

Exercise alleviates diabetic kidney disease through PPAR δ -CPT1 α pathway-dependent fatty acid β -oxidation

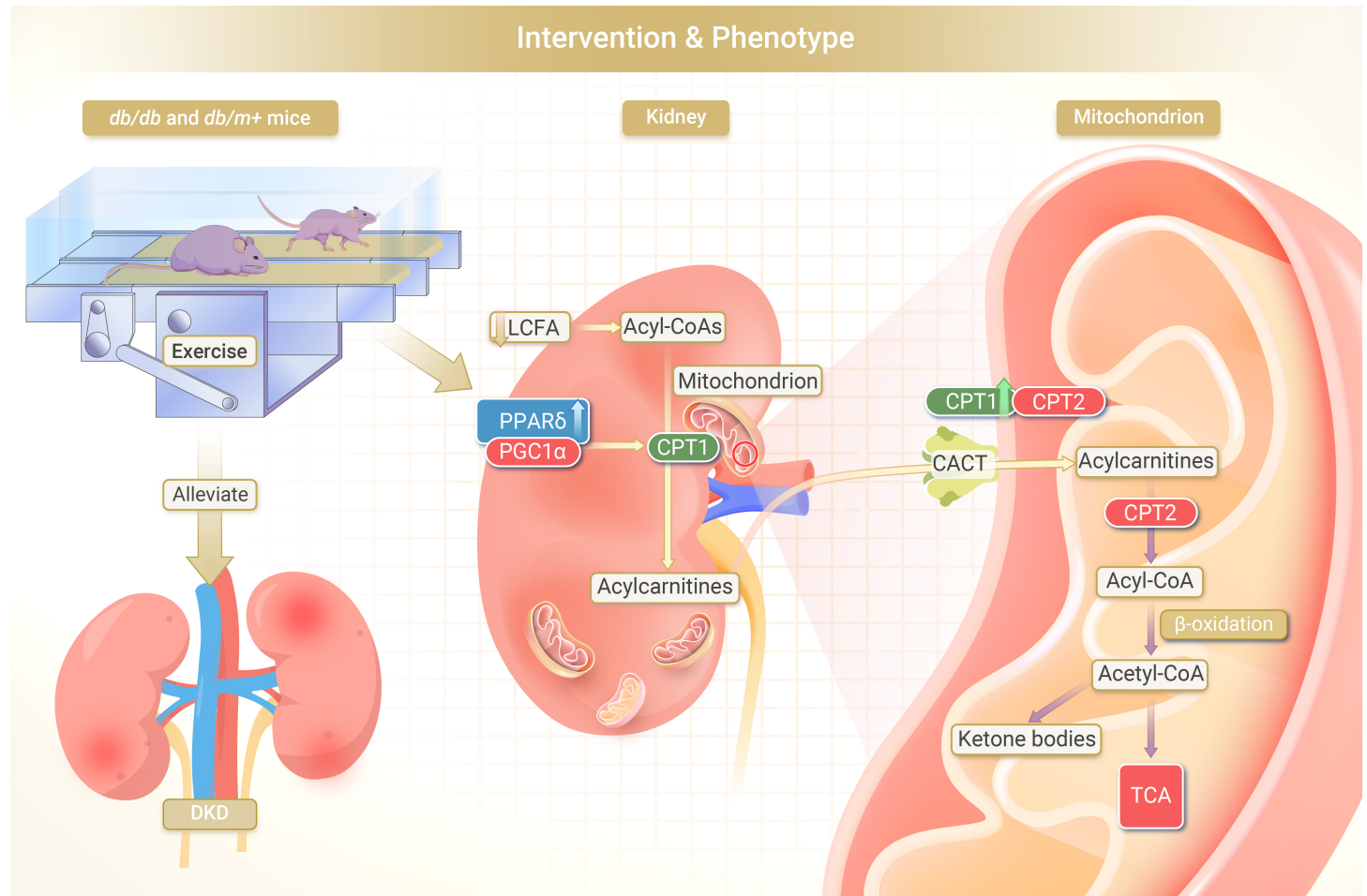
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*Correspondence: zwcai@hkbu.edu.hk (Z.C.); yu.huang@cityu.edu.hk (Y.H.)

Received: January 15, 2024; Accepted: May 14, 2024; Published Online: May 17, 2024; <https://doi.org/10.59717/j.xinn-life.2024.100065>

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GRAPHICAL ABSTRACT



PUBLIC SUMMARY

- Regular physical exercise is an effective strategy in alleviating diabetic kidney disease.
- Exercise restores renal function in diabetic mice through enhancing PPAR δ -CPT1 α -dependent fatty acid β -oxidation.
- Reveal an unappreciated link between exercise and the restoration of renal function in diabetic kidney disease.
- Could potentially become a new target for therapeutic intervention against diabetic kidney disease.



Exercise alleviates diabetic kidney disease through PPAR δ -CPT1 α pathway-dependent fatty acid β -oxidation

Li Xiang,^{1,10} Li Wang,^{2,10} Yin Xia,³ Yu Wang,⁴ Jingchun Shi,¹ Cheng-Lin Zhang,⁵ Liping Xie,⁶ Yi Ru,¹ Chak Kwong Cheng,² Yujie Pu,² Libing Hu,⁷ Junli Liu,⁸ Suowen Xu,⁹ Zongwei Cai,^{1,*} and Yu Huang^{2,*}

¹State Key Laboratory of Environmental and Biological Analysis and Department of Chemistry, Hong Kong Baptist University, Hong Kong 999077, China

²Department of Biomedical Sciences, City University of Hong Kong, Hong Kong 999077, China

³School of Biomedical Sciences, The Chinese University of Hong Kong, Hong Kong 999077, China

⁴Department of Endocrinology, Shenzhen University General Hospital, Shenzhen 518000, China

⁵Department of Pathophysiology, Shenzhen University, Shenzhen 518000, China

⁶Key Laboratory of Targeted Intervention of Cardiovascular Disease, Nanjing Medical University, Nanjing 210000, China

⁷Department of Urology, Yan'an Affiliated Hospital of Kunming Medical University, Kunming 650051, China

⁸Shanghai Diabetes Institute, Department of Endocrinology and Metabolism, Shanghai Jiao Tong University Affiliated Sixth People's Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai 200032, China

⁹Department of Endocrinology, The First Affiliated Hospital of USTC, Division of Life Sciences and Medicine, University of Science and Technology of China, Hefei 230000, China

¹⁰These authors contributed equally

*Correspondence: zwcai@hkbu.edu.hk (Z.C.); yu.huang@cityu.edu.hk (Y.H.)

Received: January 15, 2024; Accepted: May 14, 2024; Published Online: May 17, 2024; <https://doi.org/10.59717/j.xinn-life.2024.100065>

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Citation: Xiang L., Wang L., Xia Y., et al., (2024). Exercise alleviates diabetic kidney disease through PPAR δ -CPT1 α pathway-dependent fatty acid β -oxidation. The Innovation Life **2**(2): 100065.

Diabetic kidney disease (DKD), a severe diabetic complication affecting approximately one-third of diabetic patients, is the leading cause of end-stage chronic kidney disease. The benefits of regular exercise for patients with DKD have been well documented, particularly in overweight patients with DKD. However, the underlying mechanisms are incompletely understood. The present study demonstrates that exercise improves kidney function in diabetic *db/db* mice through activating PPAR δ -mediated fatty acid β -oxidation (FAO). Twelve-week treadmill running exercise improved kidney function in *db/db* mice. Metabolomics and transcriptomics profiling analysis collectively revealed that dysregulation of FAO in *db/db* mice was largely corrected by running exercise. KEGG pathway enrichment revealed that PPAR pathway, a critical signalling cascade in FAO and exercise, is involved in DKD, suggesting PPAR δ activation protects renal function through promoting FAO. Which was confirmed by improved renal function and enhanced FAO in *db/db* mice treated with PPAR δ agonist GW501516. Oppositely, PPAR δ -specific inhibitor GSK0660 abolished exercise-mediated improvement in renal function and FAO. In combining with gene expression profiling data we identified CPT1 α , the rate-limiting enzyme in fatty acid oxidation, was suppressed in the kidney of diabetic mice and reversed by exercise and the PPAR δ agonist GW501516. The findings hint at a potential role for the PPAR δ -CPT1 α pathway in exercise-induced improvements in diabetic renal function and may warrant further exploration of the PPAR δ -CPT1 α pathway as a therapeutic target in DKD.

