

## Oligonol, a low-molecular-weight polyphenol derived from lychee fruit, attenuates gluco-lipototoxicity-mediated renal disorder in type 2 diabetic *db/db* mice

Chan Hum Park<sup>1</sup>, Jeong Sook Noh<sup>2</sup>, Hajime Fujii<sup>3</sup>, Seong-Soo Roh<sup>1</sup>, Yeong-Ok Song<sup>4</sup>, Jae Sue Choi<sup>5</sup>, Hae Young Chung<sup>6</sup>, Takako Yokozawa<sup>1,6,7,\*</sup>

<sup>1</sup> College of Korean Medicine, Daegu Haany University, Daegu, Korea;

<sup>2</sup> Department of Food Science & Nutrition, Tongmyong University, Busan, Korea;

<sup>3</sup> Amino Up Chemical Company, Ltd., Sapporo, Japan;

<sup>4</sup> Department of Food Science Nutrition, Pusan National University, Busan, Korea;

<sup>5</sup> Division of Food Science & Biotechnology, Pukyong National University, Busan, Korea;

<sup>6</sup> Molecular Inflammation Research Center for Aging Intervention, Pusan National University, Busan, Korea;

<sup>7</sup> Graduate School of Science and Engineering for Research, University of Toyama, Toyama, Japan.

### Summary

Oligonol is a phenolic product derived from lychee fruit extract containing catechin-type monomers and oligomers of proanthocyanidins, produced by a manufacturing process which converts polyphenol polymers into oligomers. These proanthocyanidins have been reported to exhibit beneficial bioactivities in many studies, and so oligonol, a rich source of polyphenol, is expected to show favorable effects on various chronic diseases. This article summarizes recent work whether oligonol has an ameliorative effect on diabetic indices and renal disorders associated with gluco-lipototoxicity-mediated oxidative stress, inflammation, and apoptosis in *db/db* mice with diabetes. Oligonol was able to improve diabetic indices, prevent the development of diabetic renal disease, and preserve renal cells and the renal morphological structure via the attenuation of reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase-induced oxidative stress, inhibition of advanced glycation endproduct (AGE) generation, and prevention of apoptosis-induced cell death in *db/db* mice, being independent of changes in the body weight or serum glucose levels. The present study provides important evidence that oligonol exhibits a pleiotropic effect, representing renoprotective effects against the development of diabetic complications in type 2 diabetic *db/db* mice.

**Keywords:** Oligonol, type 2 diabetes, renoprotective effects

### 1. Introduction

The major biochemical alterations in diabetes are hyperglycemia and dyslipidemia, leading to gluco- and lipotoxicity, respectively, which directly or indirectly account for diabetic complications in various organs such as nonalcoholic fatty liver disease, chronic kidney disease, pancreatic  $\beta$ -cell apoptosis, and diabetic

cardiomyopathy (1-4).

Hyperglycemia causes oxidative stress by two mechanisms: firstly, by decreasing the regeneration of the important cellular antioxidant, reduced glutathione (GSH) from oxidized glutathione (GSSG), and, secondly, by decreasing the availability of reduced nicotinamide adenine dinucleotide phosphate (NADPH) (5). Furthermore, hyperglycemia-induced reactive oxygen species (ROS) stimulate the activation of protein kinase C (PKC), formation of advanced glycation endproducts (AGEs), and sorbitol accumulation. Also, an increase in ROS leads to the activation of nuclear factor-kappa B (NF- $\kappa$ B), and activated NF- $\kappa$ B can enhance the expression of proinflammatory cytokines, chemokines,

\*Address correspondence to:

Dr. Takako Yokozawa, Graduate School of Science and Engineering for Research, University of Toyama, 3190 Gofuku, Toyama 930-8555, Japan.

E-mail: yokozawa@inm.u-toyama.ac.jp

adhesion molecules, inflammatory receptors, and inflammatory enzymes such as inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) (6-8).

Long-term hyperlipidemia, which is associated with the abnormal expression of transcriptional factors such as peroxisome proliferator-activated receptor (PPAR)  $\alpha$  or sterol regulatory element-binding protein (SREBP) in the nucleus, increases non-esterified fatty acid (NEFA) uptake and accumulations of triglycerides and cholesterol in tissues such as the liver and kidney. In addition, critical toxicity caused by dyslipidemia is due to oxidative and carbonyl stress as a result of impaired antioxidant defense systems and increased ROS generated by the mitochondrial respiratory chain reaction, nonenzymatic glycation, and glucose autoxidation (9-12).

There have been many studies to identify effective therapeutic agents from natural sources for metabolic disorders such as obesity, diabetes mellitus, and its complications due to their absence of toxic and/or side effects (13). Currently, functional food and/or dietary ingredients with health benefits are given much attention due to the absence of adverse effects, abundant production, and application to various commercial products (14). Polyphenol-rich foods, such as wine, tea, coffee, and chocolate, have been receiving considerable attention as dietary sources of antioxidants that are valuable for human health. Polyphenols, including catechins and their derivatives, resveratrol, and curcumin, have attracted attention as functional foods with various bioactivities, such as anticancer, antimutagenic, antimicrobial, and antiviral activities (15-17).

