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Antiinflammatory and Hypoglycemic Efficacy of *Poria cocos* and *Dioscorea opposite* in Prediabetes Mellitus Rats

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Running Title: *Poria cocos* and *Dioscorea opposite* ameliorate pre-DM status in rats

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ABSTRACT

Poria cocos (Fu Ling) and *Dioscorea opposite* (Chinese Yam) were suggested to have potential benefits in blood sugar control. However, little is known about the underlying mechanisms. In this study, we investigated the antiinflammatory and hypoglycemic effects of *Poria cocos* and *Dioscorea opposite* extracts in prediabetic rats. Fifty streptozotocin-injected rats with a mimic prediabetic status were gavaged with a single dose of either *D. opposite* (0.35 g/kg/d) or or *P. cocos* (0.14 g/kg/d), a combination dose comprising single doses of the 2 herbal extracts, or vehicle for a 6-week treatment. Each group contains 10 rats. Blood and selected organ samples were collected during the study. The results indicated that administering the extracts singly or in combination for 6 wk significantly reduced the fasting blood-glucose level. The levels of interleukin-6 in plasma and selected organs decreased significantly during the treatment. The concentration of plasma free fatty acids (FFA) and the ratio of n-6/n-3 polyunsaturated fatty acids (PUFAs) were also lowered significantly after 6 wk intervention. The results indicate that administering *P. cocos* and *D. opposite* extracts produced antiinflammatory effects in prediabetic rats by reducing the levels of interleukin-6 and the FFA ratio of n-6/n-3 PUFAs.

Keywords: prediabetes, antiinflammatory, *Poria cocos*, *Dioscorea opposite,* hypoglycemic, polyunsaturated fatty acids

1. Introduction

Metabolic syndrome (MetS) is characterized by central obesity, insulin resistance, impaired glucose tolerance, abnormal lipoprotein metabolism, and hypertension. MetS has been reported to be correlated with a high disease and economic burden in patients.[1] In MetS and type 2 diabetes mellitus (T2DM), a condition that is considered to be a progressive state and an intermediate state, respectively, is prediabetes, which is characterized by impaired glucose tolerance or fasting-glucose levels.[2,3] More than 25% of patients with a prediabetes status become T2DM patients within 5 y in the United States[4] and Taiwan.[5] Patients diagnosed with diabetes have accompanying vascular complications and the dysfunction of over 50% of pancreatic β cells. Therefore, to prevent T2DM, abnormal glucose metabolism must be reversed and a reliable early predictor for preventing pre-DM must be identified.[6]

Previously, a low ratio of n-6/n-3 polyunsaturated fatty acids (PUFAs) was reported to affect the development of cardiovascular disease.[7] Current Western diets are highly caloric and feature inadequate lipid profiles and dietary ratios of n-6/n-3 PUFA.[8] N-3 PUFAs have been reported to compete with n-6 PUFAs as substrates for cyclooxygenases, and n-3 PUFAs often exert physiological effects that are the opposite of those exerted by n-6 PUFA eicosanoid products. Therefore, the use of the ratio of n-6/n-3 PUFA as a biomarker in dietetics to prevent chronic disease should be considered.

Increasing evidence indicates that elevated levels of advanced glycation end products (AGEs) contribute to the development of several related vascular diseases and to the progression of diabetes.[9] Patients with a hyperglycemia status are considered to experience chronic inflammation.[10] Glycated proteins have been reported to function as triggers that propagate non-enzymatic chain reactions leading to AGE formation[11] and activate the expression of receptors for AGEs (RAGEs). RAGEs, which participate in signal transduction by activating Nuclear Factor kappa B (NF-κB), have been implicated in the development of various disorders.[12] Activation of RAGE ligands initiates a chronic inflammatory pathway that contributes to the pathogenesis of diabetic complications.[13] In addition, AGEs and RAGEs promote the release of proinflammatory cytokines (i.e., tumor necrosis factor-α [TNF-α], interleukin-1 [IL-1], and interleukin-6 [IL-6]) by macrophages, and this triggers the recruitment of inflammatory mediators and results in an acceleration of tissue dysfunction. These findings suggest that blocking RAGE expression could serve as an effective approach in treating a range of diabetic complications[14], particularly diabetic renal dysfunction.[15]

Poria cocos (Fu Ling) and *Dioscorea opposite* (Chinese yam) are well-known and widely used Chinese herbs that have been reported to exhibit antidiabetic and antiinflammatory effects.[16-19] Certain Chinese herbs are also included in diets to control blood sugar in patients with hyperglycemia.[20] However, the potential mechanisms underlying the antidiabetic effects of *P. cocos* and *D. opposite*, which could depend on the antiinflammatory or hypoglycemic properties

of the herbs, remain unknown. Therefore, in this study, we tested the hypothesis that *P. cocos* and *D. opposite* crude extract exert antiinflammatory and hypoglycemic effects in prediabetic rats.

2. Materials and methods

2.1 Preparation of plant materials

Crude extracts of white Fu Ling (*P. cocos*) and Chinese yam (*D. opposite* Thunb; Tainong 2) were purchased from Derling Biotech Co Ltd. (Nantou, Taiwan). The selected crude extracts were prepared with partial modification as follows[21]. The fruiting bodies were washed, diced, shredded, rinsed with 3 parts of water for 8 h, and then extracted 3 times (for 2 h each) using the same volume of 70% ethanol at room temperature. After centrifugation (at 3,000 × *g* for 20 min at 5 °C), the supernatants were collected and freeze-dried to obtain a powder. The extraction rates of the *P. cocos* and *D. opposite* crude extracts were 8.96:1 and 13:1, respectively. All chemicals and solvents used in this study were obtained from Sigma (St. Louis, MO, USA) unless otherwise specified.

2.2 Animal protocols and experimental design

We used 60 7-wk-old Sprague-Dawley rats (BioLASCO Taiwan, Taipei, Taiwan) that weighed 250–300 g at the start of the study. The rats were housed in a temperature- and humidity-controlled room (22–24 °C and 60%) on a 12-h light-dark cycle (lights on 08:00–20:00). The rats

were allowed to adapt for 1 wk, were fed Rodent Laboratory Chow 5001, and then used in an 8-wk study.

