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Anti-Diabetic Effect of Alkaline-Reduced Water on OLETF Rats

Dan Jin,[1,2] Sung Hoon Ryu,[3] Hyun Won Kim,[3] Eun Ju Yang,[4] Soo Jung Lim,[4]
Yong Suk Ryang,[4] Choon Hee Chung,[5] Seung Kyu Park,[6] and Kyu Jae Lee[6,†]

[1]*Department of Microbiology, Wonju College of Medicine, Yonsei University, Wonju, Gangwon 220-701, Korea*

[2]*Department of Microbiology and Immunology, Yanbian University College of Medicine, Yanji 133000, China*

[3]*Department of Biochemistry, Wonju College of Medicine, Yonsei University, Wonju, Gangwon 220-701, Korea*

[4]*Department of Biomedical Laboratory Science and Institute of Health Science, College of Health Science,
Yonsei University, Wonju, Gangwon 220-710, Korea*

[5]*Department of Internal Medicine, Wonju College of Medicine, Yonsei University, Wonju, Gangwon 220-701, Korea*

[6]*Department of Parasitology and Institutes for Basic Medical Science, Wonju College of Medicine, Yonsei University,
Wonju, Gangwon 220-701, Korea*

**Alkalin-reduced water (ARW) is known to exert
several anti-cancer effects, as well as to scavenge
reactive oxygen species (ROS) and reduce blood-glucose
levels. This study was performed in order to determine
the effects of ARW on the control of spontaneous
diabetes in Otsuka Long-Evans Tokushima Fatty
(OLETF) rats.**

**We assigned 16 male OLETF rats (4 wk) to two
groups: an experimental group, which was given ARW,
and a control group, which received laboratory tap
water. From week 6 to 32, the body weight, lipid
composition, and glucose levels in the blood of the rats
were measured. The glucose levels of both groups
tended to increase. However, the ARW group's glucose
levels were significantly lower than those of the control
group after 12 weeks ($p < 0.05$). The total cholesterol
and triglyceride levels in the ARW group were found to
be significantly lower than those of the control group
during the experimental period.**

**These results suggest that ARW spurred the growth
of OLETF rats during the growth stage, and that long-
term ingestion of ARW resulted in a reduction in the
levels of glucose, triglycerides, and total cholesterol in
the blood.**

Key words: alkaline-reduced water (ARW); Otsuka
Long-Evans Tokushima Fatty (OLETF)
rat; diabetes; cholesterol

Alkaline-reduced water (ARW) is generated either by
electrolysis, or by a chemical reaction with alkaline
earth metals. In nature, a variety of minerals, including
Mg, Ca and Li, exhibit the capacity to change water into
alkaline water. Mg becomes magnesium hydroxide
when it reacts with water. ARW has been determined
to exert a suppressive effect on the free radical level in
living organisms, thereby resulting in disease preven-
tion.[1] ARW also exhibits an antioxidative function,[1]
scavenges reactive oxygen species (ROS),[2] accelerates
growth, and promotes metabolism.[3] Huang (2003) *et al.*
have previously demonstrated the effects of ARW on
end-stage renal disease patients, in whom the combined
use of electrolyzed reduced water during hemodialysis
caused a reduction in oxidative stress.[4]

Diabetes is a metabolic disease, which is accompa-
nied by a variety of complications, caused by either
insulin deficiency or insulin tolerance. Abnormal lipid
metabolism also constitutes a principal cause of morbid-
ity and death, and is known to be the triggering factor in
a host of microvascular and macrovascular complica-
tions.[5,6] Hyperglycemia is the primary risk factor of
atherosclerosis and, as a consequence, is a factor in
coronary heart disease (CHD).[7,8] It is important to
control hyperglycemia, as CHD associated with hyper-
glycemia is a primary cause of death in type II diabetes
patients.[9]

Hyperglycemia and hyperlipidemia are known to be
related to the ROS levels in blood vessels, tissues, and
cells.[10–12] Moreover, it has been recognized that the
scavenging of ROS and the control of lipid metabolism
are both quite relevant to the control of diabetes. For
these reasons, research into the relationship between
antioxidants and the control of lipid metabolism in
diabetes is an important field of study.[13,14]

Kim and Yokoyama (1997) have previously reported
that the administration of ARW to GK rats resulted in a

reduction in the blood levels of glucose and lipid peroxide.[15] Another researcher has also reported that ARW could substantially increase the activity of hexokinase, which is a pivotal enzyme inducing the reduction of blood glucose levels.[16]

Although ARW is believed to be effective by an antioxidative mechanism, scientific approaches designed to elucidate its functions have classically proven insufficient. Based on previous results on the anti-diabetic effects of ARW, this study was designed to confirm the lipid and glucose levels in the blood of Otsuka Long-Evans Tokushima Fatty (OLETF) rats. These rats are thought to constitute a viable model for human type II diabetes.

