Vascular Protection by Exercise in Obesity: Inflammasome-associated Mechanisms

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BASIC SCIENCES

ABSTRACT

LEE, J., J. HONG, M. UMETANI, E. C. LAVOY, J.-H. KIM, and Y. PARK. Vascular Protection by Exercise in Obesity: Inflammasome-associated Mechanisms. *Med. Sci. Sports Exerc.*, Vol. 52, No. 12, pp. 2538–2545, 2020. **Purpose:** The nodlike receptor family pyrin domain containing 3 (NLRP3) inflammasome is a critical player in vascular pathology as it regulates caspase-1-mediated interleukin (IL)-1β processing. Physical activity ameliorates obesity-induced inflammation and vascular dysfunction, but the mechanisms responsible for these positive changes are incompletely understood. Here, the protective effect of physical activity on the inflammasome-associated vascular dysfunction in obesity and its putative mechanisms were investigated. **Methods:** Mice were fed a control low-fat diet (LFD) or a high-fat diet (HFD; 45% of calories from fat) and provided with running wheel access (LF-RUN or HF-RUN) or denied wheel access for our sedentary condition (LF-SED or HF-SED). The NLRP3 inflammasome-associated pathway, including NLRP3, caspase-1, and IL-1β, in mice aorta was examined by RT-qPCR and FLICA and DAB staining. The protein expression of zonula occluden-1 (ZO-1), ZO-2, adiponectin (APN), and adiponectin receptor 1 (AdipoR1) in aortic endothelial cells was determined by immunofluorescence double staining. Intracellular reactive oxidative stress and nitric oxide (NO) production were monitored with fluorescence probes, dihydroethidium, and diaminofluorecein. **Results:** HFD increased caspase-1 and IL-1β at mRNA and protein levels in endothelial cells of the aorta, and this was attenuated by voluntary running. HFD decreased ZO-1 and ZO-2 expression and reduced APN and AdipoR1 signaling; these were restored by running. The elevated intracellular superoxide (O_2^-) production observed in HF-SED was ameliorated in HF-RUN. Finally, HF-RUN improved NO production in the aorta compared with HF-SED. **Conclusions:** Our findings suggest that voluntary running ameliorates mechanisms associated with vascular dysfunction by suppressing NLRP3 inflammasome, improving NO production, and reducing oxidative stress. Such benefits of physical activity may be, at least in part, associated with APN–AdipoR1 signaling and tight junction protein expression. **Key Words:** EXERCISE, INFLAMMATION, VASCULAR DYSFUNCTION, ADIPONECTIN, TIGHT JUNCTION PROTEIN

Obesity is a growing health concern and a major risk factor for chronic diseases, such as cardiovascular disease (1,2). Endothelial dysfunction is the earliest event of many cardiovascular diseases during metabolic disorders, such as obesity (3). Contribution of obesity to vascular dysfunction is mainly associated with decreased bioavailability of nitric oxide (NO) by increased oxidative stress leading to inactivating endothelial NO synthase (eNOS) (3,4). Chronic inflammation is one of the important contributors to the progression of vascular dysfunction (4). Previous studies have identified the detrimental roles of inflammatory cytokines, such as TNF-α and interleukin-6 (IL-6), in endothelial function (5,6); however, the mechanisms of IL-1β-associated vascular dysfunction have been relatively understudied.

Recently, inflammasomes have been found to trigger IL-1β-involved inflammatory process (7). The nodlike receptor family pyrin domain containing 3 (NLRP3) inflammasome is composed of sensor NLRP3, adaptor apoptosis-associated specklike protein containing a C-terminal caspase recruitment domain (ASC), and effector caspase-1 (7). The inflammasome plays an important role in sterile inflammation against endogenous danger-associated molecular patterns, such as free fatty acids, glucose, cholesterol crystals, etc. Excessive danger-associated molecular patterns stimulate the inflammasome, leading to the activation of caspase-1-dependent IL-1β production (7) that is an initiating mechanism for vascular pathology (8). In addition, the inflammasome signaling involves the downregulation of junction proteins zonula occluden-1 (ZO-1) and ZO-2 contributing to endothelial permeability and dysfunction (9). It has been shown that inhibiting NLRP3 inflammasome ameliorates endothelial dysfunction (10). However, whether obesity-induced

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inflammasome signaling in vascular endothelial cells is attenuated by exercise is not completely studied.

