Peer Review File

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First Round of Peer Review

Reviewer A:

The study by Dr Yang and colleagues reports that HFD protects the heart from post MI LV adverse remodeling by suppressing myocardial inflammation. Although the concept is not totally new as the myocardial protection effects of HFD in heart injury have been reported (PMID: 25197961; PMID: 25953257), evaluating the impact of HFD on post MI LV remodeling in a permanent LAD ligation model is relevant and meaningful. In particular, the authors provide a rich set of interesting observations of HFD-associated alterations in various infiltrating immune cells after MI. Overall, the manuscript is well organized and written. For revision, I have the following concerns to address:

Major

1. HFD seems protective in post MI LV remodeling, which is evidenced by both improvement in cardiac function parameters and reduction in fibrosis and inflammatory cell infiltration. However, what is the molecular mechanism(s)? How does HFD influence the infiltration of various immune cells into myocardium, and any interactions among the immune cell populations characterized in this study?

Reply: Thank you for the positive comments and the very important question. Prior studies have suggested that some adipokines secreted by adipose tissue may attenuate inflammatory responses(1,2). In addition, IL-33 levels are directly correlate with leptin level in mice and men(3), and HFD mice have higher leptin and IL-33 levels than their lean littermates(4,5). On the other hand, Tregs accumulate in infarcted myocardium to exert protective effects though the IL-33/ST2 axis(6). Therefore, the level of IL-33 would be higher in our HFD group, and IL-33 expanded Tregs through the IL-33/ST2 signaling pathway. Expanded Tregs promoted macrophages polarization into a repair-friendly phenotype and suppressed the level of cardiac inflammation.

Changes in the text: However, the mechanism by which HFD may alleviate ventricular remodeling by reducing cardiac inflammation remains unknown. Prior studies had suggested that some adipokines secreted by adipose tissue might attenuate inflammatory responses(1,2). IL-33 levels are directly correlate with leptin level in mice and men(3), and HFD mice have higher leptin and IL-33 levels than their lean littermates(4,5). On the other hand, Tregs accumulate in infarcted myocardium to exert protective effects through the IL-33/ST2 axis(6). Therefore, the level of IL-33 would be higher in HFD group, and IL-33 expanded Tregs through the IL-33/ST2 signaling pathway. (see Page 19, line 421-429)

2. Any information on the immune profile in the blood? Since immune cells are mainly interact via cytokine/chemokine network, it would be helpful to determine cytokine/chemokine changes (proteins) in the heart tissue.

Reply: Thanks for your excellent suggestions. The immune profile in the blood is indeed important to reflect the systemic inflammation, and Chemokines and cytokines play crucial roles in cardiac inflammation by directing the trafficking of infiltrating immune cells and their interactions(7,8). We only detected the expression of *Arg1, Il10, Il1b, Il6, Il2, Il4, Il23* and *Il18* in heart by RT-qPCR, and the myocardial expression of *Il1b* and *Il6* mRNA as M1 markers significantly decreased by day 7 after MI upon twelve-week HFD feeding (Figure 2F). As I am leaving the lab, we cannot supplement this part of the data. We have explained this part of the limitation in the revised manuscript.

Changes in the text: Second, the immune profile in the blood could reflect systemic inflammation. The immune cells mainly interact via cytokine/chemokine network. Chemokines and cytokines play crucial roles in cardiac inflammation by directing the trafficking of infiltrating immune cells and their interactions(8). These parameters should be taken into account in our future research. (see Page 20, line 449-454)

3. Additionally, several recent studies repot that innate immune system is likely activated via the cGAS-STING pathway and the toll like receptor (3/4/7/9) signaling pathways in post

MI LV remodeling, are those danger-sensing signaling pathways implicated in the setting of current study? The authors may not necessarily obtain all the experimental evidence but given that the key message of this current work is cardiac inflammation, the Discussion shall be in much more depth.

