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Oligonol, a Low-Molecular-Weight Polyphenol Derived from Lychee Fruit, Attenuates Diabetes-Induced Renal Damage through the Advanced Glycation End Product–Related Pathway in *db/db* Mice[1–3]

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Abstract

This study was conducted to examine whether oligonol, a low-molecular-weight polyphenol derived from lychee fruit, has an ameliorative effect on diabetes-induced alterations, such as advanced glycation end product (AGE) formation or apoptosis in the kidneys of *db/db* mice with type 2 diabetes. Oligonol [10 or 20 mg/(kg body weight · d), orally] was administered every day for 8 wk to prediabetic *db/db* mice, and its effect was compared with vehicle-treated *db/db* and normal control mice (*m/m*). The administration of oligonol decreased the elevated renal glucose concentrations and reactive oxygen species in *db/db* mice ($P < 0.05$). The increased serum urea nitrogen and creatinine concentrations, which reflect renal dysfunction in *db/db* mice, were substantially lowered by oligonol. Oligonol reduced renal protein expression of NAD(P)H oxidase subunits (p22 phagocytic oxidase and NAD(P)H oxidase-4), AGEs (except for pentosidine), and c-Jun N-terminal kinase B-targeting proinflammatory tumor necrosis factor-α ($P < 0.05$). Oligonol improved the expressions of antiapoptotic [B-cell lymphoma protein 2 (Bcl-2) and survivin] and proapoptotic [Bcl-2–associated X protein, cytochrome *c*, and caspase-3] proteins in the kidneys of *db/db* mice ($P < 0.05$). In conclusion, these results provide important evidence that oligonol exhibits a pleiotropic effect on AGE formation and apoptosis-related variables, representing renoprotective effects against the development of diabetic complications in *db/db* mice with type 2 diabetes. J. Nutr. 144: 1150–1157, 2014.

Introduction

Diabetes mellitus leads to serious complications of the kidneys, such as nephropathy that develops into end-stage renal disease. An early feature of diabetic nephropathy is the loss of podocytes through several pathologic pathways (1). The cause and consequences of renal cell loss during early diabetic nephropathy were expected to involve hyperglycemia and, consequently, increased oxidative stress and advanced glycation end product (AGE)[8] formation (2,3). Several researchers demonstrated that, in the early diabetic condition, reactive oxygen species (ROS) generation induced by NAD(P)H oxidase and the mitochondrial electron transport chain is a general event (4). Moreover, AGEs were regarded as the adverse effects of hyperglycemia (5). AGEs stimulate matrix production and inhibit mesangial proliferation, and the glycated glomerular basement membrane is more resistant to digestion by protease (6). Suzuki et al. (7) reported that the intensity of AGE staining gradually increases in parallel with the degree of mesangial expansion in patients with diabetic nephropathy. In addition, an excessive AGE–receptor for AGE (RAGE) interaction activates intracellular signaling cascades, leading to a plethora of proinflammatory and profibrotic cellular responses through various downstream pathways (8).

A decreased number of podocytes per glomerulus was found to be the strongest predictor of the progression of diabetic nephropathy, in which fewer cells predicted more rapid progression (1,9). Podocyte apoptosis is the major cause of renal cell loss and coincides with the onset of hyperglycemia, which activates proapoptotic signaling (2). It was revealed that high-level glucose, AGEs, and increased ROS generation by mitochondrial and nonmitochondrial sources were strongly associated with a signal

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[3] Supplemental Tables 1 and 2 are available from the ''Online Supporting Material'' link in the online posting of the article and from the same link in the online table of contents at http://jn.nutrition.org.
[8] Abbreviations used: AGE, advanced glycation end product; Bax, B-cell lymphoma protein 2–associated X protein; Bcl-2, B-cell lymphoma protein 2; CEL, N^{ε}-(carboxyethyl)lysine; CMA, N^{ε}-(carboxymethyl)arginine; CML, N^{ε}-(carboxymethyl)lysine; GA, glycolaldehyde; GLUT2, glucose transporter isoform 2; JNK, c-Jun N-terminal kinase; Nox-4, NAD(P)H oxidase-4; RAGE, receptor for advanced glycation end product; ROS, reactive oxygen species; SGLT, sodium-dependent glucose transporter.
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TABLE 1 Body-weight gain, food intake, water intake, and kidney weight in *m/m* mice or *db/db* mice treated with water or oligonol for 8 wk[1]

		db/db		
Item	*m/m*	Veh	O10	O20
Body-weight gain, *g/8 wk*	2.45 ± 0.50[b]	11.3 ± 1.58[a]	9.13 ± 0.55[a]	10.5 ± 0.74[a]
Food intake, *g/d*	3.10 ± 0.04[b]	5.79 ± 0.05[a]	5.82 ± 0.06[a]	5.98 ± 0.07[a]
Water intake, *mL/d*	4.52 ± 0.13[b]	13.5 ± 0.26[a]	14.5 ± 0.20[a]	13.3 ± 0.25[a]
Kidney weight, *mg/100 g body weight*	131 ± 2[a]	87 ± 4[b]	92 ± 4[b]	93 ± 2[b]

[1] Values are means ± SEMs, $n = 10$ (*db/db*) or 6 (*m/m*). Labeled means within a row without a common letter differ, $P < 0.05$ (Duncan's test). *m/m*, vehicle-treated *m/m* mice; O10, oligonol 10 mg/(kg body weight · d)–treated *db/db* mice; O20, oligonol 20 mg/(kg body weight · d)–treated *db/db* mice; Veh, vehicle-treated *db/db* mice.

pathway of apoptosis in experimental diabetes (10). Therefore, the development of new strategies to prevent diabetic nephropathy can come from a better understanding of the molecular mechanism involved in the early changes of diabetic nephropathy. Research on the preventative effects of functional foods and/or dietary ingredients against diabetic nephropathy help to prevent diabetic complications.

