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Sesame lignans suppress age-related disorders of the kidney in mice

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Abstract. – OBJECTIVE: Sesamin is a functional ingredient in sesame (*Sesamum indicum*) seeds and has many physiological effects. This study investigated whether sesame lignans, sesamin and episesamin (1:1), can suppress age-related disorders of the kidney.

MATERIALS AND METHODS: Twenty-month-old mice were divided into three groups, and each group received a regular diet (O-C), diet containing sesame lignans (O-SE), and diet containing sesame lignans and α-tocopherol (VE; O-SE+VE), respectively, for 5 months. Six-month-old young mice (Y-C) were compared to the older mice.

RESULTS: Renal lipofuscin deposition was increased in the O-C group compared to that in the Y-C group and its deposition with aging was significantly decreased in both O-SE and O-SE+VE groups. Plasma blood urea nitrogen levels in the O-C group increased compared to those in the Y-C group; however, those in both O-SE and O-SE+VE groups did not differ from those in the Y-C group. The number of podocytes in the O-C group decreased compared to that in the Y-C group and this effect was attenuated in the O-SE and O-SE+VE groups. The effect was strongest in the O-SE+VE group. Histological examinations showed that glomerular hypertrophy accompanied by mesangial hyperplasia and renal tubular degeneration was less severe in the O-SE and O-SE+VE groups than in the O-C group. Moreover, age-related increases in the mRNA expression of NADPH oxidase- and inflammation-related genes, including *p67^{phox}*, *p40^{phox}*, *TNFα*, and *IL-6*, in the kidney were suppressed in the O-SE and O-SE+VE groups.

CONCLUSIONS: Sesame lignans might be useful to suppress age-related kidney disorders, and these effects could be enhanced with VE.

Key Words:
Aging, Inflammation, Podocyte, Sesame lignans, α-tocopherol.

Abbreviations

BUN, blood urea nitrogen; GFR, glomerular filtration rate; HE, hematoxylin-eosin; HNE, 4-hydroxynonenal; IL, interleukin; MDA, malondialdehyde; NOX, NADPH oxidase; PAS, periodic acid-Schiff; qPCR, quantitative polymerase chain reaction; SEM, standard error of the mean; TNF, tumor necrosis factor; VE, α-tocopherol; WT1, Wilms' tumor 1 protein.

Introduction

Kidneys are important organs that maintain body fluid homeostasis. However, renal function declines with aging, and dysfunction leads to various diseases such as chronic kidney disease[1]. Moreover, elderly people have increased susceptibility to ischemia-reperfusion and acute renal injury[2,3] and the mortality of aged mice is increased after ischemia-reperfusion compared to that of young mice[4]. In addition to this decline in renal function, histological age-related disorders such as glomerulosclerosis, glomerular basement membrane thickness, atherosclerosis, and tubular atrophy are observed[1]. These histological changes are considered to be caused by fibrosis[5], high oxidative stress levels[6], and increased number of senescent cells[7]. Podocytes are cells that have foot processes and mesh with adjacent podocytes. The slit membrane between the foot processes acts as the final size barrier for hemofiltration and is the most important component of the glomerular filtration barrier[8]. The number of podocyte foot processes also decreases with aging[9].

Lipofuscin is a yellow-brown and autofluorescent granule that accumulates in various tissues[10]. In addition, lipofuscin is a waste product that is generated through the insufficient degradation of oxidative damaged molecules during the process of

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autophagy[11] and is considered an age-related marker[12,13]. In kidneys, lipofuscin deposition is observed mainly in proximal tubular cells but not in glomeruli of aged rats and humans[14,15]. Based on these findings, the suppression of lipofuscin deposition is considered to reflect anti-aging effects.