The lychee (*Litchi chinensis*, Sapindaceae) has been consumed since ancient times in China and the southern area of Southeast Asia. The lychee is rich in polyphenols; Brat *et al.* (18) reported that its polyphenol content per edible part is second only to strawberries. A particular feature of lychee polyphenols is a phenolic product containing catechin-type monomers and oligomers of

proanthocyanidins. Proanthocyanidins are structurally characterized as polymers of catechin and have a high molecular weight. However, their absorption in the body is low when administered orally, and so their *in vivo* activity is not as high as expected. Moreover, proanthocyanidins with a high molecular weight are practically insoluble in water and have an astringent taste, binding to salivary proteins and mucous membranes in the mouth (19), and making them difficult to use in the food industry. Tanaka *et al.* (20) were successful in converting high-molecular-weight proanthocyanidin into low-molecular-weight proanthocyanidin, which is utilizable in the food industry. Therefore, in this review, we summarize recent work on the effects of oligonol on diabetic indices and renal disorders associated with gluco-lipotoxicity-mediated oxidative stress, inflammation, and apoptosis in *db/db* mice with diabetes.

## 2. Conversion to low-molecular-weight proanthocyanidin

For a prolonged period, a thiolysis method has been used for the structural analysis of proanthocyanidin. In this method, based on a nucleophilic reaction, a compound possessing a thiol group binds to the end unit of proanthocyanidin fragmented under acidic conditions, and, accordingly, low-molecular-weight proanthocyanidin is stably obtained. However, as most compounds with a thiol group are not suitable for consumption, their application to food products is limited. Tanaka *et al.* (20) developed a technique to convert high-molecular-weight proanthocyanidins to low-molecular-weight proanthocyanidins without using thiol compounds, and have been successful in making the technology practicable. This method involves binding a compound possessing a phloroglucinol ring structure (such as catechin) as a nucleophilic compound to proanthocyanidin fragmented under acidic conditions. As shown in Figure 1, catechin

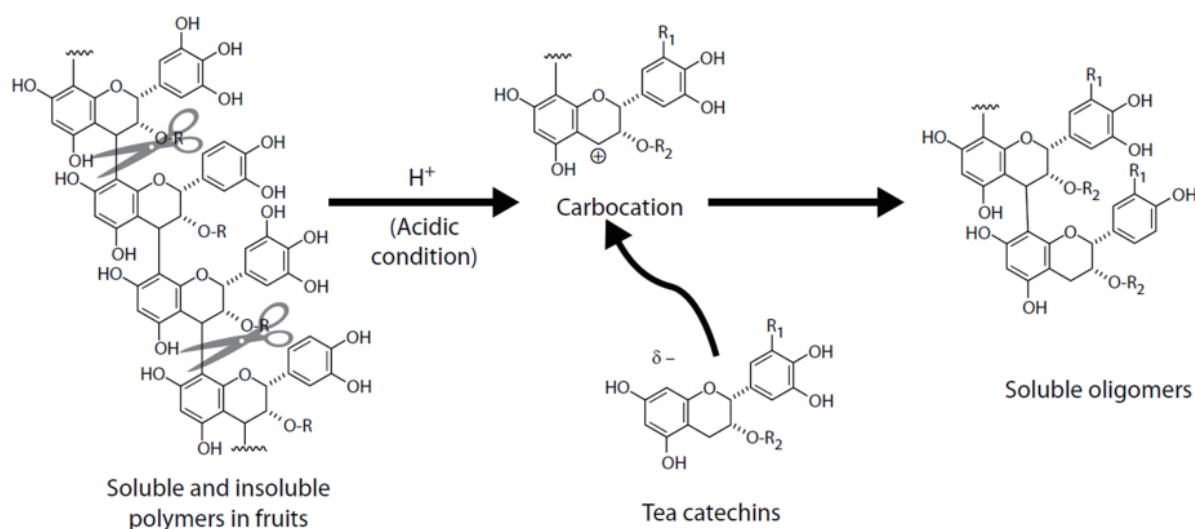


Figure 1. A conceptual diagram showing the conversion of proanthocyanidin to a low molecular weight. Ref. (20).

monomers are substituted at the C-4 position of fragmented proanthocyanidins with a high molecular weight and, as a consequence, low-molecular-weight proanthocyanidins are stably generated.

### 3. Oligonol

Oligonol is produced by oligomerizing polyphenol polymers derived from lychee fruit, as described previously (21). Briefly, dried lychee fruits, one of the richest sources of polyphenols, are extracted with 50% (v/v) ethanol. The filtrate is evaporated and passed through a DIAION HP-20 column, and eluted with ethanol. The eluate is evaporated to dryness, yielding a dark brown powder which contains a mixture of proanthocyanidins. The lychee extract is mixed with green tea extract, which provides an enriched source of monomeric procyanidins, and citric acid in water. The reaction mixture is heated at 60°C for 16 h, filtered through a DIAION HP-20 column, washed with water, and eluted with 40% (v/v) ethanol. Evaporation of the eluate yields a reddish brown powder containing the monomeric and oligomeric proanthocyanidin mixture. Oligonol comprises a polyphenol mixture of 15.3% monomers (including catechin) and 16.7% dimers (including procyanidin and catechin), whereas lychee fruit polyphenol comprises a mixture of 6.4% monomers and 9.8% dimers (Table 1). Oligonol is commercially available (Amino Up Chemical Co., Ltd., Sapporo, Japan). The safety of oligonol as a food or dietary supplement and as a pharmaceutical additive has already been confirmed (21,22).

