



Gut microbe-derived metabolite trimethylamine N-oxide activates the cardiac autonomic nervous system and facilitates ischemia-induced ventricular arrhythmia via two different pathways

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ABSTRACT

Background: We previously demonstrated the gut microbes-derived metabolite trimethylamine N-oxide (TMAO) could activate the atrial autonomic ganglion plexus and promote atrial arrhythmia. The cardiac sympathetic nervous system (CSNS) play important roles in modulating ventricular arrhythmia (VA).

Methods: Part 1: To test whether TMAO can directly activate the CSNS, we performed local injection of TMAO into the left stellate ganglion (LSG). Part 2: To test whether TMAO can indirectly activate the CSNS through the central nervous system, we performed intravenous injection of TMAO. Ventricular electrophysiology and LSG function and neural activity were measured before and after TMAO administration. Then, the left anterior descending coronary artery was ligated, and electrocardiograms were recorded for 1 h. At the end of the experiment, LSG and paraventricular nucleus (PVN) tissues were excised for molecular analyses.

Findings: Compared with the control, both intravenous and local TMAO administration significantly increased LSG function and activity, shortened effective refractory period, and aggravated ischemia-induced VA. Proinflammatory markers and c-fos in the LSG were also significantly upregulated in both TMAO-treated groups. Particularly, c-fos expression in PVN was significantly increased in the systemic TMAO administration group but not the local TMAO administration group.

Interpretation: The gut microbe-derived metabolite TMAO can activate the CSNS and aggravate ischemia-induced VA via the direct pathway through the LSG and the indirect pathway through central autonomic activation.

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1. Introduction

The trillions of microbes residing in the human gut have been shown to play important roles in shaping human health [1,2]. In recent years, considerable effort has been focused on exploring the relationship

between gut microbes and cardiovascular diseases such as hypertension, heart failure, and heart attack [3]. However, whether gut microbes associate with ventricular arrhythmia (VA) has not been reported.

VA is one of the most common clinical arrhythmias, especially under ischemic conditions. Malignant VAs, including sustained ventricular tachycardia (VT) and ventricular fibrillation (VF), are the major causes of cardiac death after myocardial infarction [4]. Evidence shows that the imbalance of the autonomic tone, especially the overactivation of sympathetic nerves, plays critical roles in the occurrence of VA [5]. Neural recordings revealed the hyperactivity of left cardiac sympathetic nerves before the onset of VA, and stimulation of the left stellate ganglion (LSG) significantly promotes the

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Research in context

Evidence before this study

Recent studies indicate the gut microbes-derived metabolite trimethylamine N-oxide (TMAO) was involved in multiple cardiovascular diseases, such as hypertension, heart attack, and heart failure, etc., most of which were associated with the hyperactivity of the cardiac sympathetic nervous system (CSNS). And the CSNS also play important roles in modulating ventricular arrhythmia (VA). On the other hand, the bidirectional interactions between the gut and the central nervous system by multiple bioactive compounds have been well recognized, and TMAO has been demonstrated to cross the blood brain barrier. Our previous study revealed TMAO could activate the atrial autonomic ganglion plexus and promote atrial arrhythmia.

Added value of this study

The present study demonstrated that TMAO could significantly promote ischemia-induced VA by facilitating autonomic remodeling within the LSG, and at least two pathways were involved in LSG remodeling: the direct pathway through local TMAO within the LSG and the indirect pathway through the “gut-brain-heart” axis.

Implications of all the available evidence

The present study provides novel insights into the development of VA. The gut microbes-derived metabolite TMAO might be involved in the development of VA through the activation of CSNS. Therefore, targeting gut dysbiosis or TMAO production might be novel therapeutic strategies for the prevention of VA in high-risk patients.

incidence of VA [6,7]. Therefore, multiple strategies targeting the LSG have been explored for the prevention and treatment of VA [5,8]. Clinically, LSG denervation has been developed for the management of life-threatening VAs such as catecholaminergic polymorphic VT, long QT syndrome, and ventricular storm [9–11].

We previously demonstrated that the gut microbe-derived metabolite trimethylamine N-oxide (TMAO) could directly activate the atrial autonomic ganglion plexus (GP) [12]. Our results indicated that a local increase in TMAO levels had a strong effect on the atrial autonomic GP, which is an important trigger for arrhythmia. In the present study, we aimed to investigate the effect of TMAO on the LSG and the incidence of VA under ischemic conditions.

Gut microbes can bidirectionally interact with the brain through autonomic neurotransmitters and metabolites; thus, the concept of the “microbiota-gut-brain” axis has been widely accepted in recent years [13]. Additionally, recent studies have shown that TMAO can cross the blood-brain barrier (BBB) [14]. Therefore, we hypothesized that TMAO might also activate the central sympathetic nervous system, which is the regulator of the sympathetic nervous tone of the whole body including the cardiac sympathetic nervous system (CSNS).

We hypothesized that there are two different pathways through which TMAO activates the CSNS (Fig. 6): the direct pathway, in which localized TMAO directly activates the LSG, and the indirect pathway, in which circulating TMAO indirectly activates the LSG through the activation of the central nervous system. We called the

indirect pathway as the “gut-brain-heart” axis. In the present study, both local and systemic administration of TMAO was used to investigate these two pathways.

2. Materials and methods

2.1. Animal preparation

All animals were supplied by the Animal Center at Renmin Hospital, Wuhan University. The Animal Ethics Committee of Wuhan University approved all of the study protocols, which complied with the “Guide for the Care and Use of Laboratory Animals” published by the US National Institutes of Health.