Sesamin is a natural ingredient of sesame (*Sesamum indicum*) seeds. Almost half of sesamin is epimerized and converted to episesamin during the refining of non-roasted sesame seed oil. We have previously demonstrated that the monocatechol metabolites of sesamin and episesamin produced by P-450 have potent antioxidative effects[16-18]. In addition to these anti-oxidative effects, sesame lignans, which contain sesamin and episesamin, exert hypocholesterolemic[19], anti-hypertensive[20], liver-protective[21], and neuroprotective[22,23] physiological effects. Furthermore, sesamin accelerates the anti-oxidative effects of α-tocopherol (VE)[24,25], whereas VE enhances the cholesterol-lowering effect of sesame lignans[26]. With respect to renal function, sesame lignans improve renal function by correcting structural abnormalities and attenuating renal oxidative stress in hypertensive rats fed a high-fat-sucrose diet[27]. Rousta et al[28] reported that sesame lignans diminish LPS-induced acute renal damage by attenuating renal oxidative stress, inflammation, and apoptosis. Sesame lignans also show protective effects against renal ischemia-reperfusion injury[29]. We previously demonstrated that a high amount of sesamin can be detected in rat kidneys after [^{14}C] sesamin injection[30]. These results suggest that treatment with sesame lignans could be renoprotective. Regarding anti-aging effects, we previously showed that sesame lignans suppress the age-related cognitive decline on SAMP10 mice[31]. However, the effect on kidney disorders and aging remains unclear. The purpose of this study was to evaluate the beneficial effect of sesame lignans against kidney aging in mice. In addition, the effect of sesame lignans and VE was also examined.

Material and Methods

Materials

Sesame lignans (comprising approximately equivalent amounts of sesamin and episesamin) were obtained from TAKEMOTO OIL & FAT Co., Ltd. (Aichi, Japan). VE was purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan).

Animals

Male mice of the C57BL/6NCrSlc strain, 6 and 20 months of age, were obtained from the animal facility of Tokyo Metropolitan Institute of Gerontology, Tokyo, Japan. Thirty mice at 20 months of age were divided into three groups and each group was fed the following chow diets for 5 months: regular diet (CRF-1, Oriental Yeast Co., Ltd., Tokyo, Japan) for the old control (O-C) group (n = 10), sesame lignans (0.2%)-mixed diet for the O-SE group (n = 10), and sesame lignans (0.2%) and VE (0.2%)-mixed diet for the O-SE+VE group (n = 10). Body weight, food consumption, and water intake were measured every 4 weeks to monitor the conditions of mice. Mice at 6 months of age were used for the young control (Y-C) group (n = 8). Blood and kidneys were obtained at the age of 6 months for Y-C and 25 months for O-C, O-SE, and O-SE+VE groups.

All protocols for animal procedures were approved by the Tokyo Metropolitan Institute of Gerontology (approval No. 15002) and the Ethics Committee of Animal Experiment of Suntory in accordance with the Internal Regulations on Animal Experiments at each organization, which are based on the Law for the Humane Treatment and Management of Animals (Law No. 105, 1 October 1973, as amended on 2 June 2017). The number of animals used was kept to the minimum necessary for meaningful interpretation of the data, and animal discomfort was minimized.

Analysis of Lipofuscin Deposition

Half of sliced kidneys were fixed in 4% formalin solution and embedded in paraffin. After slicing at 4 μm, lipofuscin was detected using microscopy (OLYMPUS BX52, Tokyo, Japan). Images of five fields were randomly selected from each mouse. Lipofuscin deposition areas were detected using LUMINA VISION (Mitani Corporation, Tokyo, Japan).

Blood Biochemistry

Plasma blood urea nitrogen (BUN) was measured using the 7180 Clinical Analyzer (Hitachi High-Technologies Corporation, Tokyo, Japan).

Histopathology

Paraffin-embedded kidneys (3-4 μm) were used for hematoxylin-eosin (HE) and periodic acid-Schiff (PAS) staining. Histopathological examinations were performed in a blinded manner. Twenty-five glomeruli per specimen were randomly selected from each specimen and the glo-

merular area was measured under a microscope (OLYMPUS BX51) and with software (OLYMPUS cellSens Ver. 01.07.01). Overall glomerular areas were averaged. The glomerular sclerosis score was measured following the method[32] reported. Briefly, 50 glomeruli on each PAS specimen were observed and injury severity scores were graded according to the percentage of the glomeruli involved as follows: 0, no lesions; 1, < 25%; 2, 25-50%; 3, 50-75%; and 4, >75%. Glomerular sclerosis scores for each mouse were obtained by multiplying the severity score (0 to 4) by the number of glomeruli displaying the same degree of injury, summing these scores, and dividing by the number of glomeruli observed.

