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# Effects of a Palatinose-Containing Diet with Exercise on Progression of Diabetic Nephropathy and Metabolic Syndrome in Obese-Diabetic Rats

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## ABSTRACT

*This study examined the effects of a long-term Palatinose (Pala)-containing diet, with or without exercise, on blood pressure (BP), body weight (BW), the progression of diabetic nephropathy (DN), and metabolic syndrome (MetS) component factors including visceral fat mass (VFM), glucose intolerance, and dyslipidemia in an obese diabetic rat model. Twenty-eight male Otsuka Long Evans Tokushima Fatty (OLETF) rats aged 24 weeks were assigned to the sedentary (OLETF-Sed), Pala-containing diet (OLETF-Pala), exercise (OLETF-Ex), and combination of Pala with exercise (OLETF-Pala & Ex) groups. The OLETF-Ex and OLETF-Pala & Ex groups were allowed to run voluntarily on a rotatory wheel. BW and BP were measured weekly from 24 to 29 weeks of age. Pre and posttreatment serum glucose, insulin, and leptin concentrations, as well as body composition were measured, and 24 h urine samples were collected for measurements of protein excretion. After treatment, measurement of serum lipid levels and kidney morphological analysis were performed. The OLETF-Sed group showed increased BW and VFM, decreased lean body mass (LBM%), and deterioration in MetS component factors. The OLETF-Ex group showed decreased VFM and increased LBM%, and improvement in all MetS component factors posttreatment. However, both groups showed increases in BP and progression of DN after treatment. The OLETF-Pala and OLETFPala and Ex groups improved in all MetS component factors measured and did not show BP increases or progression of DN. This study demonstrated that a Pala-containing diet alone or combined with exercise improved MetS component factors without progression of DN.*

**Keywords:** Diabetic nephropathy, Urinary protein excretion, Renal morphology, Palatinose

## INTRODUCTION

The concept of "slow calories" has been proposed recently, which refers to carbohydrates that are absorbed slowly without stimulating insulin secretion [1]. Palatinose (Pala, isomaltulose) is one of the "slow-calorie" carbohydrates. There are numerous reports on the beneficial effects of long-term administration of Pala on blood pressure (BP) and metabolic syndrome (MetS) component factors including visceral fat mass (VFM) [1,2] insulin resistance (IR), and prevention of diabetic complications [3-5]. Considering the beneficial effects of Pala including antihypertensive effects and improvements in IR and MetS component factors, a dietary Pala-containing regimen may be recommended for obese-diabetic patients.

On the other hand, an essential clinical approach for obese-diabetic patients is exercise and diet regimens. However, our previous study [6] showed that an exercise regimen caused progression of diabetic nephropathy (DN) in an obese-diabetes model, Otsuka Long Evans Tokushima Fatty (OLETF) rats. That study showed that exercise did not inhibit BP increases during treatment, although glucose intolerance (GI) and dyslipidemia improved. However, it is necessary to prescribe exercise in a way that does not result in the progression of renal injury in patients with obesity and diabetes mellitus, because exercise promotes aerobic capacity and increases muscle mass.

In our previous study using OLETF rats [7], both dietary restriction and exercise significantly increased BP post treatment compared with the pretreatment level, although body weight (BW) was significantly reduced in those regimens. Our results [6,7] suggested that weight loss due to dietary restriction and/or increased energy expenditure through exercise were not sufficient to decrease BP. The lack of inhibition of BP increases during treatment is thought to be one cause of DN progression in both dietary restriction and/or exercise regimens in OLETF rats.

Those findings suggested that when a Pala-containing diet regimen is combined with exercise, the progression of DN caused by exercise may be inhibited due to the BP-lowering activity of Pala and that additive improvement in GI, dyslipidemia, and other MetS component factors may be achieved. In addition, it is necessary to clarify whether a Pala-containing diet regimen alone could improve the prognosis of DN in OLETF rats.

Therefore, we examined the effects of a Pala-containing diet alone and combined with exercise on BP and progression of DN as evaluated by urinary protein excretion and kidney morphology, as well as on BW, VFM, lean body mass (LBM), and parameter-related MetS in OLETF rats.

## MATERIALS AND METHODS

### Experimental animals

Twenty-eight male OLETF rats, which exhibit IR and accumulate intraabdominal fat [8], were used in this study. Six male Long-Evans Tokushima Otsuka (LETO) rats served as controls. The experimental protocols were approved by the Committee for the Care and Use of Animals of the Jikei University School of Medicine prior to study commencement. This study was conducted under the Guidelines on Humane Use and Care of Laboratory Animals for Biomedical Research published by the National Institutes of Health (No. 83-23, Revised 1996) and the Guidelines on Animal Care of the Jikei University School of Medicine.