Physical activity and exercise are an effective intervention for improving and maintaining cardiovascular health. The mechanisms of exercise-induced vascular benefits include enhancement of eNOS signaling, antioxidant capacity (11), and anti-inflammatory actions (12). Adiponectin (APN), which is inversely correlated with obesity (13), would be another important mechanism for the beneficial effect of exercise on vascular disease. It has been known that chronic exercise increases plasma level of APN (14) and that APN increases NO production (15) and decreases oxidative stress in endothelial cells (16). A recent study reported that NLRP3 inflammasome activation is attenuated by APN treatment in cultured human monocytic cells (17). In addition, as adiponectin receptor 1 (AdipoR1) plays a dominant role in endothelial cells for downstream of APN signaling (18), it is plausible that APN–AdipoR1 signaling links to the decreased induction of inflammasome. However, no studies have yet asked if exercise increases expression of APN–AdipoR and tight junction proteins in vascular endothelial cells in obesity. Our previous work has shown the decreased endothelial inflammasome in resistance vessels (coronary arteriole) of high-fat diet (HFD) feeding mice by voluntary wheel running (19). Nevertheless, it remains uncertain as to whether physical activity induces an anti-inflammasome effect and, thus, improves inflammasome-associated vascular dysfunction in conduit vessels, such as the aorta, in obesity.

Therefore, the current study aimed to identify whether voluntary wheel running attenuates features of the NLRP3 inflammasome axis in the aorta of HFD fed mice. We further aimed to elucidate mechanisms by which voluntary running can improve vascular function in obese mice, by examining the effects of exercise on APN–AdipoR, tight junction proteins, and NO bioavailability.

MATERIALS AND METHODS

Animal care. The animal set for this study was same as our previous study (19). Simply, male C57BL/6J mice were maintained on a 12-h light–12-h dark cycle, controlled temperature and humidity and given autoclaved water. All procedures conformed to the approved guidelines set by the Institutional Animal Care and Use Committee at the University of Houston. Our experimental design/approach "primordial prevention" was described in a 2011 American Heart Association Policy Statement (20), meaning the prevention of the development of risk factors in the first place. Thus, the voluntary wheel running, a natural type of aerobic exercise in murine model, and HFD were provided at the same time point to determine the "preventive" effect of exercise rather than the "treatable" effect after obesity-associated vascular dysfunction. At 8 wk of age, all mice were randomly assigned to either low-fat diet (LFD; 4.5% calories from fat, Rodent laboratory chow; Purina, St. Louis, MO) with sedentary (LF-SED, $n = 10$) as a reference, LFD with running (LF-RUN, $n = 10$), HFD (45% calories from fat; D12451; Research Diet, New Brunswick, NJ) with sedentary (HF-SED, $n = 8$), or HFD with running (HF-RUN, $n = 10$). Running mice were singly accommodated in a cage installed with the running wheel (Run Around Exercise Wheel; KATEE, Chilton, WI) equipped with a cycling computer (BC 8.12; Sigma, Neustadt an der Weinstrasse, Germany) to record daily running distance. After 12–14 wk of treatments, mice were euthanized by bilateral thoracotomy under isoflurane sedation after a 24-h cessation of voluntary running, so as to wash out acute effect of running exercise on subsequent experiment. Isolated abdominal aortae were frozen in liquid nitrogen.

Gene expression analysis. Total RNA was extracted as described earlier (21). Simply, total RNA extraction was carried out using Trizol reagent (15596; Life Technologies, Carlsbad, CA). RNA (2 μg) treated with DNAse I was reverse-transcribed into cDNA (40 μg) using a Superscript II (18064014; Thermo Fischer, Waltham, MA). Real-time PCR reactions were conducted with 12.5 μL of SYBR Green (4309155; Life Technologies, Carlsbad, CA) and 2.5 $\mu mol \cdot L^{-1}$ of each primer in triplicate using an RT-qPCR system (ABI Prism 7900HT instrument; Applied Biosystems, Foster City, CA). Mouse primers were used as follows:

mNLRP3 (F) TACGGCCGTCTACGTCTTCT and
(R) CGCAGATCACACTCCTCAAA;
mCaspase-1 (F) CACAGCTCTGGAGATGGTGA and
(R) TCTTTCAAGCTTGGGCACTT;
mIL-1β (F) TGACGGACCCCAAAAGATG and
(R) TGGACAGCCCAGGTCAAAG;
mIL-18 (F) CCGCCTCAAACCTTCCA and
(R) CATGGCAGCCATTGTTCCT3′
meNOS, (F) GCTGCACCACAGCAAGCA and
(R) AGAATGGTTGCCTTCACACG;
mNOX2 (F) ACTCCTTGGGTCAGCACTGG and
(R) GTTCCTGTCCAGTTGTCTTCG;
and mCyclophilin, (F) GGAGATGGCACAGGAGGAA
and (R) GCCCGTAGTGCTTCAGCTT.