Adult male mongrel dogs (18 to 24 kg) used for this study were anesthetized with 3% pentobarbital sodium (1 ml/kg), followed by an additional dose of 2 ml/h for the maintenance of general anesthesia. All animals received ventilation through a positive pressure respirator with room air. The Lead 7000 Workstation (Jinjiang Inc., Chengdu, China) was used to record blood pressure and surface electrocardiograms. Thoracotomy was performed, and acute myocardial infarction (AMI) was induced by left anterior descending coronary artery occlusion (LADO). Electrocardiograms within 1 h after AMI were continuously recorded to assess the incidence of VA including ventricular premature beats (VPBs), salvo (2 consecutive VPBs), nonsustained VT (nSVT, 3 or more consecutive VPBs with a duration <30s), sustained VT (consecutive VPBs with a duration ≥30s), and VF. If VF occurred within 1 h after AMI, no defibrillation was performed and the dogs were sacrificed immediately. The time from AMI to VF was calculated as the living time within 1 h after AMI, and the living time will be calculated as 60 min if no VF occurred. At the end of the experiment, the animals were euthanized with an overdose intravenous injection of pentobarbital sodium (100 mg/kg).

2.2. Study protocol

Part 1: To test whether TMAO can directly activate the CSNS, we performed local injections of TMAO (n = 10) or saline (n = 10) into the LSG. LSG function and activity and ventricular effective refractory period (ERP) were measured before and 30 min after TMAO administration. Then, LADO was performed, and electrocardiograms were recorded for 1 h. At the end of the experiment, LSG and paraventricular nucleus (PVN) tissues were excised for molecular analyses (Fig. S1).

Part 2: To test whether TMAO can indirectly activate the LSG, we performed intravenous injections of TMAO (n = 10) or saline (n = 10) to simulate a high TMAO state under pathological conditions. The effect of TMAO on LSG function and activity, ventricular ERP was measured before and 30 min after TMAO administration. Then, LADO was performed, and electrocardiograms were recorded for 1 h. At the end of the experiment, LSG and PVN tissues were excised for molecular analyses.

2.3. TMAO injection

In part 1, 0.1 ml 1 mmol/l TMAO (Sigma, St. Louis, MO, USA) or an equivalent volume of 0.9% saline was slowly injected into the LSG at 3 different sites for approximately 1 min in each group (Fig. S2). In part 2, a total of 6 ml 100 mmol/l TMAO was intravenously injected through the femoral vein. The dose of TMAO was chosen based on our previous studies to attain high but physiological TMAO levels reported clinically [12]. The significant elevated serum TMAO concentration 30 min after intravenous injection is consistent with that caused by chronic high fat diet. Intravenous injection of TMAO also significantly increased the TMAO concentration in LSG, and local TMAO injection increased TMAO concentration in LSG to the same order of magnitude (Fig. S3).

2.4. Measurement of ventricular electrophysiological properties

Multielectrode catheters were sutured at three epicardial sites: the left ventricular apex (LVA), the left ventricular base (LVB), and the median area between the LVA and the LVB (LVM). The detailed methods for ERP measurement have been previously described [5].

2.5. Measurement of LSG function and neural activity

Multielectrode catheters were attached to the LSG, and the function of LSG was defined as systolic blood pressure (SBP)-elevating responses to high frequency stimulation (HFS; 20 Hz, 0.1 ms pulse width). Due to the significant variation in SBP-elevating responses to HFS in each dog, four incremental voltage levels (level 1: 1–5 V; level 2: 5–7.5 V; level 3: 7.5–10 V; and level 4: 10–15 V) were used for LSG stimulation. The detailed methods for neural activity measurement are described in our previous studies [15].

2.6. Immunofluorescence analysis

LSG tissues were quickly harvested and fixed in 4% paraformaldehyde. Paraffin blocks were processed and cut into 5- μ m sections for immunofluorescence analysis. Antibodies against c-fos (Abcam, Cambridge, England), IL-1 β (Abcam, Cambridge, England), IL-6 (PTG, Proteintech, USA), TNF- α (Abcam, Cambridge, England), TH (Abcam,

Cambridge, England), NMDAR1 (Genetex, Alton Parkway Irvine, USA), NMDAR2A (Genetex, Alton Parkway Irvine, USA), and NMDAR2B (Genetex, Alton Parkway Irvine, USA) and anti-rabbit secondary antibodies were used as previously described [12,15]. The expression of c-fos, inflammatory factor, and NMDARs in tyrosine hydroxylase (TH) positive neurons were quantified by testing five fields per section in a blinded manner in each slide. The commercially available software Image-Pro Plus (Media Cybernetics, Inc., Rockville, MD) was used for quantitative analysis.

2.7. Statistical analysis

All continuous data are presented as the mean \pm standard error. Unpaired *t*-tests were used to compare the differences in IL-1 β , IL-6, TNF- α , c-fos, and *N*-methyl-D-aspartate receptors (NMDARs) levels. Two-way repeated-measures analysis of variance was used to analyze the differences in ventricular ERP and LSG function between different groups at different time points. Two-way analysis of variance was used to analyze differences in LSG activity between different groups at different time points. Mann-Whitney *U* test was used for the analyses of the incidence of VPBs, salvo, nSVT, and living time 1 h after LADO among different groups. Fisher's exact test was used to analyze the incidence of SVT/VF. All data were analyzed with Prism software (version 7.0, GraphPad Software, Inc., San Diego, California). Values were considered significant at $P < 0.05$.

