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High-fat diet selectively decreases bone marrow lin⁻/CD117⁺ cell population in aging mice through increased ROS production

Yichao Xiao^{1,2}, Qingyi Zhu^{1,2}, Xuanyou Liu², Meng Jiang², Hong Hao², Hua Zhu³, Peter J. Cowan^{4,5}, Xiaoming He⁶, Qiming Liu¹, Shenghua Zhou¹, Zhenguo Liu²

¹Department of Cardiology, The Second Xiangya Hospital of Central South University, Changsha, China

²Center for Precision Medicine and Division of Cardiovascular Medicine, Department of Medicine, University of Missouri School of Medicine, Columbia, MO, USA

³Davis Heart and Lung Research Institute, The Ohio State University Medical Center, Columbus, OH, USA

⁴Department of Medicine, University of Melbourne, Melbourne, Victoria, Australia

⁵Immunology Research Centre, St. Vincent's Hospital, Melbourne, Victoria, Australia

⁶Fischell Department of Bioengineering, University of Maryland, College Park, MD, USA

Abstract

Bone marrow (BM) stem cells (BMSCs) are an important source for cell therapy. The outcome of cell therapy could be ultimately associated with the number and function of donor BMSCs. The present study was to evaluate the effect of long-term high-fat diet (HFD) on the population of BMSCs and the role of reactive oxygen species (ROS) in aging mice. Forty-week-old male C57BL/6 mice were fed with HFD for 3 months with regular diet as control. Experiments were repeated when ROS production was reduced in mice treated with N-acetylcysteine (NAC) or using mice overexpressing antioxidant enzyme network (AON) of superoxide dismutase (SOD)1, SOD3, and glutathione peroxidase. BM and blood cells were analyzed with flowcytometry for lineage negative (lin⁻) and Sca-1⁺, or lin⁻/CD117⁺, or lin⁻/CD133⁺ cells. Lin⁻/CD117⁺ cell population was significantly decreased with increased intracellular ROS and apoptosis and decreased proliferation in BM, not in blood, in HFD-treated mice without change for Sca-1⁺ or CD133⁺ cell populations in BM or blood. NAC treatment or AON overexpression effectively prevented HFD-induced intracellular ROS production and reduction of BM lin⁻/CD117⁺ population. These data

Correspondence: Zhenguo Liu, MD, PhD, Division of Cardiovascular Medicine, Center for Precision Medicine, Department of Medicine, University of Missouri School of Medicine, 1 Hospital Drive, CE306, Columbia, MO 65212, USA. liuzheng@health.missouri.edu, Shenghua Zhou, MD, PhD, Department of Cardiology, The Second Xiangya Hospital, Central South University, Changsha, Hunan 410011, China. zhoushenghua@csu.edu.cn.

AUTHOR CONTRIBUTIONS

S. Z. and Z. L. developed the concept and designed the experiment. Y. X., Q. Z., X. L., M. J., P. J. C., H. Z., and H. H prepared the experimental materials and collected the data. Y. X., X. H., Q. L., and Z. L. analyzed the data. Y. X., S. Z., and Z. L wrote and revised the manuscript.

CONFLICT OF INTEREST

There was no conflict of interest.

suggested that long-term HFD selectively decreased BM $\text{lin}^{-}/\text{CD117}^{+}$ cell population in aging mice through increased ROS production.

Keywords

bone marrow stem cells; high-fat diet; N-acetylcysteine; oxidative stress; reactive oxygen species

1 | INTRODUCTION

Adult mesenchymal stem cells (MSCs) including bone marrow (BM) stem cells (BMSCs) are an important source for cell therapy for tissue repair and regeneration (Charif et al., 2017; Mo, Wang, Zhou, Li, & Wu, 2016; Uccelli, Moretta, & Pistoia, 2008). Most patients in need of cell therapies especially those with ischemic cardiac damages are often of advanced age with metabolic abnormalities, including hyperlipidemia (Charif et al., 2017). The outcome of cell therapy could be ultimately associated with the number and function of donor BMSCs (Mezey, 2011). It is known that aging is an important factor for biological properties including proliferation, senescence, and paracrine function and thus is critical to the therapeutic potency and outcome of BMSCs (Beane, Fonseca, Cooper, Koren, & Darling, 2014). However, little is known on how the number and function of BMSCs are influenced in individuals with advanced age.