Relative mRNA levels were calculated using the double delta CT analysis (22) normalized to cyclophilin and then compared with LF-SED (defined as 1.0-fold).

Immunofluorescence staining of the aorta. Immunofluorescence experiments were performed to identify and localize proteins in the endothelium of aorta as described in our previous study (23). The freshly isolated aorta was fixed with 10% formalin and embedded in Tissue-Tek OCT and sectioned at 10 μm at −20°C in a cryostat (CM 1950; Leica, Buffalo Grove, IL). After heat-induced antigen retrieval with 10 mM Na Citrate, slides were blocked with solution (5% donkey or goat serum in phosphate-buffered saline) for 30 min. Sections were then incubated overnight at 4°C with diluted primary antibodies: APN (1:500; ab22554; abcam, Cambridge, UK), AdipoR1 (1:200; sc518030; Santa Cruz Biotechnology, Dallas, TX), NOX2 (1:200; ab129068; abcam, Cambridge, UK), and endothelial cell markers, von Willebrand factor (vWF; 1:500; sc365712; Santa Cruz Biotechnology, Dallas, TX) or platelet

endothelial cell adhesion molecule-1 (PECAM; 1:200; sc1506; Santa Cruz Biotechnology, Dallas, TX), and α smooth muscle actin (1:500; ab5694; abcam, Cambridge, UK). Sections were washed and labeled with corresponding fluorescent-conjugated secondary antibodies, Alexa Fluor® 488 and 594 (Thermo Scientific, Waltham, MA), or labeled with FLICA probes from caspase-1 FLICA^{tm} kit (#97; ImmunoChemistry Technologies, Bloomington, MN) for 1 h at room temperature. Goat IgG, mouse IgG1, mouse IgG, and rabbit IgG were used as negative controls (data not shown). Sections were mounted with Hoechst and mounting media. Images were obtained using fluorescence microscopes (BX51; Olympus, Tokyo, Japan) with 10× objective lens. Microscope and camera settings were kept constant during the process for each protein quantification using FIJI, an open-source image processing package based on ImageJ. Colocalization regions were highlighted with a white overlay mask on the original red and green images, and the generated colocalized images were used to analyze area and pixel intensity.

Measurement of endothelial IL-1β. IL-1β was measured in the aortic endothelial lining with DAB staining (SK-4100; Vector Laboratories, Burlingame, CA). The tissue sections were stained with IL-1β (1:200; ab9722; abcam, Cambrige, UK) for 1 h at 4°C after quenching endogenous peroxidases with H_2O_2 for 20 min and blocking with 10% donkey serum for 30 min. After incubation with IL-1β primary antibody diluted in phosphate-buffered saline with 5% serum, slides were incubated with antihorseradish peroxidase antibody for 1 h at room temperature. After washing, the slides were counterstained with hematoxylin for 5 min, and 50 μL of DAB was added to each section and stained for 1 min. The slides were mounted and observed under a bright field microscope (Olympus BX51). The percentage of the area of endothelium positive for indicated staining in aorta was analyzed by ImageJ software.

DAF staining for measurement of NO production. NO in the vasculature was quantitated with diaminofluorecein (DAF-FM; D23844; Invitrogen, Carlsbad, CA), a cell-permeant reagent that passively diffuses across cellular membranes. Once inside cells, it is deacetylated by intracellular esterases to become DAF-FM. Sections were preincubated with 1 μM of acetylcholine, an activator of endothelium-derived NO production, for 10 min and then coincubated with 5 μM of DAF-FM and 100 nM of acetylcholine for 15 min in 37°C under 95% O_2 and 5% CO_2. Stained tissue sections were visualized by a fluorescence microscope (Eclipse Ti2; Nikon Instruments Inc., Melville, NY) and photographed with a DS-Qi2 monochrome CMOS camera using 20× objective lens at excitation and emersion maxiam of 490 and of 515 nm, respectively. Fluorescence intensity was quantified with ImageJ software.

Measurement of intracellular superoxide (O_2^-). Redox-sensitive fluorescence dye dihydroethidium (DHE) was used to measure O_2^- production in aorta as previously measured (19). Sections were incubated with DHE (10^{-5} mol·L^{-1}; D1168 Invitrogen, Carlsbad, CA) for 15 min in an incubator maintained at 37°C under 95% O_2 and 5% CO_2. DHE-stained vessels were visualized using a fluorescence microscope (Olympus BX51) and acquired using 10× objective lens at an excitation peak of 545 nm and emersion spectral peak of 610 nm. Fluorescence intensity was evaluated with ImageJ software.