Materials and Methods

ARW. ARW was generated by Alkalogen® sticks (HDr, Korea) which contain Mg within a plastic housing. The sticks were placed into water bottles for feeding. When the Mg comes into contact with water, it reacts as follows:

$$Mg + 2(H_2O) \rightarrow Mg(OH)_2 + H_2(= 2H + 2e^-) \uparrow$$

$$\rightarrow Mg^{2+} + 2OH^- + H_2 \uparrow .$$
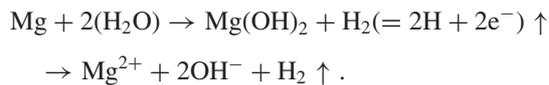
The pH value and oxidation-reduction potential (ORP) of ARW were respectively controlled within pH 10.0–10.5 and below -100 mV (Table 1).

Experimental animals. Four-week old male OLETF rats were donated by Otsuka Pharmaceuticals Co. (Japan). The rats were supplied with food (Superfeed company, Korea; Table 2) and water, and reared at a temperature of 22 °C, a humidity of $56 \pm 5\%$, and a 12-hour photoperiod until the end of this study. The rats were assigned to control ($n = 8$) and ARW ($n = 8$) groups after adaptation. The rats in the control group were given laboratory tap water with a composition of 6.45 mg/l of Ca, 0.66 mg/l of Mg, 10.02 mg/l of Na, 0.06 mg/l of Fe, and 0.68 mg/l of K, and in the ARW group were given ARW.

The study was approved by the Yonsei University

Table 2. Composition of the Diet

	Per 100 g
General analysis	
Moisture	11.00 g
Crude protein	22.30 g
Crude fat	5.90 g
Crude ash	6.70 g
Crude fiber	3.20 g
Nitrogen-free extract	50.90 g
Calories	346 kal
Mineral analysis	
Ca	1150 mg
P	750 mg
Mg	200 mg
Na	400 mg
K	880 mg
Fe	14.00 mg
Cu	1.80 mg
Zn	13.00 mg
Co	0.05 mg
Mn	10.00 mg
Cl	0.58 mg
S	0.25 mg
I	0.08 mg
Se	0.04 mg

Animal Committee, and the animals were maintained in accordance with the guidelines for the care and use of laboratory animals of Wonju College of Medicine, Yonsei University.

Changes in the body weight and blood parameters. Changes in the body weight were measured at 1-week intervals, from week 6 to week 32. Blood was taken from the tail vein of each rat at 4-week intervals, and the parameters were measured with Cholestech L.D.X® (Cholestech, U.S.A.). The blood parameters that were measured were total cholesterol, very-low-density lipoprotein (VLDL), high-density lipoprotein (HDL), low-density lipoprotein (LDL), and glucose. On week 32, we also observed glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT), both of which are important transamines, in the rat sera.

Statistics. Differences between the two groups were assessed by Student's *t*-test, using Prism version 3.0 software (GraphPad Software, U.S.A.). Each data value is expressed as the mean $\pm$ SD.

Results

Change in body weight

The body weight changes of the two groups were not significantly different until week 24. The ARW group then exhibited increased body weight, whereas the control group exhibited unchanged or reduced body weight between weeks 24 and 32. We observed a significant difference in the average body weight in both groups between weeks 24 and 32 ($p < 0.05$) (Fig. 1).
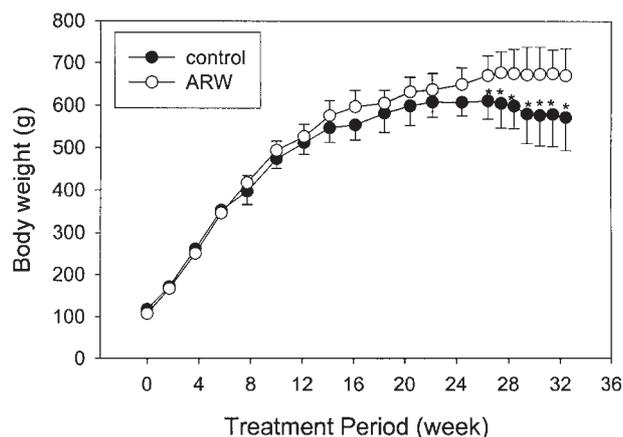
Table 1. Changes in pH and ORP Values

