Letter to the Editor

Green Tea Polyphenols Attenuate High-Fat Diet-Induced Renal Oxidative Stress through SIRT3-Dependent Deacetylation*

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Fifty male Wistar rats were fed a standard chow diet or a high-fat (HF) diet, and different concentrations of green tea polyphenols (GTPs) (0.8, 1.6, and 3.2 g/L) were administered in the drinking water. We found that the malondialdehyde (MDA) level in the HF diet group was significantly higher than that in the control (CON) group (*P*<0.05). Decreased peroxisome proliferator-activated receptor (PPAR)-α and sirtuin 3 (SIRT3) expression, and increased manganese superoxide dismutase (MnSOD) acetylation levels were also detected in the HF diet group (*P*<0.05). GTP treatment upregulated SIRT3 and PPARα expression, increased the *pparα* mRNA level, reduced the MnSOD acetylation level, and decreased MDA production in rats fed a HF diet (*P*<0.05). No significant differences in total renal MnSOD and PPAR-γ coactivator-1α (PGC1-α) expression were detected. The reduced oxidative stress detected in kidney tissues after GTP treatment was partly due to the higher SIRT3 expression, which was likely mediated by PPARα.

Lysine acetylation has emerged as a pivotal post-translational modification for the modulation of cellular metabolism and functions. Increases in metabolic fuels, such as fatty acids, glucose, and amino acids, increase the acetylation of proteins involved in mitochondrial metabolic cycles, with concomitant regulation of enzyme activities[1]. A recent study has shown that acetyl-CoA (AcCoA) generated by fatty acid oxidation directly provides acetyl units for lysine acetylation in the mitochondria[2]. These findings have led to speculation regarding non-enzymatic mitochondrial acetylation and the key roles of deacetylation. As a major NAD^+-dependent deacetylase in mitochondria, SIRT3 regulates many mitochondrial functions, including energy metabolism and homeostasis, oxidative stress, and cell survival, by regulating the deacetylation of numerous mitochondrial proteins[3].

A high-fat diet, or Western-type diet, which is associated with an increase in the prevalence of obesity, metabolic syndrome, type 2 diabetes mellitus, and cardiovascular disease, could cause oxidative renal injury[4]. Green tea polyphenols are natural antioxidants. The positive effects of GTPs include regulation of body weight, reduction of cholesterol level and cardiovascular disease (CVD) risk, and prevention of cancer and neurodegenerative disease. However, whether GTPs can attenuate high-fat diet-induced renal damage is unknown. We hypothesized that GTPs promote reactive oxygen species (ROS) clearance by upregulating the expression of SIRT3. We tested this hypothesis to gain insight into a novel anti-oxidative mechanism of GTPs.

Fifty male Wistar rats (40-60 g) were randomly divided into 5 groups after a 1-week acclimation. The control group was fed standard chow; the other 4 groups were fed modified HF chow containing 60% (w/w) standard chow, 12% sugar, 12% lard, 8% yolk powder, 6% peanut powder, and 1% milk powder. From the 4th week, 3 of the 4 HF diet groups were administered water containing different concentrations of GTPs (polyphenol 99.99%; catechin 91.21%; EGCG 71.72%) as follows: low dose (GL), 0.8 g/L; medium dose (GM), 1.6 g/L; and high dose (GH), 3.2 g/L. The rats were fed for 26 weeks.

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Body weight was recorded, and the tissues (liver, kidney, pancreas, and fat) of the sacrificed rats were removed and weighed. The serum was separated and stored at -20 °C until testing. The kidneys were frozen in liquid nitrogen and stored at -80 °C until analysis.

MDA was detected by measuring the levels of thiobarbituric acid (TBA) reactive substances (TBARS) at 532 nm (Nanjing Jiancheng Bioengineering Co. Ltd., Nanjing, China). Data were expressed as nanomoles per milligram of protein.

Protein expression was tested by immunoblotting. Kidney tissues were rapidly homogenized and lysed in cold RIPA extraction buffer and then incubated on ice for 2 h[5]. The cell debris was separated by centrifuging at 12,000 ×g for 15 min at 4 °C, and the supernatant was collected. The protein concentration was determined using Bio-Rad DC Protein Assay Reagent (Bio-Rad, Hercules, CA, USA). Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting were performed according to the method of Amersham Biosciences (Buckinghamshire, UK). The primary antibodies used included anti-PPARα (Abcam, Cambridge, MA, USA), anti-SIRT3 (CST, Billerica, MA, USA), anti-PGC1-α (CST, Billerica, MA, USA), anti-MnSOD (Santa Cruz, CA, USA), anti-acetylated-lysine (CST, Billerica, MA, USA), and anti-β-actin (Sigma Aldrich, St. Louis, MO, USA). Protein expression bands were detected using a chemiluminescent detection system (SynGen, Cambridge, UK) and analyzed with the ChemiDoc Quantity One software (Bio-Rad).