A pre-DM status was induced in 50 rats within 2 wk, and these animals were assigned to receive the following treatments: a single dose of either herb (0.35 g/kg/d of the *D. opposite* crude extract [D] or 0.14 g/kg/d of the *P. cocos* crude extract [P]) and a combination dose comprising single doses of the 2 herbal extracts (0.35 g/kg/d of the *D. opposite* crude extract and 0.14 g/kg/d of the *P. cocos* crude extract [M]); or the vehicle (0.5 mL of normal saline, [C; pre-DM control]). Each group contained 10 rats. The remaining 10 rats were assigned to the "normal" group [N] and they received no treatment during the study. Detailed procedures used for producing a pre-DM status are described elsewhere.[22,23] Briefly, the rats received a single injection of 20 mg/kg streptozotocin (STZ) intraperitoneally to induce a mimic prediabetes status. The fasting blood-sugar level of qualified STZ-treated rats was determined to be 140–200 mg/*dL* before the intervention. After a prediabetes status was confirmed, the rats were gavaged with specified amounts of herbal crude extracts, daily for 6 wk. Venous blood was collected from the tails of the rats in all the groups at baseline, after 3 and 6 wk and at the end of the experiment. The animals were provided an American Institute of Nutrition-93M (AIN-93M) diet and water, ad libitum. These conditions were maintained constant throughout the experiment. At the end of the experimental period, all the animals were euthanized using carbon dioxide, the kidney and liver were excised, and abdominal blood was collected for analyses. All chemicals used in this

study were obtained from Sigma (St. Louis, MO, USA). The animals were housed 2/cage, and all the procedures used complied with the guidelines of the Institutional Animal Care and Use Committee of Taipei Medical University (LAC-99-0271).

2.3 Analysis of the glucose metabolic index and lipid profile

Serum was analyzed to measure a glucose metabolic index (fasting glucose, insulin, and homeostasis model assessment of insulin resistance [HOMA-IR]) and lipid profile (serum triglyceride [TG], total cholesterol [TC], low-density lipoprotein-cholesterol [LDL-c], and high-density lipoprotein-cholesterol [HDL-c]). To measure fasting blood-glucose levels, overnight tail-vein blood samples were collected from the animals; the glucose levels were measured using a OneTouch® Ultra® Blood Glucose Test System Kit (LifeScan, Milpitas, CA, USA) at baseline, and after 3, 6, and 8 wk. The serum insulin level was determined using a radioimmunoassay kit (DIA Source, Lovain-La-Nueve, Belgium). Serum HbA1c was determined using an HLC-723 GHb G7 analyzer (Tosoh, Tokyo, Japan). The HOMA-IR was performed as described previously.[24] TG and TC levels were measured using an Ortho Clinical Diagnostics VITROS 950 automated analyzer (Johnson & Johnson, New Brunswick, NJ, USA). Serum LDL-c and HDL-c levels were determined using a TBA-c16000 automated analyzer (Toshiba, Tokyo, Japan).

2.4 Analysis of the proinflammatory index

Serum and tissue samples were analyzed to measure a proinflammatory index (TNF-α, IL-6, and C-reactive protein [CRP]). Serum and tissue samples were mixed or homogenized with ice-cold 0.1 M phosphate buffer (pH 7.4) and centrifuged at 12,000 × *g* for 20 min at 4 °C, and the supernatants obtained were mixed with a protease-inhibitor cocktail (Sigma). A rat TNF-α platinum-sandwich enzyme-linked immunosorbent assay (ELISA) kit (Cat. No. BMS622, eBioscience, Vienna, Austria) was used to determine the TNF-α levels in serum and selected tissues. Briefly, homogenates and standards were pipetted into 96-well microplates precoated with a TNF-α-specific monoclonal antibody. An enzyme-linked monoclonal antibody specific to TNF-α was then added to the wells. After 60 min, any unbound antibody-enzyme complex was washed out, and a color-development substrate solution was added to the wells to determine the amount of TNF-α bound in the initial step. Color development was then stopped, and the color intensity was measured within 30 min using a microplate reader (Versa Max Microplate Reader, Molecular Devices, Sunnyvale, CA, USA) set to 450 nm. IL-6 levels in the serum and selected tissues were determined using a rat IL-6 platinum ELISA kit (Cat. No. BMS625, eBioscience, Vienna, Austria). The ELISA procedures used were similar to those used to determine the TNF-α levels, and the color intensity was measured at 405 nm. The total protein content of the samples was measured using the Bradford method.[25] The serum CRP levels were determined using a TBA-c16000 automated analyzer (Toshiba, Tokyo, Japan). VCAM levels in the plasma were determined using a rat vascular cell adhesion protein 1 (VCAM-1/CD106) ELISA kit (Cat. No.

CSB-E07275r, CUSABIO, Wuhan, China). E-selectin levels in the plasma were determined using a rat soluble E-selectin (sE-selectin) ELISA kit (Cat. No. CSB-E07996r, CUSABIO, Wuhan, China). The ELISA procedures used were similar to those used to determine the TNF-α levels, and the color intensity was measured at 450nm.

2.5 Analysis of free fatty acid profiles

Detailed procedures used for analyzing plasma FFAs were described by Edward and Maes.[26] Plasma (270 μL) was extracted using distilled water (2 mL), methanol (2 mL), chloroform (2 mL), and supersaturated saline (1 mL).[27,28] After centrifugation at 3,500 × *g* for 15 min at 4 °C, the supernatants were transferred to a test tube and vacuum dried. Crude lipids in the plasma were dissolved in 200 μL of chloroform and, to separate the FFAs, were applied to a solid-phase extraction column (Bakerbond spe™ Amino Disposable Extraction Column, J.T. Baker, Center Valley, PA, USA). The FFAs extracted from each sample were transferred to test tubes that featured Teflon-lined screw caps, and were then dissolved in 200 μL of 14% boron trifluoride methanol (BF_3-methanol, Sigma) and 700 μL of methanol to methylate the fatty acids. Fatty-acid methyl esters were analyzed using a capillary gas chromatograph (Trace GC, Thermo Finnigan Trace GC, Milan, Italy) equipped with a 30-m-long, 0.32-mm-inner-diameter, 0.32-μm-df capillary column (Rtx®-2330 column, Restek, Bellefonte, PA, USA) and a flame ionization detector. Fatty-acid profiles were identified according to the retention times of appropriate standard fatty-acid methyl esters. Composition data are expressed as weight-percentages of total

fatty acids. We focused the analysis on 3 n-6 fatty acids: C18:2 linoleic acid (LA), C20:2 eicosadienoic acid (EDA), and C20:4 arachidonic acid (AA); 4 n-3 fatty acids: C18:3 α-linolenic acid (ALA), C20:5 eicosapentaenoic acid (EPA), C22:5 docosapentaenoic acid (DPA), and C22:6 docosahexaenoic acid (DHA); and the ratio of n-6/n-3 PUFAs.