Oligonol is a new dietary phenolic product derived from lychee fruit polyphenols containing catechin-type monomers and low-molecular-weight oligomers (11). In recent studies, accumulating evidence demonstrated that oligonol can exert its physiologic and biochemical effects, such as anticancer, antioxidant, anti-inflammation, and antiobesity, in vitro and in vivo (11–14). Moreover, the oral administration of oligonol regulated the adipokine-related genes in high-fat diet–fed mice (15). The results showed that oral oligonol intake had a considerable effect on the inhibition of inflammatory markers and cortisol in healthy young men (16). In a previous study, we reported that oligonol could have renoprotective effects against abnormal lipid metabolism and ROS-related AGE formation in type 2 diabetes. In particular, there is a strong correlation between AGE accumulation and the presence and severity of diabetic kidney disease (17). Accordingly, to prevent diabetic renal damage associated with structural and functional changes, it is important to reduce the development of AGEs. The impacts of AGEs were recognized over a wide range, resulting in the expression and activation of pathogenic mediators implicated in the development of diabetic complications, such as oxidative stress, cytokines, and apoptosis, via receptor-dependent and/or -independent pathways (18–21). The present study was conducted to evaluate the preventive effects of oligonol on diabetic indices and renal disorders associated with AGE formation and apoptosis in *db/db* mice with diabetes.

Materials and Methods

Oligonol. Oligonol is produced by oligomerizing polyphenol polymers derived from lychee fruit. The safety of oligonol as a food or dietary supplement and as a pharmaceutical additive has been confirmed, as described previously (11). Oligonol comprises a polyphenol mixture of 16.0% monomers (including catechin) and 13.9% dimers (including procyanidin and catechin), whereas lychee fruit polyphenol comprises a mixture of 6.4% monomers and 9.8% dimers (**Supplemental Table 1**). Oligonol is commercially available (Amino Up Chemical).

Experimental mice and treatment. The study adhered to the *Guidelines for Animal Experimentation* approved by the University of Toyama (Registration No. S-2006 INM-22). Five-wk-old male mutant diabetic C57BLKS/J (*db/db*) and age-matched normal homozygote (*m/m*) mice were purchased from Japan SLC and housed with ad libitum access to a laboratory pellet unpurified diet (CLEA Japan) and water in a controlled environment (22 ± 2°C, 50 ± 5% humidity, 12-h light/dark cycle). After an adaptation period (at age 9 wk), the glucose concentration of blood taken from the tail vein and body weight were measured, and *db/db* mice were divided into 3 groups. The *m/m* ($n = 6$) or *db/db* vehicle group ($n = 10$) was orally administered water, whereas the other 2 groups ($n = 10$ per group) were orally administered oligonol every day at a dose of 10 or 20 mg/kg body weight by gavage, respectively. The oral concentrations of oligonol administration were the same as in our previous studies using *db/db* mice (17,22). Food and water intakes were determined every day during the experimental period. After administration for 8 wk, blood samples were collected by the cardiac puncture of anesthetized mice. Serum was separated immediately after centrifugation. Subsequently, each mouse was perfused with ice-cold physiologic saline, and then the kidneys were harvested, snap-frozen in liquid nitrogen, and stored at −80°C until analysis.

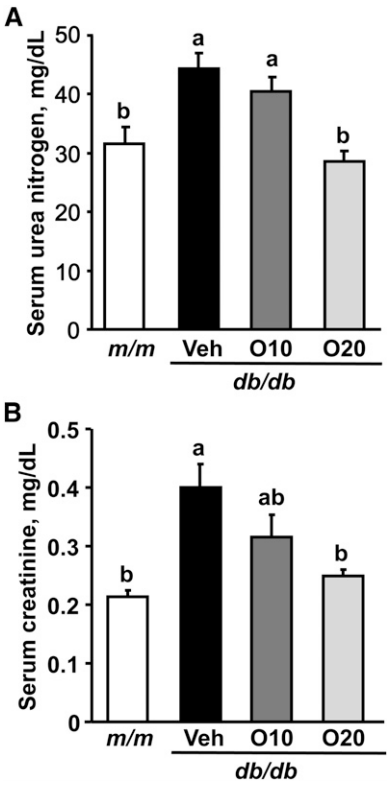

FIGURE 1 Serum urea nitrogen (*A*) and creatinine (*B*) concentrations in *m/m* mice and in *db/db* mice treated with water or oligonol for 8 wk. Values are means ± SEMs, $n = 10$ (*db/db*) or 6 (*m/m*). Labeled means without a common letter differ, $P < 0.05$ (Duncan's test). *m/m*, vehicle-treated *m/m* mice; O10, oligonol 10 mg/(kg body weight · d)–treated *db/db* mice; O20, oligonol 20 mg/(kg body weight · d)–treated *db/db* mice; Veh, vehicle-treated *db/db* mice.