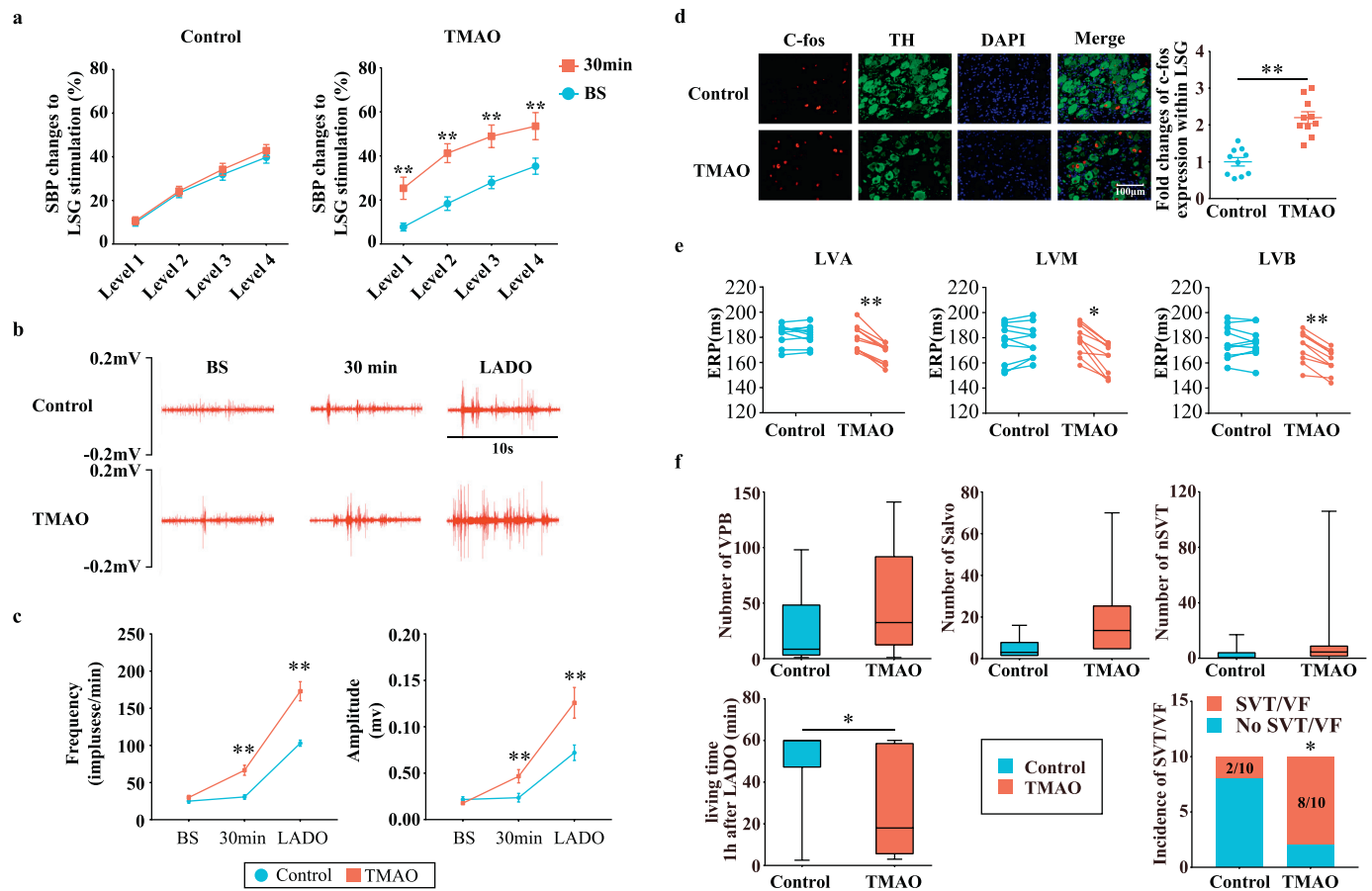


Fig. 1. Effect of local TMAO administration on LSG function and neural activity, and the incidence of VA after ischemia. **a:** Compared with the control, local administration of TMAO within the LSG significantly increased LSG function in part 1 (two-way repeated-measures analysis of variance). **b-c:** Representative examples and quantitative analysis of neural spikes at different timepoints. Compared with the control, local TMAO administration significantly increased the frequency and amplitude of neuron spikes at different time-points (two-way analysis of variance). **d:** Representative examples and relative expression of c-fos in the LSG (unpaired *t*-test). **e:** Local administration of TMAO in LSG tissues significantly increased the left ventricular ERP values (two-way repeated-measures analysis of variance). **f:** The incidence of variable forms of VAs including VPBs (Mann-Whitney *U* test), nSVT (Mann-Whitney *U* test), and SVT/VF (Fisher's exact test), as well as the survival duration within 1 h after LADO (Mann-Whitney *U* test) in part 1. * $P < 0.05$, ** $P < 0.01$.

3. Results

3.1. Part 1: Local administration of TMAO within the LSG directly increased LSG neural activity and VA incidence

3.1.1. Effect of TMAO on LSG function and neural activity

In part 1, local injection of TMAO into the LSG was performed to test whether TMAO can directly increase LSG neural activity. LSG function was defined as the systolic blood pressure (SBP)-elevating responses to high frequency stimulation simulation, which as an indicator of sympathetic nervous activity. Compared with the control group, no significant difference of LSG function was observed at baseline (Fig. 1a, left panel), but LSG function was significantly enhanced at 30 min after TMAO injection (Fig. 1a, right panel). In addition, direct neural recordings within the LSG indicated both neural firing frequency and amplitude were significantly increased in the TMAO group after local TMAO administration (Fig. 1b–c). The overexpression of *c-fos* in tyrosine hydroxylase (TH) positive neurons in the TMAO group further demonstrated that the sympathetic neurons within the LSG were significantly activated by local TMAO administration (Fig. 1d).

3.1.2. Effect of TMAO on ventricular electrophysiological properties

The ERP is an important indicator of VA inducibility. In part 1, no significant difference of ventricular ERP was observed between the control group and the TMAO group at baseline. But at 30 min after TMAO injection, ERP were significantly shortened in the local TMAO group rather than the control group (Fig. 1e).

3.1.3. Effect of TMAO on the incidence of VA after AMI

Electrocardiograms were continuously recorded for 1 h after AMI, and various types of VAs were analyzed. Compared with the control, local TMAO administration increased the number of VPBs, salvo, and nSVT episodes, although the differences were

not significant (Fig. 1f, upper panel). However, the incidence of SVT/VF, the most life-threatening forms of malignant arrhythmia, was significantly increased by local TMAO administration (Fig. 1f, right lower panel). Notably, the survival duration of dogs within 1 h after LADO was also significantly shortened in the TMAO group (Fig. 1f, left lower panel), which might explain why the difference of VPBs, salvo, and nSVT episodes incidence between the TMAO and control groups were not significant, although the mean incidence of these forms of VA were higher after local TMAO administration.