2.6 Immunohistochemistry of AGEs and RAGEs

To perform immunohistochemical staining, small pieces of rat kidney cortex were fixed by immersing them in 4% formaldehyde buffer for 3 d and then embedding them in paraffin. The kidney tissue sections cut from the paraffin blocks were deparaffinized and then rehydrated using graded alcoholic solutions and phosphate-buffered saline (PBS). Renal paraffin sections (5 μm) were stained with primary antibodies against AGEs and RAGEs (Abcam Inc. Cambridge, MA, USA) for 3 h at 37 °C. After washing with the rinse buffer, biotinylated secondary antibodies were applied (antirabbit and antimouse IgGs, to detect AGEs and RAGEs, respectively) for 60 min at room temperature. Finally, sections were exposed to the chomogen (3,3′-diaminobenzidine tetrahydrochoride solution) and counterstained using 50% hematoxylin. The control sections were processed in parallel, in which mouse non-immune IgGs were used as the primary antibodies (at the same concentrations as the AGE and RAGE antibodies). To determine the AGE and RAGE expression levels, in each section, the percentages of AGE- and RAGE-expressing cells were calculated by counting cells in 5 low-magnification microscopic fields. Finally, all the sections were examined under a light microscope.

2.7 Statistical analyses

Statistical analyses were performed using SPSS Version 18.0 software (SPSS, Chicago, IL, USA). All values are presented as means ± SEM. Data were analyzed using one-way analysis of variance (ANOVA) to examine the effects of the treatments. Duncan's post hoc test was used to identify significant differences. $P < 0.05$ was considered statistically significant except where indicated otherwise.

3. Results

3.1 Chemical component analysis of *P. cocos* and *D. opposite*

The *D. opposite* crude extract contained 1.96% phenolic compounds (steroidal saponins 1.29%, tannins 0.32% and phytosterols 0.35%) and 5.2% dietary fiber (polysaccharose glycoside), whereas the *P. cocos* crude extract contained 2.62% phenolic compounds (pachymic acid 2.12%, pachymic acid methyl ester 0.31%, poricoic acid 0.14%, eburicoic acid 0.02%, tumulosic acid 0.02%, tumulosic acid methyl ester 0.01%) and 2.9% dietary fiber (pachyman, pachymaran 2.68% and other unknowns 0.22%).

3.2 Prediabetes status and growth of animals

To investigate the prediabetes status and the growth of rats during the experiment, we recorded their body weight and dietary intake. Body-weight changes and food intake of all the groups are

shown in Table 1. No statistically significant differences in body-weight change, food intake, or feeding efficiency was detected in the pre-DM groups. Final body weights in the pre-DM groups were significantly lower than that of the normal group ($P < 0.05$). Compared with the normal group, in the pre-DM groups, food intake was significantly higher ($P < 0.05$) and feeding efficiency (%) was significantly lower ($P < 0.05$). Liver- and kidney-function indices after the intervention lack of safety consideration (Table 1).

【Please insert Table 1 here】

3.3 Glycemic response and the lipid profile

When we measured the glycemic response, we confirmed that the induced fasting-glucose levels in all the test animals were 140–200 mg/dL after treatment with a low dose of STZ (Table 2). No statistically significant differences in fasting blood-glucose levels were detected among the groups at the beginning of the experiment. As expected, the fasting sugar levels of each test group after 3, 6, and 8 wk were significantly higher than those of the normal group ($P < 0.05$ for all). Animals treated with a single dose of the *P. cocos* crude extract or the combination of *P. cocos* and *D. opposite* crude extracts exhibited a significant reduction in fasting blood-glucose levels after 8 wk ($P < 0.05$) compared with those of the other herb-treated groups. FFA levels were significantly lower in the *P. cocos* group ($P < 0.05$) than in the other herb-treated groups. No differences in the lipid profile and CRP levels were observed.

【Please insert Table 2 here】

3.4 Plasma fatty acid profiles and the ratio of n-6/n-3 PUFAs

The profiles of plasma fatty acids are shown in Table 3. No differences in saturated fatty acid (SFA) and monounsaturated fatty acid (MUFA) levels were detected among the treated groups. The PUFA levels in the groups that either received the single-dose herbal extracts or a high-dose combination of extracts were significantly higher than that in the normal group ($P < 0.05$); however, no differences were detected among the herb-treated groups. Furthermore, no differences were observed in either the ratio of n-6/n-3 PUFAs or AA/EPA ratios.

The profiles of plasma FFAs are presented in Table 3. SFA levels in the *P. cocos* group and the high-dose combination group were significantly lower than that in the control group; the MUFA levels in the single-dose and high-dose combination groups were significantly higher than that in the control group; and PUFA levels in the herb-treated groups were significantly lower than that in the control group ($P < 0.05$ for all). AA declined most noticeably in the herb-treated groups. Both the ratio of n-6/n-3 PUFAs and the AA/EPA ratios in the herb-treated groups were significantly lower than those in the control group ($P < 0.05$ for all).

【Please insert Table 3 here】

3.5 Inflammatory status in plasma and selected organs

TNF-α and IL-6 levels are shown in Figures 1 and 2, respectively. Compared with the level in the normal group, TNF-α and IL-6 levels in the control group were significantly higher after 8 wk ($P < 0.05$) in plasma and in the liver and kidneys. After the herbs were administered, the levels of TNF-α and IL-6 in the plasma and in the selected organs had decreased significantly ($P < 0.05$ for all). However, the plasma VCAM level of M group showed increase significant ($P < 0.05$). No differences of plasma e-selectin were found among the treated groups. The results revealed pre-DM induced groups with higher levels of VCAM, ICAM and E-selectin concentration. However, no alimental effects were observed in herbal-treated groups. We also measured selected organ levels of TNF-α and IL-6. The results indicated no significant differences among the herb-treated groups except for the level of renal TNF-α.

【Please insert Figures 1 and 2 here】

3.6 AGE accumulation and RAGE expression in renal tissues

Immunohistochemical staining revealed that after herbal treatment for 6 wk, AGE accumulation and RAGE expression increased in renal tissues (Figures 3 and 4). However, we did not quantify renal morphological changes. The results indicated that AGEs accumulated mainly in afferent arterioles and in the proximal area of renal vessels. AGE accumulation was the highest in the control group. After herbal treatment, AGE accumulation and RAGE expression were ameliorated in the renal vessel walls. RAGEs were highly expressed in afferent arterioles and in

the proximal area of renal vessels, especially in the control group. Both single and combination doses of the herbal extracts reduced the expression of RAGEs in renal tissues.