Time (hour)	pH		ORP (mV)	
	Experimental (ARW)	Control	Experimental (ARW)	Control
0	7.38	7.35	531.2	531.2
1	9.17	7.39	−143.7	531.1
2	9.81	7.38	−169.1	531.2
3	9.98	7.38	−175.7	531.2
4	10.12	7.39	−180.5	531.1
5	10.38	7.38	−184.8	531.1
6	10.54	7.38	−197.1	530.9
7	10.55	7.38	−197.3	530.7
12	10.51	7.39	−195.2	530.8
24	10.49	7.37	−194.7	529.4

Fig. 1. Changes in Body Weight.

Five-week-old OLETF rats were given laboratory tap water (n = 8) or ARW (n = 8) for 32 weeks. Each data value is expressed as the mean ± SEM (*$p < 0.05$).
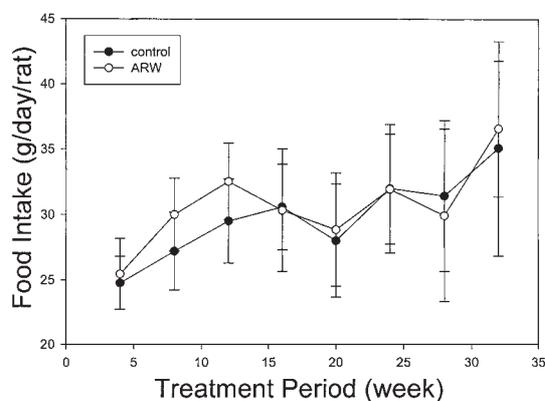

Fig. 2. Level of Food Intake.

Five-week-old OLETF rats were given laboratory tap water (n = 8) or ARW (n = 8) for 32 weeks. Each data value is expressed as the mean ± SEM.

Levels of food intake and water drunk

Neither the food intake (Fig. 2) nor water drunk (Fig. 3) was significantly different between the control group and ARW group until week 32.

Glucose, lipids, and lipoproteins in the blood

The glucose level increased in all of the rats from week 6 to 32. The glucose level in the ARW group decreased ($p < 0.05$) between weeks 12 and 32 (Fig. 4). The total cholesterol (Fig. 5) and triglyceride (Fig. 6) levels in the ARW group were significantly lower between weeks 6 and 32 than the equivalent levels in the control group.

GOT and GPT in the rat sera

The GOT level in the ARW group was determined to be significantly lower than that of the control group ($p < 0.05$) at week 32 (Fig. 7). The GPT level in the ARW group was also lower than that of the control group, although this difference was not statistically significant (Fig. 8).
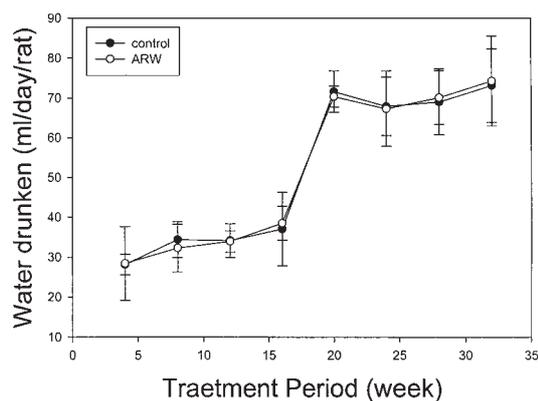

Fig. 3. Level of Water Drunk.