### Experimental design

Animals were housed in dedicated facilities under specific pathogen-free conditions with a temperature of 21.0 ± 1.5°C (± standard error; SE), with lighting from 06:00 to 18:00 throughout the experimental period. At 24 weeks of age, the animals were divided into the following groups: sedentary (OLETF-Sed, *n*=7); Pala-containing diet (OLETF-Pala, *n*=7); exercise (OLETF-Ex, *n*=7); and Pala-containing diet combined with exercise (OLETF-Pala & Ex, *n*=7). The LETO rats served as sedentary controls (LETO-Sed, *n*=6). All rats were housed individually during the treatment period. The Pala-containing diet (Mitsui Sugar Co., Ltd., Tokyo, Japan) was prepared by replacing 10% granulated sugar and 45.5% cornstarch in the CLEA purified basic formula diet (CE-2, CLEA Japan, Inc., Tokyo, Japan) with 39.6% Pala and 16.0% cornstarch. The energy densities of the Pala-containing diet and standard rat chow (CE-2) were 364.4 and 344.9 kcal/100 g, respectively. The OLETF-Pala and OLETF-Pala & Ex groups were fed a Pala-containing diet, while the OLETF-Sed, OLETF-Ex, and LETO-Sed groups were fed standard rat chow. The exercise groups were allowed to run voluntarily every day on a rotatory wheel attached to the cage (Shinano manufacturing Co., Ltd., Tokyo, Japan), and their running distance was recorded weekly. All groups were allowed access to food and tap water ad libitum. The treatment period was from 25 to 29 weeks of age.

### Measurement of BW, body composition, BP, food consumption, and running distance

During the treatment period, BW, food consumption (FC), and running distance were measured weekly at a scheduled time in the morning. The systolic BP (SBP) and diastolic BP (DBP) of all groups were measured 5 times using the tail-cuff method (BP-98, Softron Co., Ltd., Tokyo, Japan) between 10:00 and 12:00 at 24, 27, and 29 weeks of age. The measurement of BP for exercise groups was performed at least 1 h after cessation of exercise. Pre- and posttreatment, body composition including VFM, total subcutaneous fat mass (TSFM), and LBM [9,10] were measured using an X-ray computed tomography scanner (Latheta LCT-200, Aloka System Engineering Co., Ltd., Tokyo, Japan) while the rats were under isoflurane anesthesia.

### Analysis of serum glucose, insulin, and leptin concentrations and urinary excretion of proteins

At 24 (pretreatment) and 30 (post treatment) weeks of age after overnight fasting, approximately 500 µL of blood was collected from the retroorbital sinus [10,11] under diethyl ether anesthesia to measure fasting serum glucose (FSG; Glucose C II-test Wako, Wako Pure Chemical Industries, Ltd., Osaka, Japan), insulin (FSI; Rat Insulin ELISA kit, Shibayagi Co., Ltd., Japan), and leptin (FSL; Rat Leptin ELISA Kit, Yanaihara Institute Inc., Japan) concentrations.

The homeostatic model assessment of IR (HOMA-IR) was calculated from FSG and FSI values according to the following formula: HOMA-IR = FSI (μU/mL) × FSG (mg/dL)/405 [12]. At 2–3 days after the measurement of body composition pre and posttreatment, the animals were placed in metabolic cages (Shinano), and 24 h urine samples were collected in 50 mL cylinders containing liquid paraffin. The urine volume (UV) was measured, and samples were stored at –20°C until measurements of total protein (TP; Total protein-HRII, Wako) and albumin (Alb; ICN Pharmaceuticals, Inc., Aurora, OH, USA) concentrations. The urinary excretion of proteins per day ($U_{TP}$, $U_{Alb}$, mg/day) was calculated.

**Analysis of serum lipid concentrations**

At 30 weeks of age, the animals were exsanguinated by cardiac puncture under pentobarbital anesthesia after overnight fasting. Approximately 5–8 mL of blood was rapidly withdrawn and used to measure serum total cholesterol (T-cho), triglyceride (TG), and high-density lipoprotein cholesterol (HDL-C) concentrations (Hitachi 7600-210, Hitachi, , Ltd., Tokyo, Japan). Low-density lipoprotein cholesterol (LDL-C) levels were calculated using the Friedewald equation (LDL-C (mg/dl)=T-cho-HDL-C–TG/5).

**Kidney morphology**

The rat kidneys were removed and weighed. The left kidney was halved longitudinally, fixed in 20% formalin solution for 1 week, and embedded in paraffin. Paraffin sections were stained with periodic acid silver methenamine to determine glomerular size as previously described [13,14]. Glomerular (A [G]) and mesangial (A [M]) areas were measured using an automatic image analyzer (SP-500, Olympus Corporation, Tokyo, Japan) that was fitted onto a microscope [13,14]. For each rat, 50 randomly selected glomerular profiles were analyzed and averaged, and the individual mean A (G) ( $\overline{A}[G]$ ), A(M) ( $\overline{A}[M]$ ), and mean glomerular volume ( $\overline{V}[G]$ ) were calculated using the previously described equation [13,14].

To measure glomerular basement membrane (GBM) thickness, cortical tissues were extracted from the remaining half of the left kidney and immediately fixed in 2% glutaraldehyde for 1 week. The tissues were prepared and then embedded in epoxy resin following the same procedure as in our previous study [14]. Ultrathin sections were stained with uranyl acetate and lead citrate and observed using a transmission electron microscope (H-7500, Hitachi High-Technologies Corporation. Tokyo, Japan). Five representative fields of glomerular capillaries were randomly selected for each rat, enlarged to 5,000x magnification for examination, and transferred to a computer. GBM thickness was measured using color imaging analyzer software (WinROOF, MITANI CORPORATION, Fukui, Japan) on the computer. Twenty regions of a thinner portion of the GBM in each magnified image were manually selected, and the width of the GBM was measured perpendicular to the tangent of the curved portion [14].