【Please insert Figures 3 and 4 here】

4. Discussion

In the pre-DM-induction model used in this study, the expected glycemic response of the prediabetic status was maintained for only 6 wk, and the animals still deteriorated to T2DM. Comparing the changes in body weight in the low- and high-dose STZ-induced pre-DM models[23] revealed that rats treated with low-dose STZ exhibited increases in body weight that resembled the increase that occurs during the onset period of diabetes. Regarding food intake and feeding efficiency, the test animals had a physical status that was similar to that of humans with diabetes.[29] Hyperglycemia, increased food intake, and reduced body weight were observed in the test animals. In the pre-DM groups, no changes in the insulin level were detected, but the HOMA-IR index was elevated. These pre-DM conditions observed in the test animals were similar to those in patients with normal insulin levels but poor diabetic responses (i.e., insulin resistance).[30] In this study, we confirmed that the low-dose STZ-induced model mimicked the status of prediabetic responses, especially the gradual body-weight loss, elevated fasting blood sugar, and insulin resistance.

P. cocos, a saprophytic fungus, is used in traditional Chinese medicine for its diuretic, sedative, and tonic effects.[31-34] *D. opposite* is commonly used in traditional Chinese medicine to treat patients with diabetes.[17,30,35] In this study, no statistically significant differences in hyperglycemia were detected among the herbal extract-treated groups; however, the *D. opposite* group exhibited a significant increase in body-weight gain compared with that of the control group, which may be attributed to the antiglycemic property of polysaccharides.[18] Two potential components of *P. cocos*[19], polysaccharides and triterpenes (i.e., pachymic acid), were proposed to stimulate glucose uptake by enhancing glucose transporter 4 (GLUT4) gene expression and GLUT4 translocation.[36] *Dioscorea opposite*-treated rats with STZ-induced diabetes displayed increased sensitivity to exogenous insulin.[18] The results of this study indicate that *P. cocos* and *D. opposite* crude extracts exhibited potential antiglycemic properties during treatment.

An acute elevation of plasma FFA levels is a key aspect of the process of T2DM development, and it is a major factor that induces insulin resistance.[37] The study data revealed that plasma FFA levels declined in a statistically significant manner in the *P. cocos* group compared with that of the control group. Distinct FFA profiles have been proposed to produce opposite effects on the progression of insulin resistance and T2DM.[38] Elevated levels of dietary SFAs are considered to decrease insulin sensitivity and increase the risk of T2DM.[39,40] In this study, we detected elevated FFA n-6 PUFA levels, especially AA levels, in the rats with a prediabetic status. Elevated levels of SFA and n-6 PUFAs in plasma FAAs were proposed to

trigger the proinflammatory cascade in tissues.[41] In addition, the herbal treatments ameliorated both the levels and profiles of FFAs. Certain profiles of FFAs are considered more critical than the concentration of FFAs in T2DM.[42] Lowering the ratios of n-6/n-3 PUFA or AA/EPA in FFAs was positively correlated with inflammation[43] and IL-6 levels.[44] The results of this study indicate that *P. cocos* or *D. opposite* crude extracts ameliorated the ratio of n-6/n-3 PUFAs and AA/EPA ratios and concomitantly reduced inflammation. Thus, the ratios of n-6/n-3 PUFAs and AA/EPA in FFAs might serve as predictors in assessing chronic inflammation in patients with a prediabetic status.

Diabetes is considered to be a form of chronic inflammation, which is defined as a series of phenomena induced by distinct pathological stimuli and tissue injuries. Hyperglycemia enhances the formation of AGEs, which are generated through the autooxidation of glucose and certain proteins. Interactions of AGEs with their receptors, called RAGEs, might lead to the production of proinflammatory cytokines and cause inflammation.[45,46] The present results revealed that the TNF-α and IL-6 concentrations in the plasma, liver, and kidneys gradually increased with the rise in blood glucose and the progression of diabetes. We confirmed that in the process of the development of prediabetes into diabetes, the rats exhibited a chronic inflammation status, which accelerated disease development.[47]

Diabetic nephropathies are common chronic complications in diabetes. Continual hyperglycemia leads to elevated vessel pressure, glomerular filtration rate, and mesangial

proliferation. The interactions of AGEs with RAGEs induce the expression of vascular cell adhesion molecule-1 and lead to vascular endothelial damage.[48] AGE accumulation in vessel walls causes a thickening of glomerular basal membrane, elicits changes in renal physiological functions and morphology, and increases the risk of atherosclerosis or glomerulosclerosis.[49] Although we did not quantify renal morphological changes, the results indicated that AGEs and RAGEs accumulated mainly in afferent arterioles and in the proximal area of renal vessels, especially in the control group. The animals in the control group exhibited thickened renal glomerular basal membranes. Yoon et al. reported that a high concentration of glucose induced an elevated proliferation of mesangial cells, and that a water extract of *P. cocas* inhibited mesangial cell proliferation by reducing the expression of cyclins and cyclin-dependent kinases.[50] In the present study, following the herbal-extract intervention, the thickness of the glomerular basal membrane was decreased, and reduced AGE accumulation and RAGE expression were observed in the renal vessel walls. Immunohistochemical analysis revealed that the crude extracts of both *P. cocos* and *D. opposite* exerted similar protective effects against diabetic nephropathies. However, the bioactive ingredients in the extracts are not identical, and polyphenols, flavonoids, and soluble dietary fiber might play critical roles in diabetic nephropathies. The herbal extracts might potentially produce protective effects by improving fasting blood-sugar levels[20] and lowering the levels of proinflammatory cytokines[15] and cyclin regulators.[50]

This study has a few limitations. First, the pre-DM model has been used to mimic the diabetes status in certain murine species, but the period for which the pre-DM status is maintained in rats does not appear to be controllable. Second, we did not quantify renal morphologic changes. Although the results of immunohistochemical staining indicated that AGEs and RAGEs accumulated mainly in afferent arterioles and in the proximal area of renal vessels, the expression levels of AGEs and RAGEs should be quantified.

In conclusion, the results indicate that in prediabetic rats, administering *P. cocos* or *D. opposite* crude extracts produced hypoglycemic and antiinflammatory effects by reducing fasting blood-sugar levels, IL-6 levels, the FFA ratio of n-6/n-3 PUFAs, and AGE formation in kidney vessels, but the extracts did not produce synergistic effects.

Conflict of interest

The authors have no potential conflicts of interest to declare.