3.1.4. Effect of TMAO on the expression of proinflammatory markers and NMDARs

Compared with that in the control group, the expression of proinflammatory markers including IL-1 β , IL-6, and TNF- α in TH positive neurons within LSG tissues was significantly upregulated in the local TMAO administration group (Fig. 2a–c).

NMDARs are important excitatory receptors that are widely expressed in the nervous system. In the present study, NMDARs expression was detected in LSG tissues, and local administration of TMAO significantly upregulated NMDARs expression in TH positive neurons of LSG (Fig. 2d–f).

3.2. Part 2: Systemic administration of TMAO indirectly activated the LSG through the activation of the central sympathetic nervous system

3.2.1. Effect of systemic TMAO administration on LSG function and neural activity

As shown in Fig. 3a, compared with the control, systemic administration of TMAO also significantly increased the SBP-elevating responses to LSG stimulation. Neural recordings revealed that the systemic administration of TMAO also significantly activated LSG neural activity both in frequency and amplitude (Fig. 3b–c). Moreover, immunofluorescence

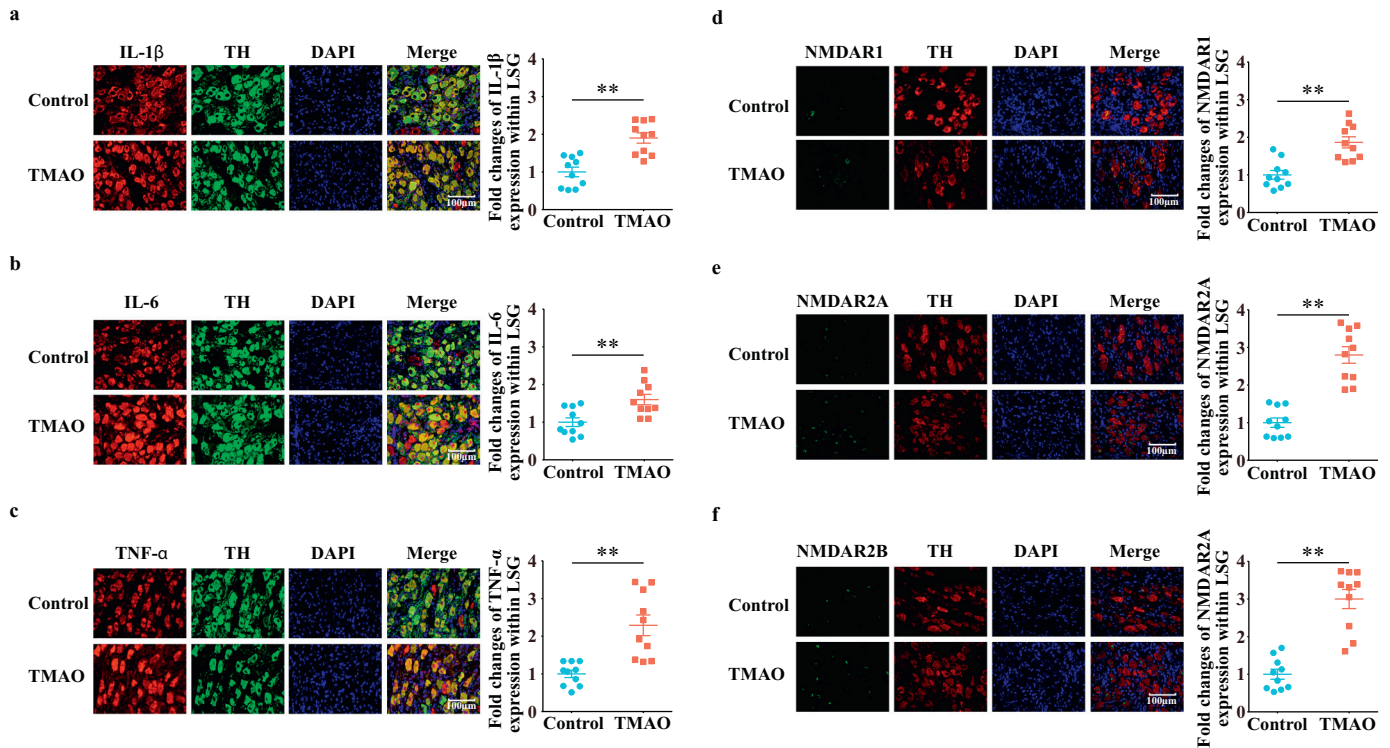


Fig. 2. Effect of local administration of TMAO on proinflammatory markers and NMDARs expression in TH positive neurons in the LSG. a–c: Representative examples and fold changes of IL-1 β , IL-6, and TNF- α expression in TH positive neurons within the LSG in part 1. Compared with the control, local administration of TMAO significantly upregulated IL-1 β , IL-6, and TNF- α expression in TH positive neurons within the LSG (unpaired *t*-test). d–f: Representative examples and fold changes of NMDAR1, NMDAR2A, and NMDAR2B expression in TH positive neurons within LSG in part 1. Compared with the control, local TMAO administration significantly upregulated NMDAR1, NMDAR2A, and NMDAR2B expression in TH positive neurons within the LSG (unpaired *t*-test). * *P* < 0.05; ** *P* < 0.01.