INTRODUCTION

Diabetic kidney disease (DKD), the common cause of end-stage renal disease, is the leading cause of morbidity and mortality in patients with type 2 diabetes (T2DM).^{1,2} Lifestyle modifications such as healthy diets and regular physical exercise are effective in the management of T2DM and chronic renal disease.³⁻⁶ Several studies revealed the pleiotropic beneficial effect of exercise in improving renal function including reducing blood pressure, blood glucose, lipid level and body weight, and improving insulin sensitivity.⁷⁻¹⁰ However, the underlying mechanisms remain incompletely understood.

Increased lipid utilization is one of the prominent metabolic events during exercise.¹¹ Renal tubular epithelial cells (TECs) rely on fatty acids as the main energy source.¹² As a result, suppressed fatty acid oxidation (FAO) in TECs results in energy crisis, which subsequently promotes apoptosis and dedifferentiation, and ultimately leads to renal fibrosis and DKD progression.¹³ Peroxisome proliferator-activated receptors (PPARs) are the master regulators of lipid metabolism. Particularly, PPAR δ agonists were revealed to mimic the beneficial effects of exercise and increase running endurance by enhancing fatty acid metabolism.^{14,15} However, how PPAR δ regulated during exer-

cise is still unknown.^{16,17} We hypothesize that PPAR δ is suppressed in diabetic kidney disease and PPAR δ activation contributes to restoration of the impaired renal function during exercise under diabetic conditions. To elucidate the underlying mechanisms of exercise-induced beneficial effect on DKD, we compared the gene expression profiles of kidneys from diabetic mice and non-diabetic mice. Both KEGG pathway analysis and GSEA analysis revealed that PPAR signalling and fatty acid metabolism pathways are significantly enriched. Consistently, the non-targeted metabolomics analysis revealed FAO was suppressed in the kidney of diabetic mice and reversed by running exercise. By using specific antagonist and agonist, we identified PPAR δ have profound impact on FAO and FAO related gene expression. Further studies revealed PPAR δ activation improved FAO and renal function. By contrast, treatment with a PPAR δ antagonist abolished the beneficial effect of exercise. A multi-omics study was carried out to identify potential PPAR target that directly associated with the FAO. As the gene expression profile and metabolic profile collectively revealed, CPT1 α , the rate-limiting enzyme of FAO, is essential for PPAR δ activation-induced improvement in FAO and renal function.¹⁸⁻²⁰

MATERIALS AND METHODS

Experimental animals

Commonly used male *db/db* C57BL/KsJ leptin receptor deficient mice were selected as mouse model of diabetic nephropathy. Age-matched male *db/m+* heterozygous (C57BL/KsJ) were used as normal control mice. Mice were supplied by the CUHK Laboratory Animal Service Center. Male *db/m+* and *db/db* mice (six to seven weeks-old) were randomly assigned to several groups. The exercise was initiated with 8 m/min, 30 min per day adaptive training for the first week, and incrementally increased to 10 m/min, 45 min per d, six days a week, and continuously for 12 weeks. Sedentary mice were kept in the stationary treadmill for 45 min per d. For PPAR δ agonist GW501516 treatment, GW501516 was dissolved in 0.5% carboxymethylcellulose and administered orally at 10 mg/kg/d,²¹ six days a week, for six consecutive weeks. Control mice were gavage with 0.5% carboxymethylcellulose. For PPAR δ antagonist GSK0660 treatment in mice with treadmill training, GSK0660 was dissolved in 0.9% NaCl solution (with 5% DMSO and 30% PGE400), intraperitoneally injected at 1 mg/kg/d, for 12 consecutive weeks. Control mice were administered with 0.9% NaCl solution (5% DMSO and 30% PGE400) without GSK0660.

End points

Various clinical indicators of renal function, such as urinary albumin, urinary creatinine, kim-1, TAG, BUN, total cholesterol was measured. Kidney fibrosis was evaluated by Periodic Acid-Schiff staining and Masson trichrome

staining. The expression levels of several proteins were measured by using immunohistochemistry or immunofluorescence. Mitochondrial respiration assays were conducted on a Seahorse XFP analyzer. Nontargeted metabolomics analysis of kidney samples was carried out on both LC-MS and GC-MS platforms.²² Targeted measurement of acylcarnitines was done using LC-MS, while targeted measurement of free fatty acids and ketone bodies was performed on LC-QqQ-MS.^{23,24} The detailed information on the animal experiments, methods and statistical analysis approaches is available in the Supplementary Methods. See supplemental information for details.

RESULTS

Running exercise improves renal function in *db/db* mice

Regular exercise is known to exert beneficial effects in patients with kidney disease.²⁵ To understand the underlying mechanism, the *db/db* mice, a common mouse model of DKD²⁶ were subjected to treadmill running exercise for 12-weeks (Figure 1A). Consistent with the previous studies in human,²⁷ running exercise increased insulin sensitivity (Figures S1A-B), reduced circulating triacylglycerol (TAG), total blood cholesterol level (Figures S1C-D), and body weight (Figure S1E). Meanwhile, exercise improved renal function as demonstrated by the reductions of blood urea nitrogen (BUN) (Figure 1B), urinary volume (Figure 1C), urinary albumin (Figure 1D), and urinary glucose (Figure 1E) in *db/db* mice subjected to running exercise. Moreover, the expression of several biomarkers in the kidneys from *db/db* mice revealed that exercise improved renal function. For example, exercise suppressed the expression of urinary lipocalin-2 (uNGAL), a marker of renal tubular injury (Figure 1F). On the other hand, the expression levels of Megalin (Figures 1G-I), an important mediator for tubular reabsorption of proteins (including albumin),²⁸ and E-cadherin, an antifibrotic marker, were increased (Figures S1F-G). Moreover, the increased levels of α -SMA and fibronectin, the two fibrotic markers, in *db/db* mice were reversed by exercise (Figures 1J-K). Furthermore, mesangial expansion, which is positively correlated to glomerular fibrosis and function,²⁹ was also attenuated by exercise (Figures S1H-I). Taken together, these results show that running exercise improves renal structure and function that are impaired in diabetic mice.

Exercise enhances fatty acid β -oxidation in kidneys of *db/db* mice

A multi-omics approach was used to investigate the underlying mechanisms of the beneficial effects of exercise. To begin with, kidneys from *db/m+* and *db/db* mice with and without running exercise were collected for nontargeted metabolomics analysis. The results show that exercise alters the metabolic profile of both normal and diabetic mice, with a greater effect in diabetic mice. (Figure S2A & Figure 2A). The differentiated metabolites in diabetic mouse kidneys were concentrated on fatty acid metabolism (Figure S2B), with less fatty acids were accumulated in kidneys from diabetic mice with exercise (Figure 2B). Analysis in detail revealed that long-chain fatty acid showed both the highest accumulation and most significant reversal with exercise (Figure 2C). Oppositely, the levels of acylcarnitines in the kidney were decreased in *db/db* mice, and significantly increased by exercise (Figure 2D), suggesting the fatty acid metabolism might be blocked during mitochondrial importation (Figure 2E). Next, we investigate the exercise-induced transcriptomic alterations. Consistently, the gene set enrichment analysis (GSEA) shows that exercise results in upregulated expression of genes involved in fatty acid metabolism (Figures 2F-G). In line with the metabolic profiling, carnitine palmitoyltransferase 1 α (CPT1 α), the rate-limiting fatty acid transporter, was induced by exercise (Figures 2G-H), which was further confirmed by the increased protein level in kidneys from diabetic mice subjected to exercise (Figures 2I). To further explore whether CPT1 α is associated with DKD in clinical kidney samples, we re-analyzed public available RNA sequencing data from DKD samples (GSE142025).³⁰ Consistent with our findings, the expression of CPT1 α is negatively correlated with the advancement of DKD (Figure 2J), which was further confirmed by the immunostaining (Figure 2K).