**Statistical analyses**

Results are expressed as mean ± SE. Analysis of the data was performed using analysis of variance (ANOVA). If ANOVA indicated a significant difference, subsequent post-hoc pairwise differences were calculated using Fisher's protected least significant difference. Student's paired *t*-test was also used to determine significant differences between variables measured before and after treatment in each group. Statistical determination of correlation coefficients was performed using Pearson's test. A 95% level of confidence was accepted as significant for all statistical tests. Cohen's parameters as an effect size were also calculated. For statistical analysis, Stat View (SAS Institute Inc., Cary, NC, USA) was used.

## RESULTS

**Changes in BW, FC, running distance, and BP**

Changes in BW and FC are shown in Table 1. The pretreatment BW of the OLETF-Pala and OLETF-Pala and Ex groups was significantly higher than that of the OLETF-Sed and OLETF-Ex groups. The OLETF-Sed group showed a gradual increase in BW, and it became the highest of all 5 groups at study completion, although it was the lowest pretreatment. The OLETF-Pala and OLETF-Ex groups maintained pretreatment BW levels throughout the study. On the other hand, the OLETF-Pala & Ex group showed a gradual decrease in BW and had the lowest BW, even though it was significantly higher among the 4 OLETF rat groups pretreatment.

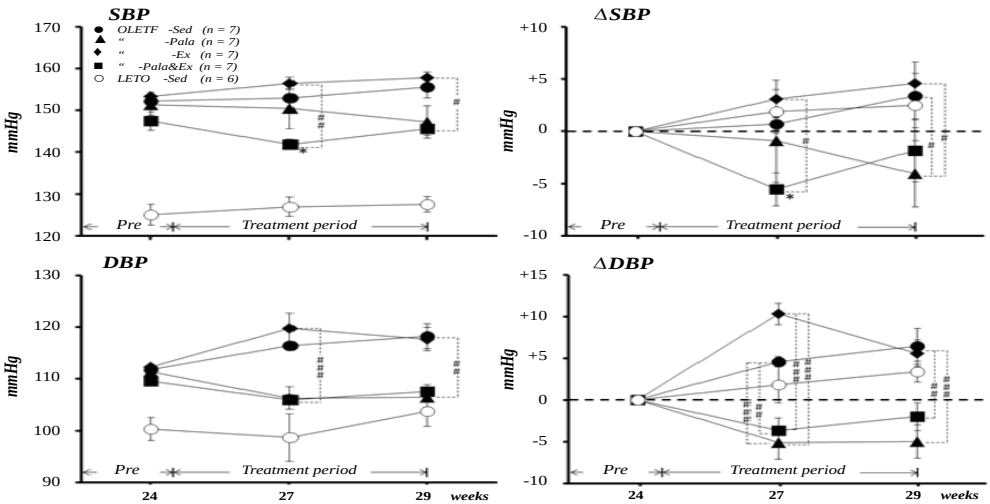
**Table 1:** Changes in body weight (BW) and food consumption (FC) throughout the experimental period.

| Group | | Pretreatment | Treatment period | | | | |
|---|---|---|---|---|---|---|---|
| | | Age (weeks) | | | | | |
| | | 24 | 25 | 26 | 27 | 28 | 29 |
| OLETF-Sed (*n*=7) | BW (g) | 530.6 ± 6.6 | 545.3 ± 7.2$^{***}$ | 586.3 ± 13.0$^{***}$ | 649.5 ± 15.9$^{***}$ | 665.4 ± 14.6$^{***}$ | 683.1 ± 13.2$^{***}$ |
| | FC (g/day) | 39.6 ± 1.5 | 38.6 ± 1.5 | 38.0 ± 1.0 | 38.1 ± 1.0 | 38.9 ± 1.4 | 39.1 ± 0.9 |
| OLETF-Pala (*n*=7) | BW (g) | 614.7 ± 6.6$^{\#\#\#}$ | 621.9 ± 12.0$^{\#}$ | 597.1 ± 15.2 | 608.0 ± 15.4$^{\#}$ | 606.4 ± 13.6 | 608.2 ± 17.4 |
| | FC (g/day) | 27.9 ± 1.1$^{\#\#\#}$ | 28.5 ± 0.9$^{\#\#\#}$ | 32.0 ± 2.8 | 32.3 ± 0.6 | 30.2 ± 1.3$^{\#\#\#}$ | 30.1 ± 0.7$^{\#\#\#}$ |
| OLETF-Ex (*n* = 7) | BW (g) | 529.3 ± 14.5 | 544.0 ± 13.2 | 545.3 ± 12.7$^{\#}$ | 522.7 ± 7.0$^{\#\#\#}$ | 513.9 ± 7.3 | 549.4 ± 6.5$^{\#\#\#}$ |
| | FC (g/day) | 25.6 ± 2.0$^{\#\#\#}$ | 26.2 ± 1.8$^{\#\#\#}$ | 27.1 ± 1.8 $^{\#\#}$ | 28.6 ± 1.2$^{\#\#\#}$ | 27.6 ± 1.5$^{\#\#\#}$ | 27.6 ± 1.0$^{\#\#\#}$ |
| OLETF-Pala and Ex (*n*=7) | BW (g) | 614.0 ± 15.0$^{\#\#\#}$ | 550.9 ± 15.1$^{***}$ | 537.8 ± 12.8$^{\#\#***}$ | 530.6 ± 11.1$^{\#\#\#***}$ | 543.6 ± 11.6$^{***}$ | 538.4 ± 10.4$^{\#\#\#***}$ |
| | FC (g/day) | 28.2 ± 3.6$^{\#}$ | 27.9 ± 3.3$^{\#}$ | 27.6 ± 3.3$^{\#\#}$ | 30.7 ± 2.4$^{\#\#}$ | 33.7 ± 1.8$^{\#}$ | 33.3 ± 1.1$^{\#\#}$ |
| LETO-Sed (*n*=6) | BW (g) | 466.2 ± 8.1$^{\#\#\#}$ | 478.8 ± 6.9$^{\#\#\#}$ | 481.8 ± 4.1$^{\#\#\#}$ | 476.5 ± 5.6$^{\#\#\#}$ | 497.8 ± 6.5$^{\#\#\#}$ | 494.8 ± 5.4$^{\#\#\#}$ |
| | FC (g/day) | 32.3 ± 1.6 | 33.3 ± 2.4 | 31.0 ± 1.4$^{\#}$ | 29.8 ± 0.9$^{\#}$ | 29.7 ± 0.6$^{\#\#\#}$ | 30.2 ± 1.0$^{\#\#\#}$ |