### 4. Function of oligonol against diabetes-induced renal damage in *db/db* mice

There is accumulating evidence that oligonol can exert some biological effects *in vitro* and *in vivo*: anticancer

(23) as well as antioxidant and anti-inflammatory effects (24), beneficial activity for nitric oxide bioavailability (25), and a regulatory effect on lipid metabolism (26,27). Indeed, dietary feeding with proanthocyanidins, which comprise oligonol, has been reported to induce a significant attenuation of tissue fat levels, without changing the total body mass of the animals compared with non-proanthocyanidin-fed animals (28). Thus, many beneficial physiological activities of oligonol have been reported, and this review was focused that oligonol has ameliorative effects on the kidney in type 2 diabetes.

Diabetic kidney damage is one of the most serious complications of diabetes mellitus and has been the most common cause of end-stage renal failure among patients undergoing chronic hemodialysis therapy since 1998 (29). Therefore, we proposed the effects of oligonol on diabetic indices and renal damage in type 2 diabetic *db/db* mice, as reported previously (30,31).

#### 4.1. Diabetic indices and renal histological examination

To investigate the effect of oligonol, *db/db* mice were used. The spontaneous mutant strain C57BLKS/J *db/db* mice have a *db* gene mutation, a splicing mutation caused by a point mutation in the downstream intron of the leptin receptor gene, and so they are unresponsive to leptin. Leptin is a peptide hormone secreted by adipocytes, and it is involved in eating behavior and energy homeostasis. For this reason, after birth, homozygous type 2 diabetic (*db/db*) mice show unrepressed eating behavior, become obese, and develop severe insulin resistance associated with hyperinsulinemia and hyperglycemia (32). In this study, *db/db* mice showed diabetic characteristics, such as excessive body weight gain, increased food and water intakes, hyperglycemia, hyperinsulinemia, and hyperleptinemia, compared with homozygous control (*m/m*) mice. However, oligonol administration did not affect the body weight gain or serum glucose and leptin concentrations in spite of elevated serum insulin. In oligonol-administered *db/db* mice, the cause of increased insulin secretion is unclear and may be related to preservation of the pancreatic  $\beta$ -cell function by oligonol treatment. In addition, there were significant reductions in the serum lipid concentration (triglycerides, total cholesterol, and NEFA), ROS, and lipid peroxidation, as well as improvements in renal function parameters such as serum urea nitrogen and creatinine. Serum adiponectin concentrations were significantly higher in the oligonol-treated than in the vehicle *db/db* group, as shown in Table 2. Low adiponectin levels are associated with insulin resistance in type 2 diabetic patients and experimental animals (33). Unfortunately, we cannot measure the insulin sensitivity regarding the influence on target tissues such as muscle and fat. In addition to biofactors such

**Table 1. Contents of oligonol and lychee fruit polyphenol**

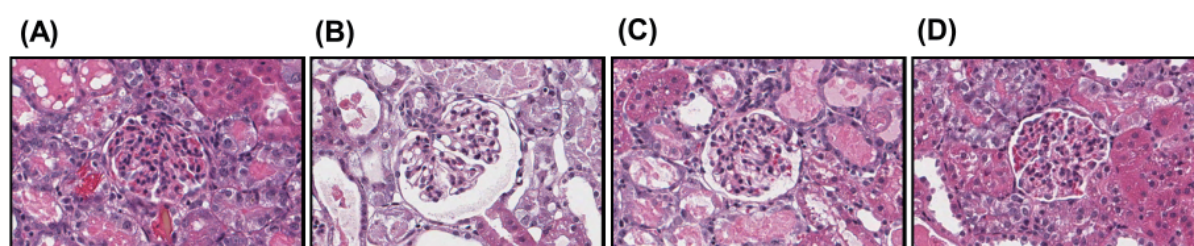
| Items                             | Oligonol | Lychee fruit polyphenol |
|-----------------------------------|----------|-------------------------|
| <b>Monomers</b>                   |          |                         |
| (+)-Catechin/(-)-Epicatechin      | 6.9%     | 6.4%                    |
| (-)-Epicatechin gallate           | 1.4%     | n.d.                    |
| (-)-Epigallocatechin gallate      | 7.0%     | n.d.                    |
| <b>Dimers</b>                     |          |                         |
| Procyanidin A1                    | 6.2%     | 4.0%                    |
| Procyanidin A2                    | 6.6%     | 3.3%                    |
| Procyanidin B1                    | 0.4%     | 0.8%                    |
| Procyanidin B2                    | 2.0%     | 1.7%                    |
| Catechin-epigallocatechin gallate | 1.5%     | n.d.                    |

Oligonol comprises a polyphenol mixture of 15.3% monomers ((+)-catechin, (-)-epicatechin, (-)-epicatechin gallate, and (-)-epigallocatechin gallate) and 16.7% dimers (procyanidin A1, A2, B1, B2, and catechin-epigallocatechin gallate), while lychee fruit polyphenol comprises a mixture of 6.4% monomers and 9.8% dimers. Ref. (31).

