Arteriosclerosis, Thrombosis, and Vascular Biology

RESEARCH LETTER

Regulation of Atherosclerosis Development by Neurotensin Derived From Lymphatic Endothelial Cells in Mice

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NTS (neurotensin) is a 13-amino acid peptide that interacts with NTSR1 (neurotensin receptor), NTSR2, or SORT1 (sortilin) proteins. Studies have shown positive correlations between NTS and the development of cardiovascular diseases.[1,2] However, constitutive depletion of *Nts* in the mice is not suitable for investigating this topic, given the diverse effects of NTS on body temperature and food intake by regulating the central nervous system.

The data that support the findings of this study are available from the corresponding author upon reasonable request.

The lymphatic system, a part of the circulation system, transports the immune cells and lipids. Lymphatic endothelial cells (LECs) also secrete various proteins, such as REELIN, which may be important for the cardiovascular system.[3] Understanding the physiological functions of these secretory proteins is a research highlight in vascular biology.

Our previous studies have shown that LECs secrete NTS in various tissues, including adipose tissues, skin, and liver.[4,5] To investigate the effects of LEC-derived NTS on atherosclerosis development, we developed an inducible *Nts* knockout mouse model (C57BL6/J) restricted to LECs (*Prox1-cre/ERT2::Nts* $^{flox/flox}$, referred as *Nts*$^{-/-}$ mice hereafter), with the same design and tamoxifen treatment strategy as previously described.[5] The experimental details were described in https://data.mendeley.com/datasets/c5yrdg5vtx/1. All the experiments were approved by the Institutional Animal Care and Use Committee of the School of Life Science at Fudan University (IDM2021006d).

We observed a depletion of *Nts* mRNA in various peripheral tissues (Figure [Ai]). Additionally, the *Nts* was found to be depleted in the LECs of mesenteric fat, but it was not detectable in the non-LECs of the liver (Figure [Aii]). As we were unable to purify the LECs from liver, we cannot rule out the possibility that the changes in *Nts* expression were due to off-target effects. To test the effects of LECs-derived NTS on the development of atherosclerosis, we injected the adeno-associated virus via the tail vein to overexpress the mutated *PCSK9*. This was done in male *Nts*$^{-/-}$ mice that were 8 weeks old, and we found that these mice had lower body weight (Figure [Bi]) but similar food intake (Figure [Bii]) when fed a Western diet (Research Diets, Inc) comparing to the 8-week-old control mice. It is worth noting that we only used male mice, as it is difficult to induce atherosclerosis in female mice. Notably, lower levels of triglyceride, total cholesterol, and low-density lipoprotein cholesterol were observed in the *Nts*$^{-/-}$ mice (Figure [C]). However, the expression of thermogenic genes in the brown adipose tissue and the level of lipid content and inflammatory/fibrotic genes in the liver were not affected by *Nts* depletion (https://data.mendeley.com/datasets/c5yrdg5vtx/1).

Our next step was to investigate whether the changes in lipid metabolism observed in the *Nts*$^{-/-}$ mice affected

Key Words: amino acid ■ atherosclerosis ■ liver ■ mice ■ neurotensin

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Arterioscler Thromb Vasc Biol is available at www.ahajournals.org/journal/atvb