Statistical approach. All statistical analyses were conducted using SPSS version 22.0 software (IBM, Armonk, NY). Molecular studies were analyzed using one-way ANOVA followed by LSD *post hoc*. All values were presented as mean ± SEM. Statistical significance was accepted at $P < 0.05$.

RESULTS

As previously shown (19), HFD significantly increased obesity-associated parameters, such as body mass, abdominal girth, and fat mass; voluntary running effectively reduced HFD-induced changes in these obesity-induced changes (see Table, Supplemental Digital Content 1, Effect of high-fat dietary feeding and voluntary wheel running on obesity, http://links.lww.com/MSS/C17; see Figure, Supplemental Content 2; Voluntary running diminishes HFD-induced weight gain in C57BL/6J mice, http://links.lww.com/MSS/C18; see Appendix, Supplemental Content 3, Supplemental methods, http://links.lww.com/MSS/C19).

NLRP3 inflammasome. HFD significantly increased mRNA levels of caspase-1 and IL-1β, but not NLRP3 and IL-18, compared with LF-SED (Fig. 1A). The HFD-induced alteration was not significantly attenuated by voluntary running, although HF-RUN showed a decreasing pattern in caspase-1 and IL-1β compared with HF-SED (Fig. 1A). The activation of the NLRP3 inflammasome triggers caspase-1 cleavage, leading to IL-1β processing (7). This was confirmed in our study, as we found increased colocalization of cleaved caspase-1 (FLICA) in vWF-labeled endothelial cells in HF-SED compared with the signal in LF-SED (Figs. 1B d, i and 1C). Consistently, immunochemistry data using DAB chromogen showed an inflammasome-induced caspase-1-mediated product in the endothelial layer of the aorta in HF-SED (Figs. 1D and 1E). These HFD-induced increases in caspase-1 and IL-1β were decreased by voluntary running (Figs. 1C and 1E).

Tight junction proteins and APN–AdipoR1. HFD decreased the expression of ZO-1 and ZO-2 on aortic endothelial cells (Figs. 2A k and 2B, and Figs. 2C k and 2D). This downregulation of ZO-1/2 induced by HFD was prevented by voluntary running, such that HF-RUN did not differ from LF-SED (Figs. 2A o and 2C o). As shown in Figures 3A–D, endothelial APN and AdipoR1 contents were much lower in the aorta of HF-SED, although these decreases were prevented by voluntary running which is similar to LF-SED.

Oxidative stress and NO production. The mRNA level of NOX2 was significantly higher in HF-SED aorta compared with LF-SED; this increase was attenuated in HF-RUN (Fig. 4A). Dual immunostaining using PECAM demonstrates where the NOX2 protein is localized in the vascular endothelial cells (Fig. 4B). NOX2 protein expression was remarkably increased in aortic endothelial cells of HFD compared with LF-SED; this was attenuated in HF-RUN (Fig. 4B). Indeed, we found that increased fluorescent intensity, indicating higher cellular O_2^- level, in HFD mice aorta was significantly ameliorated in HF-RUN (Figs. 4C and 4D). Figure 4E shows an increasing trend in running groups