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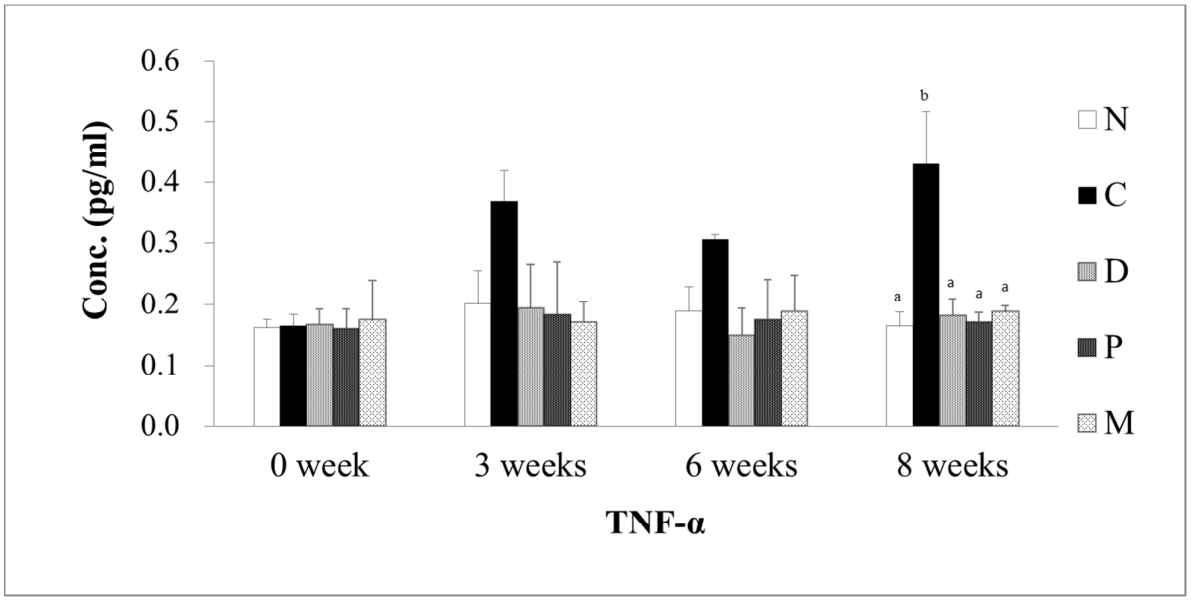
Figure 1

Plasma levels of the cytokines (a) TNF-α and (b) IL-6. N, normal; C, prediabetes mellitus control; D, *D. opposite* (1×); P, *P. cocos* (1×); M, combination of *P. cocos* (1×) and *D. opposite* (1×). TNF-α: tumor necrosis factor-α; IL-6: interleukin-6. Data are presented as means ± SEM. Values indicated by the same letter are not significantly different from one another; Duncan's multiple range test ($P < 0.05$).

(a)



(b)

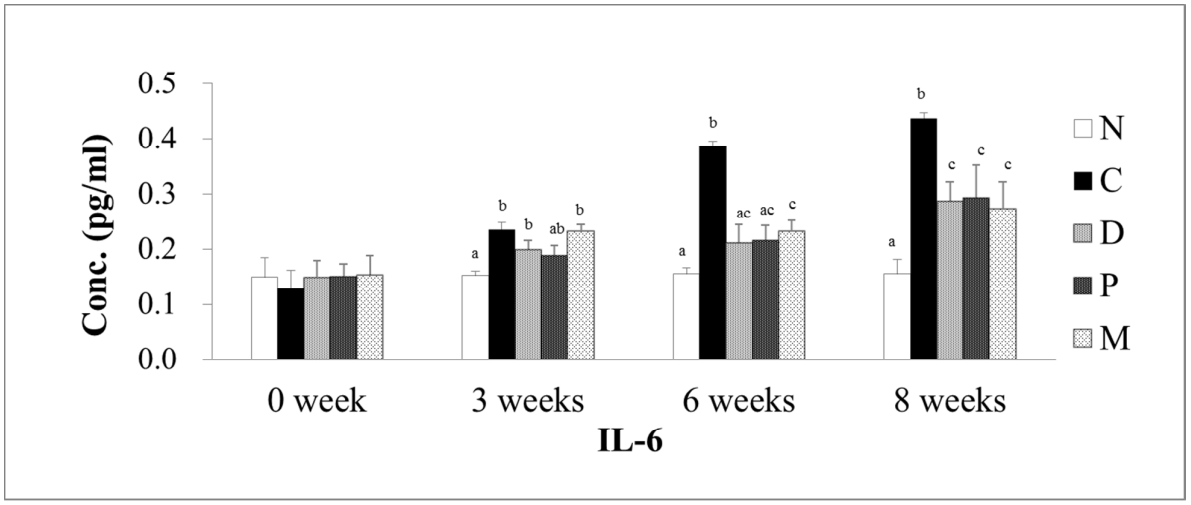
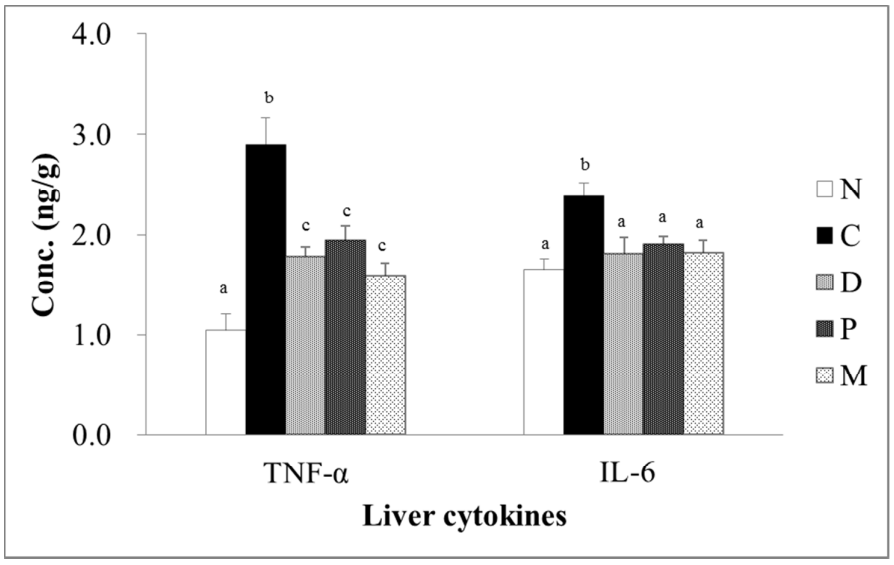


Figure 2

Concentrations of cytokines in the (a) liver and (b) kidney. N, normal; C, prediabetes mellitus control; D, *D. opposite* (1×); P, *P. cocos* (1×); M, combination of *P. cocos* (1×) and *D. opposite* (1×). TNF-α: tumor necrosis factor-α; IL-6: interleukin-6. Data are presented as means ± SEM. Values indicated by the same letter are not significantly different from one another; Duncan's multiple range test ($P < 0.05$).