$^{***}P$<0.001 compared with the pretreatment value (at 24 weeks of age), $^{\#}P$<0.05, $^{\#\#}P$<0.01, $^{\#\#\#}P$<0.001 versus OLETF-Sed.

No group exhibited significant changes in FC throughout the experimental period, although it was significantly greater in the OLETF-Sed group than in the other 4 groups. Changes in calorie intake calculated from FC also showed the same changes as FC throughout the experimental period. The cumulative running distance during the treatment period was significantly higher in the OLETF-Ex group (85.2 ± 2.1 km) than that in the OLETF-Pala and Ex group (60.2 ± 7.7 km).

Changes in BP are shown in Figure 1. Mean pretreatment SBP/DBP values in all OLETF rats (n=28) were 151.1 ± 0.9/109.6 ± 1.5 mmHg, and there were no significant differences in SBP/DBP in the 4 OLETF rat groups, although SBP/DBP was significantly higher than in the LETO rat group (125.0 ± 2.5/100.3 ± 2.2 mmHg). In Figure 1, the left panel shows changes in absolute SBP/DBP values, and the right panel shows net changes in SBP/DBP (ΔSBP/ΔDBP) from the pretreatment values. Compared with pretreatment SBP values, there was a trend toward an increase in SBP in the OLETF-Sed and OLETF-Ex groups at 27 and 29 weeks of age. On the other hand, the OLETF-Pala and OLETF-Pala & Ex groups showed a tendency toward decreased SBP at 27 and 29 weeks of age. ΔSBP in the OLETF-Pala & Ex group at 27 weeks of age was significantly lower than the pretreatment level. Furthermore, ΔSBP in the OLETF-Pala and OLETF-Pala & Ex groups was significantly lower than that in the OLETF-Sed and OLETF-Ex groups at 27 and 29 weeks of age. There was no significant difference in pretreatment DBP among the 4 OLETF rat groups, although they had significantly higher DBP than the LETO rat group. Changes in DBP were similar to those in SBP, and changes in ΔDBP were significantly lower in the OLETF-Pala and OLETF-pala & Ex groups than in the OLETF-Sed and OLETF-Ex groups during the study.

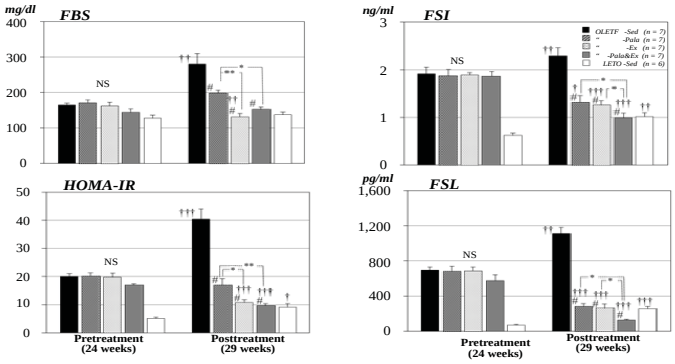



Values are mean ± SE. Left panel shows the absolute change in SBP and DBP. Right panel shows the net change in SBP (ΔSBP) and DBP (ΔDBP) from the pretreatment (Pre) values (at 24 weeks of age). *$P$ < 0.05 compared with pretreatment level; #$P$ < 0.05, ##$P$ < 0.01, ###$P$ < 0.001 between two groups.

**Figure 1:** Changes in SBP and DBP during the experimental period.

### Changes in FBS, FSI, HOMA-IR, and FSL levels

As shown in **Figure 2,** no significant differences in pretreatment glucose metabolism-related parameters and FSL level were observed among the 4 OLETF rat groups. However, levels of all 4 variables increased significantly in the OLETF-Sed group post treatment. The OLETF-Pala group had significantly decreased FSI and FSL values, even though this group showed no significant changes in FBS and HOMA-IR post treatment. Both the OLETF-Ex and OLETF-Pala & Ex groups significantly improved all 3 glucose metabolism-related parameters and FSL level, and improvements in FSI and FSL were marked in the exercise combination groups posttreatment.