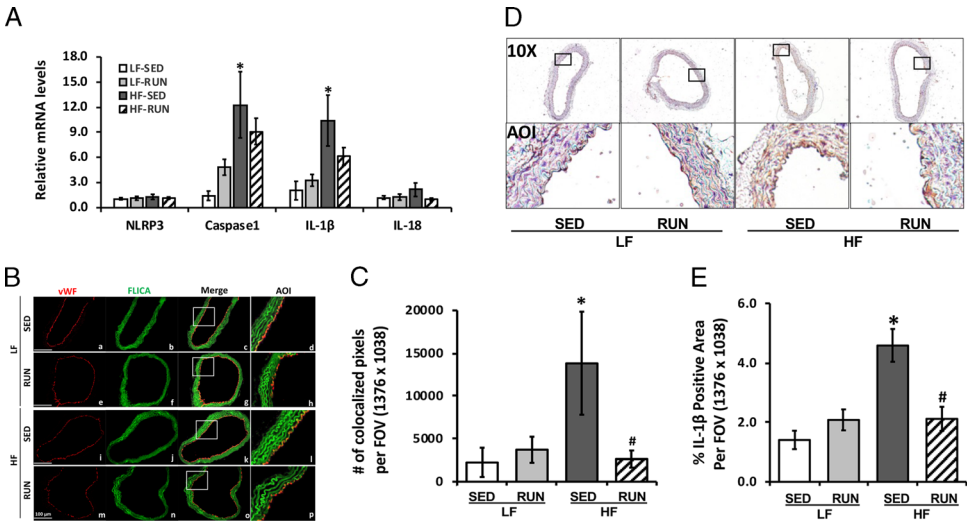

FIGURE 1—Effect of voluntary running on NLRP3 inflammasome in HFD mice aorta. A, Relative mRNA level of NLRP3, and caspase-1, IL-1β, and IL-18 analyzed in mice aorta (n = 4–6). B and C, Mice aortic sections stained with FLICA, a *green fluorescent probe* specific for active caspase-1 and Alexa594-conjugated antibodies against an endothelial cell marker von Willebrand factor (vWF). The merged images displaying *yellow dots or patches* indicating the colocalization of FLICA (*green*) and vWF (*red*). Summarized data showing colocalized pixels numbers (n = 5–7). D and E, Immunohistochemical analysis of IL-1β expression (*dark brown precipitate*) in the endothelial layer of the aorta. Summarized data showing area percentage of the endothelium positive for IL-1β in aorta (n = 4–6). Values are presented as mean ± SEM. *P < 0.05 vs LF-SED; #P < 0.05 vs HF-SED.

compared with corresponding control given the same diet. The green fluorescent NO signal by DAF-FM was diminished in HF-SED mice in comparison with the signal in LF-SED. Voluntary running significantly opposed HFD-induced decreases in NO fluorescent intensity in the HF-RUN cohort (Figs. 4F and 4G).

DISCUSSIONS

The current study provides the first evidence that voluntary running attenuated NLRP3 inflammasome signaling, including caspase-1 activation and IL-1β process, in endothelial cells

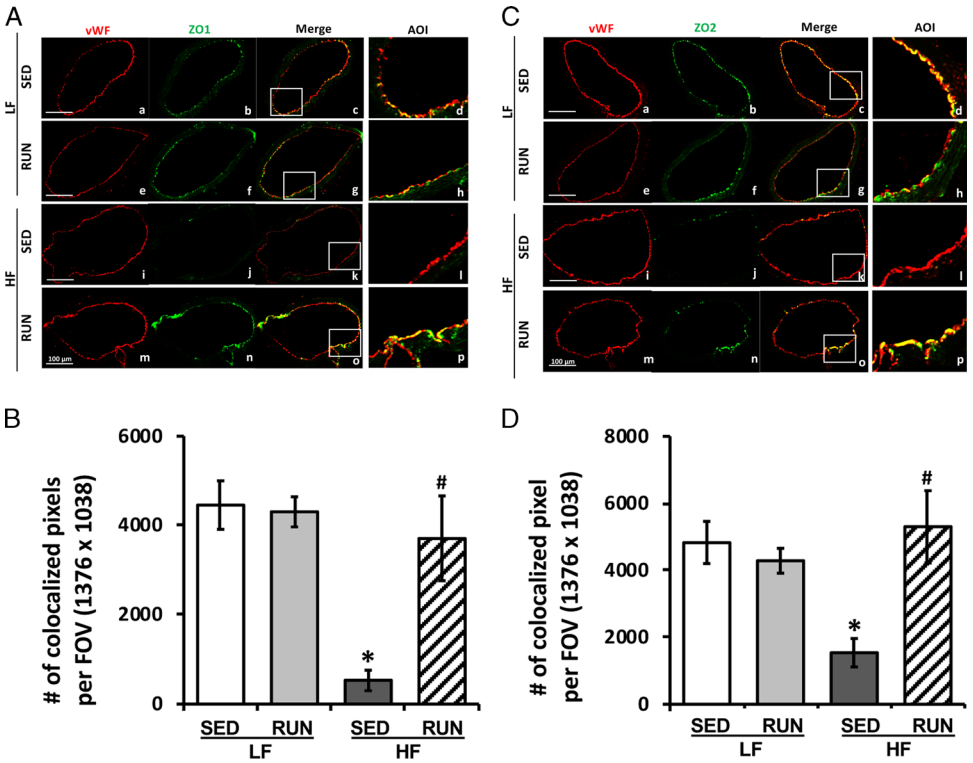

FIGURE 2—Running effect on HFD-induced disruption of tight junction. A and C, Representative fluorescent images stained with Alexa488-conjugated antibodies against either ZO-1 or ZO-2, and with an Alexa594 against endothelial cell marker vWF, respectively. Yellow dots or patches in the merged images indicating the colocalization of ZO-1 and ZO-2 (*green*) and vWF (*red*). B and D, Quantification data displaying colocalized positive signals of ZO-1/2 in endothelial cells (n = 4–6). Values are presented as mean ± SEM. *P < 0.05 vs LF-SED; #P < 0.05 vs HF-SED.