**Table 2. Hematological analyses**

| Items                     | <i>m/m</i>     | <i>db/db</i> |              |               |
|---------------------------|----------------|--------------|--------------|---------------|
|                           |                | Veh          | O10          | O20           |
| Glucose (mg/dL)           | 153 ± 12***    | 594 ± 29     | 571 ± 61     | 668 ± 41      |
| Insulin (ng/mL)           | 0.17 ± 0.08*** | 1.77 ± 0.18  | 1.71 ± 0.22  | 2.86 ± 0.32** |
| Leptin (ng/mL)            | 3.0 ± 0.4***   | 19.8 ± 0.3   | 19.5 ± 0.2   | 19.3 ± 0.4    |
| Urea nitrogen (mg/dL)     | 31.5 ± 2.9**   | 44.4 ± 2.4   | 40.4 ± 2.4   | 28.6 ± 1.7*** |
| Creatinine (mg/dL)        | 0.23 ± 0.02*   | 0.35 ± 0.04  | 0.37 ± 0.04  | 0.26 ± 0.02*  |
| Triglyceride (mg/dL)      | 76 ± 6***      | 220 ± 18     | 197 ± 30     | 161 ± 20*     |
| Total cholesterol (mg/dL) | 76 ± 4***      | 157 ± 13     | 139 ± 8      | 124 ± 7*      |
| NEFA (mEq/L)              | 0.74 ± 0.10*** | 1.41 ± 0.08  | 1.18 ± 0.13  | 1.10 ± 0.09*  |
| Adiponectin (ng/mL)       | 6.12 ± 0.62*** | 2.42 ± 0.23  | 2.93 ± 0.20* | 3.12 ± 0.12*  |
| ROS (fluorescence/min/mL) | 171 ± 27*      | 321 ± 42     | 214 ± 24*    | 145 ± 24**    |
| TBARS (nmol/mL)           | 5.7 ± 0.2***   | 14.9 ± 1.3   | 13.4 ± 1.1   | 7.1 ± 0.3***  |

Values are mean ± S.E.M. Significance: \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$  vs. vehicle-treated *db/db* group. *m/m*, vehicle-treated *m/m* mice ( $n = 6$ ); *db/db*-Veh, vehicle-treated *db/db* mice ( $n = 10$ ); *db/db*-O10, oligonol 10 mg/kg body weight-treated *db/db* mice ( $n = 10$ ); *db/db*-O20, oligonol 20 mg/kg body weight-treated *db/db* mice ( $n = 10$ ). Ref. (30).



**Figure 2. H/E staining of renal tissue.** Vehicle-treated *m/m* mice (A), vehicle-treated *db/db* mice (B), oligonol 10 mg/kg body weight-treated *db/db* mice (C), oligonol 20 mg/kg body weight-treated *db/db* mice (D). Images are at 200x magnification. Ref. (31).

as the lipid profile and oxidative stress, kidney damage was controlled under type 2 diabetic conditions with oligonol administration. Figure 2 shows representative microphotographs of renal hematoxylin and eosin (H/E) staining. When compared with *m/m* mice (Figure 2A), the marked glomerular enlargement observed in *db/db* mice (Figure 2B) was clearly improved on oligonol treatment (Figures 2C and 2D). These results indicate that oligonol protects against renal injury induced in type 2 diabetic *db/db* mice.

To investigate the effects of oligonol on renal damage induced by hyperglycemia and abnormal lipid synthesis, the influence of hyperglycemia and hyperlipidemia in the kidneys of *db/db* mice was also examined. The renal contents of triglycerides and total cholesterol were significantly decreased by the administration of oligonol. These results indicate that the biological activities of oligonol in the serum of *db/db* mice are associated with lipid metabolism, such as synthesis or deposition for energy production.

Lipid homeostasis is regulated by a family of membrane-bound transcription factors called SREBPs. Up-regulations of SREBP-1 and SREBP-2 were reported in leptin-resistant mice such as *ob/ob* and FVB<sup>*db/db*</sup> mice (34,35). In our study, the increase in renal SREBP-1 and SREBP-2 in *db/db* mice was down-regulated by the administration of oligonol. This was probably related to the inhibition of renal triglyceride and total cholesterol accumulation. Furthermore, PPARs,

with three isoforms ( $\alpha$ ,  $\delta$ , and  $\gamma$ ), are also involved in the long-term regulation of lipid metabolism, and their activity is modulated by endogenous lipid-derived ligands. When PPAR $\alpha$  is activated, it promotes fatty acid oxidation, ketone body synthesis, and glucose-sparing (36) and ameliorates diabetes, insulin resistance, albuminuria, glomerular hypertrophy, and mesangial expansion in *db/db* mice (37). The decreased renal PPAR $\alpha$  level in *db/db* mice was significantly increased on oligonol administration. These results clarify the effect of oligonol on regulations of both PPAR $\alpha$  and SREBP protein expressions.

#### 4.2. Oligonol attenuates diabetes-induced renal damage triggered by ROS-related pathway

Hyperglycemia and elevated NEFA levels result in the generation of ROS, and, consequently, increase oxidative stress. ROS not only directly damage cells by oxidizing DNA, proteins, and lipids, but also indirectly damage them by activating a variety of stress-sensitive intracellular signaling pathways such as NF- $\kappa$ B, p38 mitogen-activated protein kinase (MAPK), NH<sub>2</sub>-terminal Jun kinase/stress-activated protein kinase, hexosamines, PKC, and AGE/receptor for AGE (RAGE). Activation of these pathways results in the increased expression of numerous gene products that cause cellular damage and play a major role in the etiology of later-stage complications of