Immunohistochemistry

Paraffin-embedded kidneys (3-4 μm) were used for the staining of Wilms' tumor 1 protein (WT1). Sections were incubated with rabbit Anti-WT1 antibody (1:100 dilution, ab89901, Abcam, Cambridge, UK) overnight at 4°C. Sections were then incubated with Histofine® Simple Stain Mouse MAX PO (Nichirei Biosciences Inc., Tokyo, Japan) and 3,3'-diaminobenzidine, tetrahydrochloride in accordance with the manufacturer's protocol. Twenty glomeruli per specimen were used to measure the glomerular area and detect WT1-positive areas using Lumina Vision (Mitani Corporation, Fukui, Japan).

Quantitative Polymerase Chain Reaction (qPCR)

Kidneys were infused with RNAlater (Thermo Fisher Scientific, Waltham, MA, USA) and stored at 4°C until use. RNA was extracted using the RNeasy mini kit (Qiagen, Hilden, Germany) and cDNA was synthesized using High-Capacity cDNA Reverse Transcription Kits (Applied Biosystems, Foster City, CA, USA) according to the manufacturers' protocols. The qPCR was conducted using TaqMan Universal PCR Master Mix (Applied Biosystems). The following primers were used: *Cybb* (Mm01287743_m1), *Ncf1* (Mm00447921_m1), *Ncf2* (Mm00726636_s1), *Ncf4* (Mm00476300_m1), *Rac2* (Mm00485472_m1), *Tnf* (Mm00443260_g1), *Il6* (Mm00446190_m1), *Il1b* (Mm00434228_m1), and *Ccl2* (Mm00441242_m1) (Applied Biosystems). Each relative gene expression level was normalized to 18S ribosomal RNA (Hs99999901_s1) expression (Applied Biosystems, Foster City, CA, USA). All reactions were performed with a StepOnePlus™ System (Applied biosystems, Foster City, CA, USA).

Statistical Analysis

Results are expressed as means ± standard errors of the mean (SEMs). Statistical analyses were performed using one-way ANOVAs followed by a Tukey-Kramer post-hoc analysis. All analyses were performed using IBM SPSS statistics 25 software (IBM, Armonk, NY, USA). *p*-values less than 0.05 were regarded as statistically significant.

Results

Lipofuscin Deposition In Kidneys

Two animals in the O-C group and two animals in the O-SE group died during the experiment. A total of 34 animals were finally analyzed (eight in the Y-C group, eight in the O-C group, eight in the O-SE group, and 10 in O-SE + VE group). Lipofuscin deposition is known to accumulate in kidneys with age[14,15]. We detected and measured renal lipofuscin deposition as an age-related marker. In the O-C group, high lipofuscin deposition was detected compared to that in the Y-C group and the percentage of areas with lipofuscin deposition was significantly higher than that in the Y-C group. Lipofuscin deposition in the O-SE and O-SE+VE groups was significantly decreased compared to that in the O-C group (Figure 1A, 1B).

Evaluation of Renal Function

To evaluate renal function, we measured plasma BUN levels in O-C, O-SE, O-SE+VE, and Y-C groups. The BUN level in the O-C group was higher than that in the Y-C group; however, the BUN levels in the O-SE and O-SE+VE groups did not differ from that in the Y-C group (Figure 1C).

Number of Podocytes In Kidneys

WT1 is known to be a key regulator of podocyte function and its reduced expression levels cause crescentic glomerulonephritis and mesangial sclerosis[8]. Therefore, to evaluate podocyte function, we immunostained for WT1 in kidney sections from the O-C, O-SE, O-SE+VE, and Y-C groups and measured the number of podocytes per glomerular sectional area in each group. The number of podocytes in the O-C group was less than that in the Y-C group. The decrease in podocyte count was attenuated in the O-SE and O-SE+VE groups and was greater in the O-SE+VE group (Figure 1D).