Exercise promotes FAO by activating PPAR δ

KEGG pathway enrichment reveals that PPAR pathway, the master regulator of lipid metabolism, were significantly altered in the kidney of diabetic mice (Figure 3A).³¹ To identify which PPARs family members contribute to

CPT1 α inhibition-mediated suppression of FAO, we examined the expression of CPT1 α in human kidney 2 (HK-2) and mouse kidney proximal tubule epithelial cells (TKPTS) treated with different PPAR antagonists (PPAR δ antagonist GSK0660, PPAR α antagonist GW6471, and PPAR γ antagonist GW9662). As the results revealed, PPAR δ inhibitor GSK0660 but not other PPAR antagonists suppressed the expression of CPT1 α in both cells (Figure 3B). Moreover, PPAR δ promoted FAO as revealed by PPAR δ specific agonist GW501516 increased the level of medium- to long-chain acylcarnitines (Figure 3C) and reduce the level of upstream free fatty acid (Figure S3A), and PPAR δ specific antagonist GSK0660 reduced the levels of medium- to long-chain acylcarnitines (Figure 3D), and elevated the levels of free fatty acids (Figure S3B). Moreover, PPAR δ activation shift the energy source from glucose to FAO as revealed by GW501516 suppressed lactate, the indicator of anaerobic glycolysis (Figure S3C), and increased the glucose level (Figure S3D). Reversely, GSK0660 displayed the opposite effect (Figure S3E). Seahorse analysis further revealed GW501516 shifts the metabolism from glucose to fatty lipid metabolism (Figures 3E-F). Consistently, PPAR δ protein level is reduced in the kidney from diabetic mice and reversed by exercise (Figures 3G-I).

PPAR δ activation improves FAO efficiency and renal function in *db/db* mice

To examine whether PPAR δ activation improves renal function, we utilized the PPAR δ agonist GW501516. This compound has received approval for its positive effects in various cellular processes, including the enhancement of fatty acid oxidation in muscle tissue,³² reduction of adiposity, and improved adaptation to training,³³ etc. GW501516 is a selective agonist with a high affinity for PPAR δ , exhibiting over 1000-fold selectivity relative to PPAR α and PPAR γ .³⁴ Although there have been reports of potential adverse effects, such as carcinogenesis in multiple organs, research on the role of GW501516 in tumors remains controversial, with some studies suggesting that GW501516 enhances colitis-associated colorectal cancer,³⁵ yet others finding that GW501516 inhibits undifferentiated nasopharyngeal carcinoma.³⁶ These divergent outcomes may be influenced by factors such as the timing and dosage of drug application, the multifaceted pathogenesis of tumors, and the heterogeneity of cancer types. To clarify the specific mechanisms by which GW501516 affects tumor growth, more comprehensive studies are required. Thus, considering the high selectivity of PPAR δ , in this study, we treated *db/db* mice with PPAR δ agonist GW501516 via oral gavage for 6 weeks. PPAR δ expression was significantly induced by GW501516 in kidneys from *db/db* mice (Figures 4A-B & Figure S4A). The levels of the FAO intermediates acylcarnitines, were elevated in both *db/m+* and *db/db* mice treated with GW501516 (Figure 4C). Consistently, the accumulation of free fatty acids in *db/db* mouse kidneys was markedly reduced (Figure 4D). Moreover, the reduced level of free carnitine which is the raw material in FAO pathway was restored by GW501516 in *db/db* mouse kidneys (Figure 4E). These results clearly show that activation of PPAR δ improves FAO efficiency in the kidneys of diabetic mice.

Several lines of evidence revealed that PPAR δ agonist GW501516 significantly improved the kidney function in T2DM. Mesangial expansion is the most important structural change in T2DM.³⁷ GW501516 treatment inhibited mesangial expansion in the kidneys of *db/db* mice (Figures S4B-C), reduced the urine volume, and suppressed the expression of renal injury markers uNGAL and BUN (Figures 4F-H), while it elevated Megalin levels (Figure 4I). In addition, the level of E-cadherin was also increased (Figures S4D-E). The elevated levels of fibronectin and α -SMA in *db/db* mouse kidneys were reversed by a 6-week treatment of GW501516 (Figure 4J & Figures S4F-G). These results demonstrate that similar to the effect of exercise, pharmacological activation of PPAR δ is effective in ameliorating renal dysfunction, injury, and fibrosis in diabetic mice.

PPAR δ mediates the beneficial effects of exercise on FAO and renal function in *db/db* mice

In order to determine the role of PPAR δ in exercise-induced FAO and renal function improvement, we treated *db/db* mice subjected to running exercise with PPAR δ antagonist GSK0660 (Figure 5A). The results showed that exer-