**Table 3. Biomarkers associated with oxidative stress in the kidney**

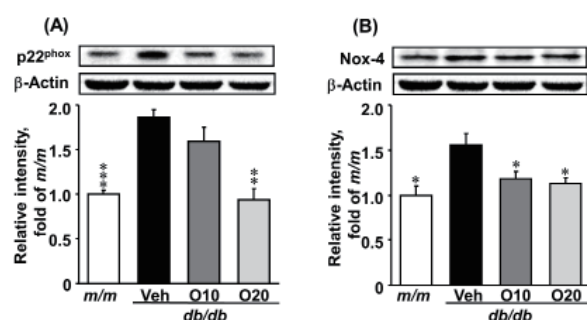
| Items                             | <i>m/m</i>    | <i>db/db</i> |              |              |
|-----------------------------------|---------------|--------------|--------------|--------------|
|                                   |               | Veh          | O10          | O20          |
| ROS (fluorescence/min/mg protein) | 1057 ± 94**   | 3069 ± 327   | 1970 ± 282*  | 764 ± 112*** |
| TBARS (nmol/mg protein)           | 1.11 ± 0.05** | 1.51 ± 0.07  | 1.37 ± 0.05  | 1.18 ± 0.09* |
| GSH (μmol/mg protein)             | 3.02 ± 0.18   | 2.95 ± 0.09  | 3.23 ± 0.33  | 3.08 ± 0.17  |
| GSSG (μmol/mg protein)            | 1.40 ± 0.03** | 1.63 ± 0.04  | 1.47 ± 0.05* | 1.52 ± 0.06  |
| GSH/GSSG ratio                    | 2.17 ± 0.13*  | 1.80 ± 0.04  | 2.19 ± 0.18  | 2.05 ± 0.15  |

Values are mean ± S.E.M. Significance: \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$  vs. vehicle-treated *db/db* group. *m/m*, vehicle-treated *m/m* mice ( $n = 6$ ); *db/db*-Veh, vehicle-treated *db/db* mice ( $n = 10$ ); *db/db*-O10, oligonol 10 mg/kg body weight-treated *db/db* mice ( $n = 10$ ); *db/db*-O20, oligonol 20 mg/kg body weight-treated *db/db* mice ( $n = 10$ ). Ref. (30).

diabetes (38). Thus, the up-regulation of endogenous antioxidative systems and suppression of oxidative stress are important factors ameliorating diabetes and its complications.

We investigated ROS generation and lipid peroxidation, as biomarkers associated with oxidative stress, and also measured GSH and GSSG as indicators of an endogenous antioxidative system. Lipid peroxidation leads to oxidant production from many molecules, and, thus, amplifies oxidative damage (39). Our results showed that the level of ROS generation and that of lipid peroxidation in the serum and kidney were increased in *db/db* mice, which suggests that these mice show increased oxidative damage due to an elevation of ROS generation induced by hyperglycemia and hyperlipidemia. However, oligonol treatment exerted antioxidant activity, promoting decreased serum ROS and 2-thiobarbituric acid-reactive substance (TBARS) levels with corresponding effects on renal tissue in *db/db* mice (Tables 2 and 3). Regarding GSH/GSSG ratios, the *db/db* vehicle group showed a significant reduction compared with the *m/m* group, which resulted from the marked increase in the GSSG concentration in the kidney. However, oligonol treatment did not significantly alter the renal GSH level and GSH/GSSG ratio (Table 3). This suggests that the administration of oligonol would ameliorate oxidative stress under type 2 diabetes through the inhibition of ROS generation and lipid peroxidation, and, thus, it would result in the improvement of renal disorders caused by oxidative stress.

Many researchers have demonstrated that catechin and its derivatives exhibited a favorable antioxidative effect and attenuated oxidative stress under diverse, chronic, degenerative conditions (40). Furthermore, there is increasing evidence of the potential benefits of polyphenols containing catechin in the regulation of cellular processes such as redox control and the inflammatory response based on established animal models and clinical studies (41,42). Recently, our studies have shown that epigallocatechin gallate ameliorates glucose toxicity and renal injury, thereby alleviating renal damage caused by abnormal glucose metabolism-associated oxidative stress in diabetic nephropathy (43). Since oligonol contained catechin-



**Figure 3. Renal p22<sup>phox</sup> (A) and Nox-4 (B) protein expressions.** Values are mean ± S.E.M. Significance: \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$  vs. vehicle-treated *db/db* group. *m/m*, vehicle-treated *m/m* mice ( $n = 6$ ); *db/db*-Veh, vehicle-treated *db/db* mice ( $n = 10$ ); *db/db*-O10, oligonol 10 mg/kg body weight-treated *db/db* mice ( $n = 10$ ); *db/db*-O20, oligonol 20 mg/kg body weight-treated *db/db* mice ( $n = 10$ ). Ref. (31).

based oligomers, we assumed that it could attenuate oxidative stress induced by chronic hyperglycemia and/or direct NADPH oxidase-dependent ROS generation in type 2 diabetes. In the kidney cortex of *db/db* mice, protein expressions of NADPH oxidase-4 (Nox-4) (NADPH oxidase homologs) and p22<sup>phox</sup>/p47<sup>phox</sup> (subunit of NADPH oxidase) were increased, and Nox-4 was a major source of renal ROS through the p38 MAPK-dependent pathway (44). Our study demonstrated that oligonol decreased the renal ROS concentration as well as down-regulated the expression of NADPH oxidase, as shown in Table 3 and Figure 3. These results suggest that the attenuation of oxidative stress in renal tissue by oligonol is associated with the reduction of NADPH oxidase activity.