Reactive oxygen species (ROS) has long been considered as a major contributor to inflammatory response and plays an important role in oxidative stress and cell function including cell-fate signaling, proliferation, and gene transcription and expression, as well as cell injury and death (Cui et al., 2015; Lee et al., 2012; Vasconcelos et al., 2009). ROS and related oxidative stress are critically involved in the regulation of the number and/or function of stem cells and progenitors including BMSCs (Cui, Jia, et al., 2015; Lee et al., 2012; Shi, Hu, Lv, & Tu, 2016; Vasconcelos et al., 2009). However, excessive ROS production and related oxidative stress could significantly impair the number and number of stem cells, thus decreasing the effectiveness of stem/progenitor cell therapy.

ROS is a natural product in vivo from a variety of sources including energy metabolisms (Dymkowska, Drabarek, Podrzywalow-Bartnicka, Szczepanowska, & Zablocki, 2014; Finkel & Holbrook, 2000; Lyu et al., 2019). Western diet is known to be rich of animal fats, and high-fat diet (HFD) has been shown to significantly increase the production of proinflammatory cytokines like tumor necrosis factor (TNF)- α and induce tissue oxidative stress (Herieka & Erridge, 2014; Weinberg, Maymon, & Weinreb, 2014). In the present study, experiments were designed to test the hypothesis that HFD could alter the population of BMSCs in aging mice through increased ROS production. The objectives of the present study included the following: (1) evaluating the effect of long-term treatment with HFD on the populations of BMSCs in aging mice and (2) determining the role of ROS in mediating the effect of HFD on the populations of BMSCs by reducing ROS production with antioxidant N-acetylcysteine (NAC) treatment or using transgenic mice with concomitant overexpression of antioxidant enzyme network (AON) of superoxide dismutase (SOD)1, SOD3, and glutathione peroxidase (GPx). The reason to use NAC as the agent of choice to

block ROS production was that it is a Food and Drug Administration (FDA)-approved drug with long history of safety record clinically and readily available for clinical use if needed.

2 | MATERIALS AND METHODS

2.1 | Animal model

All the animal experiments were performed in accordance with the Guidelines of the Animal Care Committee of the University of Missouri, Columbia, Missouri, USA. The experimental protocols for the present study were reviewed and approved by the Animal Care Committee of the University of Missouri, Columbia, Missouri, USA. Aging male wild-type (WT) C57BL/6 mice (40 weeks old) were fed with HFD or regular diet (for control) for 3 months. Mouse serum lipid profile was quantitatively determined using a lipid profile analyzer and cassette (Cholestech LDX, CA, USA) to confirm the establishment of hyperlipidemic state after 3 months of HFD treatment. To evaluate the role of ROS in mediating the effect of HFD on BM cell population, experiments were repeated when ROS production was blocked in WT mice treated with NAC (1 mg/ml in the drinking water, Sigma-Aldrich, St. Louis, MO, A7250) as described (Cui et al., 2015) for 24 h before initiation of HFD and continuously for the rest of the experiments with phosphate-buffered saline (PBS) as the control or using age-matched transgenic mice with C57BL/6 mouse background and concomitant overexpression of AON of SOD1, SOD3, and GPx with decreased ROS production as described (Cui, Jia, et al., 2015).