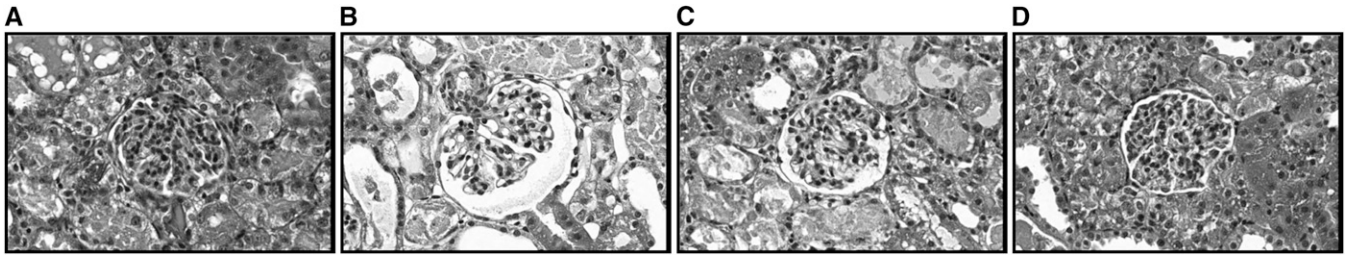

FIGURE 2 Hematoxylin–eosin staining of renal tissue in *m/m* mice and in *db/db* mice treated with water or oligonol for 8 wk: vehicle-treated *m/m* mice (*A*), vehicle-treated *db/db* mice (*B*), oligonol (10 mg/kg body weight · d)-treated *db/db* mice (*C*), oligonol (20 mg/kg body weight · d)-treated *db/db* mice (*D*). Images are at 200× magnification.

Measurement of renal function variables. Urea nitrogen and creatinine concentrations were determined using commercial kits (BUN Kainos and CRE-EN Kainos; Kainos Laboratory).

Light microscopic study. Renal sections for the observation of morphologic changes were prepared by perfusing with cold saline, fixing in 10% neutral-buffered formalin, embedding in paraffin, and cutting into 4-μm intervals. The sections stained with hematoxylin–eosin were observed by light microscopy. The glomerular area was determined as the mean area of a total of 30 glomeruli in each kidney sample (n = 6–10), cut at their vascular poles, by tracing along the outline of the capillary loop using a VH analyzer (VH-H1A5; Keyence).

Determination of glucose and ROS concentrations in the renal tissue. To measure glucose concentration in the kidney, tissue was prepared as described previously (23), and glucose concentration was evaluated using a Wako kit (Glucose CII-Test). ROS generation was measured by using the method of Ali et al. (24).

Western blot analyses. The preparation of nuclear and postnuclear fractions was described previously (17,22). Postnuclear and nuclear proteins (15 μg) were electrophoresed in 8–13% SDS–polyacrylamide gel. Separated proteins were transferred, blocked, and then incubated with primary and secondary antibodies. Each antigen–antibody complex was visualized using ECL Western Blotting Detection Reagents and detected by chemiluminescence with an LAS-4000 camera (Fujifilm). Band densities were calculated using an image analyzer (ATTO densitograph; ATTO) and normalized to β-actin or histone. The protein expression amounts were represented relative to those of *m/m* mice (represented as 1).

Statistical analysis. Data are expressed as means ± SEs. Statistical comparisons were performed by 1-factor ANOVA, followed by Duncan's multiple-range test. Statistical analysis was conducted using SAS (release 9.2; SAS Institute), and $P < 0.05$ was considered significant.

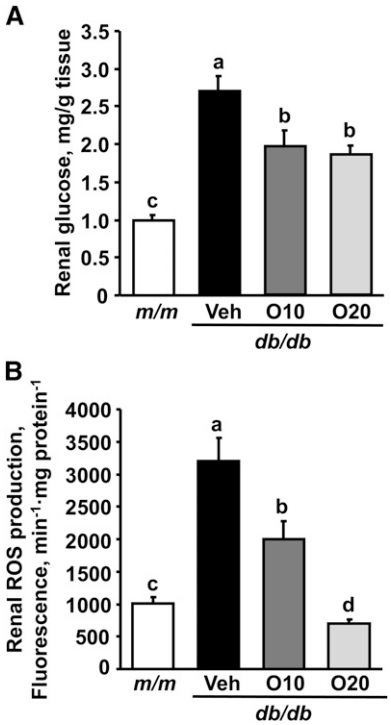

FIGURE 3 Renal glucose concentration (*A*) and ROS production (*B*) in *m/m* mice and in *db/db* mice treated with water or oligonol for 8 wk. Values are means ± SEMs, n = 10 (*db/db*) or 6 (*m/m*). Labeled means without a common letter differ, $P < 0.05$ (Duncan's test). *m/m*, vehicle-treated *m/m* mice; O10, oligonol 10 mg/(kg body weight · d)–treated *db/db* mice; O20, oligonol 20 mg/(kg body weight · d)–treated *db/db* mice; ROS, reactive oxygen species; Veh, vehicle-treated *db/db* mice.