#### 4.3. Oligonol acts as a regulator of diabetes-induced renal damage through an AGE-related pathway

AGEs are elevated under chronic hyperglycemia and excessive glucose uptake in tissues, and, furthermore, their formation can be accelerated by increased oxidative stress (45). Some polyphenols, including green tea catechins and epicatechins, chlorogenic acids, quercetin, and naringenin, could inhibit the absorption of glucose from the intestine *via* the inhibition of sodium-dependent glucose transporter (SGLT) 1 and SGLT2 (46). Glucose transporter isoform 2 (GLUT2)-

mediated glucose transport was inhibited by apigenin, quercetin, myricetin, and tea catechins (47,48). Polyphenol also shows a wide range of target tissue and is distributed in various organs, including the liver and kidney. Our study shows that the increased glucose concentration in the kidney of *db/db* mice is decreased by oligonol treatment. Thus, the decreased renal glucose concentration may be due to SGLT1, SGLT2, and GLUT2 inhibition.

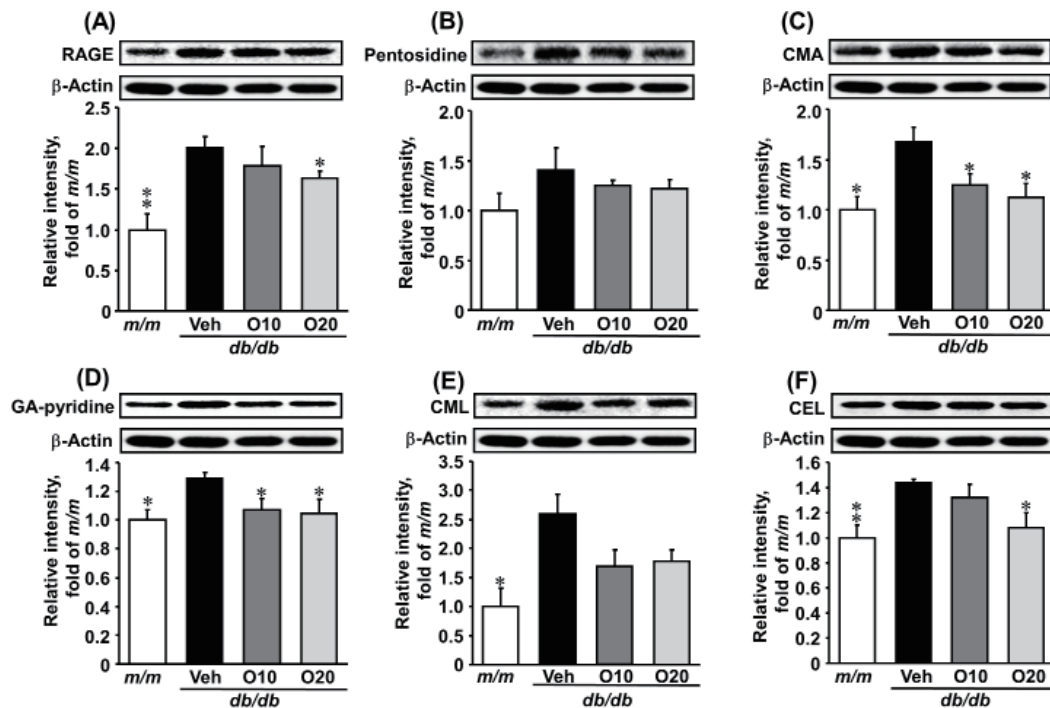
AGEs are complex compounds formed *via* a nonenzymatic reaction between reducing sugars and amine residues on proteins, lipids, or nucleic acids (49). The intracellular production and accumulation of AGEs are closely linked to diabetic complications such as neuropathy, retinopathy, and nephropathy (50,51). Especially, there is a strong correlation between AGE accumulation and the duration and degree of severity of diabetic kidney disease (52). AGEs can interact with certain receptors, such as RAGE, to induce intracellular signaling, which leads to enhanced oxidative stress and the production of key proinflammatory and pro-sclerotic cytokines. Recently, attention has been focused on the essential roles of AGEs, that is, AGEs alter the structure and function of matrix tissue proteins, and AGE-modified proteins stimulate a variety of cellular responses *via* a specific cell surface receptor, resulting in the expression and activation of pathogenic mediators, *e.g.*, the extracellular matrix, oxidative stress, cytokines, and growth factors, implicated in the development and stimulation of diabetic renal diseases (53). We evaluated the protein expressions of AGEs and receptors, such as pentosidine, *N*<sup>ε</sup>-(carboxymethyl)arginine (CMA), glycolaldehyde (GA)-pyridine, *N*<sup>ε</sup>-(carboxymethyl)lysine (CML), *N*<sup>ε</sup>-(carboxyethyl)lysine (CEL), and RAGE, in the kidney. These products are not only derived from intermediates of glucose metabolism and metabolites of glycolysis, but also serve as general bio-markers of oxidative stress resulting from carbohydrate and lipid oxidation reactions (54). Pentosidine, a cross-link between arginine and lysine, is a well-characterized AGE (55). Recently, serum pentosidine concentrations were shown to be closely associated with peripheral arterial disease in type 2 diabetes mellitus patients (56). Odani *et al.* (57) suggested that CMA was a new marker of diabetic complications, and that its serum concentration was increased in diabetic patients with non-renal failure compared with a healthy control group. CMA production increased in parallel with the incubation time, and its yield was greater than that of pentosidine (58). GA was formed either as a fragmentation product in the Maillard reaction or as a result of the myeloperoxidase-hydrogen peroxide-chloride reaction (59,60). GA-pyridine, a specific GA-derived AGE, has been described in foam cells and in the extracellular matrix of human atherosclerotic fibrotic lesions, and also reported to show mesangial