(a)



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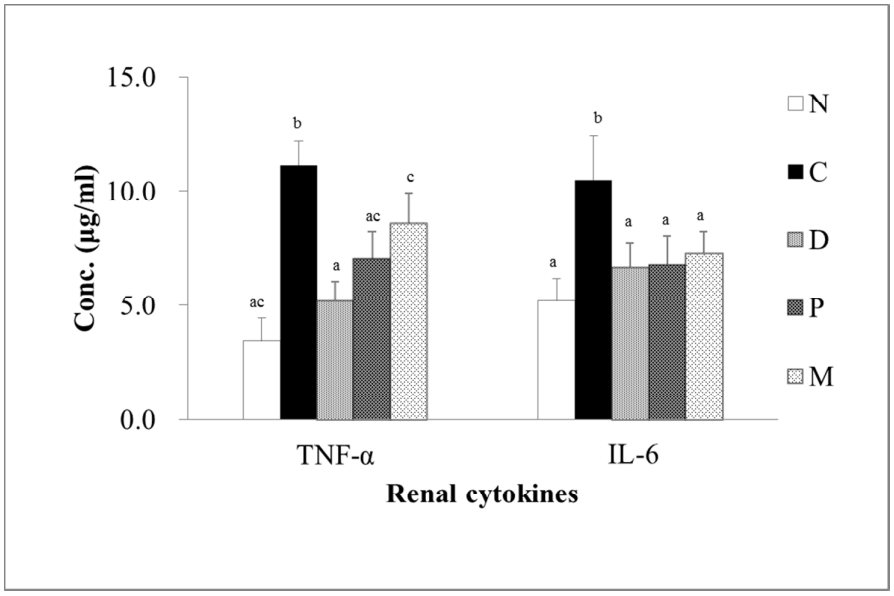
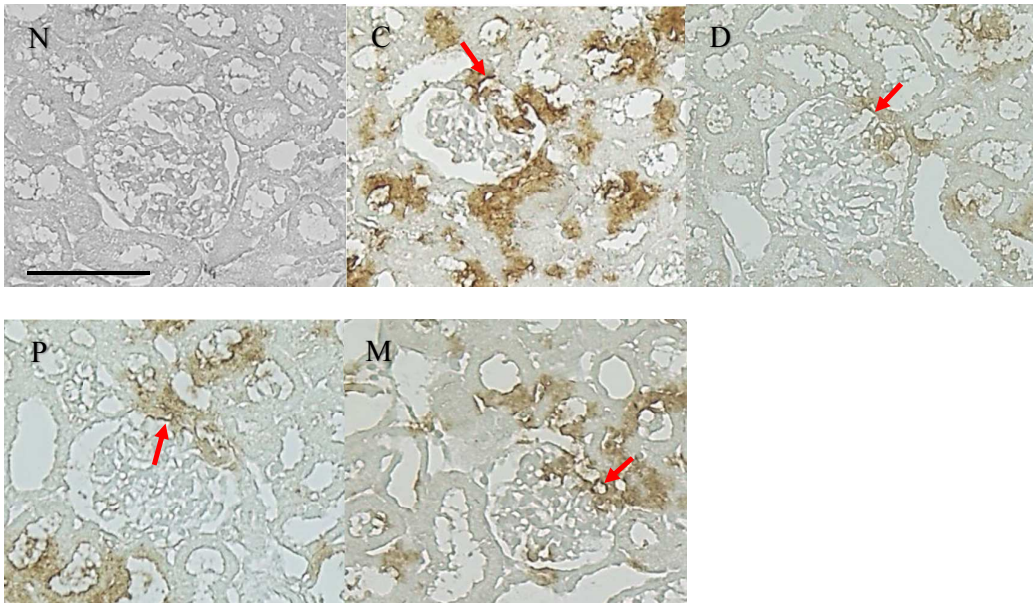


Figure 3

Histological analysis of the kidneys in the 5 groups: advanced glycation end products (AGEs). N, normal; C, prediabetes mellitus control; D, *D. opposite*; P, *P. cocos*; M, combination of *P. cocos* and *D. opposite*. Expression of AGEs in (I) renal glomerulus (200×) and (II) renal tubules (200×). The scale bars represent 75 μm.

(I) Renal glomerulus



(II) Renal tubules

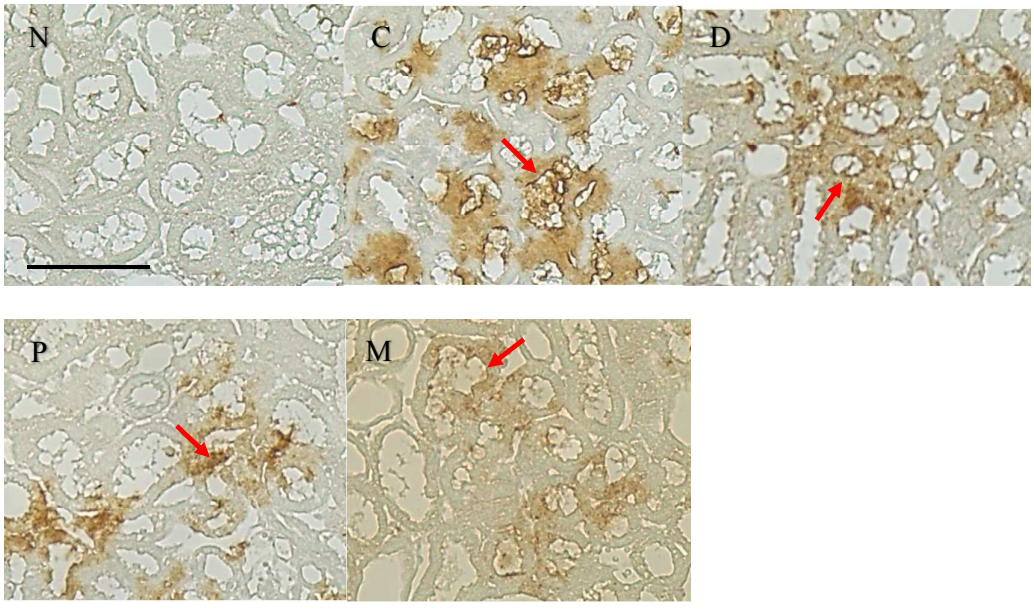
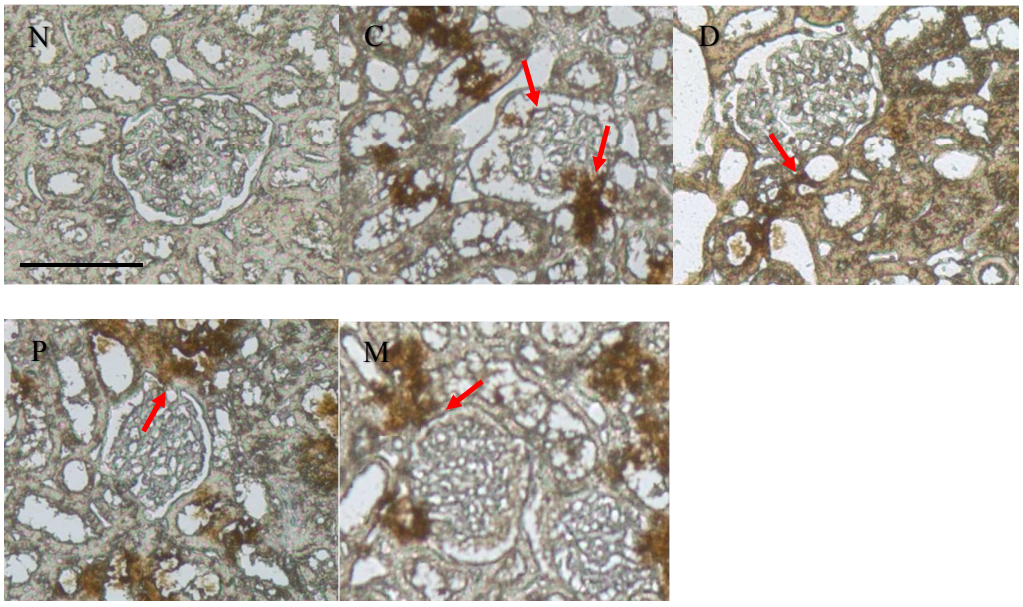