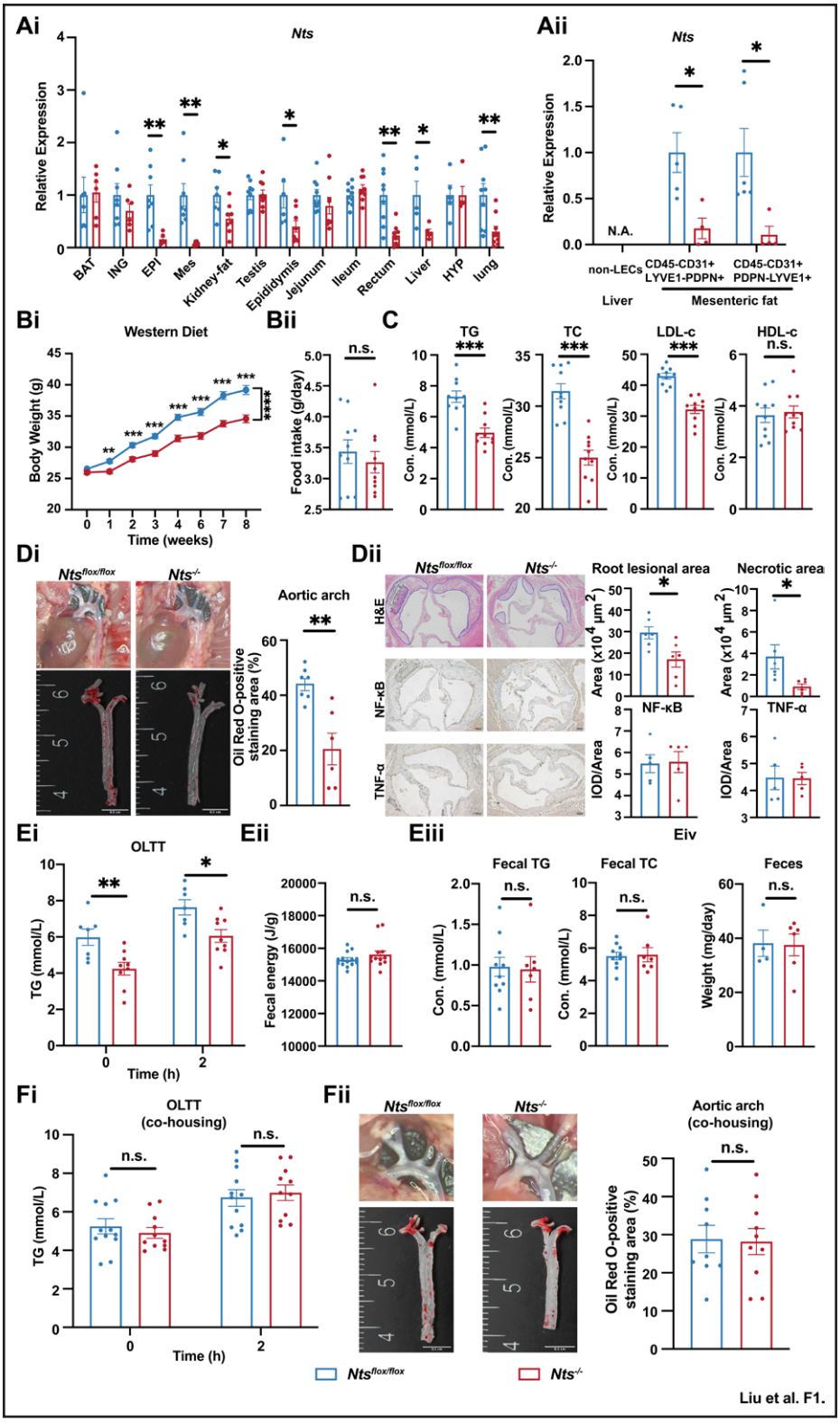

Figure. The lymphatic endothelial cell (LEC)–derived NTS (neurotensin) regulates the development of atherosclerosis. **Ai**, *Nts* expression in various tissues. N=6 to 9. Levels of significance: epididymal adipose tissue (EPI): *P*=0.0029; mesenteric fat (Mes): *P*=0.0033; kidney fat: *P*=0.0224; epididymis: *P*=0.0336; rectum: *P*=0.0018; liver: *P*=0.0302; lung: *P*=0.0099. **Aii**, *Nts* expression in non-LECs from liver and LECs from fat. N=4 to 6. Levels of significance: CD45−CD31+LYVE1-PDPN+: *P*=0.0169; CD45−CD31+ PDPN-LYVE1+: *P*=0.0283. Body weight (**Bi**) and food intake (**Bii**) of mice fed by a Western diet. N=8 to 10. Two-way ANOVA was used for significant test. **C**, The serum lipid profile of the mice fed by Western diet. N=8 to 10. Levels of significance: triglyceride (TG): *P*=0.0001; total cholesterol (TC): *P*<0.0001; low-density lipoprotein cholesterol (LDL-c): *P*<0.0001; high-density lipoprotein cholesterol (HDL-c): *P*=0.2822. Atherosclerotic lesions of aortic arch (**Di**) or aortic root (**Dii**) of mice fed by a Western diet. N=5–7. Blue circle indicates the (*Continued*)