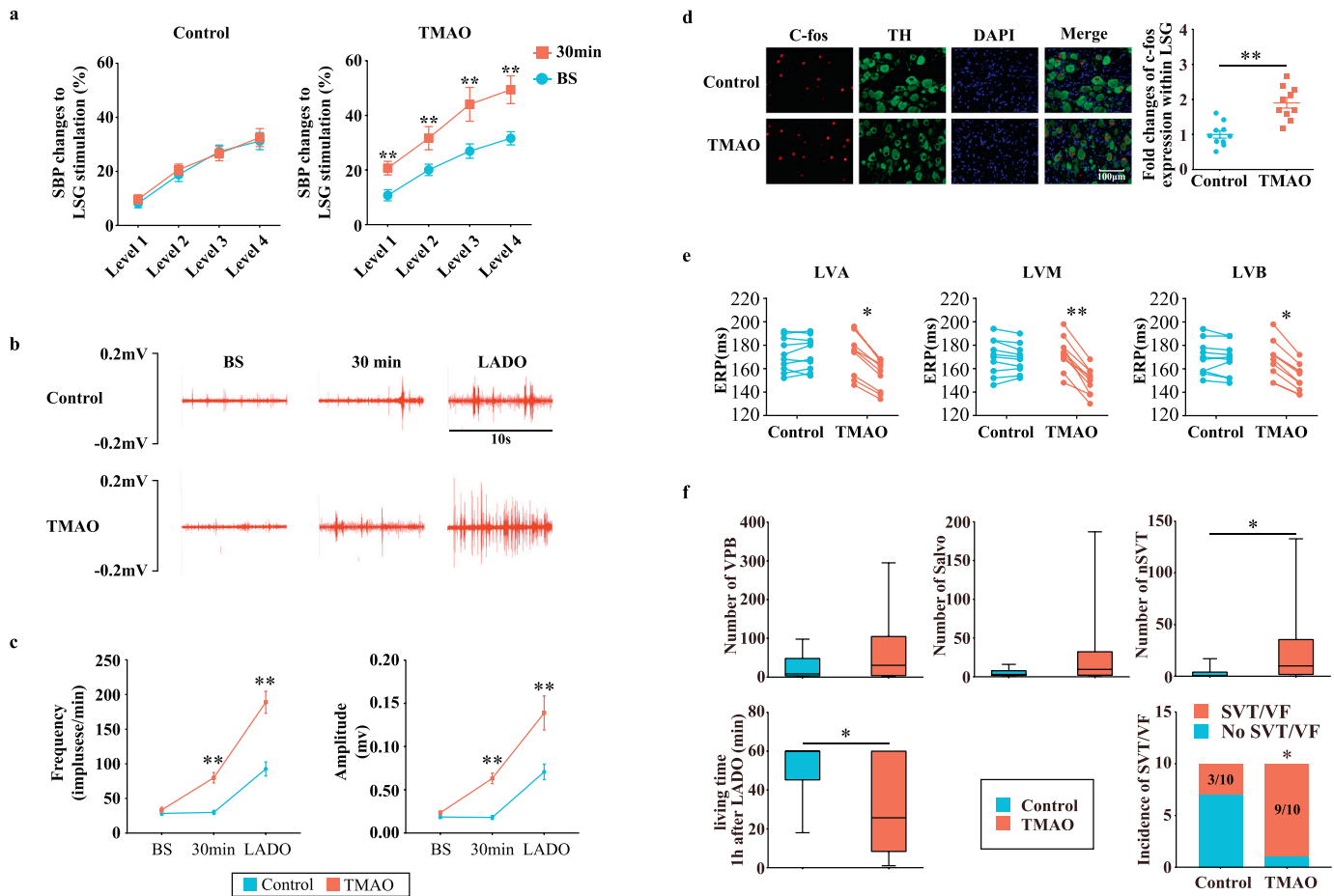


Fig. 3. Effect of systemic administration of TMAO on LSG function, neural activity within LSG, and the incidence of VA after ischemia. **a:** Compared with the control, systemic administration of TMAO significantly increased LSG function in part 2 (two-way repeated-measures analysis of variance). **b–c:** Representative examples and quantitative analysis of neural spikes at different time-points (two-way analysis of variance). **d:** Representative examples and quantitative analysis of c-fos expression in TH positive neurons in the LSG (unpaired t-test). **e:** Compared with the control, systemic administration of TMAO significantly increased the left ventricular ERP values (two-way repeated-measures analysis of variance). **f:** The incidence of variable forms of VAs including VPBs (Mann–Whitney U test), nSVT (Mann–Whitney U test), and SVT/VF (Fisher's exact-test), as well as the survival duration within 1 h after LADO (Mann–Whitney U test) in part 2. * $P < 0.05$; ** $P < 0.01$.

analysis indicated that c-fos expression in TH positive neurons was significantly upregulated by systemic TMAO administration (Fig. 3d).

3.2.2. Effect of systemic TMAO administration on ventricular electrophysiological properties and the incidence of VA after AMI

Compared with the control, systemic administration of TMAO significantly shortened the ventricular ERP, a surrogate of VA inducibility (Fig. 3e). Compared with the control, systemic administration of TMAO also increased the number of VPBs, and salvo, but the differences were not significant (Fig. 3f, upper panel). However, the incidence of nSVT and SVT/VF were significantly increased after systemic TMAO administration (Fig. 3f, right upper and right lower panel). Notably, the survival duration of dogs within 1 h of LADO in the systemic TMAO administration group was significantly shortened (Fig. 3f, left lower panel).

3.2.3. Effect of systemic TMAO administration on the expression of proinflammatory markers and NMDARs

Compared with the control, systemic administration of TMAO significantly upregulated the expression of proinflammatory markers such as IL-1 β , IL-6, and TNF- α within LSG tissues in TH positive neurons (Fig. 4a–c). Furthermore, the overexpression of NMDARs in TH positive neurons in LSG and PVN tissues was observed after the systemic administration of TMAO (Fig. 4d–f).