accumulation in human renal disease (61). The hydroxy-amino acid L-serine is oxidized and converted to reactive carbonyl compounds, such as GA which is highly reactive with proteins, leading to the formation of CML (60). Taking the above-mentioned studies together, AGE formation, accumulation, and interaction with RAGE were increased and aggravated under the diabetic condition, consistent with our findings such as the up-regulated expression of these proteins in the kidney of type 2 diabetic mice. However, oligonol administration effectively down-regulated AGE-related protein expressions (RAGE, CMA, GA-pyridine, CML, and CEL) (Figure 4). These results suggest that the inhibitory activity of oligonol on AGE formation and its receptor expressions shows clinical potential, including its delay of AGE-related disorder development in diabetes. The reno-protective effects of oligonol against diabetes were not attributable to improved renal glycemic adjustment alone, but also likely reflected its antioxidant activity. The combined antioxidant and reduction of the glycation reaction by oligonol should be particularly advantageous and perhaps even synergistic in preventing renal injury and other diabetic complications.

#### 4.4. Oligonol attenuates diabetes-induced renal damage by ROS-sensitive pathway of inflammation and apoptosis

A possible pathway of ROS-associated apoptosis was suggested by the observation that increased oxidative stress activates c-Jun N-terminal kinase (JNK) and p38 MAPK through apoptosis signal-regulating kinase 1 (62). JNK activation has been suggested to be involved in the regulation of tumor necrosis factor- $\alpha$  (TNF- $\alpha$ )-induced c-Jun kinase and apoptosis (63). Otherwise, the suppression of JNK and p38 MAPK mediated anti-apoptotic signaling (64). We evaluated the expression of pro-apoptotic cellular signal-related protein in diabetic renal tissues. The renal protein expressions of JNK, phosphor-JNK (p-JNK), c-Jun, and TNF- $\alpha$  were augmented in the diabetic *db/db* mice compared with normal *m/m* mice. These up-regulated proteins were markedly down-regulated by oligonol treatment, as reported previously (31). This indicates that the down-regulation of TNF- $\alpha$  or oxidative stress-mediated JNK signaling affected the renal apoptotic response.

Therefore, we subsequently examined renal apoptosis-related protein expression in the kidney of *db/db* mice with oligonol administration. Podocyte loss caused by detachment or apoptosis will lead to a decrease in the density of podocytes. Reduction of the podocyte density is an important determinant of progressive diabetic nephropathy and precedes the development of renal dysfunction and albuminuria in diabetic patients and animal models of diabetes mellitus (65). The marked increase in podocyte apoptosis observed during the early stage of diabetes



**Figure 4. Renal RAGE (A), pentosidine (B), CMA (C), GA-pyridine (D), CML (E), and CEL (F) protein expressions.** Values are mean  $\pm$  S.E.M. Significance: \*  $p < 0.05$ , \*\*  $p < 0.01$  vs. vehicle-treated *db/db* group. *m/m*, vehicle-treated *m/m* mice ( $n = 6$ ); *db/db*-Veh, vehicle-treated *db/db* mice ( $n = 10$ ); *db/db*-O10, oligonol 10 mg/kg body weight-treated *db/db* mice ( $n = 10$ ); *db/db*-O20, oligonol 20 mg/kg body weight-treated *db/db* mice ( $n = 10$ ). Ref. (31).

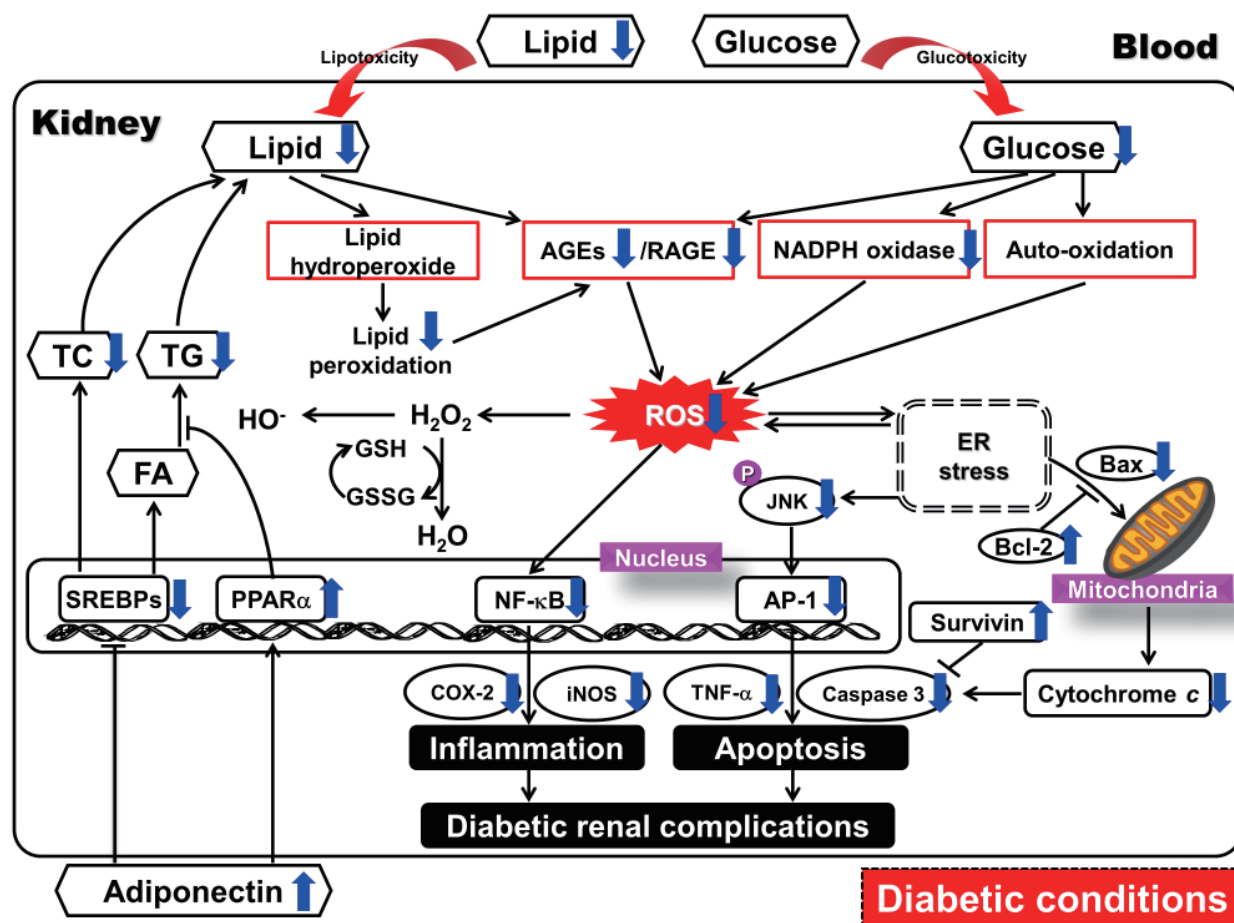
in *db/db* mice is mediated to a large extent by the activation of NADPH oxidase and its contribution to the generation of ROS (65). Our results support the idea that the decrease in NADPH oxidase expression affected the proapoptotic protein expression. Oligonol administration led to a significant increase in Bcl-2 and survivin and reduction of Bax, cytochrome *c*, and caspase-3 protein in the renal tissue of *db/db* mice, as shown in a previously report (31). These data suggest that the attenuation of diabetic renal apoptosis is influenced by decreased NADPH oxidase-dependent oxidative stress and/or JNK-related signaling.