Reply: Thanks for your excellent suggestions. High fat diet could activate cGAS-STING signaling, NF-κB signaling(9,10) and TLRs (2/3/4) signaling(11-13). cGAS as the primary cytosolic DNA sensor, recognizes various pathogen-associated molecular patterns to initiate and enhance inflammation(14). It has been proved that cGAS-sting(15-17) and TLRs(18) promote NF-κB activation. Besides, the transcription factor NF-κB is crucial for the expression of FoxP3 and the development of Tregs(19,20). So HFD may activate cGAS-STING signaling or TLRs signaling to enhance the expression of FoxP3. We have added these data in the revised manuscript.

Changes in the text: Besides, High fat diet could activate cGAS-STING signaling, NF-κB signaling(9,10) and TLRs (2/3/4) signaling(11). cGAS as the primary cytosolic DNA sensor, recognizes various pathogen-associated molecular patterns to initiate and enhance inflammation(14). It has been proved that cGAS-sting(15) and TLRs(18) promote NF-κB activation. The transcription factor NF-κB is crucial for the expression of FoxP3 and the development of Tregs(20). So HFD may activate cGAS-STING signaling or TLRs signaling to enhance the expression of FoxP3. (see Page 20, line 429-436)

4. Mortality, mostly caused by cardiac rupture, is typically 30~50% in the mouse permanent MI model, even shown in several recent reports (PMID: 15639486; PMID: 30830156; PMID: 34646369). This important pathological event may be complicated by HFD feeding, however, was omitted in this study.

Reply: This is indeed a very important question. We add this information in this new manuscription. There are fifteen mice both in HFD and ND group underwent cardiac left anterior descending coronary artery ligation. Eight survived representing a survival rate of 53.3% in ND group, and nine survived representing a survival rate of 60% in HFD group until the end of the experiment. All the mice died within seven days of surgery. The difference in survival

rate and time to death were not significant in the two groups. We have added these data in the revised manuscript.

Changes in the text: There were fifteen mice both in HFD and ND group underwent cardiac left anterior descending coronary artery ligation. Eight survived representing a survival rate of 53.3% in ND group, and nine survived representing a survival rate of 60% in HFD group until the end of the experiment. All the mice died within seven days of surgery. (see Page 6, line 121-125)

5. It would be helpful to provide a table presenting key cardiac function parameters for different groups of mice at all time points, including the baseline. Animal heart weight and/or cardiomyocyte size would provide insight on the effects of HFD on myocardial hypertrophy.

Reply: Thanks for this practical suggestion. We added this data in in the revised manuscript.

Changes in the text:

Supplementary Table I. Echocardiography characterization of ND and HFD mice

	ND			HFD		
	Before MI	7 days MI	28 days MI	Before MI	7 days MI	28 days MI
LVEF (%)	74.24±2.072	21.22±0.7953	17.65±2.920	67.11±2.705	29.99±3.260*	26.26±1.998*
FS (%)	42.19±1.978	9.801±0.3810	8.097±1.380	36.49±2.172	14.21±1.676*	12.12±0.9385*
LVESV (μL)	10.94±1.483	123.2±10.55	132.4±20.82	13.54±1.443	76.81±9.347**	77.45±14.88*
LVEDV (μL)	42.53±4.202	156.0±12.52	156.4±20.62	41.24±2.835	107.2±9.300**	90.63±14.17*

Echocardiography was performed on the mice before MI and at the indicated time after MI.

Unpaired t test compared to ND, Values are means ± SEM: *$P < 0.05$; ** $P < 0.01$.

ND, normal diet; HFD, high fat diet; LVEF, left ventricular ejection fraction; FS, fractional shortening; LVESV, left ventricular end systolic volume; LVEDV, left ventricular end diastolic volume. (see Page 12, line 257 and 267)

6. Surgery procedure of opening the chest and isolation of LAD per se causes trauma and subsequently inflammatory response. However, the important data set on SHAM control group is missing in the manuscript even though the authors briefly mentioned SHAM surgery in the M&M.

Reply: Thank you for the insightful question. The surgery of myocardial infarction does cause a severe inflammatory response. It would be much better if there is a sham control group. In our study, we try to get a definitive answer to know the effect of 12 weeks of HFD on myocardial infarction and changes, and the difference between ND and HFD groups is dietary conditions. In the future we will design the sham group for an in-depth discussion on the mechanism how HFD improved adverse ventricular remodeling post-myocardial infarction by alleviating local inflammation. We have explained this part of the limitation in the revised manuscript.