Five-week-old OLETF rats were given laboratory tap water (n = 8) or ARW (n = 8) for 32 weeks. Each data value is expressed as the mean ± SEM.
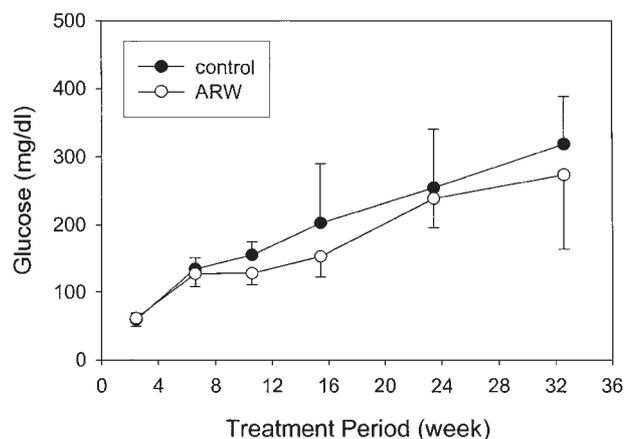

Fig. 4. Difference in Blood Glucose.

Five-week-old OLETF rats were given laboratory tap water (n = 8) or ARW (n = 8) for 32 weeks. The blood glucose level was measured at 4-week intervals by Cholestech L.D.X® (U.S.A.) using blood extracted from the tail vein. Each data value is expressed as the mean ± SEM.
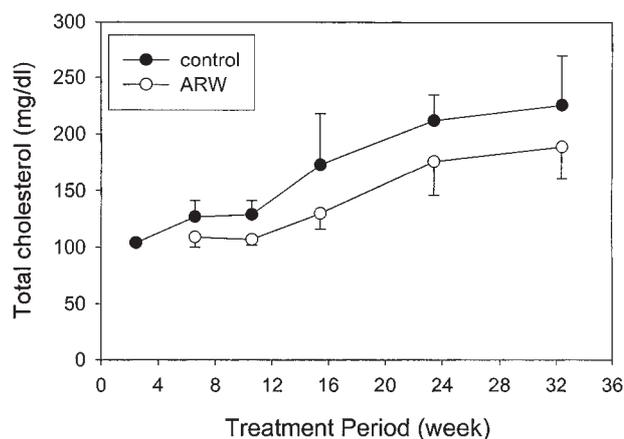

Fig. 5. Difference in Total Cholesterol Level.

Five-week-old OLETF rats were given laboratory tap water (n = 8) or ARW (n = 8) for 32 weeks. The total cholesterol level was measured at 4-week intervals by Cholestech L.D.X® (U.S.A.), using blood obtained from the tail vein. Each data value is expressed as the mean ± SEM.
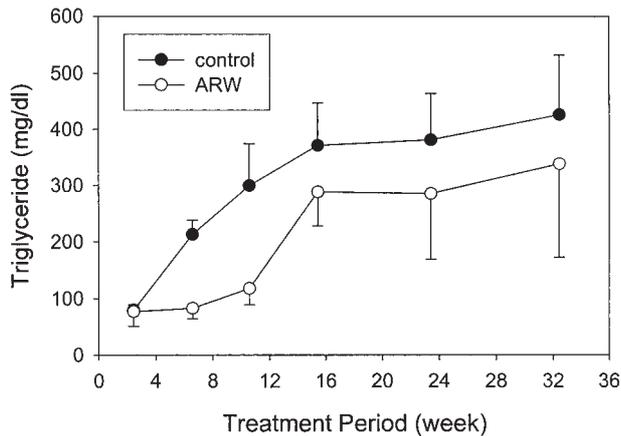
Fig. 6.　Difference in Triglyceride Level.
　　Five-week-old male OLETF rats were given laboratory tap water (n = 8) or ARW (n = 8) for 32 weeks. The triglyceride level was measured at 4-week intervals by Cholestech L.D.X® (U.S.A.), using blood obtained from the tail vein. Each data value is expressed as the mean ± SEM.
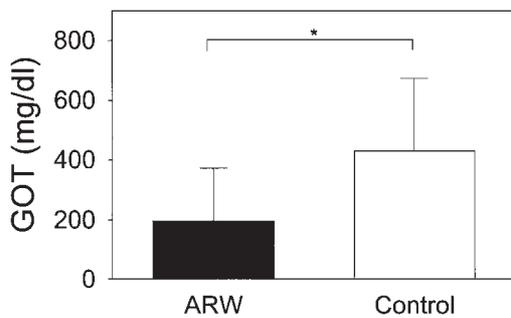

Fig. 7.　Concentration of GOT in the Serum.
　　Five-week-old male OLETF rats were given laboratory tap water (n = 8) or ARW (n = 8) for 32 weeks. On week 32, the glutamic oxaloacetic transaminase (GOT) level in the rat serum was determined. Each data value is expressed as the mean ± SEM ($*p < 0.05$).