Under type 2 diabetes, the redox-sensitive intracellular signaling pathway is altered. In particular, one major intracellular target of hyperglycemia and oxidative stress is the transcription factor NF- $\kappa$ B. NF- $\kappa$ B can be activated by a wide array of exogenous and endogenous stimuli, including hyperglycemia, elevated NEFA, ROS, TNF- $\alpha$ , interleukin-1 $\beta$ , other proinflammatory cytokines, AGE-binding RAGE, and p38 MAPK. The activation of NF- $\kappa$ B induces the inflammation-related proteins COX-2 and iNOS, and the subsequent production of prostaglandin and nitric oxide (NO), respectively. NO reacts very rapidly with superoxide to form peroxynitrite and other NO-derived oxidants capable of damaging DNA and proteins (66). There is a vicious cycle involving NF- $\kappa$ B, oxidative stress, and inflammation under the diabetic condition. Therefore, the inhibition of NF- $\kappa$ B transcription plays a central role in regulating the pathophysiology of diabetic complications. Elevated protein expressions of

NF- $\kappa$ Bp65 and iNOS in the liver of *db/db* mice were markedly down-regulated by oligonol administration (31). Oligonol administration could adjust inflammation through the inhibition of the NF- $\kappa$ B pathway.

## 5. Conclusion

Oligonol, which is now available commercially as a new dietary ingredient, is an optimized phenolic product derived from lychee fruit polyphenols containing catechin-type monomers and low-molecular-weight oligomers (21). Based on recent studies, there is accumulating evidence that oligonol can induce some physiological and biochemical alterations *in vitro* and *in vivo*, such as the induction of apoptosis in cancer cells (23), antioxidant and anti-inflammatory effects in mice (24), and beneficial subjective effects on the feeling of fatigue in young athletes (67). Moreover, the oral administration of oligonol improves the regulation of genes for adipokines in white adipose tissue of mice on administering a high-fat diet (26). The dietary feeding of proanthocyanidins, which comprise oligonol, has been reported to induce the significant attenuation of tissue fat levels, without changing the total body mass of animals compared with non-proanthocyanidin-fed animals (28). In this review, we summarize our recent work: oligonol was able to improve diabetic indices, prevent the development of diabetic renal disease, and preserve renal cells and the renal morphological structure *via* the attenuation of NADPH oxidase-induced oxidative stress, inhibition of AGE generation, and prevention



**Figure 5. Predicted mechanism in renal tissues on administering oligonol against oxidative stress, AGE formation, and apoptosis.** Oligonol attenuated glucose and lipid accumulation, the regulation of transcription factors for lipid metabolism, and subsequently decreased NADPH oxidase-derived ROS production in the kidney. Furthermore, oligonol ameliorated the values of AGE-related protein expressions (RAGE, CMA, GA-pyridinine, and CEL), anti-apoptotic protein expressions (Bcl-2 and survivin), and apoptotic protein expressions (JNK, p-JNK, AP-1, Bax, cytochrome *c*, and caspase-3 proteins).

of apoptosis-induced cell death in *db/db* mice, being independent of changes in the body weight or serum glucose levels (Figure 5). Accordingly, oligonol may be a promising dietary supplement for the prevention or delaying of diabetic renal complications. With respect to the current research, more clinical studies are needed on the management of hyperglycemia and inflammation in both healthy subjects and metabolic syndrome patients and its pharmaceutical kinetics in order to develop therapeutic agents.

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