2.2 | Analysis of BMSCs population and in vivo cell proliferation

After treatment of mice with regular diet or HFD with or without NAC for 3 months, mouse BM cells were collected and prepared for BMSCs analysis using flow cytometry after red blood cells (RBCs) were eliminated with RBC lysis as described previously (Rosenkranz et al., 1992). For in vivo BMSC proliferation analysis, mice were treated with 1-mg BrdU (via intraperitoneal injection) 12 h before BM cell collection as described (Baldrige, King, Boles, Weksberg, & Goodell, 2010). After staining with lineage cocktail (components including anti-mouse CD3, clone 17A2; anti-mouse Ly-6G/Ly-6C, clone RB6-8C5; anti-mouse CD11b, clone M1/70; anti-mouse CD45R/B220, clone RA3-6B2; and anti-mouse TER-119/Erythroid cells, clone Ter-119), Pacific Blue, Sca-1 PE-Cy5, CD117, and CD133 PE antibodies (all antibodies were purchased from Bio-logend, San Diego, CA, USA), cells were permeabilized and stained with anti-BrdU FITC using the BrdU Flow Kit according to the manufacturer's instruction (559619, Becton Dickinson and Company BD Biosciences, San Jose, CA, USA). For BMSC population analysis, BM lineage negative/Sca-1 positive (LS), lineage negative CD133 positive (Lin⁻/CD133⁺), and lin⁻/c-Kit⁺ cell populations were carefully compensated (each cell population percentile was further confirmed with single antibody staining) and determined using flow cytometry as described (Traverse et al., 2012). For all fluorescence-activated cell sorting (FACS) data, at least 5×10^4 cells were counted for each sample with the total of at least 1×10^6 cells per sample.

2.3 | Analysis of cell apoptosis

BM and blood cells were harvested from WT mice both with and without NAC treatment, as well as transgenic mice with AON overexpression after 3 months of HFD treatment. The

apoptosis rate of BMSCs was determined with FACS using an apoptosis kit from BD Pharmingen (CA, USA) as per manufacturer's protocol. Early apoptotic cells were defined as Annexin V FITC positive cells, whereas the late apoptotic cells were defined as Annexin V FITC and propidium iodide (PI) double positive cells as described (Jindal, Minhas, Prabhakar, & Anand, 2014).

2.4 | Analysis of intracellular ROS formation

BM and blood cells were harvested from WT mice both with and without NAC treatment, as well as transgenic mice with AON overexpression after 3 months of HFD treatment. RBCs were eliminated using RBC lysis as described above. The level of intracellular ROS formation in BM cells was determined using the ROS Detection Reagents-FITC (Invitrogen) as described (Rosenkranz et al., 1992). The cells were incubated with the reagent for 10 min at 37°C. The labeled cells were washed twice with PBS and then suspended in warm PBS for analysis using flow cytometry. The fluorescence-positive cells were quantitatively evaluated using an LSRII (BD Bioscience, CA, USA) at the wavelength of 525 nm.

2.5 | Statistical analysis

All the data were presented as means $\pm$ standard deviation (SD) and statistically analyzed using unpaired Student t test (two-sided) for comparison between two groups of data or one-way analysis of variance (ANOVA) followed by post hoc Tukey's test for comparison for three or more groups of data to minimize Type I error (PRISM Version 4.0; GraphPad Software, Inc., San Diego, CA, USA). The differences were considered statistically significant when a two-tailed $p < 0.05$.

3 | RESULTS

3.1 | HFD increased intracellular ROS production and selectively decreased BM $\text{lin}^{-}/\text{c-Kit}^{+}$ cell population in aging mice

After 3 months of HFD, serum lipid level was significantly increased in aging WT mice (Table 1), confirming that the animal model was successful. Flow cytometry analysis demonstrated that intracellular ROS production was significantly increased in the BM cells from mice with 3 months of HFD treatment as compared with the control animals with normal diet (Figure 1). Flow cytometry analysis also showed that treatment with HFD for 3 months significantly decreased the population of $\text{lin}^{-}/\text{c-Kit}^{+}$ cells by 26% in BM, but not in blood, as compared with the control group, whereas the populations of $\text{lin}^{-}/\text{Sca-1}^{+}$ or $\text{lin}^{-}/\text{CD133}^{+}$ cells in BM and blood were similar between HFD-treated mice and control animals (Figure 2).