Discussion

　Diabetes is a metabolic disease that is accompanied by a host of complications, most of which are attributable to continuous hyperglycemia. The causes of the disease in diabetic patients include insulin deficiency and insulin tolerance. Diabetes gives rise to both acute and chronic complications. The acute complications can be triggered by metabolic disorders, including ketoacidosis and non-ketotic coma and infections, but these symptoms can be relatively well controlled. However, the chronic complications tend to worsen as the diabetes progresses. Chronic complications associated with diabetes include macroangiopathies such as coronary artery disease and cerebrovascular disease, and microangiopathies such as neuropathy, orthostatic hypotension, retinopathy and nephropathy.
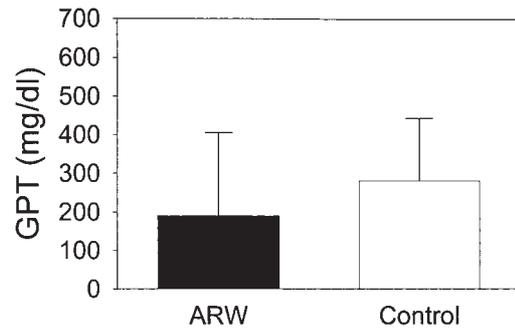
　Macroangiopathy is triggered by multiple factors,

Fig. 8.　Concentration of GPT in the Rat Serum.
　　Four-week-old male OLETF rats were given laboratory tap water (n = 8) or ARW (n = 8) for 32 weeks. On week 32, the glutamic pyruvic transaminase (GPT) level in the rat serum was determined. Each data value is expressed as the mean ± SEM.

such as increased LDL-C, hypertriglycemia, and decreased HDL-C. Dysfunctions of the capillary circulatory system, an abnormal increase in glucose metabolism, and genetic susceptibility also all exert significant effects on microangiopathy. These complicated pathological causes are believed to be related to the principal mechanisms for ROS and oxidative stress. Increases in oxygen radicals and lipid peroxide due to monosaccharidic oxidation induce oxidative stress in a variety of tissues, and also induce oxidative stress to DNA in diabetes patients. Nitric oxide (NO) generated within angio-endothelial cells can also inhibit the aggregation and adhesion of platelets, weaken the adhesive function of monocytes, and suppress the proliferation of vascular smooth muscle cells. Hyperglycemia directly suppresses the activation of NO synthase. A great deal of research has recently demonstrated that ROS are directly related to diabetic complications.

　The effects of ARW have only recently been observed in studies of diabetes. Moreover, it has only recently been suggested that ARW might have some effects on blood glucose and lipid metabolism.

　We observed in this study the effect of ARW on OLETF rats. OLETF rats can be used as an animal model for type II diabetes, the symptoms of which also constitute the principal risk factors for atherosclerosis, including obesity, hyperglycemia, hypertension, and hyperlipidemia.[17,18]

　The glucose level of the control group was 202.5 ± 96.5 mmol/dl at week 18, while that of the ARW group only reached 202.5 ± 96.5 mmol/dl on week 26. The blood glucose level in the ARW group was consistently lower than that the control group. These results indicate that ARW induced a reduction in the blood glucose level. This is believed to be attributable to up-regulation of the hexokinase activity by ARW.[11]

　Strawn has reported that the possibility of both microvascular and macrovascular complications worsened when combined with hypercholesterolemia. He also determined that ROS triggered diabetic and atherosclerotic complications, by linking hyperglycemia

and hypercholesterolemia to such complications. Oxidative stress associated with angiotensin II functions as a causative factor in an endothelial dysfunction, by triggering both hyperglycemia and hypercholesterolemia. The endothelial dysfunction then results from suppression and inactivation of NO generation in the endothelium. Other researchers have also confirmed that angiotensin II played an important role in the development of both atherosclerosis and glomerulosclerosis.[10] Harrison *et al.* have reported that angiotensin II increased the incidence of cardiovascular diseases, including hypertension, hypercholesterolemia, atherosclerosis, coronary artery disease, left ventricular hypertrophia, heart failure, and diabetes. Angiotensin II has also been implicated in the activation of NAD(P)H oxidase, which is one of the principal factors in the generation of ROS within vascular cells.[19]