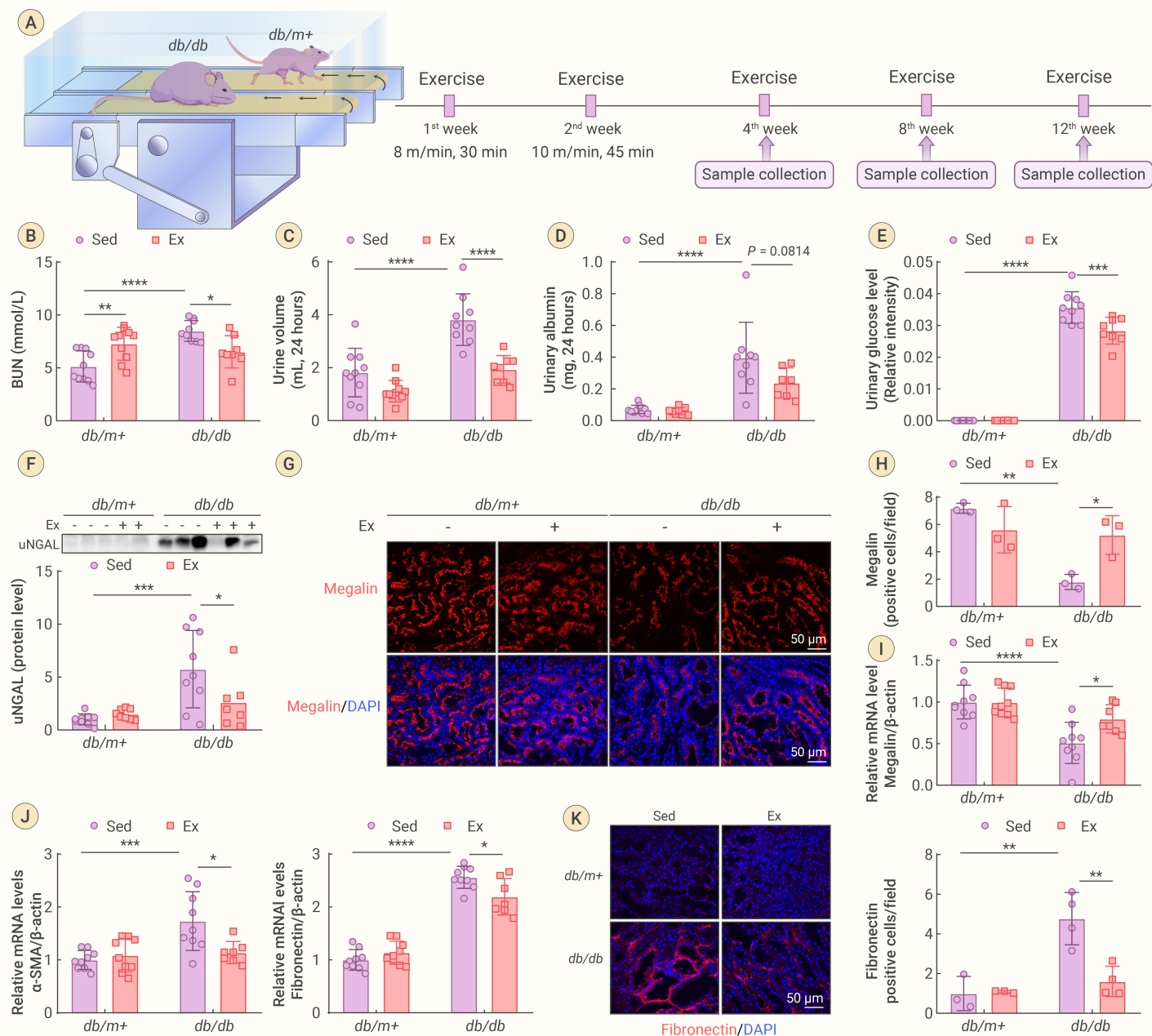


Figure 1. Regular exercise training improves renal function in *db/db* mice (A) schematic overview of exercise training and sample collection. (B) blood urea nitrogen (BUN) level in response to 12-week exercise in *db/m+* and *db/db* mice, $n=8-10$. (C) urine volume, $n=8-10$. (D) urinary albumin levels in response to 12-week exercise in *db/m+* and *db/db* mice, $n=8-10$. (E) urinary glucose level, $n=8-10$. (F) urinary lipocalin-2 (uNGAL) levels, $n=7-9$. (G) representative immunofluorescence staining of megalin in kidney samples of *db/m+* and *db/db* mice, $n=3$. Scale bar, 50 μm . (H) statistical analysis of (G). (I) mRNA levels of megalin in kidney samples of *db/m+* and *db/db* mice, $n=7-9$. (J) mRNA levels of α -SMA and fibronectin in kidney samples of *db/m+* and *db/db* mice, $n=7-9$. (K) representative immunofluorescence staining analysis of fibronectin in kidney samples, $n=3-4$. Scale bar, 50 μm . Data were analyzed by one-way ANOVA in (B)–(K) with Tukey's post-hoc tests, and indicated by $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, $****P < 0.0001$. Data are shown as mean $\pm$ SD. Sed, sedentary. Ex, exercise.

cise did not affect intraperitoneal glucose tolerance test (IPGTT) results, however, exercise failed to increase insulin sensitivity when treated with GSK0660 (Figure S5A). Moreover, exercise-induced PPAR δ expression was reversed by GSK0660 treatment (Figures S5B–C). As expected, PPAR δ inhibition by GSK0660 increased the level of upstream free fatty acids and reduced downstream acylcarnitines (Figures 5B–C). PPAR δ inhibitor abolished the exercise-induced improvement in renal function. PPAR δ inhibitor ameliorated the exercise-mediated reduction of mesangial (Figures S5D–E). Consistently, GSK0660 infusion reversed the exercise-induced reduction of uNGAL (Figure 5D) and urinary volume (Figure 5E), elevation of BUN (Figure 5F), and kidney injury molecule-1 (Kim-1, Figure 5G). Moreover, exercise-induced expression of megalin (Figure 5H) and E-cadherin (Figures S5F–G) as well as fibrotic markers (Figures 5I–J) were all reversed by GSK0660 treatment in the kidneys of *db/db* mice. Furthermore, the decreases in collagen deposition

induced by exercise were also eliminated by GSK0660 (Figure S5H). Collectively, these results indicate that PPAR δ activation is critical for the exercise-induced improvement in renal structure and function in diabetic mice.

PPAR δ enhances FAO through increasing CPT1 α expression

To further clarify the role of PPAR δ in the renal FAO pathway, we first determined the effect of PPAR δ agonist GW501516 in HK-2 cells. We found that in addition to oxidative phosphorylation pathway, fatty acid metabolism pathway, which is also a key pathway involved in cellular energetics in renal tubular cells,³⁸ was significantly enriched (Figure 6A). We next studied the regulatory effect of PPAR δ on fatty acid metabolism. The gene set enrichment analysis confirmed that this pathway was strongly enriched (Figure 6B). Additionally, the most significantly up-regulated genes in response to PPAR δ agonist is the rate-limiting enzyme of FAO, CPT1 α (Figure 6C), which was