3.2.4. Effect of systemic TMAO administration on the central sympathetic nervous system

As recent evidence indicates that TMAO can cross the BBB, systemic administration of TMAO was performed to test whether the central sympathetic nervous system is involved in the progression of cardiac sympathetic overactivation by TMAO. The results indicated that c-fos expression in TH positive neurons in PVN, an important central sympathetic nucleus, was significantly upregulated by systemic but not local TMAO administration (Fig. 5a–b). The difference of c-fos expression in PVN is consistent with that serum TMAO concentration was significantly increased only by intravenous TMAO injection but not local TMAO injection (Fig. S3a–b). Furthermore, in PVN tissues, systemic administration of TMAO significantly increased the expression of excitatory NMDARs in TH positive neurons (Fig. 3c–e), whose overexpression has been proven to increase the sympathetic tone and promote poor cardiac outcomes.

4. Discussion

The relationship between gut microbes and cardiovascular diseases has been extensively investigated in recent years. Preclinical and clinical studies indicate that TMAO, a gut microbes-derived metabolite, plays important roles in mediating cardiovascular diseases and even serves as an independent predictor for major

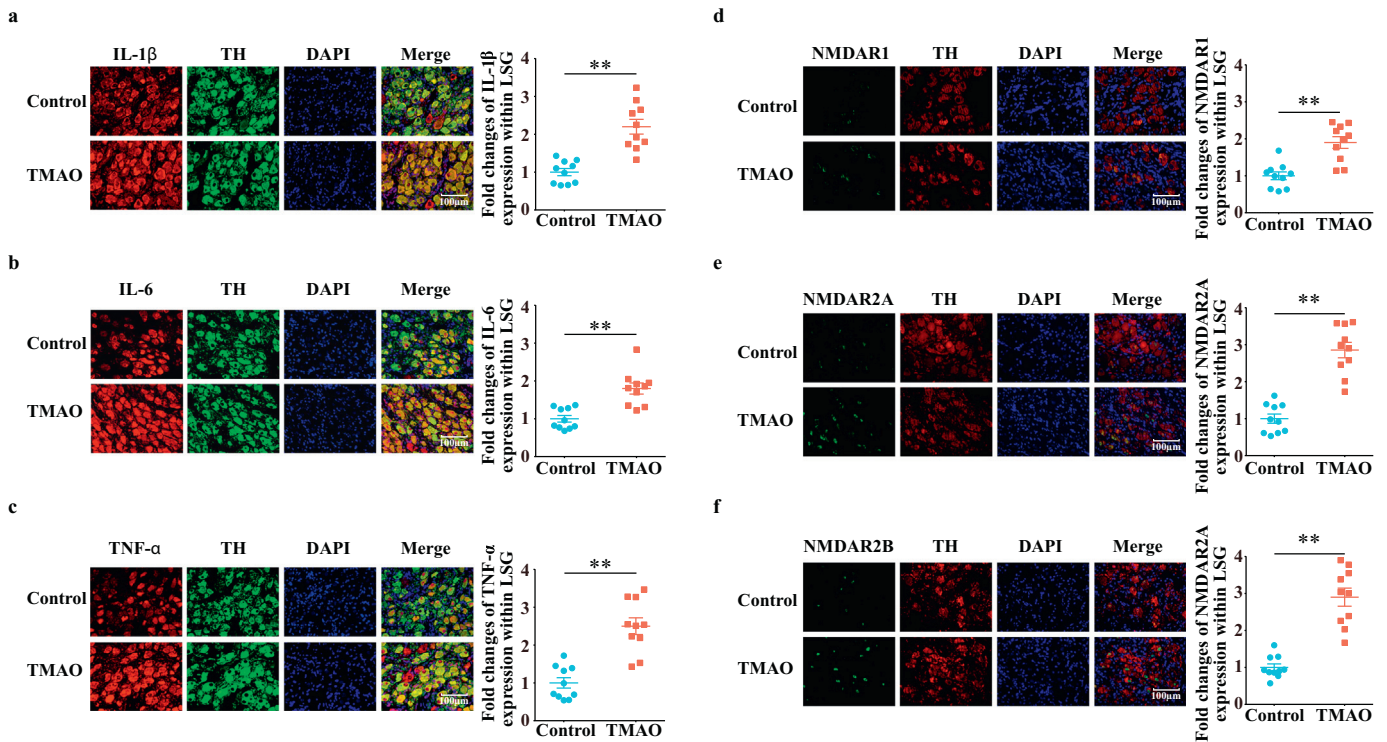


Fig. 4. Effect of systemic administration of TMAO on proinflammatory markers and NMDARs expression in TH positive neurons in the LSG. a-c: Representative examples and fold changes of IL-1 β , IL-6, and TNF- α expression in TH positive neurons within the LSG in part 2. Compared with the control, systemic administration of TMAO significantly upregulated IL-1 β , IL-6, and TNF- α expression in TH positive neurons within the LSG (unpaired t-test). d-f: Representative examples and fold changes of NMDAR1, NMDAR2A, and NMDAR2B expression in TH positive neurons within LSG in part 2. Compared with the control, systemic TMAO administration significantly upregulated NMDAR1, NMDAR2A, and NMDAR2B expression in TH positive neurons within the LSG (unpaired t-test). * $P < 0.05$; ** $P < 0.01$.

cardiovascular adverse events and heart failure [16,17]. Subsequent studies revealed that TMAO could promote atherosclerosis, enhance platelet hyperreactivity and thrombosis risk, and promote inflammation, which result in poor outcomes of hypertension, heart failure, and heart attack, etc. [16,18–21]. However, whether TMAO can promote VA has rarely been reported.

Multiple studies have shown that gut microbes and their products activate the autonomic nervous system (ANS), which plays crucial roles in the modulation of VA. Our previous study provided the first evidence that TMAO could directly activate the cardiac GP [12]. Therefore, we hypothesized that TMAO might promote VA by activating the LSG. In the present study, microinjection of TMAO into the LSG was performed, and TMAO significantly increased the cardiac sympathetic tone and ventricular electrophysiological instability. And the incidence of VA after LADO was significantly increased by TMAO administration.