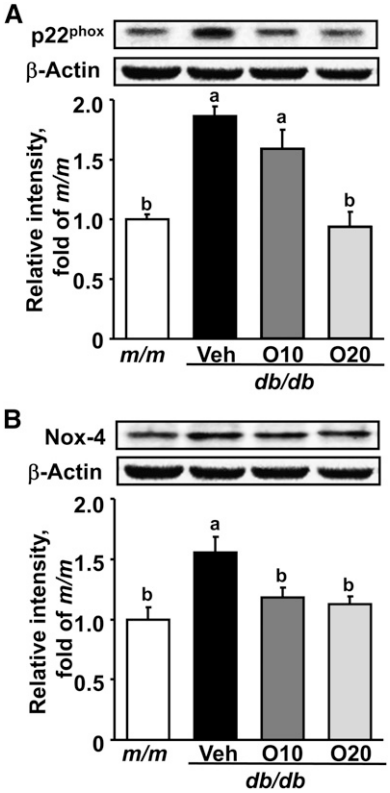

FIGURE 4 Renal $p22^{phox}$ (*A*) and Nox-4 (*B*) protein expressions in *m/m* mice and in *db/db* mice treated with water or oligonol for 8 wk. Values are means ± SEMs, n = 10 (*db/db*) or 6 (*m/m*). Labeled means without a common letter differ, $P < 0.05$ (Duncan's test). *m/m*, vehicle-treated *m/m* mice; Nox-4, NAD(P)H oxidase-4; O10, oligonol 10 mg/(kg body weight · d)–treated *db/db* mice; O20, oligonol 20 mg/(kg body weight · d)–treated *db/db* mice; $p22^{phox}$, p22 phagocytic oxidase; Veh, vehicle-treated *db/db* mice.

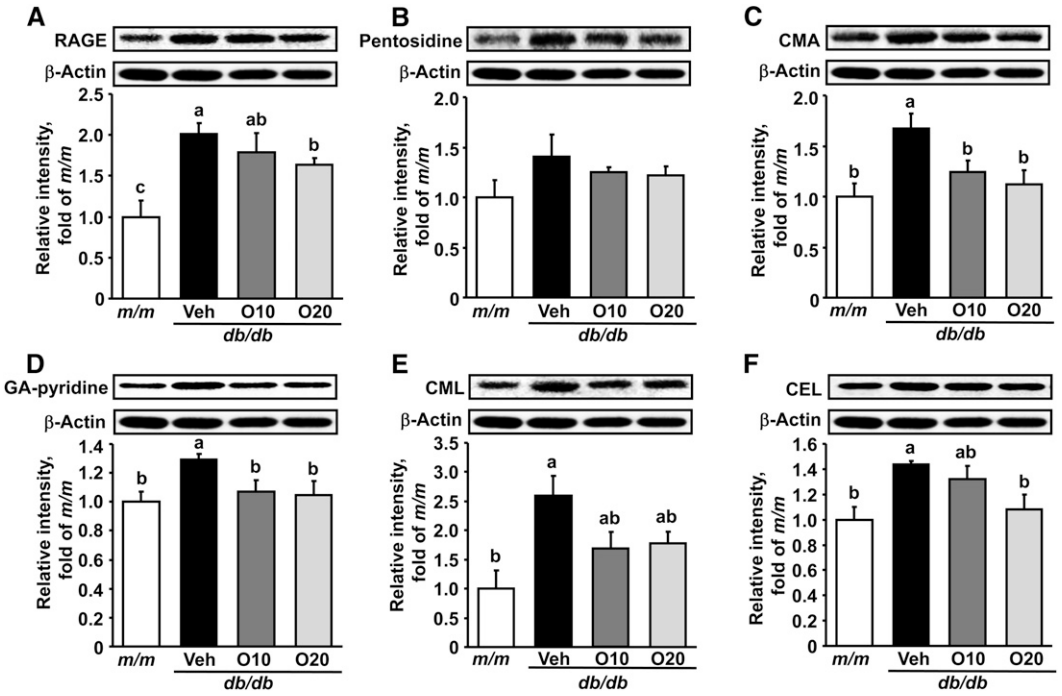

FIGURE 5 Renal RAGE (*A*), pentosidine (*B*), CMA (*C*), GA-pyridine (*D*), CML (*E*), and CEL (*F*) protein expressions in *m/m* mice and in *db/db* mice treated with water or oligonol for 8 wk. Values are means ± SEMs, $n = 10$ (*db/db*) or 6 (*m/m*). Labeled means without a common letter differ, $P < 0.05$ (Duncan's test). CEL, N^{ϵ}-(carboxyethyl)lysine; CMA, N^{ϵ}-(carboxymethyl)arginine; CML, N^{ϵ}-(carboxymethyl)lysine; GA, glycolaldehyde; *m/m*, vehicle-treated *m/m* mice; O10, oligonol 10 mg/(kg body weight · d)–treated *db/db* mice; O20, oligonol 20 mg/(kg body weight · d)–treated *db/db* mice; RAGE, receptor for advanced glycation end product; Veh, vehicle-treated *db/db* mice.

Results

Change in body-weight gain, food intake, water intake, and kidney weight. The body-weight gain and food and water intakes of the *db/db* mice after 8 wk were substantially higher than those of the age-matched *m/m* mice (**Table 1**). There were no differences in body-weight gain or food and water intakes among the *db/db* mouse groups. The kidney weight was lower in *db/db* than *m/m* mice; however, oligonol administrations led to no substantial differences in the *db/db* groups.