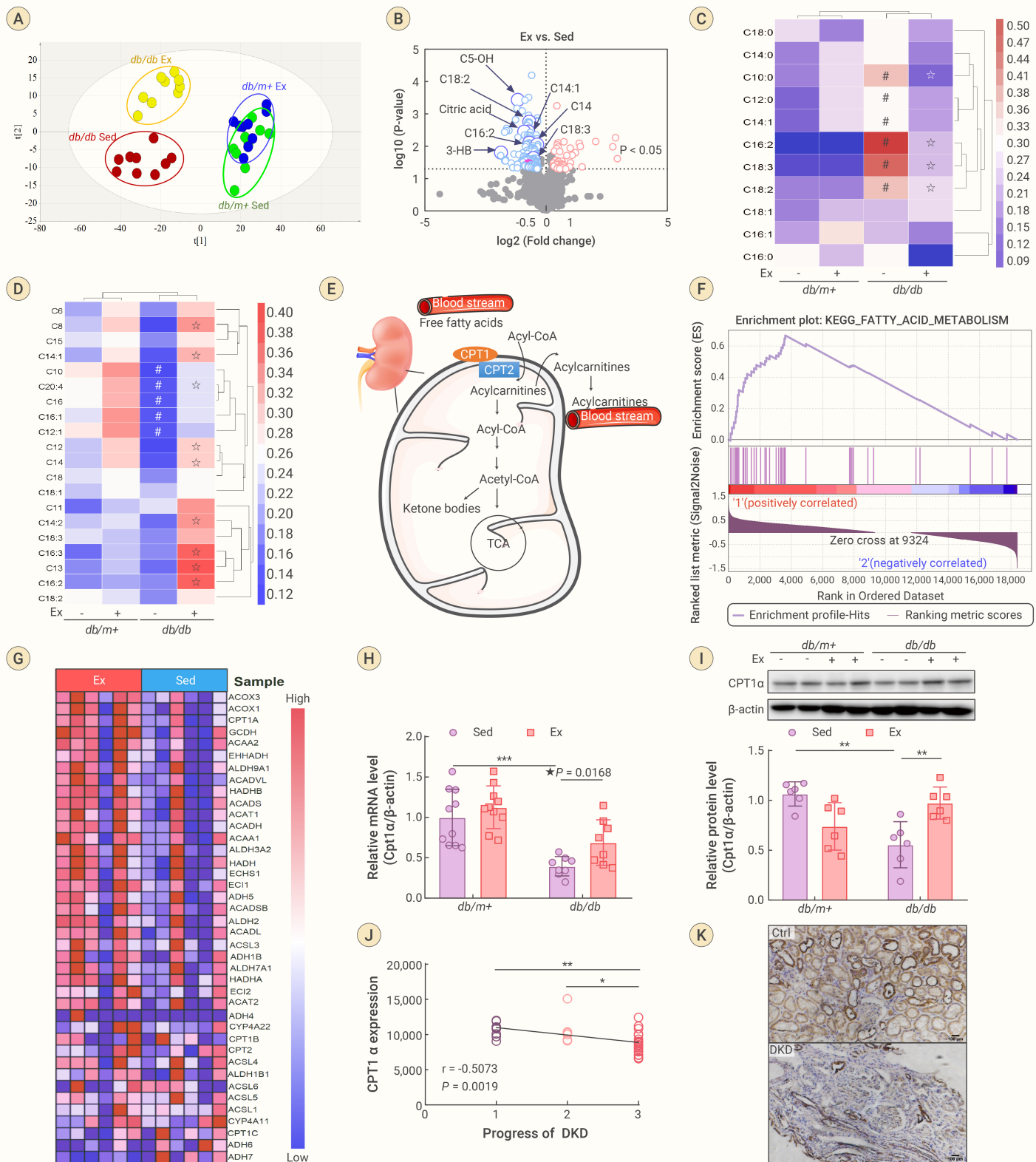


Figure 2. Running exercise improves FAO efficiency in kidney of *db/db* mice (A) PLS-DA score plot of *db/m+* and *db/db* mice in response to exercise. Data were normalized by the percentage of total ions. (B) volcano plot of detected metabolites in *db/db* mice in response to exercise. C5-OH, hydroxy-conjugated valeric acid. C18:2, linoleic acid. C14:1, tetradecenoic acid. C14, tetradecanoic acid. C18:3, α -linolenic acid. C16:2, hexadecadienoic acid. (C) heatmap analysis of free long-chain fatty acids in kidney in response to exercise, $n=7-8$. (D) heatmap analysis of acylcarnitines in kidney in response to 12-week of exercise, $n=8-10$. (E) schematic diagram of FAO metabolism pathway. (F) gene set enrichment analysis reveals strong enrichment of FAO pathway in samples obtained from *db/db* in response to 12-week of exercise, $n=6$. (G) heatmap analysis of transcripts related to fatty acid metabolism, $n=6$. (H) mRNA expression of *Cpt1a* in response to exercise in kidney on *db/m+* and *db/db* mice, $n=8-10$. (I) protein expression of CPT1 α in response to 12-week of exercise in kidney of *db/m+* and *db/db* mice, $n=6$. (J) correlation analysis of CPT1 α with the pathological progress of diabetic nephropathy in human samples. Data are originated from GSE142025 and re-analyzed. "1" represents normal group, $n=9$, "2" represents early diabetic nephropathy group, $n=6$, "3" represents advanced diabetic nephropathy group, $n=20$. (K) representative immunohistochemistry staining analysis of CPT1 α in kidney of healthy and diabetic nephropathy patient samples. Scale bar, 100 μ m. A two-tailed unpaired Student's *t*-test [(B) and (H)] or one-way ANOVA with Tukey's post-hoc [(C), (D), (H), (J) and (I)] were used for statistical analysis, and indicated by * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; #, $P < 0.05$, *db/db* Sedentary group vs. *db/m+* sedentary group. ☆, $P < 0.05$, *db/db* exercise group vs. *db/db* sedentary group. ★*P*, student's *t*-test. Data are shown as mean $\pm$ SD. Sed, sedentary. Ex, exercise.

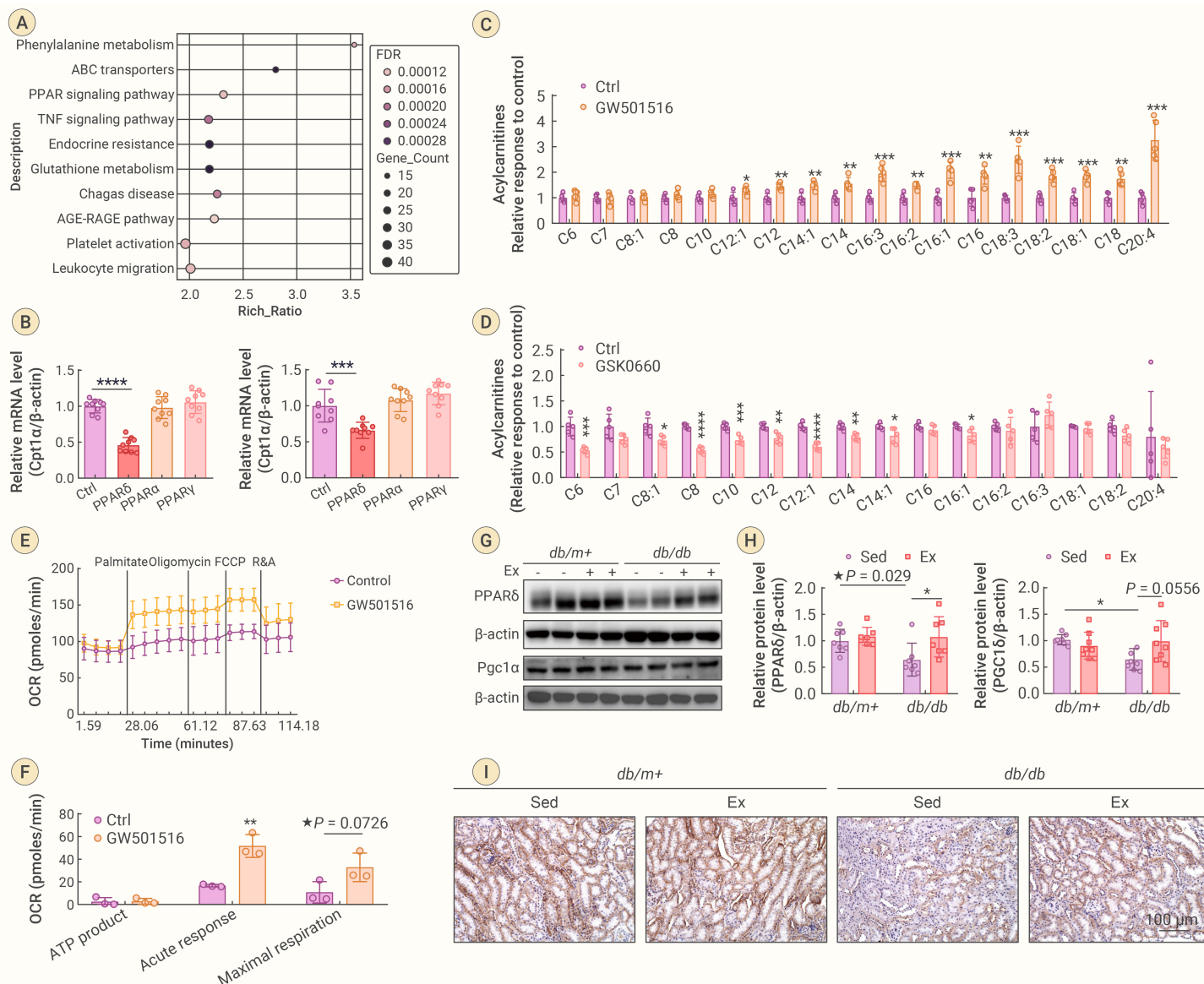


Figure 3. PPARδ was enhanced by exercise and contribute to FAO efficiency (A) enrichment analysis of changed genes based on RNA sequence analysis, *db/db* sedentary vs. *db/m+* sedentary, *n*=6. (B) RNA expression of CPT1α in response to PPARδ antagonist GSK0660, PPARα antagonist GW6471, and PPARγ antagonist GW9662 in HK-2 (upper) and TKPTS (down) cells, *n*=9. (C-D) acylcarnitines in response to PPARδ agonist GW501516 (0.5 μM, incubate for 2 hours) and PPARδ antagonist GSK0660 (1 μM, incubate for 8 hours) in HK-2 cells, respectively. Abbreviations of acylcarnitines (C6 to C20:4) are listed in Table S2. (E) oxygen consumption rate (OCR) in HK-2 cells, *n*=3. (F) summary data of (E). Data were corrected by the total protein of each sample, *n*=3. (G) protein expression of PPARδ and PGC1α in response to exercise in kidney. (H) summary data of (G). (I) representative immunohistochemistry analysis of PPARδ in kidney samples of *db/m+* and *db/db* mice. Scale bar, 100 μm. A two-tailed unpaired Student's *t*-test [(C), (D), (F) and (H)] or one-way ANOVA with Tukey's post-hoc [(B) and (H)] were used for statistical analysis, and indicated by **P* < 0.05, ***P* < 0.01, ****P* < 0.001, *****P* < 0.0001. ★*P*, student's *t*-test. Data are shown as mean ± SD. Sed, sedentary. Ex, exercise.

confirmed by up-regulated protein level of CPT1α (Figure 6D) by PPARδ agonist GW501516, and down-regulated by PPARδ antagonist GSK0660 (Figure 6E) in HK-2 cells. Consistently, the in vivo experiments showed that GW501516 infusion significantly increased the expression of CPT1α (Figure 6F). Oppositely, GSK0660 abolished exercise-induced expression of CPT1α (Figure 6G & Figure S6B), indicating PPARδ mediated the exercise-induced CPT1α expression in kidney.