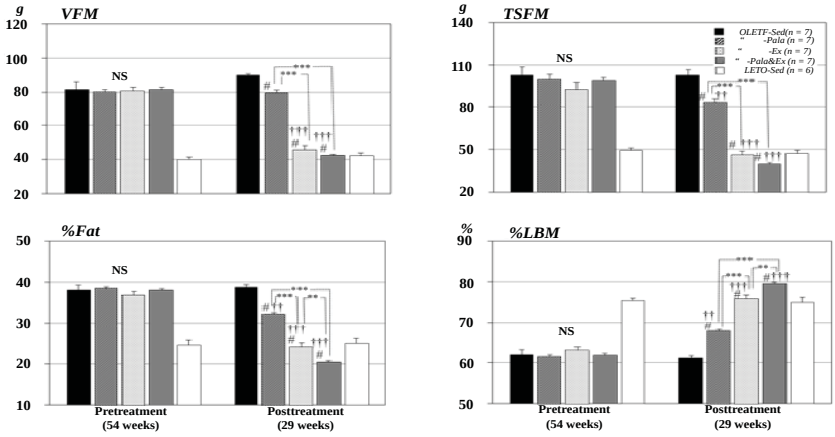



Values are mean ± SE. †$P < 0.05$, ††$P < 0.01$, †††$P < 0.001$ compared with pretreatment levels; #$P < 0.05$ compared with the OLETF-Sed group; *$P < 0.05$, **$P < 0.01$ between two groups. NS, not significant among the 4 OLETF rat groups.

**Figure 2:** Changes in FBS, FSI, HOMA-IR, and FSL concentrations pre and posttreatment.

### Changes in body composition

Changes in VFM, TSFM, %Fat, and %LBM are shown in **Figure 3**. %Fat and %LBM were calculated according to the following equations: Fat%= [(VFM+TSFM) × 100/body weight]; LBM%=(LBM×100/body weight), respectively. There were no significant pretreatment differences in VFM, TSFM, %Fat, and %LBM among the 4 OLETF rat groups. However, the LETO-Sed group exhibited significantly lower %Fat and significantly higher LBM% than the 4 OLETF rat groups. Post treatment, the OLETF-Sed group showed a tendency for increased VFM, TSFM, and Fat%, although this did not reach statistical significance, and showed no change in LBM%. The OLETF-Pala group exhibited a significant decrease in TSFM and Fat% and a significant increase in LBM% compared with pretreatment levels. This group had significantly lower VFM, TSFM, and Fat%, and significantly higher LBM% than the OLETF-Sed group post treatment. Therefore, it appears that the Pala-containing diet significantly reduces Fat% and increases LBM%. The OLETF-Ex group showed marked reductions in VFM, TSFM, and Fat%, and increased LBM% compared with the OLETF-Pala group. Notably, the OLETF-Pala & Ex group exhibited lower Fat% and higher LBM% when compared with the OLETF-Pala and/or OLETF-Ex groups posttreatment.

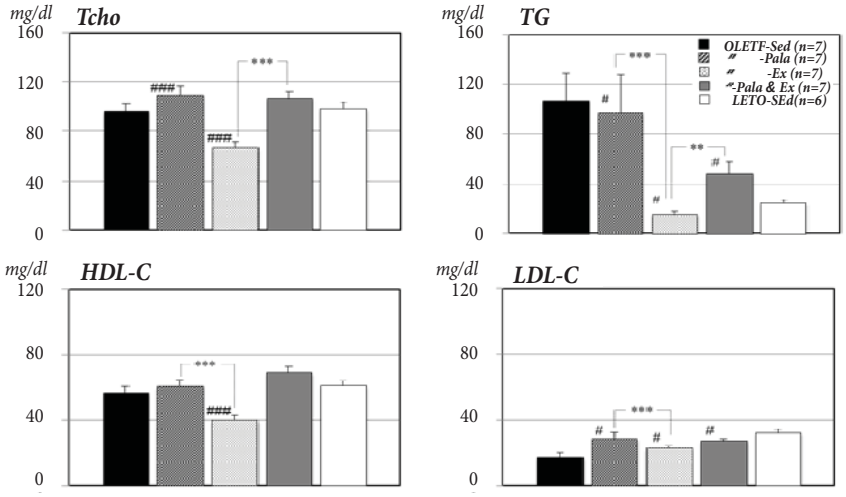



Values are mean ± SE. ††$P < 0.01$, †††$P < 0.001$ compared with pretreatment levels; #$P < 0.05$ compared with the OLETF-Sed group; **$P < 0.01$, ***$P < 0.001$ between two groups. NS, not significant among the 4 OLETF rat groups.

**Figure 3** Changes in VFM, TSFM, %Fat, and %LBM pre and posttreatment.

### Serum lipid concentrations post treatment

Serum lipid concentrations are shown in Figure 4. The OLETF-Pala group showed significantly higher levels of serum T-cho and LDL-C, although this group had a significantly lower TG concentration than that in the OLETF-Sed group post treatment. The OLETF-Pala and Ex group showed a significantly lower TG level than the OLETF-Sed and OLETF-Pala groups, whereas the TG level was significantly higher than in the OLETF-Ex group. The OLETF-Ex group showed significantly lower levels of T-cho, TG, LDL-C, and HDL-C than the other OLETF rat groups.