3.2 | HFD significantly suppressed in vivo proliferation of $\text{lin}^{-}/\text{c-Kit}^{+}$ cells in BM

Experiments were then conducted to determine if the decreased population of $\text{lin}^{-}/\text{c-Kit}^{+}$ cells in BM in the mice with HFD treatment was due to impaired in vivo proliferation of the cells using in vivo BrdU assay. Flow cytometry analysis demonstrated that HFD significantly decreased the in vivo proliferation rate of the $\text{lin}^{-}/\text{c-Kit}^{+}$ cells by 19% as compared with the control, whereas no significant change in cell proliferation was observed

in $\text{lin}^-/\text{Sca-1}^+$ or $\text{lin}^-/\text{CD133}^+$ cells in the BM or blood between HFD-treated mice and controls (Figure 3a).

3.3 | HFD significantly increased apoptosis of BM $\text{lin}^-/\text{c-Kit}^+$ cells in vivo

Experiments were also conducted to determine if increased apoptosis in vivo might significantly contribute to the decreased population of $\text{lin}^-/\text{c-Kit}^+$ cells in BM in HFD-treated aging mice. Flow cytometry analysis showed that HFD indeed significantly increased the in vivo apoptosis of $\text{lin}^-/\text{c-Kit}^+$ cells by twofold over the control, whereas no significant change in cell apoptosis was observed in $\text{lin}^-/\text{Sca-1}^+$ or $\text{lin}^-/\text{CD133}^+$ cells in the BM or blood between HFD-treated mice and the controls (Figure 3b).

3.4 | NAC treatment or overexpression of AON effectively prevented HFD-induced ROS production and preserved the population of BM $\text{lin}^-/\text{c-Kit}^+$ cells in mice with HFD

To determine the role of ROS production in mediating the effect of long-term HFD treatment on $\text{lin}^-/\text{c-Kit}^+$ cells in BM, ROS production was inhibited by treating the mice with antioxidant NAC or using triple transgenic mice with concomitant overexpression of SOD1, SOD3, and GPx with reduced ROS production. As expected, NAC treatment or AON overexpression effectively prevented HFD-induced ROS production in the aging mice (Figure 1) and preserved the population of $\text{lin}^-/\text{c-Kit}^+$ cells in BM and reversed the changes in cell proliferation and apoptosis in HFD-treated aging mice (Figures 2 and 3). Of note, NAC treatment also significantly decreased the levels of serum low-density lipoprotein (LDL) and triglycerides (TRG) in the mice with HFD feeding without significant changes in high-density lipoprotein (HDL) and total cholesterol (TC, Table 1).

4 | DISCUSSION

In the present study, we demonstrated that long-term treatment with HFD selectively decreased the population of $\text{lin}^-/\text{c-Kit}^+$ cells in BM, not in blood, in association with increased ROS production in aging mice. We also demonstrated that HFD treatment significantly decreased the proliferation of BM $\text{lin}^-/\text{c-Kit}^+$ cells in vivo and increased their apoptosis, thus contributing to decreased population of BM $\text{lin}^-/\text{c-Kit}^+$ cells. When ROS production was blocked with NAC treatment or AON overexpression, HFD-induced reduction of cell proliferation and increase in apoptosis of $\text{lin}^-/\text{c-Kit}^+$ cells were effectively prevented with restoration of the population of BM $\text{lin}^-/\text{c-Kit}^+$ cells in the aging mice. These data suggested that HFD selectively decreased the population of $\text{lin}^-/\text{c-Kit}^+$ cells in BM through both decreased proliferation and increased apoptosis with a mechanism associated with increased ROS production in aging mice.