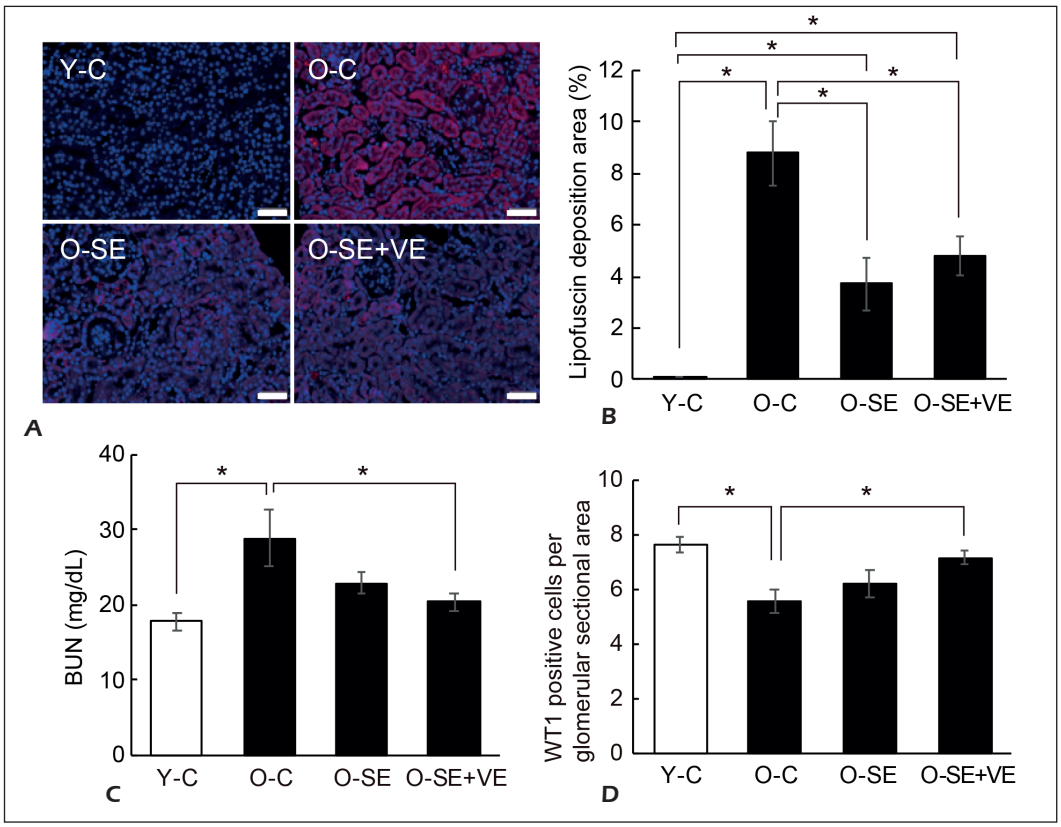

Figure 1. Effects of sesame lignans and α-tocopherol (VE) on lipofuscin deposition, plasma blood urea nitrogen (BUN) levels, and number of podocytes. Twenty-month-old mice were divided into three groups and each group was a fed regular diet (O-C), sesame lignans mixed diet (O-SE), and sesame lignans and VE-mixed diet (O-SE+VE), respectively, for 5 months. Six-month-old mice were used for the young control (Y-C) group. **A**, Representative images of lipofuscin deposition in kidneys. White bar represents 50 μm. **B**, Lipofuscin deposition area. **C**, Plasma BUN levels. **D**, Number of WT1-positive cells per glomerular sectional area. Values represent the mean ± SEM. *$p<0.05$.