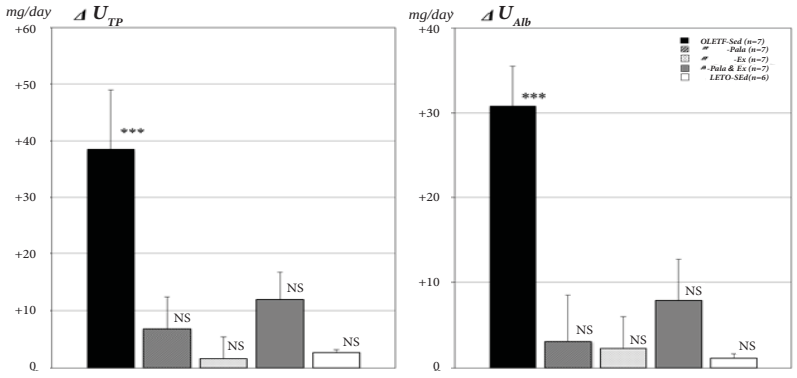



Values are mean ± SE. #$P$ < 0.05, ###$P$ < 0.001 compared with the OLETF-Sed group; **$P$ < 0.01, ***$P$ < 0.001 between two groups.

**Figure 4:** Serum lipid concentrations measured posttreatment.

### Changes in urinary protein excretion and morphological findings of the kidney

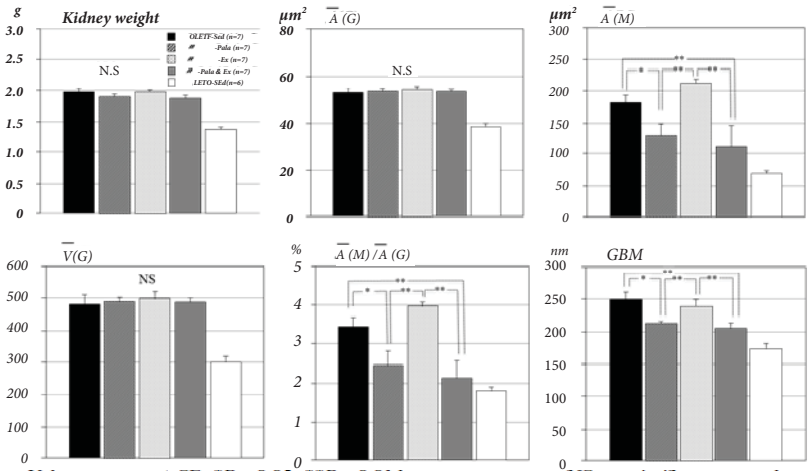
There were no significant differences in $U_{TP}$ and $U_{Alb}$ levels among the 4 OLETF rat groups pretreatment, with mean levels of 47.8 ± 5.0 and 25.8 ± 3.3 mg/day, respectively. These levels in the OLETF rat groups were significantly higher than those in the LETO rat group ($U_{TP}$, 4.6 ± 1.9 and $U_{Alb}$, 0.5 ± 0.6 mg/day, $P$<0.001 with effect size 2.41 and $P$<0.001 with effect size 1.94, respectively,). Net changes in $U_{TP}$ ($\Delta U_{TP}$) and $U_{Alb}$ ($\Delta U_{Alb}$) pre- and posttreatment are shown in Figure 5. The OLETF-Sed group exhibited a significant post treatment increase in $\Delta U_{TP}$ and $\Delta U_{Alb}$, although the other 3 OLETF and LETO rat groups showed no significant change in urinary protein excretion.




Values are mean ± SE. ***$P$ < 0.001 compared with pretreatment levels. NS, not significant compared with pretreatment level.

**Figure 5:** Net changes in urinary excretion of total protein ($\Delta U_{TP}$) and albumin ($\Delta U_{Alb}$) pre- and posttreatment.

Morphological findings of the kidney analyzed post treatment are shown in Figure 6. There were no significant differences in kidney weight, $\overline{A}(M)$ , and $\overline{V}(G)$ among the 4 OLETF rat groups. However, the OLETF-Pala and OLETF-Pala and Ex groups exhibited a significantly lower $\overline{A}(M)$ and $\overline{A}(M)/\overline{A}(G)$ , and a significantly thinner GBM than those in the OLETF-Sed and OLETF-Ex groups. No significant differences in these morphological findings were observed between the OLETF-Pala and OLETF-Pala and Ex groups.




Values are mean ± SE. *$P$ < 0.05, **$P$ < 0.01 between two groups. NS, not significant among the 4 OLETF rat groups.

**Figure 6** Morphological findings of the kidney analyzed posttreatment.

**Relationships among morphological findings in the kidney, urinary protein excretion, and BP**

When the relationships among posttreatment morphological findings in the kidney, $U_{TP}$, $U_{Alb}$, and SBP/DBP were examined, significant positive correlations were observed between SBP and GBM thickness (r=0.622, P<0.001 with effective size 0.93) and between SBP and $\bar{A}(M)$ (r=0.624, P<0.001 with effective size 0.05). However, no significant relationships were observed between urinary protein excretion and morphological findings.