Figure 4

Histological analysis of the kidneys in the 5 groups: receptors of advanced glycation end products (RAGEs). N, normal; C, prediabetes mellitus control; D, *D. opposite*; P, *P. cocos*; M, combination of *P. cocos* and *D. opposite*. Expression of RAGEs in (I) renal glomerulus (200×) and (II) renal tubules (200×). The scale bars represent 75 μm.

(I) Renal glomerulus



(II) Renal tubules

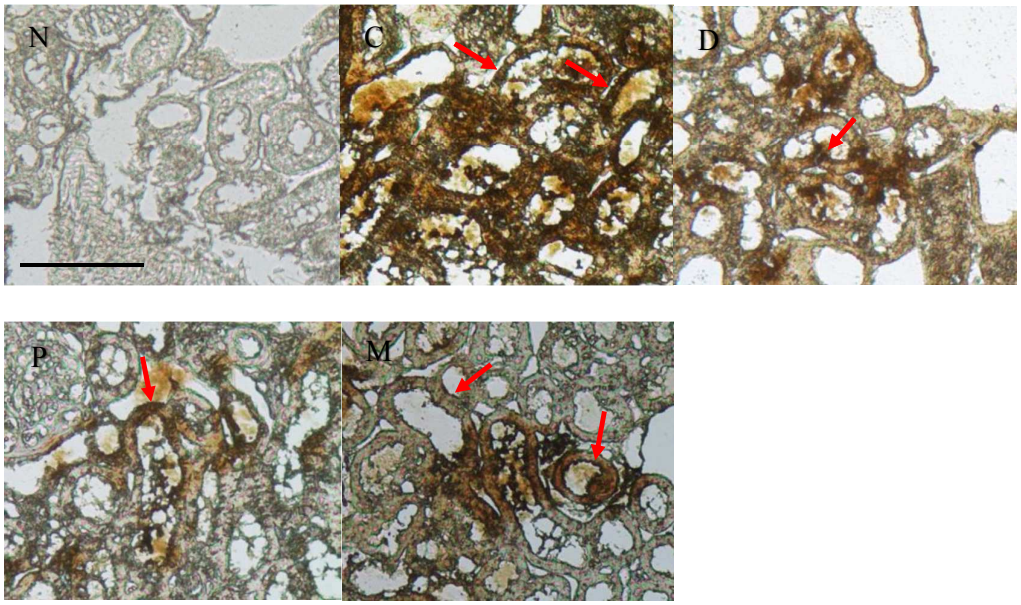


Table 1. Assessment of body weight gain and selected organs

	N	C	D	P	M
Initial body weight (g)	294.3±3.0	292.4±6.2	293.7±3.2	293.8±3.3	294.1±1.9
Final body weight (g)	463.4±14.9^{a}	286.3±8.3^{b}	319.7±13.9^{c}	308.8±6.3^{bc}	302.8±4.4^{bc}
Body-weight gain (g)	169.1±15.2^{a}	-6.1±9.5^{b}	26.0±14.5^{b}	15.0±8.1^{b}	8.6±4.9^{b}
Food intake (g/d)	21.7±0.4^{a}	30.8±0.3^{b}	29.7±0.4^{b}	30.8±0.3^{b}	30.0±0.4^{b}
Feeding efficiency (%)	13.9±1.1^{a}	-0.4±0.2^{b}	1.6±0.9^{b}	0.9±0.4^{b}	0.6±0.2^{b}
Liver weight (g)	12.0±0.8^{a}	9.7±0.5^{b}	9.1±0.5^{b}	8.9±0.4^{b}	8.3±0.4^{b}
sGOT (U/L)	98.1±2.9	105.6±9.4	98.1±5.8	98.1±2.7	99.6±3.5
sGPT (U/L)	32.2±0.9^{a}	46.0±2.8^{b}	43.0±3.0^{b}	42.4±3.3^{b}	46.1±4.3^{b}
Kidney weight (g)	2.68±0.09^{a}	3.22±0.10^{b}	3.19±0.21^{b}	2.96±0.12^{a}	3.03±0.13^{a}
BUN (mg/dL)	9.8±0.7^{a}	39.0±3.1^{b}	36.8±9.5^{b}	31.1±3.0^{c}	37.0±3.4^{b}
Creatinine (mg/dL)	0.38±0.02	0.36±0.01	0.37±0.08	0.39±0.03	0.41±0.03

N, normal; C, prediabetes mellitus control; D, *D. opposite* (1×); P, *P. cocos* (1×); M, combination of *D. opposite* (1×) and *P. cocos* (1×). Feeding efficiency (%) = (body-weight gain/total food intake) × 100%. Data are presented as means ± SEM. Each group contained 10 rats. Values in a row that are indicated by the same letter do not differ significantly from one another; Duncan's multiple-range test ($P < 0.05$).