Inflammatory cytokines have been demonstrated to directly or indirectly activate the sympathetic tone [22]. For example, the injection of IL-1 β and TNF- α into the PVN has been shown to significantly increase renal sympathetic nerve activity [23]. IL-6 could enhance neural activity by increasing calcium influx [24]. Our previous study demonstrated that increased inflammation in the LSG significantly aggravated cardiac sympathetic remodeling [15]. In the present study, significant overexpression of proinflammatory markers in TH positive neurons was observed after TMAO administration, which might contribute to the effects of TMAO on LSG remodeling and VA after ischemia.

Furthermore, these proinflammatory markers have been shown to increase the expression of NMDARs, which are important excitatory receptors widely expressed in neurons [25,26]. IL-1 β has been demonstrated to upregulate NMDAR expression and increase NMDAR-mediated calcium influx via the activation of tyrosine kinases [27]. Wheeler et al. showed that TNF- α could modulate the trafficking of NMDARs and increase NMDA-evoked calcium influx [28]. Abundant evidence indicates that NMDARs are also expressed in multiple peripheral

organs, such as the lung, gut, and heart [29]. Lu et al. observed the up-regulated NMDAR expression was associated with myocardial necrotic injury and sympathetic nerve sprouting [30]. Particularly, studies in vivo have also shown that the acute activation of NMDARs can promote VA [31].

In the present study, we found that TMAO significantly upregulated the expression of proinflammatory markers, such as IL-1 β , IL-6, and TNF- α in TH positive neurons, as well as NMDARs, which might further result in sympathetic hyperactivation within the LSG and promote the incidence of VA.

Accumulating evidence supports the interaction between the central nervous system and gut microbes, which is referred to as the “gut-brain” axis. The gut microbes produce multiple bioactive compounds that serve as signals to communicate with the host brain [32]. Recent studies have shown that TMAO can cross the BBB [12,14]. Therefore, we hypothesized that systemically administered TMAO might cross the BBB and then activate the central sympathetic nervous system to subsequently activate the CSNS.

To determine whether the central sympathetic nervous system is involved in CSNS activation by TMAO, we performed systemic administration of TMAO and evaluated c-fos expression in TH positive PVN neurons in the present study. The PVN is an important central sympathetic nucleus implicated in sympathetic excitation and cardiovascular dysfunction [33]. Evidence shows that the injection of proinflammatory cytokines including IL-1 β and TNF- α into the PVN significantly enhanced sympathetic activity in different animal models [23]. It has also been proven that the activation of NMDARs within the PVN could increase renal sympathetic nerve activity, blood pressure, and heart rate in a heart failure model, while the NMDAR antagonist exerted significant opposite effects [34]. Shi et al. demonstrated that the activation of NMDARs significantly reduced heart rate variability (HRV), promoted cardiac electrical remodeling and increased susceptibility to VA [35].