BM is traditionally considered as the primary site of hematopoiesis with abundant adipose tissues and hematopoietic stem cells produce white blood cells, RBCs, and platelets. Over the past 20 years, BM has been identified as a major source for MSCs that can develop into bone, fat, cartilage, endothelial cells, or muscle cells. BM-derived MSCs and progenitor cells are a mixture of heterogeneous subpopulations with different phenotypic features and vary in their regenerative capacity and potency (Chen, Johnson, McKay, & Robey, 2018; Herrmann et al., 2019). MSCs including BMSCs are capable of differentiating into cells of

multiple cell lineages, exhibit potent paracrine function, and thus are considered an ideal source for cell therapy in regenerative medicine because of their easy preparation and low immunogenicity without ethical concerns. However, the outcomes of clinical studies with MSCs have been inconsistent and inconclusive for unknown reasons (Majka, Sulkowski, Badyra, & Musialek, 2017; Yau et al., 2019). Age could reduce overall cell yield of all MSC populations, and age of the donors appears to be an important factor for the function and therapeutic potency of BMSCs (Beane et al., 2014; Charif et al., 2017; Li et al., 2015). It has been reported that BM MSCs are differentially impaired by aging with decreased proliferation, senescence, and chondrogenic response, whereas stem cells derived from muscle and adipose tissues are not negatively affected by aging (W. J. Du, Chi, et al., 2016; Garcia-Prat & Munoz-Canoves, 2017; Hwang & Brack, 2018; Mo et al., 2016). MSCs are also very heterogeneous in terms of their phenotype, biological behavior, and function, and not all MSCs are multipotent in their capability of differentiation (Baksh, Song, & Tuan, 2004; Mo et al., 2016; Pittenger et al., 1999). A variety of cell markers have been used to characterize and isolate MSCs and progenitors including Sca-1, CD133, and CD117 (Deans & Moseley, 2000; Jurczynski et al., 2014; Okada et al., 1992), and no standard cells markers have been used to define the nature of the cells in each patient in the clinical studies. Other factors including (but not limited to) the donor's physical conditions, lifestyle (smoking vs. nonsmoking, with or without alcohol use, and dietary preference), and disease conditions (clinical and subclinical) may also contribute to the inconsistency on the outcomes of clinical studies with MSCs (Altaner et al., 2013; Deans & Moseley, 2000; Schuler, Adams, & Goto, 2013).

The data from the present study showing that 3 months of HFD diet selectively decreased the population of $\text{lin}^{-}/\text{c-Kit}^{+}$ cells in BM in aging mice could certainly make the situation on cell therapy even more complicated. CD117 is expressed on hematopoietic stem cells and progenitor cells but not on lymphohematopoietic differentiated cells. $\text{Lin}^{-}/\text{CD117}^{+}/\text{Sca-1}^{+}$ cells consists of 0.08% of BM nucleated cells. It has been shown that BM-derived CD117⁺ cells are a potential source for cell-based tissue repair and regeneration therapy (Banik, Prabhakar, Kalra, & Anand, 2014; M. Du, Schmult, et al., 2016; Jia et al., 2012). Thus, HFD could be another significant factor for the outcome of cell therapy with BMSCs. However, it is important to point out that the present study was conducted with only male mice. Future studies are needed to determine if a gender difference could be present for the effect of HFD on the population of $\text{lin}^{-}/\text{c-Kit}^{+}$ cells in BM because substantial gender differences exist for the prevalence, progression, and outcome in cardiovascular diseases (Kander, Cui, & Liu, 2017). It is also important to determine if HFD could have different effects on $\text{lin}^{-}/\text{CD117}^{+}$ cell populations in circulation and potential mechanisms because of significant differences in the microenvironment of blood and BM.

It is well known that ROS and related oxidative stress play an important role in regulating the number and function of stem cells including BMSCs (Lee et al., 2012; Vasconcelos et al., 2009). It is also known that HFD feeding could induce the expression of gp91, a NADPH oxidase subunit, and promote ROS production in both colonic epithelial cells and lamina propria cells (Alimchandani et al., 2013; Kikuchi, Hikage, Miyashita, & Fukumoto, 2000; Li et al., 2019). Another potential source of increased ROS with HFD feeding could be the increased level of LDL as shown in Table 1. LDL could be converted in vivo to oxidized