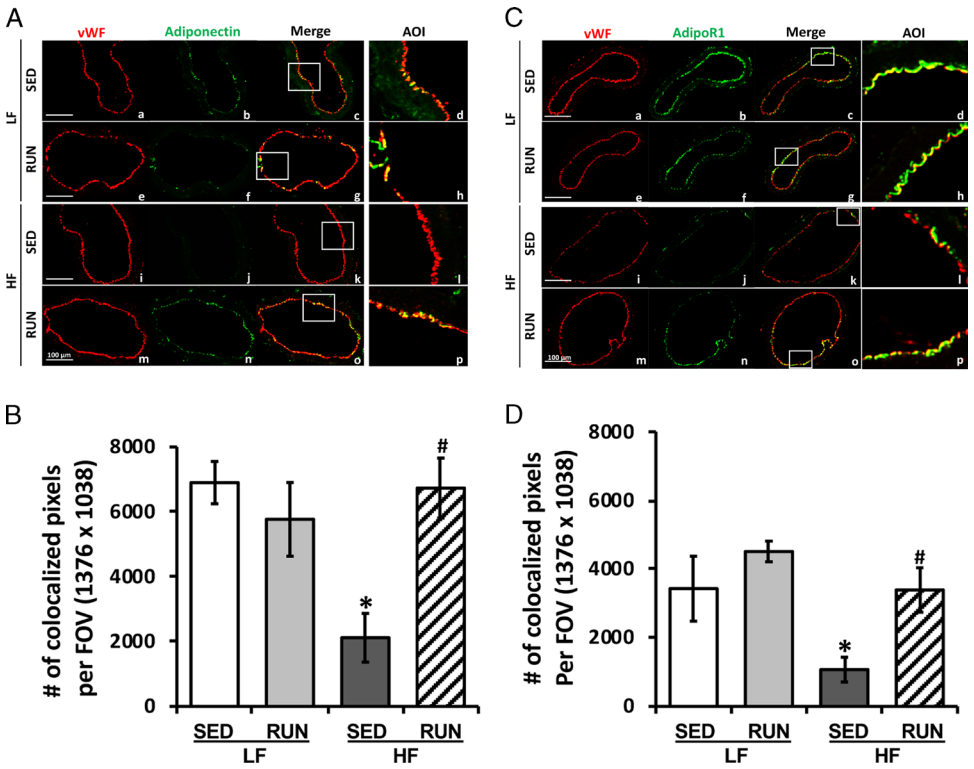

FIGURE 3—Restored APN and AdipoR1 by voluntary running in HFD mice aorta. A and C, Representative fluorescent images depicting the effect of voluntary running on the reduced expression of APN and AdipoR1 in endothelial cells of HFD mice. APN and AdipoR1, and an endothelial cell marker vWF labeled with Alexa488- and Alexa594-conjugated antibodies, respectively. The merged images showing *yellow dots or patches* indicating the colocalization of APN and AdipoR1 (*green*) and vWF (*red*). B and D, Quantification data displaying colocalization of APN and AdipoR1 positive signals with endothelial cells (n = 4–6). Values are presented as mean ± SEM. *P < 0.05 vs LF-SED; #P < 0.05 vs HF-SED.

of aorta from obese mice. Second, we found that voluntary running recovered the tight junction proteins ZO-1/2 that may be responsible for vascular homeostasis in obese aortae. Finally, we demonstrate novel findings that exercise fully restored the HFD-induced decreased content of APN and AdipoR1 in mouse aortic endothelial cells. We propose that this is linked to both the decreased inflammasome signaling and the increased NO production in HF-RUN. In combination, we suggest that reduced inflammasome signaling by voluntary running ameliorates vascular dysfunction-associated mechanisms in aorta of obese