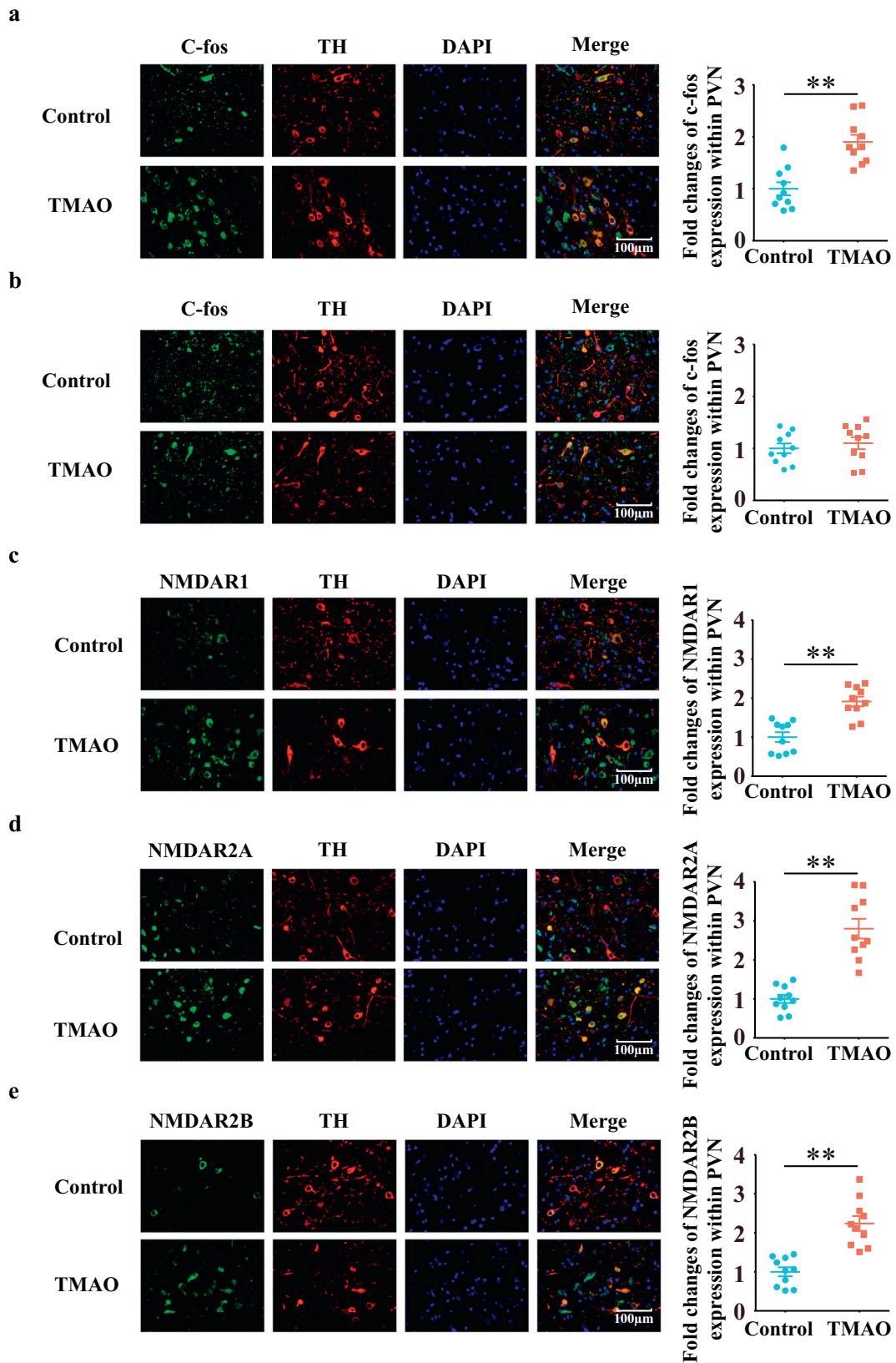


Fig. 5. Effect of TMAO administration on c-fos and NMDARs expression in TH positive neurons within the PVN. a-b: Compared with the control, systemic but not local administration of TMAO significantly upregulated c-fos expression in TH positive neurons in the PVN. (unpaired t-test) c-e: Representative examples and quantitative analysis of expression of NMDAR1, NMDAR2A, and NMDAR2B within the in TH positive PVN neurons in part 2 (unpaired t-test). * $P < 0.05$; ** $P < 0.01$.

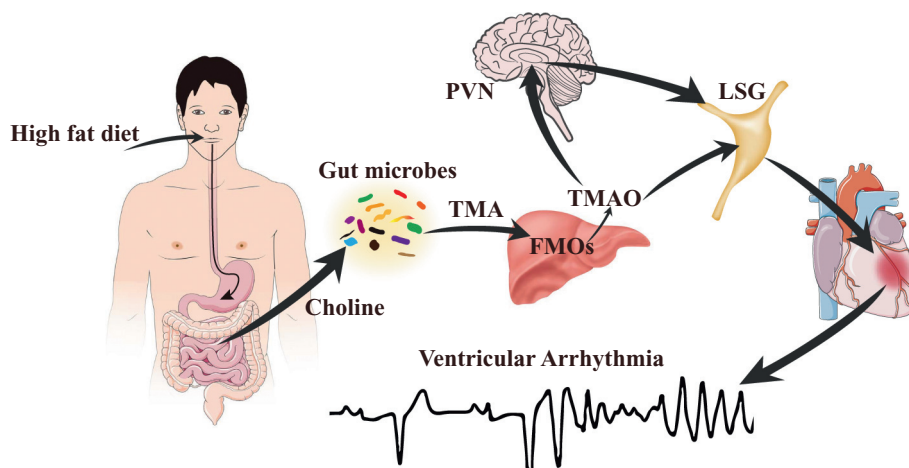


Fig. 6. The direct and indirect pathways through which TMAO activates the LSG. High-fat diet-induced gut dysbiosis increases the production of trimethylamine (TMA), which is absorbed and further oxidized in the liver by the flavin-containing monooxygenase (FMO) family of proteins (primarily FMO3) to TMAO. TMAO might activate the CSNS and promote VA through two different pathways: the direct pathway, whereby local TMAO within the LSG might directly activate the CSNS and promote VA, and the indirect pathway, whereby TMAO might cross the BBB and activate the central sympathetic nucleus, such as the PVN, which might further activate the CSNS and promote VA.

Immunofluorescence staining indicated that the expression of c-fos in TH positive PVN neurons was significantly upregulated in the systemic TMAO administration group but not in the local TMAO administration group, indicating that systemic but not local TMAO administration significantly activates the sympathetic neurons in PVN. This hyperactivation of PVN sympathetic neurons might further increase LSG neural activity and aggravate malignant VA after ischemia. In the present study, we found that TMAO also significantly increased the expression of NMDARs in TH positive PVN neurons. And the overexpression of NMDARs in TH positive PVN neurons by TMAO might contribute to these effects. These data indicate that the central sympathetic nervous system might be involved in the overactivation of the LSG by the systemic administration of TMAO.

There are several limitations in the present study: although we demonstrated systemic administration of TMAO significantly upregulated the expression of c-fos in the PVN, the activation of which has already been shown to increase cardiac sympathetic tone and aggravate cardiac outcomes by others, blockade of the efferent sympathetic nerves between the PVN and LSG may provide more convincing results; The animal model used in the present study was a model of AMI with direct TMAO application in a short time, so a high-fat diet-induced chronic gut dysbiosis model that results in high circulating levels of TMAO will be more helpful to demonstrate the effect of TMAO on autonomic imbalance and VA in the future.

In conclusion, the present study demonstrated that TMAO significantly promoted ischemia-induced VA by facilitating autonomic remodeling within the LSG, and at least two pathways were involved in LSG remodeling: the direct pathway through local TMAO within the LSG and the indirect pathway through the “gut-brain-heart” axis (Fig. 6). Therefore, strategies targeting gut dysbiosis or TMAO production will be helpful for the prevention of VA in high-risk patients.

Acknowledgements

None.

Conflict of interest

None declared.

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Author contributions

Study concept and design: Hong Jiang, Lilei Yu, Guannan Meng, Xiaoya Zhou. Animal experiments: Guannan Meng, Zhenya Wang, Meng wang, Yuhong Wang, Jielin Deng, Zhen Zhou, Yifeng Zhang, Yanqiu Lai, Qianqian Zhang, Xiaomeng Yang. Molecular biological detection: Xiaoya Zhou, Menglong Wang, Liping Zhou. Analysis and interpretation of data: Guannan Meng, Menglong Wang, Liping Zhou. Drafting of the manuscript: Guannan Meng, Xiaoya Zhou. Critical revision of the manuscript: Hong Jiang, Lilei Yu. Guannan Meng and Xiaoya Zhou contributed equally to the present.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ebiom.2019.03.066>.

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