LDL, which is a potent oxidant and releases ROS spontaneously. NAC treatment could block the bio-transformation of native LDL to oxidized LDL and attenuate ROS release from oxidized LDL (Cui, Narasimhulu, et al., 2015; Lu et al., 2010), thus leading to decreased ROS level in the aging mice with HFD feeding. In addition, NAC treatment could lower the level of serum homocysteine, which is considered an important source of ROS formation, inflammation, and oxidative stress (Hildebrandt et al., 2015; Ostrakhovitch & Tabibzadeh, 2019; Wiklund et al., 1996). Thus, the data from the present study showed that HFD increased ROS production in $\text{lin}^{-}/\text{c-Kit}^{+}$ cells in BM in aging mice and contributed to the decreased population of $\text{lin}^{-}/\text{c-Kit}^{+}$ cells in BM. The data also demonstrated that NAC treatment effectively prevented HFD-induced ROS formation and preserved the population of BM $\text{lin}^{-}/\text{c-Kit}^{+}$ cells in mice with HFD. Future studies are warranted to determine if HFD could impair the therapeutic efficacy of BMSCs from aging mice and to determine if antioxidant NAC could enhance the outcome of cell therapy with BMSCs in aging mice with HFD. Of note, NAC treatment significantly decreased the levels of serum LDL and TRG in the mice with HFD feeding in the present study, whereas other studies showed that NAC treatment had no significant impact on lipid profiles in hyperlipidemic mice with HFD (Cui, Narasimhulu, et al., 2015; Hildebrandt et al., 2015; Wiklund et al., 1996). The difference on the effect of NAC on lipid levels could be very likely due to the difference in animal models used (WT mice vs. LDL receptor knockout).

5 | CONCLUSION

The present study demonstrated that chronic HFD selectively decreased BM $\text{lin}^{-}/\text{CD117}^{+}$ cell population in aging mice through increased ROS production.

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FIGURE 1.

High-fat diet (HFD) increased intracellular reactive oxygen species (ROS) production in bone marrow (BM) $\text{lin}^{-}/\text{c-Kit}^{+}$ cells. Intracellular ROS production was measured in the mice after exposure to HFD for 3 months. Flow cytometry analysis showed that intracellular ROS level was significantly increased in BM $\text{lin}^{-}/\text{c-Kit}^{+}$ cells in the mice with HFD. N-Acetylcysteine (NAC) treatment effectively blocked ROS production in BM $\text{lin}^{-}/\text{c-Kit}^{+}$ cells in mice with HFD. Increased ROS production was effectively prevented in the aging mice with HFD with NAC treatment or overexpressing antioxidant enzyme network (AON). WT + ND, wild-type (WT) C57BL/6 mice with normal diet for 3 months; WT + HFD, WT C57BL/6 mice with HFD for 3 months; WT + HFD + NAC, WT C57BL/6 mice with HFD and NAC for 3 months; TG + ND, TG mice with normal diet for 3 months; TG + HFD, TG mice with HFD for 3 months. $*p < 0.05$, $n = 8$

FIGURE 2.

High-fat diet (HFD) selectively decreases the bone marrow (BM) $\text{lin}^-/\text{c-Kit}^+$ cell population in aging mice. BM and blood cells were collected for BM stem cells (BMSCs) population analysis after HFD treatment. Flow cytometry analysis showed that HFD significantly decreased the population of $\text{lin}^-/\text{c-Kit}^+$ cells in C57BL/6 mice by 26% in BM, not in blood, as compared with the control group (c), whereas no significant change was observed in Sca-1^+ (b) or CD133^+ cell populations (a) in the BM or blood. HFD-induced reduction of population of $\text{lin}^-/\text{c-Kit}^+$ cells in BM was effectively prevented with N-acetylcysteine (NAC) treatment or overexpression of antioxidant enzyme network (AON). WT + ND, wild-type (WT) C57BL/6 mice with normal diet for 3 months; WT + HFD, WT C57BL/6 mice with HFD for 3 months; WT + HFD + NAC, WT C57BL/6 mice with HFD and NAC for 3 months; TG + ND, TG mice with normal diet for 3 months; TG + HFD, TG mice with high-fat diet for 3 months. $*p < 0.05$, $n = 8$

FIGURE 3.