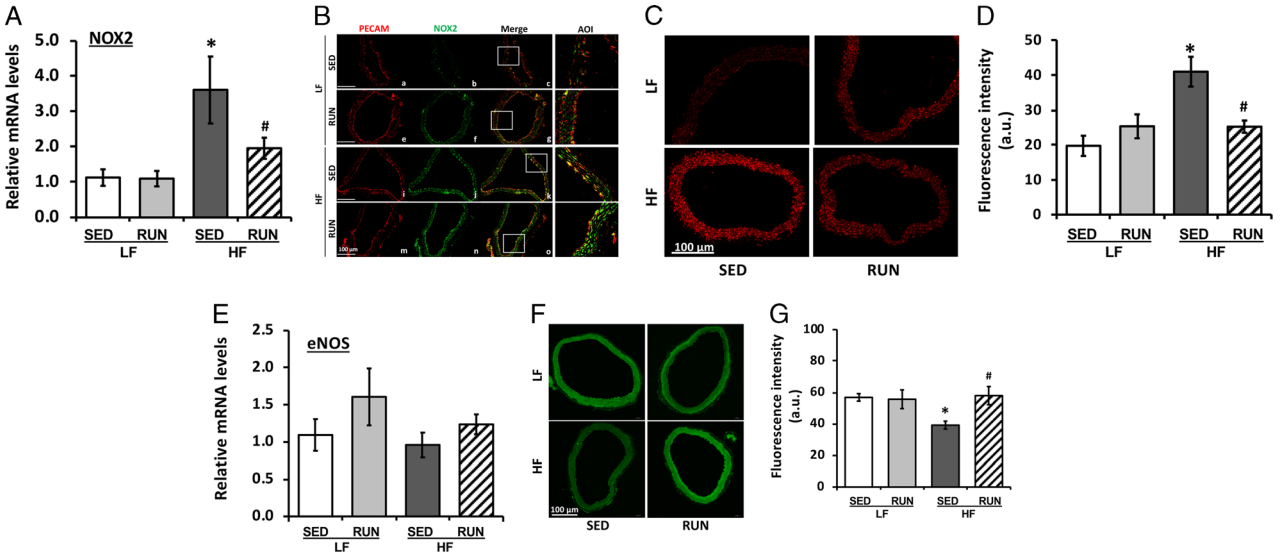

FIGURE 4—Voluntary running-induced improvement of oxidative stress and NO production in HFD. A, Relative mRNA expression levels of NOX2 in the aorta (n = 4–6). B, The merged florescent images showing *yellow dots or patches* indicating the colocalization of NOX2 (*green*) and PECAM (*red*). C and D, DHE staining to assess intracellular O_2^- fluorescence intensity in aortic sections and summarized data (n = 4). E, Aortic mRNA expression levels of eNOS (n = 4–6). F and G, Representative fluorescence image of DAF staining to test NO production in aortic section and summarized data (n = 6–8). Values are presented as mean ± SEM. *P < 0.05 vs LF-SED; #P < 0.05 vs HF-SED.

mice. Hence, it seems that APN–AdipoR1 and tight junction protein-associated mechanisms, at least partially, along with the restoration of NO bioavailability and decrease of oxidative stress by voluntary running, facilitate the maintenance of vascular function.

The inhibition of NLRP3 inflammasome can prevent the inflammasome-associated vascular pathology (10). Exercise has also been shown to reduce the protein levels of the inflammasome components in ovariectomized mice brain (24) and in adipose tissue from obese mice (25). The current study adds to this scant literature as we demonstrated a significant decrease in active caspase-1 levels in aortic endothelial cells in HF-RUN compared with HF-SED, and that elevated IL-1β in inner wall of the aorta is markedly diminished by voluntary running. Along with our recent study observing inflammasome axis in the coronary resistance vessels (19), the current findings provide a more comprehensive understanding that voluntary running could suppress the inflammasome activation triggering caspase-1 cleavage-dependent IL-1β processing in both large conduit vessel and resistance vessels in obese mice. Our approach aligns with the issue highlighted by a National Institutes of Health workshop (26) to aim to understand basic endothelial biology about vascular beds-specific diversity for disease treatment. Although others have demonstrated some differences in the molecular response in different vascular loci to exercise in humans (27) and rodents (28), our similar findings from large conduit aorta and small resistance coronary arterioles (19) suggest that regular exercise could be a potential therapy to attenuate inflammation in the vascular system.

The disruption of tight junction proteins ZO-1 and ZO-2 increased by HFD contributes to vascular permeability in mice myocardium (9). Our data are in alignment with this, as we found that HFD significantly decreased ZO-1/2 expression in endothelial cells of aorta from obese mice. Ischemia-induced oxidative stress during acute exercise might be a cause of decreased tight junctions protein and increased barrier dysfunction in intestinal wall (29). However, it remains unknown about the effects of long-term exercise on ZO-1/2 levels in obese vasculature. We demonstrate for the first time that downregulated ZO-1/2 levels in obese aorta were fully restored by voluntary running. NO level is affected by mechanotransducers PECAM and VE-cadherin through an endothelial shear stress (30), and this mechanism is likely linked to ZO-1 that regulates VE-cadherin-dependent endothelial junction (31). This could account for the fact that the maintenance of ZO-1 levels in aortic endothelial cells of HF-RUN may involve the increased NO production. Thus, we presume that voluntary running could preserve endothelial permeability and the NO pathway in obese vasculature via protection against the disruption of ZO-1/2 proteins. Endogenous NO can negatively regulate NLRP3 inflammasome signaling (32). In the present study, running exercise fully recovered NO levels and attenuated the inflammasome signaling in the aorta of HFD fed mice. In addition, ROS production is a crucial mechanism for the initiation of the inflammasome (33). It was found that knocking down common NADPH subunit p22phox decreased IL-1β (34). In this context, our findings regarding decreased O_2^- level and NOX2 expression in obese mice aorta suggest that exercise not only decreases oxidative stress but also plays a protective role against inflammasome-associated aortic endothelial dysfunction in obesity. Taken together, inhibitory mechanisms by which exercise attenuates the inflammasome signaling in obese aorta may be, in part, related to restored NO production and/or reduced oxidative stress.