Renal functional variables. Serum urea nitrogen and creatinine concentrations in *db/db* vehicle mice were elevated compared with *m/m* mice (141% and 152%, respectively), whereas these augmented concentrations were substantially decreased in oligonol-administered *db/db* mice (**Fig. 1***A*, *B*).

Renal histologic examination. **Figure 2** shows representative microphotographs of renal hematoxylin–eosin staining. When compared with *m/m* mice (Fig. 2*A*), the marked glomerular enlargement observed in *db/db* mice (Fig. 2*B*) was clearly improved on oligonol treatment (Fig. 2*C*, *D*).

Renal glucose and ROS concentrations. The glucose and ROS concentrations in the kidneys of the *db/db* vehicle group had >2.58-fold (**Fig. 3***A*) and 2.90-fold (Fig. 3*B*) increases, respectively, compared with those of the *m/m* group. These increases were substantially attenuated by oligonol administration. Specifically, obesity-induced diabetes markedly increased oxidative stress in the renal tissue, and oligonol treatment decreased ROS formation dose-dependently.

Renal p22 phagocytic oxidase and NAD(P)H oxidase-4 protein expressions. To investigate renal NAD(P)H oxidase activity, p22 phagocytic oxidase and NAD(P)H oxidase-4 (Nox-4) protein expressions were examined by Western blot analyses. The protein expressions of both proteins were augmented in *db/db* vehicle mice compared with *m/m* mice; however, the 20 mg/kg oligonol treatment group had a downregulation of NAD(P)H oxidase expression (**Fig. 4***A*, *B*).

Renal AGE-related protein expressions. To evaluate RAGE, pentosidine, anti–N^{ϵ}-(carboxymethyl)arginine (CMA), glycolaldehyde (GA)-pyridine, anti–N^{ϵ}-(carboxymethyl)lysine (CML), and anti–N^{ϵ}-(carboxyethyl)lysine (CEL) protein expressions in the kidneys, we performed Western blot analyses. Renal protein expressions of RAGE and most AGEs (except pentosidine) were elevated in *db/db* vehicle mice compared with *m/m* mice. However, the oligonol-treated group had a substantial downregulation of these AGEs (except pentosidine) and their receptors (**Fig. 5***A*–*F*).

Renal c-Jun N-terminal kinase, phosphorylated c-Jun N-terminal kinase, c-Jun, and TNF-α protein expressions. The *db/db* vehicle group had a substantial increase in the protein expression amounts of c-Jun N-terminal kinase (JNK), phosphorylated JNK, and TNF-α in the cytoplasm (**Fig. 6***A*, *B*, *D*) and proto-oncogene c-Jun in the nuclear fraction (Fig. 6*C*), which were substantially reversed by oligonol treatment.

Renal apoptosis-related protein expressions. Renal protein amounts of the antiapoptotic B-cell lymphoma protein 2 (Bcl-2) and survivin, the proapoptotic Bcl-2 family member Bcl-2–associated X protein (Bax), and also the apoptotic inducers cytochrome *c* and caspase-3 were examined in the cytoplasm. The renal protein expressions of Bcl-2 and survivin were substantially lower in *db/db* vehicle mice compared with *m/m* mice, whereas these reduced expressions were substantially enhanced by 100 mg/kg oligonol treatment nearly to the normal amounts (**Fig. 7***A*, *B*). Moreover,

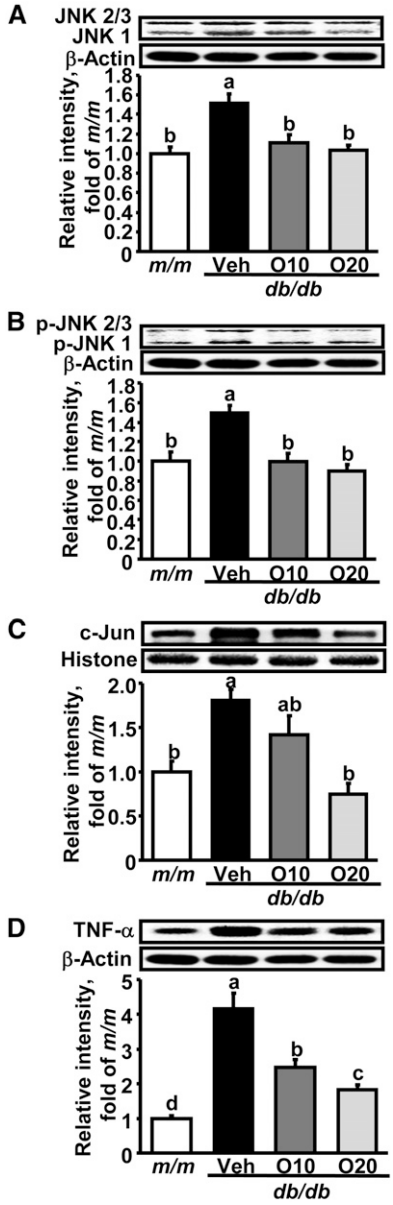

FIGURE 6 Renal JNK (*A*), p-JNK (*B*), c-Jun (*C*), and TNF-α (*D*) protein expressions in *m/m* mice and in *db/db* mice treated with water or oligonol for 8 wk. Values are means ± SEMs, $n = 10$ (*db/db*) or 6 (*m/m*). Labeled means without a common letter differ, $P < 0.05$ (Duncan's test). JNK, c-Jun N-terminal kinase; *m/m*, vehicle-treated *m/m* mice; O10, oligonol 10 mg/(kg body weight · d)–treated *db/db* mice; O20, oligonol 20 mg/(kg body weight · d)–treated *db/db* mice; p-JNK, phosphorylated c-Jun N-terminal kinase; Veh, vehicle-treated *db/db* mice.