Changes in the text: Fifth, the sham control group should be included in the study, given that the surgery of myocardial infarction can cause a severe inflammatory response. (see Page 21, line 459-461)

7. The data on Tregs and DCs appear puzzling. How to explain the increase of Tregs while DCs were reduced/impaired?

Reply: Thank you for the valuable suggestions. Prior work has shown that restriction of DCs-mediated immunity is beneficial to ventricular remodeling after MI(21,22). Besides, Tregs are known to suppress inflammation by downregulating costimulatory molecules CD80 and CD86 of DCs (23-25), which may be an additional effector function of Tregs(23-25). We observed reduced recruitment of moDCs and cDCs into the infarcted myocardium. Maturation and antigen-presenting capacity of DCs are related to the high surface expression of MHCII and costimulatory molecules, which are essential for DCs-dependent T cell activation(26). Of note, the expressions of MHCII and costimulatory molecules CD40 and CD86 on cDCs and moDCs in mLN were downregulated after MI in the background of twelve-week HFD feeding. Our

findings suggested that the reduction of cDCs and moDCs in the heart and their dysfunction in mLN, as indicated by down-regulation of MHCII and costimulatory molecules, maybe partly contribute to the changes of macrophages caused by Tregs upon twelve-week HFD feeding. We have some imformation in the revised manuscript. (see Page 19, line 407-420)

Minor

1. Minor language editing is needed, e.g. in line 256 it should be "two groupS"; line 330-331 may be better rephrased to something like "… neutrophils infiltrating the heart, and thus, ameliorate adverse ventricular remodeling."

Reply: Thank you for your patient advice. We have corrected language editing mistake. We also carefully checked and corrected the whole manuscript.

Changes in the text: even though the frequency and the absolute number of T cells remained the same between the two groups. (see Page 14, line 305)

Our finding indicated twelve-week HFD protects mice from excessive neutrophils infiltrating the heart, and thus could ameliorate adverse ventricular remodeling. (see Page 18, line 389-391)

2. Figure citation in line 241 is not accurate. Please check through the manuscript.

Reply: Thank you for your careful advice. We also carefully checked and corrected all the figures citation.

Changes in the text: while the frequency of $CD206^{+}$ M2 was increased in HFD mice (Figure 2D and S3). (see Page 13, line 288)

Reviewer B

In this study, the experimental obese group of male C57BL/6J mice was created by 8 weeks' high-fat feeding and the control group of mice fed with normal chow was raised and compared. The authors observed post-infarction cardiac remodeling, function, and tissue fibrosis at 7- and 28-days and immune cells stratification at 7 days of post-MI and did the two groups comparison. The results demonstrated favorable effects from high-fat diet regarding post-MI focal inflammation and cardiac remodeling.

1. The statement is not true “the relative research on the impact of obesity upon inflammation post-MI appears very sparse”. The effects of diet-induced obesity on inflammation and remodeling after myocardial infarction has been reported clearly by Thakker et al in 2006 (Am J Physiol Heart Circ Physiol. 2006 Nov;291(5):H2504-14). With this, the novelty of current manuscript is weak.

Reply: Thanks for your valuable comment and suggestion. There are many literatures showed obesity paradox in myocardial infarction(27-30), as well as several articles explaining the negative impact of obesity upon inflammation post-MI. For example, obesity was induced by feeing mice with HFD for 24 weeks, which is detrimental to left ventricular (LV) remodeling after Ischemia/Reperfusion (I/R) injury. At 72 h after reperfusion, the macrophage density increased and the neutrophils decreased in the obese group compared with lean group (PMID: 16731644, Am J Physiol Heart Circ Physiol. 2006 Nov;291(5):H2504-14). However, the aim of our study was to investigate the profile of inflammation in the obesity paradox in a model of myocardial infarction. The revision we revised the statement that “the relative research on the impact of obesity upon inflammation post-MI appears very sparse” to “the relative research on the impact of obesity upon inflammation post-MI appears inconsistent.”