Figure Continued. lesional area. Green circle indicates the necrotic area. Levels of significance: aortic arch: P=0.0018; root lesional area: P=0.0199; necrotic area: P=0.0365. Intestinal TG absorption capability (**Ei**), fecal energy content (**Eii**), fecal lipid content (**Eiii**), and amount of feces (**Eiv**) in the mice fed a Western diet. N=7 to 9. Levels of significance: 0 hour: P=0.0076; 2 hour: P=0.0116. Intestinal TG absorption capability (**Fi**) and atherosclerotic lesions of aortic arch (**Fii**) in the mice kept in the same cage and fed a Western diet. N=9 to 10. *P<0.05. **P<0.01. ***P<0.001. ****P<0.0001 from Student t test with 2-way ANOVA correction, unless otherwise specified. BAT indicates brown adipose tissue; Con., concentration; HYP, hypothalamus; ING, inguinal adipose tissue; IOD, integral optical density; LYVE1, lymphatic vessel endothelial hyaluronan receptor 1; N.S., not significant; OLTT, oral lipid tolerance test; and PDPN, podoplanin.

the development of atherosclerosis. Interestingly, we found that the *Nts*$^{-/-}$ mice developed less severe lesions in the aortic arch (Figure [Di]) and aortic root (Figure [Dii]). However, the level of inflammatory markers at the aortic root was comparable to that of the control mice (Figure [Dii]). All the lesion sizes were measured on multiple slides. The definition of lesion size is throughout the aortic root, and there is no bias to a certain region. To further explore the mechanism behind these changes, we assessed the absorption capability of triglycerides in the *Nts*$^{-/-}$ mice, which was found to be lower than that of the control mice (Figure [Ei]). Surprisingly, the fecal energy contents (Figure [Eii]), lipid contents (Figure [Eiii]), and amount of feces (Figure [Eiv]) were similar between the 2 groups of mice. Further investigation revealed changes in lipid absorption (Figure [Fi]) and atherosclerosis (Figure [Fii]) were eliminated when the *Nts*$^{flox/flox}$ and *Nts*$^{-/-}$ mice were kept in the same cages. Therefore, it is possible that NTS regulates lipid metabolism and atherosclerosis via its effects on gut microbiota.

Both NTS and its receptor SORT1 have been identified as important factors for lipid metabolism. Based on the results of both experimental and epidemiological investigations, it is reasonable to hypothesize that LEC-derived NTS regulates the development of atherosclerosis. The observed relief of atherosclerosis in the *Nts*$^{-/-}$ mice further underscores the importance of NTS in the context of cardiovascular diseases. Given the correlation between NTS and gut microbiota identified in our study, it is worthwhile to gain a deeper understanding of how LEC-derived NTS affects intestinal lipid absorption.

Our study has also contributed to our understanding of the connections between the lymphatic system and the development of cardiovascular diseases. In addition to directly regulating the transport of immune cells and lipids, the lymphatic system may also affect the progression of cardiovascular diseases via secretory proteins. Further studies are necessary to elucidate the details about the production, processing, and secretion of NTS in the LECs. For example, it would be important to understand how the transcription of *Nts* in LECs is regulated under physiological conditions, which enzymes are responsible for processing the NTS peptide, and how the secretion of NTS from the LECs can be precisely controlled. These questions need to be answered in the future.

In summary, our study highlights the importance of the LEC-derived NTS in the development of atherosclerosis and suggests that NTS may be a potential therapeutic target for cardiovascular diseases.

ARTICLE INFORMATION

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Acknowledgments

C. Liu, X. Xiong, and J. Li participated in conceptualization, investigation, analysis, writing, and data visualization. X. Xiong and J. Li performed funding acquisition and supervision.

Sources of Funding

This work was supported by MOST (Ministry of Science and Technology) 2020YFA0803600, 2018YFA0801300, NSFC (Natural Science Foundation Committee) 32071138 and SKLGE-2118 to J. Li, NSFC 82022013 and 81970740 to X. Xiong.

Disclosures

None.

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