DISCUSSION

In the current study, we investigated the mechanisms responsible for the exercise-induced beneficial effects on renal function in the mouse model of DKD. We found that 12-week running exercise improves renal function in *db/db* mice as evidenced by the reduction in the expression of renal injury and renal fibrosis markers.

Unbiased multi-omics studies reveal that the fatty acid metabolism pathway and PPARs, known to be critical regulators of FAO, were significantly

altered in the kidneys of *db/db* mice. Previous studies indicated that intracellular lipid accumulation plays a crucial role in the progression of renal fibrosis by inducing lipotoxicity.^{39,40} One of the important factors causing lipid accumulation in TECs is insufficient FAO. Furthermore, FAO is the key contributor of intracellular ATP levels in TECs.¹² Insufficient FAO contributes to renal fibrosis,^{19,41,42} probably due to increased apoptotic rate when cellular ATP supply is inadequate. Conversely, restoring FAO in the kidney protects against renal fibrosis.^{12,43} The present study shows that running exercise enhances FAO, leading to decreased accumulation of free fatty acids, and increased circulating levels of fatty acid metabolites (acylcarnitines) in the kidney.

Regular exercise activates a number of serine-threonine kinases and transcriptional regulators. In addition to AMPK, PPARs are the master transcriptional regulators of fatty acid metabolism. Previous studies reported that PPARδ overexpression in transgenic mice enhanced running endurance.⁴⁴ Compared with PPARδ, AMPK activation alone is insufficient to activate the fatty acid metabolism pathway.¹⁵ Contrarily, PPARδ activation markedly

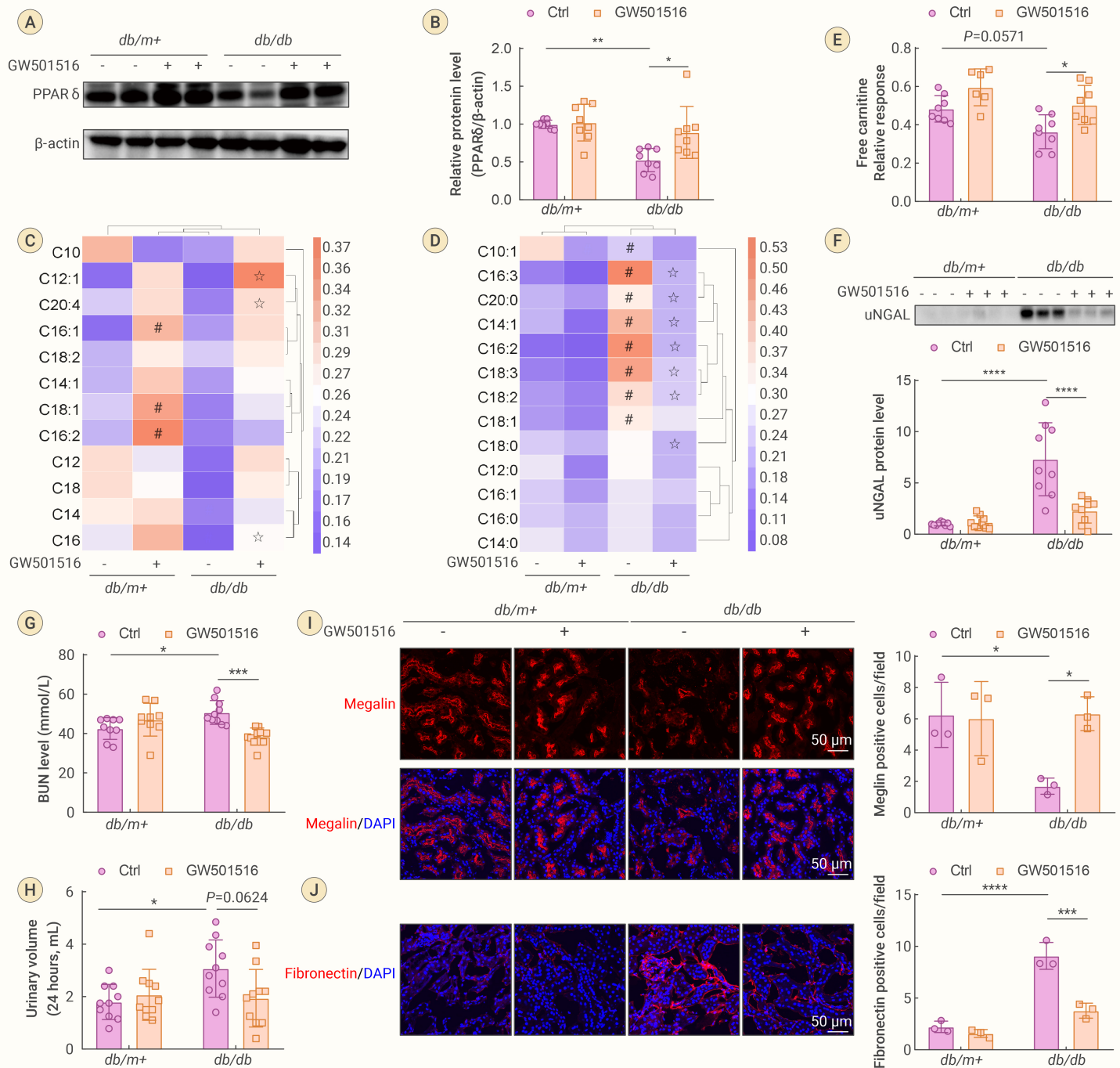


Figure 4. Activation of PPAR δ enhances FAO efficiency and renal function in *db/db* mice (A) representative immunoblots of PPAR δ in kidney in response to 6-week of administration of PPAR δ agonist GW501516 (10 mg/kg/day). (B) statistical analysis of the protein expression of PPAR δ in kidney of *db/m+* and *db/db* mice, $n=8$. (C) heatmap of acylcarnitines in response to GW501516 in kidney of *db/m+* and *db/db* mice, $n=6-8$. Abbreviations were listed in Table S2. (D) heatmap of free fatty acids in response to GW501516 in the kidney of *db/m+* and *db/db* mice, $n=7-9$. Abbreviations were listed in Table S3. (E) free carnitine in response to GW501516 in kidney of *db/m+* and *db/db* mice, $n=6-8$. (F) protein expression of lipocalin-2 in urine (uNGAL) of *db/m+* and *db/db* mice, $n=9$. (G) BUN levels in response to GW501516 in *db/m+* and *db/db* mice, $n=9-10$. (H) urine volume (fasting for 24 hours) $n=10$. (I-J) immunofluorescence staining analysis of megalin and fibronectin in kidney of *db/m+* and *db/db* mice, respectively, $n=3$. Scale bar, 50 μ m. One-way ANOVA with Tukey's post-hoc [(B), (E)-(J)] were used for statistical analysis, and indicated by * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$, # $P < 0.05$, vs. *db/m+* control group. ☆ $P < 0.05$, vs. *db/db* control group. Data are shown as mean $\pm$ SD.

increased metabolic substrate shift from glucose to fatty acids in skeletal muscle.¹⁵ We also observed the same metabolic substrate shift in renal TECs both *in vitro* and *in vivo*. PPAR δ activation remarkably increased the efficiency of FAO, accompanied by alleviated renal dysfunction in diabetic mice.