Immunohistochemistry was used to detect the expression and localization of SIRT3. Kidney tissues were fixed in 4% paraformaldehyde, dehydrated, and embedded in paraffin. The sections were incubated with the corresponding antibody, counterstained with hematoxylin, dehydrated with ethanol, and then covered with cover slips. Samples were analyzed using an Olympus BX6 microscope and Image-Pro Plus 6.0 software (Media Cybernetics, Inc. Rockville, MD, USA).

The mRNA of PPARα was measured by qPCR. Total RNA was extracted with TRIzol reagent (Invitrogen, Carlsbad, CA, USA) and then reverse transcribed into cDNA using a reverse transcription reaction kit (Toyobo, Osaka, Japan). The cDNA was then subjected to qPCR with specific primers for *pparα* (sense: TGA AAG ATT CGG AAA CTG C; antisense: TTC CTG CGA GTA TGA CCC) and β-actin (sense: ACG TTG ACA TCC GTA AAG AC; antisense: TAG GAG CCA GGG TAA). Changes in relative gene expression were determined the comparative Ct method, with β-actin as the endogenous reference gene.

The results were presented as the mean±SD. Data were analyzed using one-factor ANOVA with the Student-Newman-Keuls or Dunnett's T3 test, depending on the homogeneity of the variance. The accepted level of significance was $P<0.05$.

The levels of MDA and Ac-MnSOD in renal tissue homogenates were significantly higher in the HF group than in the CON group; GTP treatment significantly decreased the MDA and Ac-MnSOD levels ($P<0.05$; Figure 1A and 1D). GTPs significantly alleviated the decrease in SIRT3 expression in kidney tissue induced by the HF diet ($P<0.05$; Figure 1B). No significant differences were observed in total renal MnSOD expression (Figure 1C).

Immunohistochemistry analysis suggested that the HF diet decreased SIRT3 expression in kidney tissues and that GTP treatment restored SIRT3 levels ($P<0.05$; Figure 2A-F). The immunohistochemical results were consistent with the immunoblotting results. They also showed an uneven distribution of SIRT3 in rat renal tissues. The point-like or stripped distribution shown in the pictures probably resulted from the transverse and longitudinal sectioning of rat renal vessels.

GTPs significantly relieved the downregulation of PPARα expression and *pparα* mRNA levels induced by the HF diet ($P<0.05$; Figure 3A and 3B). No significant differences in total renal PGC1-α expression were observed among the 5 groups (Figure 3D). GTP treatment significantly relieved the downregulation of HMGCS2 induced by the HF diet in a dose-dependent manner ($P<0.05$; Figure 3C).

A decrease in the MnSOD acetylation level greatly enhances its ability to reduce cellular ROS and oxidative stress during calorie restriction (CR)[6]. We found that a HF diet led not only to a decrease in rat renal SIRT3 expression but also to hyperacetylation of MnSOD, a major downstream mediator of SIRT3 in the reduction of cellular ROS. GTPs relieved the downregulation of SIRT3 and the hyperacetylation of MnSOD induced by the HF diet. These results suggested that the mechanism by which GTPs reduce oxidative stress was SIRT3-related and similar to the mechanism active in CR.

The effects of GTPs on the regulation of SIRT3 remain unknown. PGC-1α, an important regulator of mitochondrial function and biogenesis, upregulates