High-fat diet (HFD) suppressed in vivo proliferation and increased in vivo apoptosis of bone marrow (BM) $\text{lin}^{-}/\text{c-Kit}^{+}$ cells. To explore the mechanism for decreased BM stem cells (BMSCs) population by HFD, in vivo BMSCs proliferation rate was determined. Flow cytometry analysis showed that HFD significantly decreased in vivo proliferation rate of $\text{lin}^{-}/\text{c-Kit}^{+}$ cells by 19% as compared with the control, whereas no significant change in cell proliferation was observed in Sca-1^{+} or CD133^{+} cells in the BM or blood (a). To determine if increased apoptosis could contribute to decreased BMSCs population in mice with HFD, in vivo BMSCs apoptosis rate was measured. HFD significantly increased the in vivo apoptosis rate of $\text{lin}^{-}/\text{c-Kit}^{+}$ cells by twofold over the control, whereas no significant change in apoptosis was observed in Sca-1^{+} or CD133^{+} cells in the BM or blood. The increased apoptosis of $\text{lin}^{-}/\text{c-Kit}^{+}$ cells was effectively reversed with N-acetylcysteine (NAC) treatment or antioxidant enzyme network (AON) overexpression in the aging mice with

HFD treatment (b). HFD-induced reduction of proliferation of $\text{lin}^{-}/\text{c-Kit}^{+}$ cells was effectively prevented with NAC treatment or overexpression of AON. WT + ND: wild-type (WT) C57BL/6 mice with normal diet for 3 months; WT + HFD: WT C57BL/6 mice with HFD for 3 months; WT + HFD + NAC: WT C57BL/6 mice with HFD and NAC for 3 months; TG + ND: TG mice with normal diet for 3 months; TG + HFD: TG mice with HFD for 3 months. $*p < 0.05$, $n = 8$

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TABLE 1

Mouse serum lipid profile with and without HFD for 3 months

	WT + ND	WT + HFD	WT + HFD + NAC	TG + ND	TG + HFD
TC (mg/dl)	114.67 ± 18.11	130.42 ± 12.24 [*]	118.33 ± 10.04 ^{**}	119.52 ± 20.14	124.11 ± 16.73
HDL	100.83 ± 14.95	91.43 ± 7.98	93.21 ± 11.29	101.54 ± 19.71	98.59 ± 18.76
TRG	67.67 ± 19.51	99.03 ± 29.46 [*]	71.02 ± 22.19 ^{**}	68.42 ± 20.01	75.68 ± 22.89
LDL	13.02 ± 9.85	26.87 ± 11.23 [*]	11.99 ± 8.92 ^{**}	11.08 ± 10.11 ^{***}	13.57 ± 8.96
Non-HDL	13.83 ± 10.60	42.39 ± 19.21 [*]	25.73 ± 10.43 ^{**}	17.98 ± 11.20 ^{***}	25.52 ± 15.91 ^{****}
TC/HDL	1.13 ± 0.10	1.45 ± 0.21 [*]	1.28 ± 0.18	1.18 ± 0.15	1.26 ± 0.19 ^{****}

Note: Wild-type aging C57BL/6 mice with or without NAC treatment and age-matched transgenic mice with AON expression were fed with normal diet or high-fat diet (HFD) for 3 months. Serum lipid profile was determined for each mouse.

Abbreviations: HDL, high-density lipoprotein; LDL, low-density lipoprotein; TC, total cholesterol; TG + ND, TG mice with normal diet for 3 months; TRG, triglycerides; WT + HFD, WT C57BL/6 mice with high-fat diet for 3 months; WT + HFD + NAC, WT C57BL/6 mice with high-fat diet and NAC for 3 months; WT + ND, WT C57BL/6 mice with normal diet for 3 months.

^{*} $p < 0.05$ (WT + ND vs. WT + HFD).

^{**} $p < 0.05$ (WT + HFD + NAC vs. WT + HFD).

^{***} $p < 0.05$ (WT + ND vs. TG + ND).

^{****} $p < 0.05$ (TG + ND vs. TG + HFD); $n = 8$.