Considering a critical role of FAO in energy supply in the kidney, and defective FAO in TECs associated with renal fibrosis,¹² we propose that PPAR δ activation is required for exercise to promote FAO, and thus improves renal function. We demonstrate that the effect of exercise on improving FAO efficiency and restoration of the impaired renal function is largely reversed by

treatment with a PPAR δ antagonist GSK0660. Therefore, the present study identifies PPAR δ as a mediator of exercise-induced benefits on FAO and renal function in diabetic mice.

Notably, a previous study reported that pharmacological activation of PPAR α also alleviated DKD, as reflected by reductions in glomerular hypertrophy, mesangial matrix expansion, and urinary albumin excretion,⁴⁵ while enhancing FAO.⁴⁶ In addition, PPAR γ , another subtype of PPARs, which is less expressed in the kidney compared to the other two types,⁴⁷ has also been suggested as a therapeutic target for the treatment of diabetic nephropathy

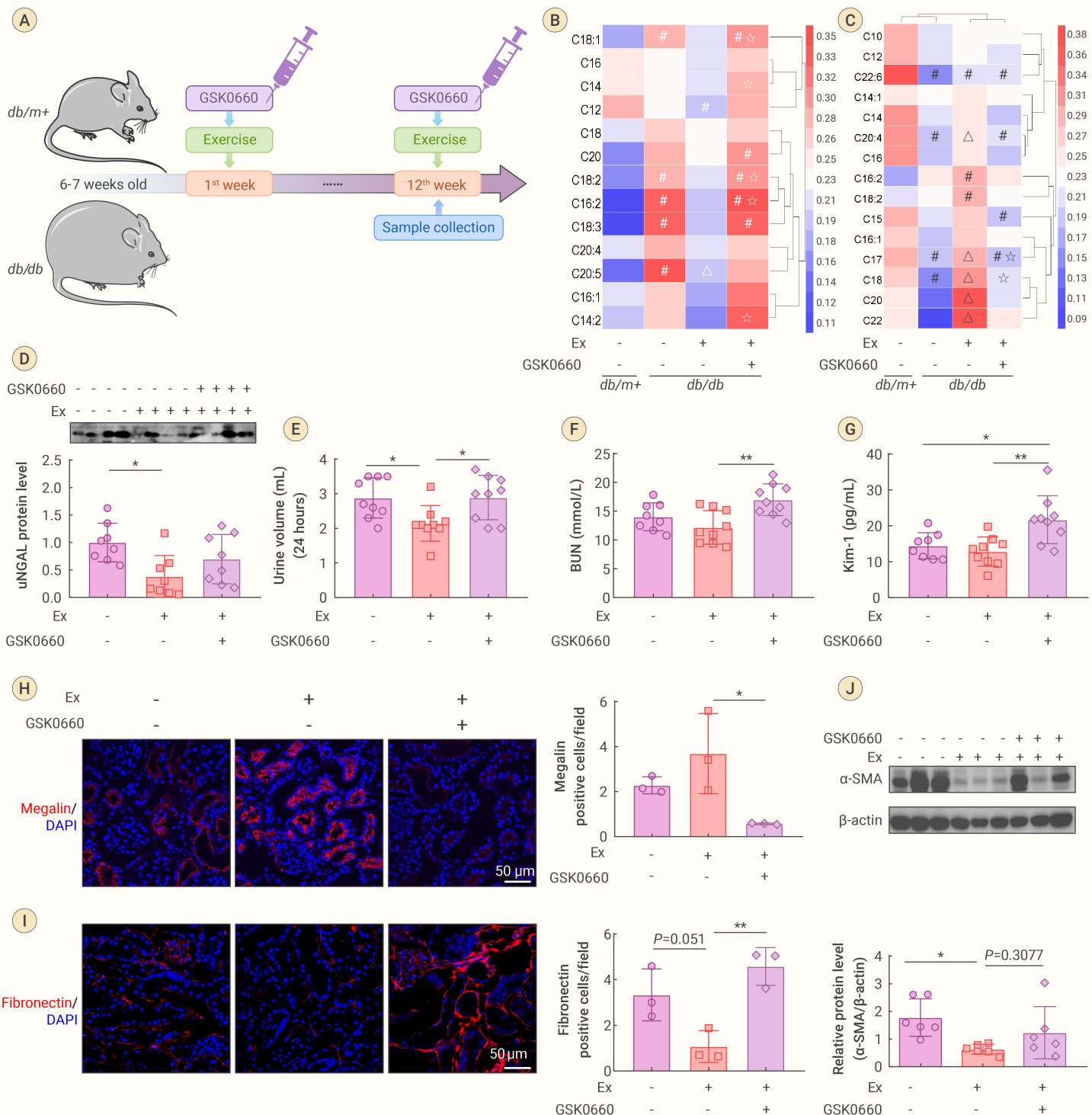


Figure 5. Inhibition of PPAR δ eliminates the beneficial effects of exercise on renal function and FAO efficiency in DKD mice (A) schematic diagram of 12-week of exercise with treatment of PPAR δ antagonist GSK0660 (1 mg/kg/day). (B) free fatty acids in kidney in response to exercise and GSK0660 on *db/db* mice, $n=8-10$. (C) acylcarnitines in kidney in response to exercise and GSK0660 on *db/db* mice, $n=8-9$. (D) urinary lipocalin-2 levels (uNGAL) in response to exercise and GSK0660 on *db/db* mice, $n=8$. (E) urine volume in response to exercise and GSK0660 on *db/db* mice, $n=9$. (F) BUN levels in response to exercise and GSK0660 on *db/db* mice, $n=8-9$. (G) Plasma Kim-1 levels in response to exercise and GSK0660 on *db/db* mice, $n=8-9$. (H) representative immunofluorescence staining analysis of megalin in response to exercise and GSK0660 in kidney of *db/db* mice, $n=3$. Scale bar, 50 μ m. (I) representative immunofluorescence staining analysis of fibronectin in kidney, $n=3$. Scale bar, 50 μ m. (J) protein expression of α -SMA in response to exercise and GSK0660 on *db/db* mice, $n=6$. One-way ANOVA with Tukey's post-hoc [(B)-(J)] were used for statistical analysis, and indicated by * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$; # $P < 0.05$, vs. *db/m+* Sed; ☆ $P < 0.05$, vs. *db/db* Ex; △ $P < 0.05$, vs. *db/db* Sed. Data are shown as mean $\pm$ SD.

and other DKD.⁴⁸ However, the present study shows that CPT1 α expression was down-regulated by PPAR δ antagonists, but not by PPAR α and PPAR γ antagonists in both HK-2 and TKPTS cells. These results argue against a significant involvement of PPAR α and PPAR γ in FAO in the kidney of diabetic mice. Therefore, future studies are required to determine the role of PPAR α

and PPAR γ in response to physical exercise and their potential in alleviating renal dysfunction caused by DKD. Taken together, the present study reveals an important role of the PPAR δ -FAO pathway in mediating exercise-induced benefits against DKD in diabetic mice. PPAR δ may be a potential target for treating DKD associated with diabetes or other risk factors.