marked increases in protein expressions of proapoptotic and apoptotic factors in the *db/db* group were attenuated by oligonol treatment (Fig. 7*C–E*).

Discussion

In the present study, *db/db* mice with type 2 diabetes had increased renal injury associated with NAD(P)H oxidase–derived oxidative stress, AGE formation, and apoptosis-related protein expressions. However, these unfavorable outcomes were reversed by oligonol administration in *db/db* mice. Oligonol treatment in the type 2 diabetic animal model improved overall renal functions, such as serum urea nitrogen and creatinine, and morphologic characteristics. However, oligonol treatment did not affect body weight and serum glucose concentration despite elevated serum insulin. In oligonol-administered *db/db* mice, the cause of increased insulin secretion is unclear and may be related to the preservation of pancreatic β-cell function by oligonol treatment (**Supplemental Table 2**), consistent with previous research (17). In the study by Kanda et al. (25), *db/db* mice exhibited apoptosis of pancreatic β-cells, which were readily detectable at age 8 wk, and decreased cell proliferation at age 12 wk with reduced insulin content in pancreatic islets. Additionally, in our previous study, serum adiponectin concentrations were substantially higher in the oligonol-treated group than in the vehicle *db/db* group (17). Low adiponectin concentrations are associated with insulin resistance in patients with type 2 diabetes and in experimental animals (26). Unfortunately, we cannot measure insulin sensitivity regarding the influence on target tissues, such as muscle and fat. However, other than serum glucose and insulin concentrations, other biofactors, such as oxidative stress, AGE formation, apoptosis, and especially kidney damage, were favorable under type 2 diabetic conditions with oligonal administration.

Many researchers demonstrated that catechin and its derivatives exhibited a favorable antioxidant effect and attenuated oxidative stress under diverse chronic degenerative conditions (27). 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 from established animal models and clinical studies (28,29). In a clinical study, oligonol had a high absorption rate and a maintained level in the serum (30). Recently, our studies showed that epigallocatechin gallate ameliorates glucose toxicity and renal injury, thereby alleviating renal damage caused by abnormal glucose metabolism–associated oxidative stress in diabetic nephropathy (31). Because oligonol contained catechin-based oligomers, we assumed that it could attenuate oxidative stress induced by chronic hyperglycemia and/or direct NAD(P)H oxidase–dependent ROS generation in type 2 diabetes. In the kidney cortex of *db/db* mice, protein expressions of Nox-4 [NAD(P)H oxidase homologs] and p22 phagocytic oxidase/p47 phagocytic oxidase [subunit of NAD(P)H oxidase] were increased, and Nox-4 was a major source of renal ROS with the p38 MAPK–dependent pathway (32). Our present study demonstrated that oligonol decreased the renal ROS concentration and downregulated expression of NAD(P)H oxidase. These results suggested that the attenuation of oxidative stress in renal tissue by oligonol is probably associated with the reduction of NAD(P)H oxidase activity.

Besides its beneficial effects on oxidative stress, our study shows that the increased glucose concentrations in the kidneys of *db/db* mice are decreased by oligonol treatment. There is accumulating evidence that some polyphenols, including green tea catechins and epicatechins, chlorogenic acids, quercetin, and naringenin, interact with the absorption of glucose from the intestine via the inhibition of sodium-dependent glucose transporter (SGLT) 1 and SGLT2 (33). Glucose transporter isoform 2 (GLUT2)–mediated glucose transport is inhibited by apigenin, quercetin, myricetin, and tea catechins (34,35). Polyphenols also have a wide range of target tissues and are distributed in various organs, including the liver and kidneys. Thus, it can be assumed that the decreased renal glucose concentration in the present experiment may be attributable to the reduced SGLT1, SGLT2, and GLUT2.

In a previous study, we evaluated AGE actions with receptors related to intracellular responses and the renal AGE (CEL and CML) amount characterized physicochemically by neither crosslinking