Here, we show for the first time that voluntary running fully recovered positive signals to endothelial AdipoR1, known as the primary receptor of APN signaling in endothelial cells (18), in obese mice aorta. These findings are similar to a previous result that treadmill running improved APN protein expression in whole aorta of diabetic mice (35), and a second study that exercise upregulated AdipoR1/2 proteins in skeletal muscle of obese mice (36). It has also been shown that overexpressed AdipoR1/2 potentiated an anti-inflammatory effect in endothelial cells with subeffective APN levels (37). A significant novel finding of the present study is that voluntary running protected obesity-induced loss of APN and AdiopR1 in mice aorta, suggesting that physical activity could be the therapeutic approach to correct obesity-associated vascular pathology via restored APN–AdipoR1 axis. Moreover, our finding of the restoration of APN–AdipoR1 is more meaningful when considering that voluntary running improves eNOS-dependent vasodilator response of mesentery arteries (see Figure, Supplemental Digital Content 4, Voluntary running improves endothelium-dependent vasodilation in HFD mice mesentery artery, http://links.lww.com/MSS/C20; see Figure, Supplemental Digital Content 5, Voluntary running restores ACh-induced eNOS-dependent vasorelaxation response in mesentery artery of HFD mice, http://links.lww.com/MSS/C21; see Appendix, Supplemental Digital Content 3, Supplemental methods, http://links.lww.com/MSS/C19). This is supported by previous findings that APN administration rescued endothelial dysfunction with restored NO and oxidative stress in obese rat mesentery artery (38), and that increased eNOS-dependent vasorelaxation in mice aorta is dependent on AdipoR1 under physiologic APN level (5 $\mu g \cdot mL^{-1}$) (18). The restored APN–AdipoR1 signaling by voluntary running relates to inflammasome data in the current study because activated inflammasome sufficiently impairs endothelial function (10) and APN deficiency increases the inflammasome activity in endothelial dysfunction in diabetes (39). APN inhibits ROS production, thus leading to suppression of inflammasome activation (17). Collectively, our findings of improved APN–AdipoR1 and NO, and attenuated ROS and inflammasome in HF-RUN, indicate that voluntary running could inhibit inflammasome activity-induced endothelial dysfunction in obese vasculature possibly, in part, through APN-associated protective mechanism.

One limitation in this project is the absence of data about serum APN concentrations. However, the changes in serum concentration of APN cause robust physiological modification, and thus it is anticipated that changes in the local production are important, especially in the vascular function. For more precise knowledge

about role of APN–AdipoR1-dependent mechanism by which voluntary running improves vascular dysfunction in obesity, more experiments using AdipoR knockout models will be warranted. In addition, exercise alone did not induce any vascular benefit in many variables despite changes in obesity-associated factors (see Table, Supplemental Digital Content 1, Effect of high-fat dietary feeding and voluntary wheel running on obesity), which are closely related to vascular health. This finding may be attributed to young and healthy wild-type mice with active behavior. Nonetheless, physiological adaptations in resting heart rate, endurance capacity, and angiogenesis in LF-RUN mice would happen with voluntary running pattern, which met a threshold, ~10 $km \cdot d^{-1}$ for 4–8 wk, reported in previous studies (2,40,41). The other limitation is that we excluded female rodents to reduce possible influence by sex hormone, such as estrogen that has the anti-inflammatory effect. Also, mice in running groups were singly housed, but the corresponding control sedentary mice were group housed. Future study is warranted to treat all mice in the same condition.

CONCLUSIONS

The findings of the present study document that voluntary running ameliorates the inflammasome-associated mechanisms for vascular dysfunction in HFD mice aorta possibly via multiple protective mechanisms, including restored tight junctions, APN–AdipoR1, NO production, and decreased oxidative stress. Voluntary running could, therefore, be an effective therapeutic strategy to maintain vascular health in obesity.

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