Changes in the text: Though there is a close relationship between obesity and inflammation, the relative research on the impact of obesity upon inflammation post-MI appears inconsistent. (see Page 5, line 92-94)

2. Missing of blood pressure data in current manuscript is a major deficit. Hypertension affects post-MI cardiac remodeling and fibrosis largely. The interactions of obese and hypertension on post-MI outcomes have also been reported (J Am Heart Assoc. 2021 Mar 16;10(6):e018212). It is shown that “When hypertension accompanies obesity, favorable metabolic pathways associated with obesity are attenuated and post-MI cardiac function and remodeling are adversely impacted.”

Reply: Thank you for the insightful comment. High-fat feeding results in increased blood pressure in mice, rats and rabbits(31-35). Hypertension does affect cardiac remodeling and fibrosis(36,37). We have added this limitation to the discussion.

Changes in the text: Many literatures reported that High-fat feeding results in increased blood pressure in mice, rats and rabbits(31-35). Hypertension does affect cardiac remodeling and fibrosis(36,37). We have added this part of the limitation in the revised manuscript.

Changes in the text: Sixth, many studies reported that High-fat feeding results in increased blood pressure(35). Hypertension affects cardiac remodeling and fibrosis(36,37). The relationship between blood pressure, cardiac fibrosis and HFD in our work is unknown. (see Page 21, line 461-464)

Reviewer C

The authors report that "mildly" obese mice show better preserved LV-EF and LV volume than mice on a standard diet. This was accompanied by less leukocyte infiltration, a higher frequency of CD206+ macrophages and more regulatory T cells in the myocardium.

Major comments:

1. The data are mainly descriptive. The authors describe less inflammation in the heart. The observation that there is less inflammation in the heart could account for the observed effects and it would be very interesting to study how metabolic changes associated with obesity dampen inflammation in the heart.

Reply: Thank you for the good question. It is indeed very interesting to study how metabolic changes associated with obesity dampen inflammation in the heart.

HFD feeding increases the mRNA expression of the sarcolemmal fatty acid translocase CD36 (38), and enhances fatty acid oxidation (FAO)(38,39). FAO stabilize Foxp3 expression, possibly by inhibiting HDAC-mediated suppression of Foxp3 gene expression and Foxp3 protein deacetylation(40), modulates Tregs differentiation, stabilization and function(41,42). CD36 functions as a receptor for long chain fatty acid (FA), and DAMPs/PAMPs as these are most relevant to innate and adaptive immunity (43) (44), and mediates metabolic adaptation supports regulatory T cell survival and function. Therefore, in our work, HFD could supports regulatory T cell survival and function though FA-CD36 interaction. We have added possible mechanistic explanations to the manuscript.

Changes in the text: In addition, increased fatty acid oxidation (FAO) and CD36 in HFD group

may stabilize Foxp3 expression(38,40), modulating Tregs differentiation, stabilization and function(40). (see Page 20, line 436-438)

2. However, an alternative reason for less inflammation could be that the initial injury was less in obese animals. Unfortunately, there are no data on early infarct sizes (e.g. on day 1) available.

Reply: Thank you for the good suggestion. In our pilot experiments, we have confirmed by echocardiography and TTC staining that the size of the myocardial infarction is the same in the ND and HFD groups on the first day after MI (Figure). But limited by the management of the animal room, we did not confirm it in our formal experiments.