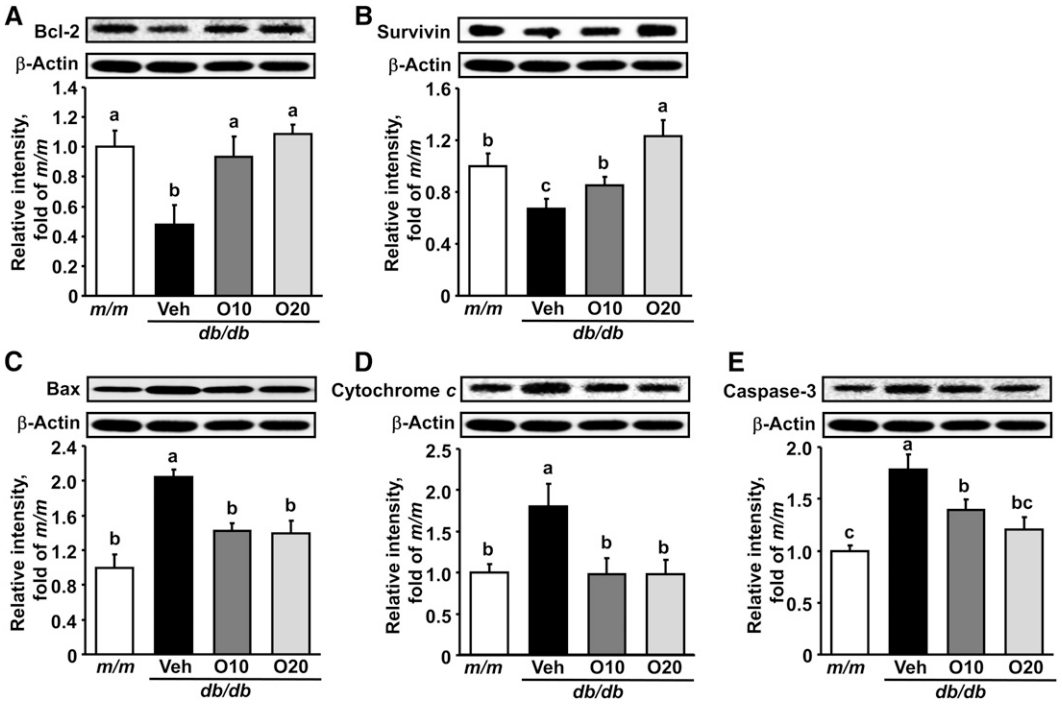

FIGURE 7 Renal Bcl-2 (*A*), survivin (*B*), Bax (*C*), cytochrome *c* (*D*), and caspase-3 (*E*) protein expressions in *m/m* mice and in *db/db* mice treated with water or oligonol for 8 wk. Values are means ± SEMs, $n = 10$ (*db/db*) or 6 (*m/m*). Labeled means without a common letter differ, $P < 0.05$ (Duncan's test). Bax, B-cell lymphoma protein 2–associated X protein; Bcl-2, B-cell lymphoma protein 2; *m/m*, vehicle-treated *m/m* mice; O10, oligonol 10 mg/(kg body weight · d)–treated *db/db* mice; O20, oligonol 20 mg/(kg body weight · d)–treated *db/db* mice; Veh, vehicle-treated *db/db* mice.

nor fluorescence (17). To add to these findings, the aim of the present study was to investigate the effect of oligonol on the possible mechanisms of AGE formation from glucose via the Maillard reaction. We evaluated the protein expressions of AGEs and receptors, such as pentosidine, CMA, GA-pyridine, CML, CEL, and RAGE. Many different AGEs are formed in various pathways (**Fig. 8**). Pentosidine, a crosslink between arginine and lysine, is a well-characterized AGE (36). Recently, serum pentosidine concentrations were shown to be strongly related to peripheral arterial disease in patients with type 2 diabetes mellitus (37). From Odani et al. (38), CMA was suggested as a new marker of diabetic complications, in which its serum concentration was increased in patients with diabetes with non-renal failure compared with the normal group. CMA production was increased with the incubation