## DISCUSSION

Hypertension is one of the causes of nephropathy progression in humans and animal models [15,16], and antihypertensive drugs attenuate the progression of renal damage [17,18]. Weight loss by restriction of calorie intake does not always improve diabetes-associated hypertension and also lowers LBM [19,20]. Although exercise regimens can improve insulin sensitivity [21], they can sometimes lead to the progression of DN [6,14].

Considering the numerous reports on the benefits of long-term administration of Pala on BP, MetS component factors, IR [1,2], and prevention of diabetic complications [3-5], combination treatment with a Pala-containing diet and exercise may be recommended as a regimen for patients with obesity and diabetes mellitus. Although several groups investigated the effects of long-term administration of Pala on MetS component factors [4,22,23], the effects of long-term combination treatment with Pala and exercise on DN progression have yet to be elucidated.

In the present study, grouping was performed so that BP was approximately equal among the 4 OLETF rat groups. As a result, significant differences in pretreatment BW were observed between groups. Although the pretreatment BW of both the OLETF-Sed and OLETF-Ex groups was significantly lower than that in the other 2 OLETF rat groups, the OLETF-Sed group showed a gradual increase in BW during the treatment and the highest BW level among the 4 OLETF rat groups posttreatment.

The OLETF-Ex group showed a decreased Fat% and an increased LBM% level, as well as inhibited BW gain. The OLETF-Pala group showed no significant change in BW during the treatment period, although its pretreatment BW was 15.5% greater than that in the OLETF-Sed group. Consequently, post treatment BW in the OLETF-Pala group was 74.9 g (12.3%) lower than that in the OLETF-Sed group. The OLETF-Pala group showed significantly lower Fat% and significantly higher LBM% compared with the OLETF-Sed group, although these changes were less than those in the OLETF-Ex and OLETF-Pala and Ex groups. Posttreatment, the greatest changes in body composition were observed in the OLETF-Pala and Ex group.

FC was significantly higher in the OLETF-Sed group than in the OLETF-Pala group during treatment, but no change was seen in FC throughout the treatment period in these 2 groups. These findings suggest that FC may not have directly affected BW change in these groups, in turn suggesting that the suppression of BW gain in the OLETF-Pala group was not caused by a decrease in FC.

The OLETF-Pala group showed significant improvements in FBS, FSI, HOMA-IR, FSL, and serum TG levels, although not as pronounced as those in the OLETF-Ex and OLETF-Pala and Ex groups. The mechanisms underlying the suppression of BW gain observed in the OLETF-Pala group are unclear. Food-induced thermogenesis may be higher on a Pala-containing diet than that on standard rat chow, although their energy densities were approximately the same. This suppression of BW gain and inhibition of insulin secretion caused by Pala intake [3-5] may have resulted in improved glucose metabolism-related parameters in the OLETF-Pala group, although the improvements were not as marked as in the OLETF-Ex and OLETF-Pala and Ex groups. Moreover, the OLETF-Pala group exhibited lower concentrations of serum TG and FSL, although this group showed a slightly yet statistically significantly higher concentration of T-cho and LDL-C compared with the OLETF-Sed group posttreatment.

The OLETF-Pala and Ex group ran a significantly shorter cumulative distance over the 5-week treatment period than the OLETF-Ex group. Oosthuyse et al. [24] reported that humans who orally ingested Pala solution before exercise experienced severe gastrointestinal symptoms during prolonged or high-intensity exercise, and that exercise performance was significantly lower compared with that after ingesting fructose-maltodextrin solution. Moreover, they suggested that gastrointestinal distress induced by Pala ingestion might result in poorer exercise performance. Based on their suggestion, it is possible that gastrointestinal distress might have limited FC and resulted in BW loss in the present study.

However, there was no significant difference in FC between the OLETF-Ex and OLETF-Pala and Ex groups during the treatment period, and FC tended to increase in both groups in the latter half of the period. Therefore, it is unlikely that the poorer performance of the OLETF-Pala and Ex group was caused by gastrointestinal distress due to the Pala-containing diet. There was no significant change in BW during the treatment between the 2 groups, although pretreatment BW in the OLETF-Pala and Ex group was greater than that in the OLETF-Ex group. The cause of the difference in running distance is unknown.

The OLETF-Ex group showed a significant increase in BP during the treatment period, as shown in our previous studies [6,7,14], whereas all MetS component factors measured in this study improved. In addition, expansion of $\overline{A}(M)$ and thickened GBM were observed in the OLETF-Ex group despite no apparent increase in $U_{TP}$ or $U_{Alb}$.

Early changes in pathomorphological findings including glomerular enlargement, thickened GBM, and mesangial proliferation are observed in the first (prenephropathy) and second (early nephropathy) diabetes stages [25,26], although an increase in $U_{Alb}$ does not always occur. It was shown that pathomorphological changes in the kidney precede an increase in $U_{Alb}$ in these stages. OLETF rats used in this study develop GI from around 10 weeks of age and exhibit an increase in $U_{Alb}$ after 20 weeks [6,27]. The present study commenced from the age of 25 weeks, corresponding to the first and second diabetes stages in OLETF rats. It is thought that an increase in proteinuria following DN might be due to a decline in the charge-selective barrier caused by decreased content of heparansulfate proteoglycan in the kidney GBM [28] and to intraglomerular hypertension caused by a systemic BP increase [29]. It is also known that systemic hypertension can be caused by both increased extracellular fluid volume and enhanced sympathetic nervous activity due to hyperinsulinemia in diabetes mellitus [30].