Histological Analysis of Kidneys

HE and PAS staining images of mouse kidney sections from the O-C, O-SE, O-SE+VE, and Y-C groups are shown in Figure 2A. In HE staining images, notable glomerular hypertrophy accompanied by mesangial hyperplasia was observed in the O-C group. These effects were diminished in the O-SE and O-SE+VE groups compared to those in the O-C group. In PAS staining images, renal tubular degeneration was observed in the O-C group and was greater than that observed in the O-SE and O-SE+VE groups. Glomerular sclerotic scores and the average glomerular area in the O-C group were higher than those in the Y-C group and were slightly lower in the O-SE and O-SE+VE groups compared to those in the O-C group (Figure 2B, 2C).

Gene Expression In Kidneys

We next analyzed mRNA gene expression levels of NADPH oxidase (NOX)-related and inflammation-related genes including NOX2 (*Cybb*), $p47^{phox}$ (*Ncf1*), $p67^{phox}$ (*Ncf2*), $p40^{phox}$ (*Ncf4*), Rac family small GTPase 2 (*Rac2*), tumor necrosis factor (TNF)-α (*Tnf*), interleukin (IL)-6 (*Il6*), IL-1β (*Il1b*), and C-C motif chemokine 2 (*Ccl2*) in the kidneys from the O-C, O-SE, O-SE+VE, and Y-C groups (Figure 3). In the O-C group, the expression of all genes was higher than that in the Y-C group. Among them, *$p67^{phox}$*, *$p40^{phox}$*, *TNFα*, and *IL-6* gene expression was lower in the O-SE and O-SE+VE groups than in the O-C group, and levels were lower in the O-SE+VE group than that in the O-SE group.

Discussion

In this study, we found that sesame lignans suppress renal lipofuscin deposition, which increased with aging. Moreover, increased plasma BUN and decreased podocyte levels were ameliorated in the aged group fed sesame lignans. Renal histological examinations revealed that increased glomerular

