± 564	2683 ± 842	0.62
E_A (mm Hg/µL)	2.3 ± 0.4	4.3 ± 2.2	0.07	4.8 ± 1.5	2.8 ± 0.9	**0.05**
PHT (ms)	5.1 ± 1.21	5.1 ± 0.6	0.62	4.3 ± 0.6	4.9 ± 0.6	0.14
Systolic						
ESP (mm Hg)	73 ± 12	82 ± 16	0.33	84 ± 6	81 ± 13	0.62
ESV (µL)	28 ± 11	29 ± 8	0.81	39 ± 13	45 ± 12	0.46
EF (%)	56 ± 10	44 ± 10	0.09	35 ± 15	43 ± 10	0.33
dP/dt_{MAX} (mm Hg/ms)	6248 ± 1938	6948 ± 1997	0.62	8870 ± 1254	7224 ± 1160	**0.05**
SB-E_{ES} (mm Hg/µL)	1.9 ± 0.3	3.0 ± 0.5	**0.019**	3.2 ± 0.9	2.0 ± 0.6	**0.05**
Diastolic						
EDP (mm Hg)	-0.2 ± 1.9	0.2 ± 2.1	0.81	1.8 ± 1.9	2.7 ± 4.2	1.00
EDV (µL)	61 ± 14	54 ± 20	0.62	58 ± 10	77 ± 16	**0.05**
Tau (ms)	10.5 ± 2.1	10.0 ± 1.3	1.00	8.3 ± 1.4	9.4 ± 1.2	0.14
$-dP/dt_{MIN}$ (mm Hg/ms)	-5393 ± 1446	-6249 ± 1581	0.14	-7808 ± 1369	-6592 ± 995	0.22
SB-E_{ED} (mm Hg/µL)	0.12 ± 0.03	0.11 ± 0.03	0.81	0.20 ± 0.11	0.11 ± 0.02	**0.027**

Mice were fed a low-fat diet (LFD) for 12 weeks and cardiac function was determined. 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= 4-5 per group). HR, heart rate; SV, stroke volume; CO, cardiac output; SW, stroke work; E_A, arterial elastance (afterload); PHT, pressure half time; ESP, end-systolic pressure; ESV, end-systolic volume; EF, ejection fraction; dP/dt_{MAX}, maximal rate of pressure increase; SB-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; SB-E_{ED}, end-diastolic elastance.