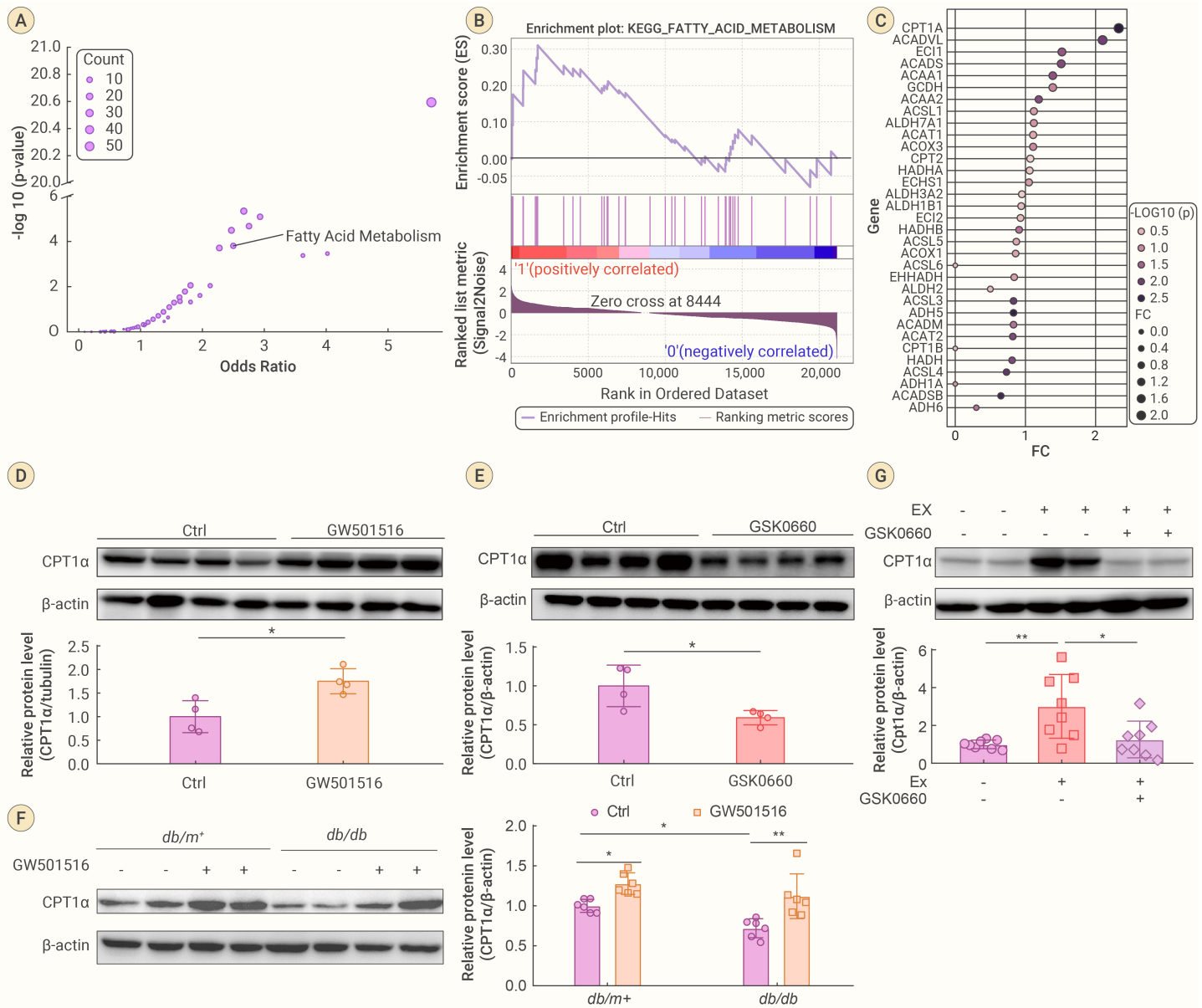


Figure 6. PPAR δ enhances CPT1 α expression in kidney cells (A) Scatter plot of pathway enrichment analysis based on up-regulated genes by the treatment of GW501516 in HK-2 cell (24h, 1μM), based on MSigDB Hallmark 2020 Database. (B) gene set enrichment analysis reveals strong enrichment of genes in fatty acid metabolism pathway stimulated by GW501516 in HK cells (24h, 1μM). Enrichment score, 0.31. Normal P -value < 0.001. (C) scatter plot of genes in fatty acid metabolism pathway in response to the treatment of GW501516 (24h, 1μM). (D) protein expression of CPT1 α in response to PPAR δ agonist GW501516 (8h) in HK-2 cells, $n=4$. (E) protein expression of CPT1 α in response to PPAR δ antagonist GSK0660 in HK-2 cells, $n=4$. (F) protein expression of CPT1 α in response to 6-week treatment of PPAR δ agonist GW501516 (1 mg/kg/day) in kidney of *db/m+* and *db/db* mice, $n=6$. (G) protein expression of CPT1 α in response to 12-week of exercise and treatment of PPAR δ antagonist GSK0660 in kidney of *db/db* mice, $n=8$. A two-tailed unpaired Student's t-test [(D), (E)] or one-way ANOVA with Tukey's post-hoc [(F), (G)] were used for statistical analysis, and indicated by * P < 0.05, ** P < 0.01, *** P < 0.001, **** P < 0.0001. Data are shown as mean $\pm$ SD. Sed, sedentary. Ex, exercise.

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FUNDING AND ACKNOWLEDGMENTS

We thank members of the Y.H. and ZW. C. groups for the helpful discussions. This work was supported by Hong Kong Research Grants Council (T12-101/23-N, SRFS2021-4S04, C4024-16W, GRF-14102620, AoE/M/707-18, R4012-18) and CityU Start-up Fund. The funders had no role in study design, data collection and analysis, decision to publish or preparation of the manuscript. Some figure panels were created with BioRender.com.

AUTHOR CONTRIBUTIONS

Li Xiang and Li Wang contributed equally to this work. Yu Huang and Zongwei Cai were responsible for funding acquisition; Yu Huang and Zongwei Cai were responsible for supervision of the manuscript; Li Xiang, Li Wang, Yu Wang, Jingchun Shi, Chak Kwong Cheng, Libing Hu and Junli Liu were responsible for investigation; Xiang Li, Li Wang and Yin Xia were responsible for the formal analysis; Cheng-Lin Zhang, Liping Xie, Yi Ru and Yujie Pu were responsible for data curation; Li Xiang wrote the original draft; Li Wang, Yin Xia, Cheng-Lin Zhang, Yujie Pu, Suowen Xu, Chak Kwong Cheng, Yu Huang and Zongwei Cai reviewed and edited the manuscript.

DECLARATION OF INTERESTS

Z.C. is an Editorial Board member of The Innovation Life. He was blinded from reviewing or making final decisions on the manuscript. The article was subject to the journal's standard procedures, with peer review handled independently of this Editorial Board member and their research groups. The other authors declare that they have no conflicts of interest.

ETHICAL STATEMENT AND PATIENT CONSENT

The use of animals was approved by the Animal Research Ethical Committee, Chinese University of Hong Kong and under the rules of the guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (the ethical number is AN-STA-00000501). Human kidney samples for immunohistochemistry staining analysis was approved by the Ethics Board at Kunming Medical University (the approval number is 2019-047-01), and was registered in the Chinese Clinical Trial Registration (the registration number is: ChiCTR1900025504).

DATA AND CODE AVAILABILITY

The data underpinning this study are accessible within the article and its Supplemental Information. For further inquiries or to request additional data for reanalysis, please reach out to the lead contact.

SUPPLEMENTAL INFORMATION

It can be found online at <https://doi.org/10.59717/j.xinn-life.2024.100065>

LEAD CONTACT WEBSITE

Yu Huang: <https://www.cityu.edu.hk/bms/profile/yuhuang.htm>
Zongwei Cai: <https://chem.hkbu.edu.hk/cai>