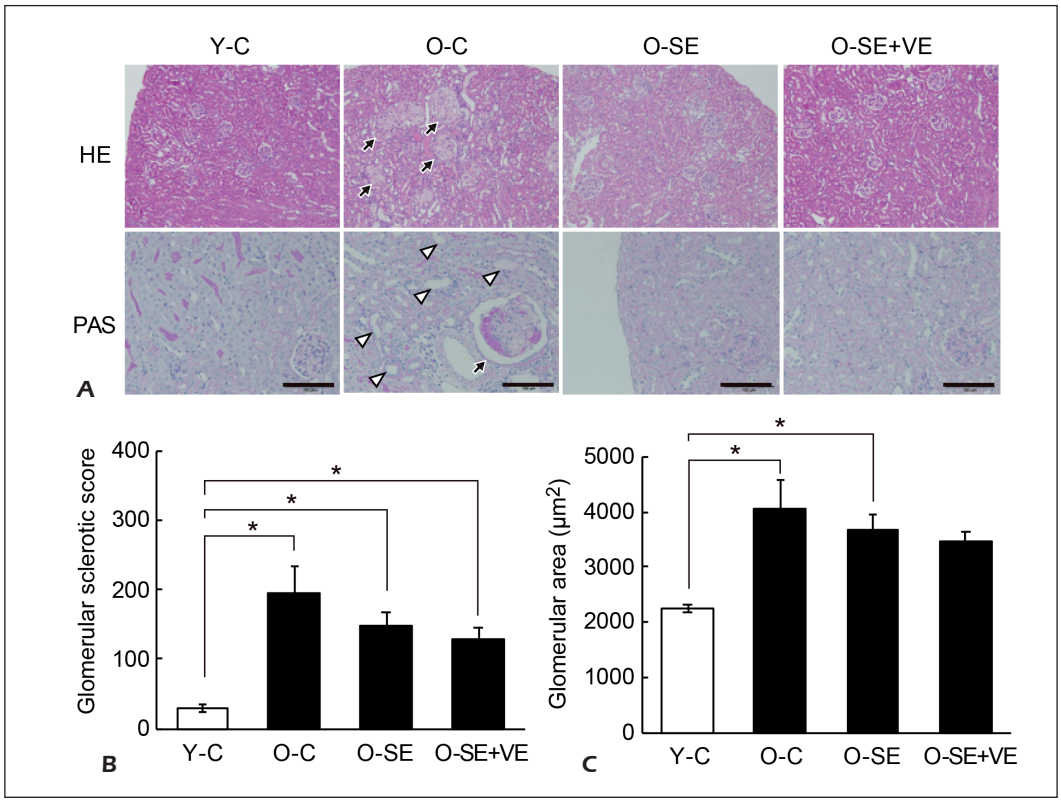

Figure 2. Histological analysis of kidneys. **A**, Hematoxylin and eosin (HE) and periodic acid-Schiff (PAS) staining images of kidney sections from regular diet (O-C), sesame lignans-mixed diet (O-SE), sesame lignans and α-tocopherol (VE)-mixed diet (O-SE+VE), and young control (Y-C) groups. Black arrows indicate glomerular hypertrophy. White arrowheads indicate renal tubular degeneration. Black bar represents 100 μm. **B**, Glomerular sclerotic score. C, Glomerular area. Values represent the mean ± SEM. *$p<0.05$.

hypertrophy accompanied by mesangial hyperplasia and renal tubular degeneration with aging was suppressed in the group fed sesame lignans. Furthermore, these effects of sesame lignans on age-related kidney disorders were enhanced with VE.

Lipofuscin is a highly cross-linked aggregate composed of oxidized proteins (30-58%) and lipids (19-51%)[33] and accumulates in various organs under high oxidative stress conditions with aging[34-36]. One of the mechanisms of lipofuscin formation is lysosomal dysfunction[37,38]. The lysosome is an organelle containing various kinds of hydrolases and plays a crucial role in taking up oxidized and unfolded proteins and damaged mitochondria, and then degrading them to supply materials for the synthesis of new proteins. Increased oxidative stress with aging leads to a decline in lysosomal function mediated by the oxidation of lysosomal membranes. In this process, highly reactive products such as 4-hydroxynonenal (HNE) and malondialdehyde (MDA), which form cross-linked proteins, result in lipofuscin accumulation[12,39]. Lipofuscin has a toxic effect on cells because it has redox-reactive surfaces that bind metals such as iron[33]. In addition, occupation of the intracellular volume inhibits intracellular lysosomal trafficking[40] and inhibits the proteasome[41], thereby resulting in deterioration of cell function and subsequently triggering apoptosis[42]. In the kidney, lipofuscin deposition with aging is observed in the proximal tubule[14], in accordance with our results, probably due to the abundant mitochondria in the proximal tubules[15,16]. In this study, sesame lignans markedly suppressed renal lipofuscin deposition, suggesting that sesame lignans might exhibit antioxidant activity in the kidneys.

We showed glomerular hypertrophy accompanied by mesangial hyperplasia and tubular epithelial desquamation, which is consistent with previous studies. Increased plasma BUN levels in aged mice might be caused by decreased filtration function of the glomerulus accompanied by chronic glomerular injury with aging. Furthermore, we found that the number of podocytes in kidneys from aged mice decreased. Podocytes are the most important component of glomerular fil-

