# Study on the anti-hypertensive mechanism of hesperidin in spontaneously hypertensive rats and vascular endothelial cells

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（高血圧自然発症ラットおよび血管内皮細胞におけるヘスペリジンの高血圧予防機構に関する研究）

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## Thesis Summary

Hypertension, the most prevalent chronic medical disorder, is a major worldwide public-health challenge and the leading contributor to mortality and disability-adjusted life years lost. Nowadays, medications targeting the renin-angiotensin system (RAS) are used to treat hypertension, but their long-term side effects, such as headaches, dizziness, constipation, coughing, and angioneurotic edema, should be noted. In this regard, diet and lifestyle changes are the first-line strategy for hypertension management. A high intake of plant-derived foods containing high naturally occurring polyphenols., e.g., hesperidin, a citrus peel flavanone, has been proved to be beneficial for modulating high blood pressure. Previous human studies revealed that daily consumption of hesperidin-containing Mikan tea significantly reduced the elevated systolic blood pressure (SBP) in mildly hypertensive volunteers. However, the underlying anti-hypertensive mechanism(s) through, e.g., the suppression of renin, angiotensin I-converting enzyme (ACE) and angiotensin II receptors ($AT_{1/2}$ R), or activation of ACE2/angiotensin (1-7)/Mas receptor (MasR) axis, remains unclear. The present study, thus, aimed to get insights into the anti-hypertensive effect and mechanism of hesperidin by focusing on the RAS regulation, using spontaneously hypertensive rats (SHRs) and human umbilical vein endothelial cells (HUVECs).

Firstly, a long-term (20 weeks) administration study of hesperidin (50 mg/kg/day) and hesperidin-containing Mikan tea (50 mg/kg/day) was performed in 8-week-old SHRs. At the end of the protocol, hesperidin and Mikan tea groups showed a significant ($P < 0.05$) SBP reduction of approximately 60 mmHg in SHRs. No changes in the heart rate and parameters related to the circulating and local RAS activity, such as ACE activity and angiotensin metabolites, were observed between groups. In contrast, hesperidin ameliorated the impaired vasomotor response in vessels of 28-week-old SHRs and was found to exert a significant upregulation of MasR expression in the aorta, but not angiotensin II type 1/2 receptor ($AT_{1/2}R$) expression. An increase in cyclic adenosine monophosphate (cAMP) level was obtained in the aorta of hesperidin-administered SHRs, indicating that the anti-hypertensive effect of hesperidin may be involved in the upregulation of MasR axis.

Then, the mechanism of hesperidin-elevated aortic MasR expression in SHRs was investigated,

using HUVECs. HUVECs were cultured with 1 μM hesperidin for 2 h, following the measurements of nitric oxide (NO) production in the cell culture medium and vasomotor-related receptors' expression in cells. Hesperidin treatment promoted NO production and only caused a significant increase in MasR expression among the vasomotor-related receptors of $AT_{1/2}R$, estrogen receptor (ER), and bradykinin B2 receptor ($BK_2R$), while a MasR antagonist did not alter the hesperidin-induced NO production and MasR expression. This indicates that hesperidin was not an agonist for MasR. By inhibitor-aided experiments a transient receptor potential vanilloid 1 (TRPV1) inhibition caused a significant reduction of MasR expression and NO production by hesperidin, suggesting that hesperidin may play a role in HUVECs by playing TRPV1 agonist. The agonistic effect of hesperidin was confirmed by increasing intracellular $Ca^{2+}$ level and TRPV1-knocked down HUVECs. The results that the inhibitions of $Ca^{2+}$/calmodulin-dependent kinase II (CaMKII) and p38 mitogen-activated protein kinase (p38 MAPK) caused an abolishment of MasR elevated by hesperidin indicate that hesperidin was in the axis of TRPV1/CaMKII-mediated p38 MAPK/MasR expression. Phosphorylation of endothelial NO synthase (eNOS) by hesperidin and reduced NO production by eNOS inhibitor revealed that hesperidin was alternatively involved in the activation of TRPV1/CaMKII-mediated eNOS/NO production pathway. The structure-activity relationship analysis of flavonoids demonstrated that the B ring of the twisted flavonoid skeleton with a hydroxy group at the 3' position was a crucial factor for agonistic TRPV1 binding of hesperidin.

In conclusion, the present study demonstrates for the first time that hesperidin exerted an anti-hypertensive effect *via* the activation of aortic MasR/NO axes through the agonistic TRPV1 binding in aortic endothelium.