Cai *et al.* have emphasized that NAD(P)H oxidase in blood vessels might be a principal factor in the cure of cardiovascular diseases.[20] Over several years of research, they have confirmed the existence of a novel NAD(P)H oxidase system, now referred to as the non-phagocytic NAD(P)H oxidase protein. They have also confirmed that cardiovascular diseases including atherosclerosis and hypertension resulted from ROS generated within the blood vessels by this enzyme. ROS generated by lipid metabolism in the blood vessels has been regarded as a principal factor in the control of diabetes, as it is often observed in diabetic patients.

The total cholesterol and triglyceride levels in the ARW group were determined in this study to be significantly different from the levels in the control group, a difference that persisted for several weeks. We assume that ARW induced a reduction in the blood glucose level, and that this affected the lipid metabolism in turn. A high level of VLDL could be corrected after normalizing hyperglycemia.[18]

The levels of cholesterol, triglycerides, and glucose in the ARW group were lower than the corresponding levels in the control group. Although the precise mechanisms underlying these results, depending on the experimental period, could not be confirmed, we believe that ARW functioned as an antioxidant involved in changes to the total lipid metabolism, thereby causing the difference in body weight between the two groups. The changes in body weight throughout the experiment are consistent with the report by Watanabe that ARW induced enhanced growth during the growth period. He reported that this growth-stimulating effect could be observed in the change in body weight, and in the development of various organs in the rats receiving ARW during the nursing period.[3]

We confirmed in this study that the body weight of the ARW group was higher than that of the control group. This suggests that ARW had a significant growth-accelerating effect, and also induced a reduction in the lipid level in the blood.

We confirmed throughout this study that the administration of ARW could relieve diabetic parameters in the blood, including the levels of glucose, triglycerides, and cholesterol. In particular, ARW was confirmed to exert a ROS-scavenging effect.[2,4]

Hanaoka has reported that antioxidants dissolved in reduced water exhibited superoxide dismutase activity. He suggested that an increase in the superoxide dismutase activity, like that seen with a proton donor such as L-ascorbic acid, *d*-catechin or quercetin, was attributable to an increase in the dissociation activity of water, whereas the scavenging activity seen in conjunction with hydrogen peroxide was attributable to activated dissolved H_2 in the reduced water. According to his results, the dissociation constant of reduced water was increased 1.46-fold.[1]

Diabetic subjects have been shown to have increased oxidative stress and a decreased antioxidant level.[21–23] Moreover, disturbance of the antioxidant defense system has been shown with diabetes: alteration in antioxidative enzymes,[24] impaired glutathione metabolism,[25] and a decreased ascorbic acid level.[26] Several studies have reported that some substances having antioxidative activity had the effect of controlling the blood glucose and complications in animal models and patients with diabetes.[27–30] For example, Sreemantula *et al.* have indicated that L-ascorbic acid, as a well-known antioxidant, produced hypoglycemic activity in a dose-dependant manner with a diabetic condition.[30] There have been reported many similar cases of the antioxidative function reducing the serum lipid level.[31,32] Our previous study has also shown that ARW had antioxidative activity, and that this antioxidative activity of ARW was like that of L-ascorbic acid (unpublished data). We therefore consider the effects of ARW on the OLETF rats in this study to have been due to the antioxidative activity.

GOT and GPT comprise the most important amino transferases in humans. When the coronary artery is blocked by lipid deposition, serious oxygen deficiency follows, and the heart muscles become partially degenerated as a consequence. Simultaneously, GOT and GPT are secreted from the damaged heart cells into the blood. We determined in this study that the GOT and GPT values in the ARW group were lower than those of the control group at week 32. This suggests that ARW had a significant effect on the prophylaxis of coronary artery diseases, as well as heart diseases caused by diabetic complications. Although, the difference in GOT concentration between the control and ARW group reached statistical significance ($p = 0.0325$), this was not the case with the GPT concentration. This was due to the fact that GOT was secreted into the blood vessels before GPT.

We conclude that ARW exerted important effects in preventing and controlling diabetic complications, and further investigations into its mechanisms, especially as related to diabetic diseases, are clearly warranted.

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