Table 2. Glycemic, lipid and inflammatory response after intervention

	N	C	D	P	M
Glucose metabolism					
FBG (mg/dL)	129.8±5.4^{a}	286.3±17.4^{b}	226.1±11.7^{c}	232.6±13.6^{c}	247.5±14.2^{c}
Insulin (μIU/mL)	0.31±0.01	0.35±0.02	0.37±0.04	0.37±0.05	0.36±0.04
HbA1c (%)	6.5±0.7^{a}	11.7±0.4^{b}	8.2±0.4^{c}	7.8±0.8^{c}	7.7±0.7^{c}
HOMA-IR	2.43±0.11^{a}	5.96±0.42^{b}	5.03±0.60^{b}	5.37±0.70^{b}	5.44±0.90^{b}
CRP (μg/dL)	6.09±2.41	7.76±1.56	5.95±1.38	6.74±1.93	6.57±1.06
Blood lipid					
TG (mg/dL)	47.9±2.9	51.1±4.2	53.0±5.6	52.4±6.3	50.9±5.2
TC (mg/dL)	100.3±5.2	159.3±15.1^{a}	149.6±12.9^{a}	143.5±9.5^{a}	137.9±4.6^{a}
HDLc (mg/dL)	13.9±1.4	16.4±1.2	15.1±1.9	15.8±2.7	14.7±1.4
LDLc (mg/dL)	3.4±0.7	5.8±0.6	4.8±0.9	4.7±0.7	4.2±0.3
Free fatty acid (mmol/L)	0.037±0.010^{a}	0.041±0.011^{b}	0.037±0.008^{ab}	0.032±0.007^{a}	0.036±0.012^{ab}
Inflammatory status					
TNF-α (pg/mL)	0.14±0.03^{a}	0.42±0.08^{b}	0.16±0.04^{a}	0.15±0.02^{a}	0.14±0.03^{a}
IL-6 (mg/mL)	0.13±0.04^{a}	0.43±0.02^{b}	0.28±0.03^{a}	0.29±0.04^{a}	0.27±0.04^{a}
VCAM (ng/mL)	51.4±14.6^{a}	104.9±13.5^{b}	94.6±11.3^{a}	86.0±19.3^{a}	116.2±21.5^{b}
ICAM (ng/mL)	36.9±15.1^{a}	156.3±27.4^{b}	130.3±32.9^{b}	110.7±21.4^{b}	127.4±26.2^{b}
e-Selectin (pg/mL)	373±38	455±32	423±170	404±105	676±221

N, normal; C, prediabetes mellitus control; D, *D. opposite* (1×); P, *P. cocos* (1×); M, combination of *D. opposite* (1×) and *P. cocos* (1×). FBG: fasting blood glucose, HbA_{1C}, glycosylated Hb, HOMA-IR: homeostatic model assessment-insulin resistance (HOMA-IR =(AC ×(insulin)/22.5), CRP: C-reactive protein, TG: triglyceride, TC: total cholesterol, HDLc: high-density lipoprotein cholesterol, LDLc: low-density lipoprotein cholesterol, TNF-α: tumor necrosis factorα, IL-6: interleukin-6, VCAM 1, vascular cell adhesion molecule 1, ICAM-1, Intercellular Adhesion Molecule 1. Data are presented as means ± SEM. Each group contained 10 rats. Values in a row that are indicated by the same letter do not differ significantly from one another; Duncan's multiple-range test ($P < 0.05$).

Table 3. Fatty acid profile of plasma

		N	C	D	P	M
Plasma	**SFAs**	41.42±1.19	40.43±0.99	39.47±0.63	41.29±0.68	39.60±0.96
	MUFAs	42.21±1.27	40.35±1.00	41.64±0.64	39.26±0.75	40.85±1.14
	PUFAs	16.37±0.52^{a}	19.23±0.45^{b}	18.89±0.55^{b}	19.45±0.64^{b}	19.55±0.56^{b}
	n-6	15.00±0.46^{a}	17.61±0.43^{b}	17.36±0.49^{b}	17.56±0.62^{b}	17.88±0.43^{b}
	C20:4 (AA)	6.69±0.42^{a}	7.34±0.22^{b}	7.05±0.36^{a}	7.68±0.61^{b}	6.57±0.35^{a}
	n-3	1.37±0.09^{a}	1.62±0.08^{a}	1.52±0.15^{a}	1.89±0.17^{b}	1.66±0.18^{a}
	C20:5 (EPA)	0.10±0.03	0.09±0.03	0.09±0.03	0.11±0.02	0.09±0.02
	n-6/n-3	11.18±0.58	11.15±0.70	10.73±0.62	10.57±1.27	11.47±0.96
	AA/EPA	66.93±8.33	81.53±8.44	78.12±6.77	69.58±7.63	73.22±7.51
Plasma Free FAs	**SFAs**	40.55±0.74^{ab}	43.34±0.65^{a}	40.00±0.40^{ab}	39.17±0.88^{b}	40.91±1.68^{ab}
	MUFAs	52.58±0.67^{ab}	50.54±0.68^{a}	55.05±0.54^{bc}	56.12±0.94^{cd}	53.92±1.86^{ab}
	PUFAs	6.87±0.19^{a}	6.12±0.34^{b}	4.96±0.32^{c}	4.71±0.23^{c}	5.18±0.37^{c}
	n-6	1.56±0.05^{a}	4.80±0.30^{b}	2.00±0.18^{a}	1.58±0.08^{a}	2.53±0.28^{c}
	C20:4 (AA)	0.42±0.03^{a}	0.82±0.03^{b}	0.22±0.05^{c}	0.16±0.03^{c}	0.37±0.02^{a}
	n-3	5.31±0.18^{a}	1.32±0.15^{b}	2.96±0.31^{c}	3.31±0.28^{c}	2.65±0.16^{c}
	C20:5 (EPA)	0.027±0.012	0.016±0.004	0.030±0.015	0.014±0.004	0.031±0.012
	n-6/n-3	0.30±0.01^{a}	2.72±0.21^{b}	0.76±0.16^{a}	0.55±0.08^{a}	0.79±0.09^{a}
	AA/EPA	14.03±4.01^{a}	50.25±3.83^{b}	9.16±3.03^{a}	11.40±5.21^{a}	11.93±3.52^{a}

N, normal; C, prediabetes mellitus control; D, *D. opposite* (1×); P, *Poria cocos* (1×); M, combination of *D. opposite* (1×) and *P. cocos* (1×). SFAs, saturated FAs; MUFAs, monounsaturated FAs; PUFAs, polyunsaturated FAs; AA, arachidonic acid; EPA, eicosapentaenoic acid. Data are presented as means ± SEM. Each group contained 10 rats. Values in a row that are indicated by the same letter do not differ significantly from one another; Duncan's multiple-range test ($P < 0.05$).