The present study and our previous ones [6,7,14] showed that the increase in $U_{TP}$ and $U_{Alb}$ during the treatment period was significantly lower in the OLETF-Ex group than in the OLETF-Sed group, suggesting that exercise repaired the damaged charge-selective barrier of the GBM due to improved GI and dyslipidemia, and consequently, $U_{TP}$ and $U_{Alb}$ were merely inhibited. However, the OLETF-Ex group showed increased and thickened GBM. This deterioration of renal morphological findings might be caused by a failure to inhibit BP increase $\overline{A}(M])$ during the treatment period. Cooper et al. [31] induced diabetes by streptozotocin administration to spontaneously hypertensive (SHR-DM) and normotensive Wistar-Kyoto (WKY-DM) rats and reported that the SHR-DM group showed increased BP, whereas BP in the WKY-DM rats remained at normal levels after the 32-week experimental period. Moreover, urinary protein excretion increased during that period, and marked GBM thickness and mesangial proliferation were observed at 32 weeks in the SHR-DM group, although higher blood and urinary glucose levels during the period were similar in both groups. Cooper et al.'s findings [31] suggest that diabetes mellitus complicated with hypertension promotes DN. We reconfirmed that exercise is useful in improving GI and dyslipidemia but it did not inhibit the progression of DN in OLETF rats, as shown in our previous studies [6,7,14].

The OLETF-Pala and OLETF-Pala and Ex groups showed an inhibited BP increase during the treatment period and

an inhibited $\overline{A}(M)$ expansion, thickened GBM, and a decrease in the $\overline{A}(M)/\overline{A}(G)$ ratio post treatment. These renal morphological findings in the OLETF-Pala and OLETF-Pala and Ex groups were similar to those observed in our previous study [14] assessing the effects of long-term administration of the calcium channel blocker azelnidipine in OLETF rats. However, the mechanisms underlying the renal-protective effects of Pala administration are unknown.

Angiotensin II (Ang II) constricts efferent arterioles in the kidney [32-34], resulting in an increase in intraglomerular pressure, which induces renal tissue damage including mesangial cell proliferation and GBM thickening [20,35]. On the other hand, it is known that glucagon-like peptide-1 (GLP-1) reduces sodium reabsorption at renal proximal tubules, renal plasma flow, and the glomerular filtration rate, and lowers plasma Ang II and aldosterone levels [30]. Furthermore, GLP-1 is also known to inhibit Ang II-induced mesangial cell damage [36] and to improve insulin sensitivity [37]. Although GLP-1 was not measured in this study, Pala stimulates GLP-1 secretion [37].

In the present study, the improved IR, inhibited $\overline{A}(M)$ expansion and GBM thickening, and reduced $\overline{A}(M)/\overline{A}(G)$ ratio observed post treatment in the OLETF-Pala and OLETF-Pala and Ex groups might have been due to improved insulin sensitivity [37] and attenuation of the intraglomerular pressure increase caused by Pala-induced GLP-1 secretion [30,36,37]. The inhibition of $U_{TP}$ and $U_{Alb}$ and renal morphological damage observed post treatment in both the OLETF-Pala and OLETF-Pala and Ex groups might be due to the inhibited BP increase caused by Pala-induced GLP-1 secretion. These assumptions are supported by the significant correlations between SBP and GBM thickness and between SBP and $\overline{A}(M)$ in this study, with a trend toward decreased BP during the treatment period observed in the OLETF-Pala and OLETF-Pala and Ex groups.

Although the post treatment reduction in body fat mass in the OLETF-Pala group was less than that in the OLETF-Ex group, similar levels of reduced FSL concentrations were observed in both groups. Okuno et al. [38] suggested that Pala-induced improvement of insulin sensitivity could be attributed to a reduction in serum leptin concentrations. Leptin administration stimulates the sympathetic nervous system [39], whereas administration of Pala was shown to reduce serum leptin levels [12]. Pala-induced GLP-1 secretion and reduction of serum leptin concentrations may improve IR and inhibit BP increases.

In the present study, although the exercise regimen alone did not inhibit the progression of DN, combined treatment with Pala and exercise inhibited its progression and markedly reduced VFM, increased LBM, and improved GI and dyslipidemia compared with rats in the exercise regimen-alone and/or Pala-containing diet regimen-alone groups. These results suggest that the progression of DN was inhibited due to improved insulin sensitivity, reduced leptin secretion, inhibition of BP increase due to suppression of sympathetic nervous system stimulation, and increased GLP-1 secretion caused by Pala administration.

## CONCLUSION

This study demonstrated that a Pala-containing diet alone or combined with exercise improved MetS component factors including hypertension, VFM, GI, and dyslipidemia without the progression of DN, although the decrease in BW and %Fat and increase in %LBM was significantly higher in the combined treatment than in the Pala-containing diet alone group in the OLETF obese diabetic rat model.

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## DISCLOSURE

MS, DS, KG and YO have no conflict of interest to declare. KM and MM are employees of Mitsui Sugar Co., Ltd.. MS was responsible for conceiving the study and its design; DS and KG performed the experiments, analyzed data, and prepared tables and figures; YO drafted the manuscript; KM and MM edited and revised the manuscript; and MS interpreted the experimental results and approved the final version of the manuscript.

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