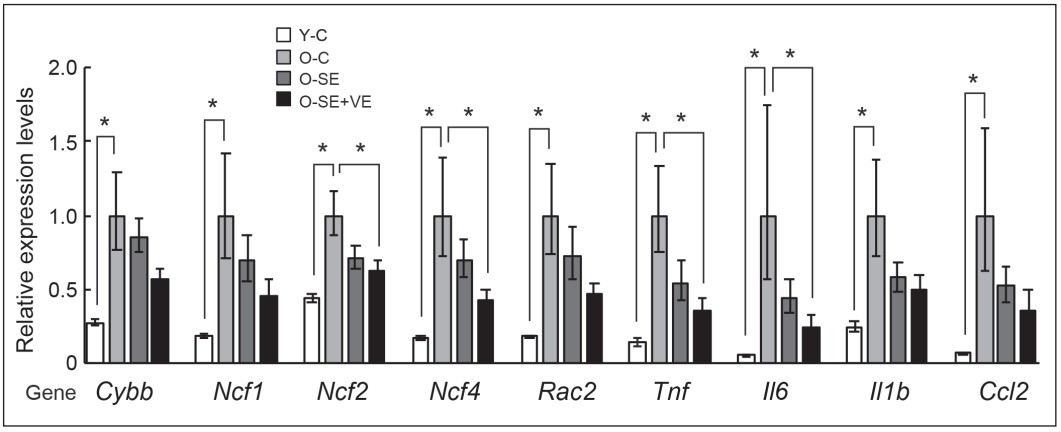

Figure 3. Effects of sesame lignans and α-tocopherol (VE) on gene expression in kidneys. NOX-related and inflammation-related gene expression levels in the kidneys from regular diet (O-C), sesame lignans-mixed diet (O-SE), sesame lignans and α-tocopherol (VE)-mixed diet (O-SE+VE), and young control (Y-C) groups. Each relative gene expression level was normalized to 18S ribosomal RNA expression. Values represent the mean ± SEM. *$p<0.05$.

tration barriers as they are the final size barrier for hemofiltration[8]. In patients with diabetes[43] and chronic renal disorder[44], as well as in the elderly[9], the number of podocyte foot processes is decreased and macromolecules, such as proteins, are excreted in the urine. Because podocytes have no ability to proliferate and regenerate[45], maintaining the function of podocytes is very important to maintain glomerular filtration. In the present study we confirmed that sesame lignans suppress age-related kidney disorders.

Sesame lignans have potent antioxidant effects after metabolism in the liver[16-18], and its monocatechol metabolites have also been found to be highly distributed in the kidney[30]. Sesame lignans have previously been shown to maintain renal function by suppressing oxidative stress in several kidney injury models[27-29], suppress the accumulation of abnormal proteins in *Drosophila*, and suppress the accumulation of HNE in aged SAMP10 mice[31]. Taken together, sesame lignans suppress age-related kidney dysfunction by increasing BUN and decreasing podocyte cell levels by suppressing oxidative stress, which is consistent with decreased NOX-related and inflammation-related gene expression.

The effects of sesame lignans on chronic changes in the kidneys associated with aging were enhanced by the combined intake of VE. A previous study reported that sesame lignans and VE synergistically enhance each other's effects[24-26]. Moreover, in a clinical study of patients with metabolic syndrome, the consumption of sesame oil alone or that with VE reduced the expression of several biochemical markers, and especially, the antioxidant and anti-inflammatory effects were enhanced by the combined intake of VE[46]. Therefore, VE might enhance the effects of sesame lignans on aging kidneys. However, since we did not have a VE group alone in this study, further research is needed to clarify the contribution of each component.

These results showed that increased oxidative stress and inflammation result in decreased podocyte numbers and morphological changes in the glomeruli, which lead to a significant increase in BUN levels. These changes are consistent with the situation of chronic kidney injury associated with aging in humans. In an increasingly elderly society, using food ingredients to prevent chronic kidney injury with aging could be a beneficial and safe precautionary strategy.

Conclusions

The above results showed that sesame lignans suppress age-related disorders, such as lipofuscin deposition, increases in plasma BUN levels, and decreases in the number of podocytes, and VE enhances these effects. Thus, sesame lignans with VE might be useful to suppress age-related decline in renal function.

Author Contributions

SS, DK, YK, AA, AS, YO, TR, HS, and AI: designed the research; SS, DK, YK, AA, YO, TR, HS, and AI: conducted the experiments; SS, DK, YK, AA, AS, YO, TR, HS, and AI: analyzed the data; SS, DK, YK, AA, AS, YO, TR, HS, and AI: wrote the manuscript and are primarily responsibility for the final content of the manuscript. All authors read and approved the final manuscript.

Conflict of Interests

SS, DT, YO, TR, and HS are employees of Suntory Wellness Ltd., which is the sponsor of this study and a manufacturer of foods that contain sesame lignans.

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