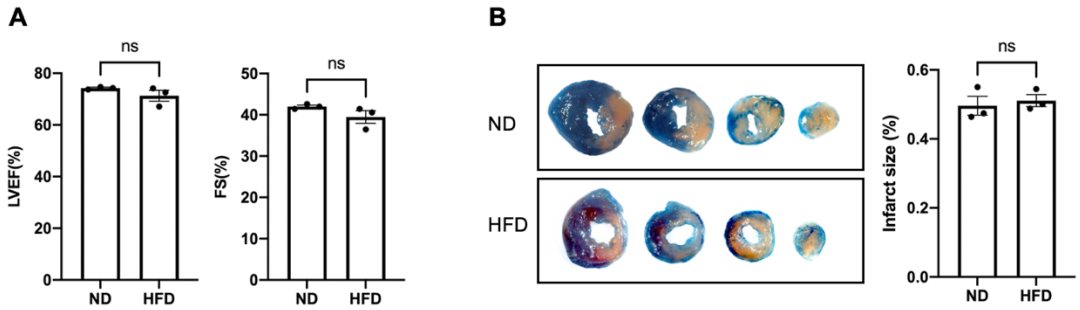

Figure. Evaluation of cardiac function and myocardial infarction size 1 day after MI. A. Group data for LVEF and from ND and HFD-treated mice1 day after MI. B. Representative TTC staining of cardiac tissue sections in ND and HFD-treated mice 1 day after MI. Group quantitation of infarct size (% area) in mice hearts treated with ND or HFD. N=3/group. All error bars denote the mean ± s.e.m. ns means no statistical significance. Scale bar, 1000 μm. MI, myocardial infarction; ND, normal diet; HFD, high fat diet; LVEF, left ventricular ejection fraction; FS, fractional shortening.

3. The data are at odds with previous reports from Thakker et al. who showed greater susceptibility to ischemia and more pronounced remodelling in obese mice. These conflicting data need to be discussed.

Reply: Thank you for the suggestion. There were mainly three differences in the design between our study and theirs, the calories of HFD, the model and the time of HFD. The difference in calories and time of HFD directly contributed to the huge difference in weight gain between

the two groups of mice (see table). It has been well documented that the obesity paradox applies only to mild obesity(45,46), which was similar to our study. Obesity in Thakker et al.'s study was more pronounced(47), so it is explainable that obesity in their study was detrimental to left ventricular (LV) remodeling after Ischemia/Reperfusion (I/R) injury, whereas it was opposite in ours.

Table: The differences in design between two studies

	Thakker et al's	Ours
Calories of HFD	42%	60%
model	I/R	MI
Time of HFD before surgery	24 weeks	8 weeks
Time of HFD after surgery	7 days	4 weeks
Weight gain, compared with ND group	69% (ND vs HFD: 27.45 ± 1.26 g vs. 46.41 ± 2.42 g)	27.7 % (ND vs HFD: 30.48 ± 1.19 g vs. 38.93 ± 0.51 g)

ND, normal diet; HFD, high fat diet; Ischemia/Reperfusion, I/R; myocardial infarction, MI.

Minor comments:

1. The manuscript needs a thorough language editing

Reply: Thanks for your suggestion. We have checked the manuscript thoroughly and corrected the typos and language errors. We also invited an English-speaking scientist to help revise the language of our manuscript.

2. Experimental details should be reported in more details according to the ARRIVE guidelines. It would be especially necessary to provide information on how many animals per group were operated and on survival and drop out rates during MI induction in obese vs lean mice.

Reply: Thank you for the practical questions. In the revision, we add more detail data in the method Part. There were fifteen mice both in HFD and ND group underwent cardiac left anterior descending coronary artery ligation. Eight survived representing a survival rate of 53.3% in ND group, and nine survived representing a survival rate of 60% in HFD group until the end

of the experiment. All the mice died within seven days of surgery. The difference in survival rate and time to death were not significant in the two groups. We have added these data in the revised manuscript.

Changes in the text: There were fifteen mice both in HFD and ND group underwent cardiac left anterior descending coronary artery ligation. Eight survived representing a survival rate of 53.3% in ND group, and nine survived representing a survival rate of 60% in HFD group until the end of the experiment. All the mice died within seven days of surgery. (see Page 6, line 121-125)