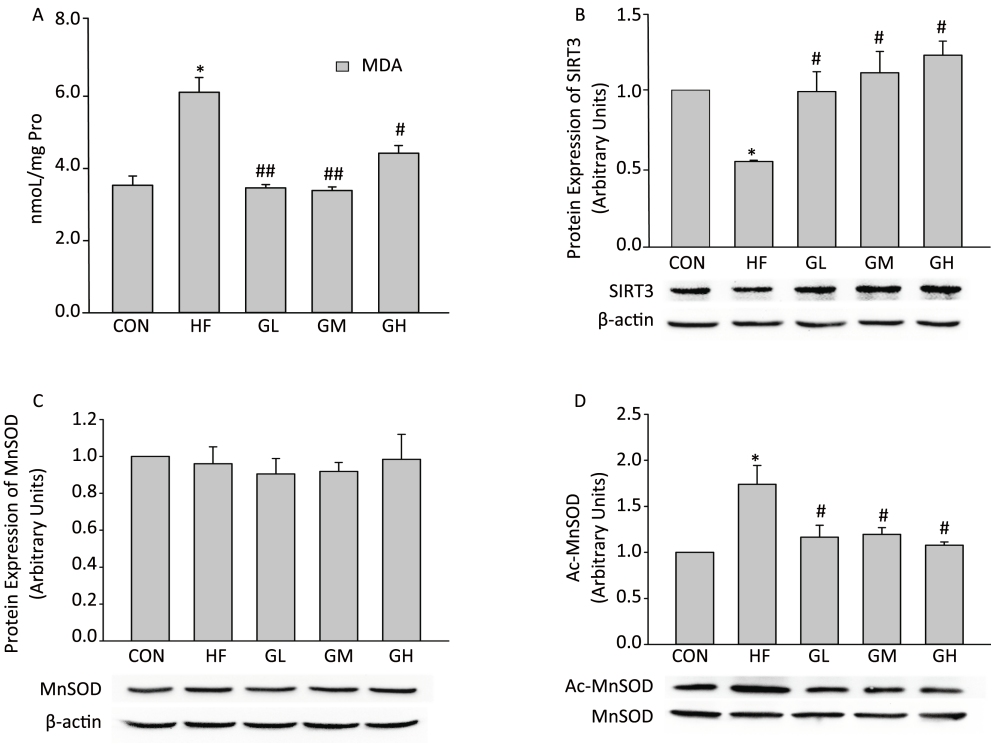

Figure 1. Effects of GTPs on renal MDA, SIRT3, MnSOD, and Ac-MnSOD levels in rats fed a HF diet. (A) MDA levels. (B) SIRT3 expression. (C) MnSOD expression. (D) Ac-MnSOD levels. ($^{*}P$<0.05 compared with the CON group; $^{\#\#}P$<0.01 and $^{\#}P$<0.05 compared with the HF diet group). MDA, malondialdehyde; SIRT3, sirtuin 3; MnSOD, manganese superoxide dismutase; Ac-MnSOD, MnSOD acetylation level; CON, control group; HF, high-fat diet group; GL, low dose GTPs; GM, medium dose GTPs; GH, high dose GTPs.

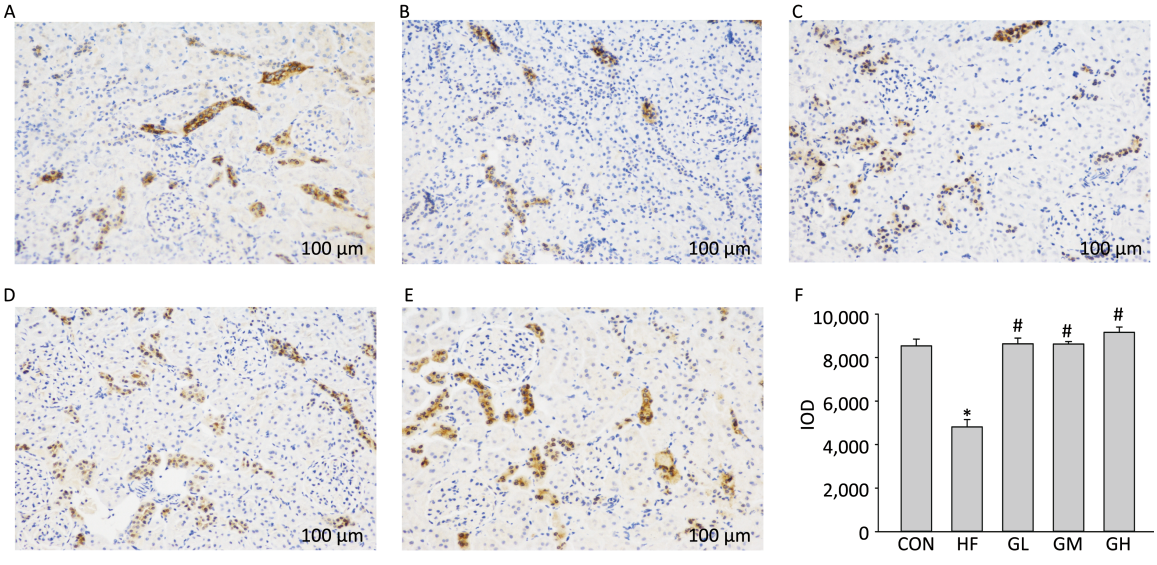

Figure 2. Immunohistochemistry staining results for SIRT3 (brown areas) in the rat kidney. (A) CON. (B) HF. (C) GL. (D) GM. (E) GH. (200× magnification, bars=100 μm). (F) The IOD values in the 5 groups. The IOD values, which represent the expression of SIRT3, were determined from the size and intensity of the brown areas. $^{*}P$<0.05 compared with the CON group; $^{\#}P$<0.05 compared with the HF group. IOD, integral optical density.