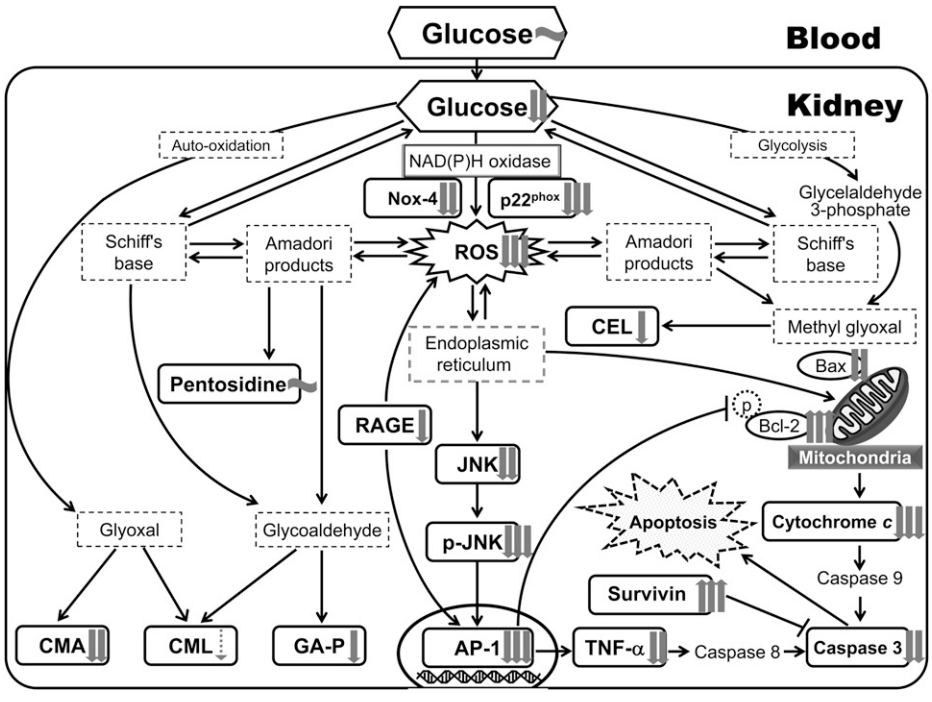

FIGURE 8 Predicted mechanism in renal tissues on administering oligonol against oxidative stress, AGE formation, and apoptosis. The oligonol attenuated glucose accumulation and subsequently decreased NAD(P)H oxidase–derived ROS production in the kidneys. Furthermore, oligonol ameliorated the values of AGE-related protein expressions (RAGE, CMA, GA-pyridine, and CEL), antiapoptotic protein expressions (Bcl-2 and survivin), and apoptotic protein expressions (JNK, p-JNK, AP-1, Bax, cytochrome *c*, and caspase-3 proteins). AGE, advanced glycation end product; AP-1, activator protein 1; Bax, B-cell lymphoma protein 2–associated X protein; Bcl-2, B-cell lymphoma protein 2; CEL, N^{ε}-(carboxyethyl)lysine; CMA, N^{ε}-(carboxymethyl) arginine; CML, N^{ε}-(carboxymethyl)lysine; GA-P, glycolaldehyde-pyridine; JNK, c-Jun N-terminal kinase; Nox-4, NAD(P)H oxidase-4; p-JNK, phosphorylated c-Jun N-terminal kinase; $p22^{phox}$, p22 phagocytic oxidase; RAGE, receptor for advanced glycation end product; ROS, reactive oxygen species.

time in parallel, and its yield was greater than that of pentosidine (39). GA was formed either as a fragmentation product in the Maillard reaction or as a result of the myeloperoxidase–hydrogen peroxide–chloride reaction (40,41). GA-pyridine, a specific GA-derived AGE, was described in foam cells and the extracellular matrix of human atherosclerotic fibrotic lesions and was also found in mesangial accumulation in human renal disease (42). 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 (41). Taking the abovementioned studies together, AGE formation and accumulation and interaction with RAGE were increased and aggravated under the diabetic complication, consistent with our findings, including the upregulated expression of these proteins in the kidneys of mice with type 2 diabetes. However, oligonol administration effectively downregulated AGE-related protein expression (RAGE, CMA, GA-pyridine, CML, and CEL). These results help to elucidate its potential therapeutic value, including the delay of AGE-related disorder in diabetes. The renoprotective effects of oligonol in diabetes were not only attributable to improved renal glycemic adjustment alone but also likely reflected its antioxidant activity. This antioxidant activity combined with the reduction in glycation by oligonal should be particularly advantageous and perhaps even synergistic in preventing renal injury and other diabetic complications.

A possible pathway of ROS-associated apoptosis was suggested by the observation that increased oxidative stress activates JNK and p38 MAPK through apoptosis signal-regulating kinase 1 (43). JNK activation was implicated in the regulation of TNF-α–induced JNK and apoptosis (44). Otherwise, the suppression of JNK and p38 MAPK mediated antiapoptotic signaling (45). In the present study, we evaluated the expression of proapoptotic cellular signal-related protein in diabetic renal tissues. The renal protein expressions of JNK, phosphorylated JNK, c-Jun, and TNF-α were augmented in the *db/db* mice with diabetes compared with normal *m/m* mice. These upregulated proteins were markedly downregulated by oligonol treatment. This result indicates that the downregulation of TNF-α or oxidative stress–mediated JNK signaling affected the renal apoptotic response.

Therefore, we subsequently examined renal apoptosis-related protein expression in the kidneys 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 podocyte density is an important determinant of progressive diabetic nephropathy and precedes the development of renal dysfunction and albuminuria in patients with diabetes and in animal models of diabetes mellitus (2). The marked increase in podocyte apoptosis observed during the early stage of diabetes in *db/db* mice is mediated to a large extent by the activation of NAD(P)H oxidase and its contribution to the generation of ROS (2). Our results support the idea that the decrease in NAD(P)H oxidase expression affect the proapoptotic protein expression. Oligonol administration led to a substantial increase in Bcl-2 and survivin and a reduction of Bax, cytochrome *c*, and caspase-3 protein in the renal tissue of *db/db* mice. These data suggest that the attenuation of diabetic renal apoptosis is probably influenced by decreased NAD(P)H oxidase–dependent oxidative stress and/or JNK-related signaling.

In conclusion, these studies showed that oligonol, a low-molecular-weight polyphenol treatment, was able to improve diabetic indices, prevent the development of diabetic renal disease, and preserve renal cells and renal morphologic structure via attenuation of NAD(P)H oxidase–induced oxidative stress, inhibition of AGE generation, and prevention of apoptosis-induced cell death in the diabetic process of *db/db* mice, which was independent of changes in body weight or serum glucose concentrations (Fig. 8). Accordingly, oligonol may be a promising dietary supplement for the prevention or delaying of diabetic renal complications.

Acknowledgments

T.Y. designed the research; J.S.N. and C.H.P. performed the experiments; J.S.N. and C.H.P. analyzed the data; T.Y., J.S.N., and C.H.P. wrote the manuscript; J.S.N. and T.Y. had primary responsibility for final content. All authors read and approved the final manuscript.

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