3. Tregs should be better defined by Foxp3 and CD25 expression.

Reply: Thank you for the suggestion. Traditionally, Tregs are identified by the coexpression of Foxp3 and CD25, but the strategy does not represent the true inhibitory function of Treg cells(48). In this work, Tregs are defined by CD3, CD4 and FoxP3, consistent with previous studies(7,49,50). First, FoxP3, a key transcription factor for the development and function of natural $CD4^+$ regulatory T cells, is stably expressed in mouse $CD25^+CD4^+$ Treg cells, but not in naive $CD25^-CD4^+$ T cells or in activated $CD4^+$ T cells(51,52). Clearly, however, the reverse does not apply, because lack of CD25 expression in a cell population cannot be taken for absence of regulatory cells. This reservation is critical, in view of previous claims that regulatory cells can differentiate in the periphery from naive CD4 cells, defined as $CD25^-$ (53).The potent suppressive capacity of both $CD25^+$ and $CD25^-Foxp3^+$ T cell subsets supports a dedicated function of Foxp3 in Treg cell differentiation(54). Second, surface expression of CD25 is labile in differentiated Foxp3-expressing Tregs, may serve as a word of caution to interpret that $Foxp3^+CD25^-CD4^+$ T cells constitute a reservoir of committed regulatory cells that regain CD25 expression upon homeostatic expansion(53). Third, in addition to the $CD25^{hi}CD4^+$ T cell subset, some $CD4^+$ T cells with a low level of CD25 or lacking CD25 express Foxp3(55).

4. I wonder if CD64+CD11c+ cells are really DCs or rather macrophages.

Reply: Thank you for your question. In our study, we defined moDCs as $CD11c^+$ $MHCII^+$ $CD64^+$ and macrophages as $Ly6G^-$ $CD64^+$(56). CD11c is a type I transmembrane protein that is

expressed on monocytes, granulocytes, a subset of B cells, dendritic cells, and macrophages. $CD11c^+$ $CD64^+$ cells mainly contain monocytes, moDCs and macrophages derived from monocytes(56,57).

Second Round of Peer Review

Reviewer A:

Still, I would say that the data are mostly descriptive and partly conflicting to reports from studies conducted in similar experimental settings. I agree with the authors that differences in the experimental conditions might account for conflicting results. Therefore, a clinical study monitoring the impact of body weight on healing and early remodeling processes after MI would be highly appreciated.

Reply: Thanks for your excellent suggestions. There were several studies have shown that obesity has a contradictory beneficial effect on the outcome of acute coronary syndromes, a phenomenon called the obesity paradox (1). For example, a study found when compared with healthy weight, there was an inverse association between overweight and obesity with all-cause mortality after acute MI (2). Recently, a study by Vojko and colleagues reported that moderate weight gain with age improves long-term survival after MI and that the magnitude of this "protective" weight gain is greater in older compared to younger patients. However, excessive weight gain (obesity grade III) is particularly harmful in the oldest age group (3). As I am leaving the lab, we cannot supplement such clinical data in our study.

Reviewer B:

I went through the manuscript and noticed the infarct size data in the rebuttal (which has errors in data presentation) was not included in the Supplemental file.

Reply: Thank you for your careful comments. We only confirmed that the size of the myocardial infarction is the same in the ND and HFD groups on the first day after MI by TTC staining (Figure), in our pilot experiments. Due to the limitation by the management of the animal room, we did not do it in our formal experiments. So, the infarct size data were not included in the Supplemental file.

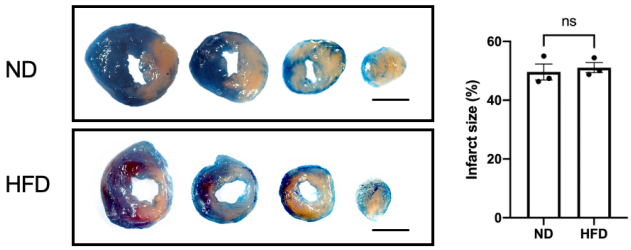

Figure. Evaluation of myocardial infarction size 1 day after MI. Representative TTC staining of cardiac tissue sections in ND and HFD-treated mice 1 day after MI. Group quantitation of infarct size (% area) in mice hearts treated with ND or HFD. N=3/group. All error bars denote the mean ± s.e.m. ns means no statistical significance. Scale bar, 2 mm. MI, myocardial infarction; ND, normal diet; HFD, high fat diet.