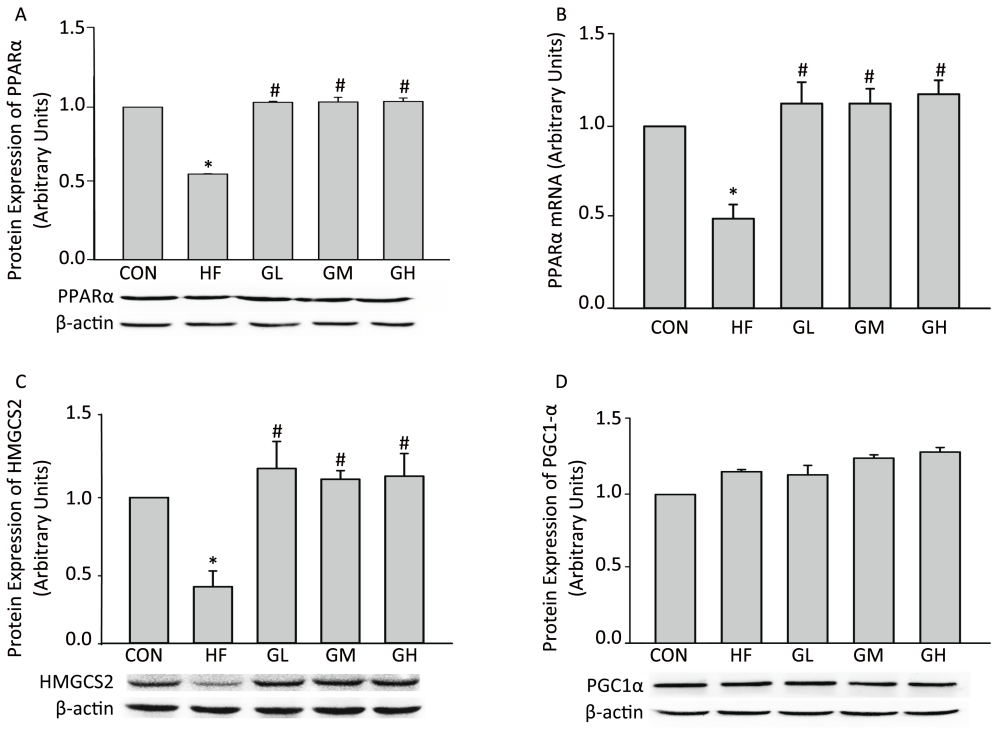

Figure 3. Effects of GTPs on the expression of PPARα, HMGCS2, and PGC1-α in the rat kidney. (A) Expression of PPARα. (B) *pparα* mRNA levels. (C) Expression of HMGCS2. (D) Expression of PGC1-α. $^{*}P<0.05$ compared with the CON group; $^{\#}P<0.05$ compared with the HF diet group. HMGCS2, mitochondrial 3-hydroxy-3-methylglutaryl CoA synthase 2; PGC1-α, peroxisome proliferator-activated receptor γ coactivator-1α; PPARα, peroxisome proliferator-activated receptor-α.

mouse SIRT3 gene expression in muscle cells and hepatocytes[7]. No differences in PGC-1α expression were observed in any of the groups in our study. GTP treatment upregulated PPARα expression and the *pparα* mRNA level. PPARα, a ligand-activated transcription factor, regulates cellular metabolism by altering the expression of a large number of target genes. On one hand, prior activation of liver X receptor (LXR) upregulated SIRT3 expression in fed and fasted wild-type mice, but this response was completely blocked in fasted PPARα-null mice, indicating that the level of LXR activation and the degree of PPARα activation influenced SIRT3 expression in skeletal muscle under starvation conditions[8]. On the other hand, mitochondrial 3-hydroxy-3-methylglutaryl CoA synthase 2 (HMGCS2), a control enzyme in ketogenesis, contains a PPARα responsive element[9]. Additionally, we found that GTPs relieved the downregulation of rat renal HMGCS2 induced by a HF diet (Figure 3C). Based on these findings, we propose that GTPs protect the rat kidney from the oxidative damage induced by a HF diet via a SIRT3/MnSOD pathway, which is likely mediated by PPARα. Further studies to confirm the effects of GTPs in a PPARα knockout mouse will help substantiate this conclusion.

In conclusion, we observed that the expression of SIRT3 decreased in the kidneys of rats fed a HF diet. The decrease in SIRT3 expression was accompanied by an increase in MDA and in MnSOD acetylation levels. The reduced oxidative stress detected in kidney tissues after GTP treatment was partly due to the higher SIRT3 expression, which was likely mediated by PPARα. Therefore, increasing SIRT3 expression might be a novel therapeutic strategy for pathologies related to oxidative stress.

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