**Supplementary File 1. Supplementary methods**

**Animals and reagents**

C57BL/6 female mice were obtained from Chongqing Medical University (Chongqing, China) and were maintained in a specific pathogen-free facility. The animal studies were approved by the Animal Ethics Committee of Second Affiliated Hospital of Chongqing Medical University. TDF was kindly gifted from Guangshengtang Pharmaceutical Co., Ltd (Fujian, China). TDF was dissolved in a carboxymethylcellulose sodium solution and stored at -20 ℃. Then, 8-week-old mice were treated with TDF 45.5 mg/kg /day was given by oral gavage for 4 months to simulate the long-term use of TDF in human (approximate ten years), and control mice received an equivalent volume of a carboxymethylcellulose sodium solution. The dose of TDF was calculated by extrapolation from human dosages and normalization to the body surface area.1,2 The body weight of each mouse was measured weekly. Following treatment, mice were sacrificed after fasting overnight. Venous blood was collected from the retro-orbital by removing the eyeball, after which tissues were collected. Lactate levels were measured in clinical laboratory using the HITACHI 7600 analyzer (HITACHI High-Technologies, Tokyo, Japan).

**Glycolytic enzyme activity and mitochondrial complex enzyme activity**

Phosphofructokinase (PFK) and pyruvate dehydrogenase (PDH) activity was analyzed using kits from Sigma-Aldrich (St. Louis, MO, USA) according to the manufacturer’s instructions. Lactate dehydrogenase-A (LDHA) activity was determined by measuring the decrease in absorbency at 340 nm when NADH was oxidized to NAD in the presence of pyruvate as previously described.3 The enzymatic activity of mitochondrial complex II was detected using a kit from Abcam (Cambridge, UK) according to the manufacturer’s instructions. The activities of mitochondrial complex III and complex IV were determined using kits from BioVision (Cambridge, UK) according to the manufacturer’s instructions. Prior to the measurement of mitochondrial complex activity, mitochondria from skeletal muscle, heart, liver, and kidney were isolated using a mitochondria isolation kit (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer’s instructions.

**Pyruvate and ATP content**

The pyruvate concentration in skeletal muscle and heart was determined using a kit from Sigma-Aldrich according to the manufacturer’s instructions. The ATP levels in the mitochondria of skeletal muscle, heart, liver, and kidney were measured using a kit from BioVision (Cambridge, UK) according to the manufacturer’s instructions.

**Mitochondrial DNA copy number**

Total DNA was extracted following the standard phenol-chloroform method using a mammalian genomic DNA extraction kit (Beyotime, Beijing, China) according to the manufacturer’s instructions. The mitochondrial DNA copy number was determined by comparing the levels of mitochondrial DNA to nuclear DNA. The expression levels of 16s ribosomal RNA (rRNA) and 18s rRNA were used to estimate the mitochondrial DNA and nuclear DNA levels, respectively.

**Quantitative real-time PCR**

Total RNA was extracted using TRIzol reagent (Invitrogen, Waltham, MA, USA), and cDNA was synthesized using reverse transcriptase (Takara, Shiga, Japan). Real-time PCR was performed using SYBR Green (Takara, Shiga, Japan) to examine the relative mRNA levels of the indicated genes. The expression of 18s rRNA was used as an internal control. The primers used for real-time PCR are shown in Supplementary Table 1.

**Western blotting**

Total protein was extracted using radioimmunoprecipitation assay (RIPA) buffer containing 50 mM Tris/HCl (pH 7.4), 150 mM NaCl, 1% SDS, 0.1% Triton X-100, 0.5% sodium deoxycholate, protease inhibitor cocktail and phosphatase inhibitor PhosSTOP (Roche, Basil, Switzerland). The homogenates were sonicated and centrifuged at 12,000 × *g* for 15 m at 4℃. Then, the supernatants were collected for western blotting. The total protein concentration was measured using the bicinchoninic acid (BCA) method. A total of 20 to 70 μg of protein was separated by SDS-PAGE and then transferred to a PVDF membrane (Millipore Corporation, Burlington, MA, USA). After being blocked with 5% bovine serum albumin (BSA), the membranes were incubated with the following primary antibodies overnight at 4°C: anti-phosphor-cAMP response element–binding protein (CREB) and anti-CREB (Cell Signaling Technology, Danvers, MA, USA), anti-PGC1𝛼 and anti-mitochondrial transcription factor B1 (TFB1M) (Abcam, Cambridge, UK), anti-β-Tubulin (Sigma-Aldrich, St. Louis, MO, USA), anti-phosphoenolpyruvate carboxykinase (Pepck; Cell Signaling Technology, Danvers, MA, USA) and anti-glucose 6-phosphatase (G6pase; Sigma-Aldrich, St. Louis, MO, USA). Then, the membranes were incubated with an HRP-conjugated secondary antibody (BBI, Shanghai, China), and the immunoreactive bands were visualized using the ECL reagent (Advansta, San Jose, CA, USA) to analyze protein expression.

**Periodic acid-Schiff staining and immunohistochemistry**

Skeletal muscle, heart, liver, and kidney tissues obtained from mice were fixed in 4% paraformaldehyde (PFA), embedded in paraffin, and sliced into 4 µm sections. Then, periodic acid-Schiff (PAS) staining was performed following the manufacturer’s instructions (Beijing Leagene Biotechnology Co., Ltd, Beijing, China) to determine the glycogen content. Simultaneously, deparaffinized liver and kidney tissue sections were stained using the following primary antibodies: anti-Pepck (Cell Signaling Technology, Danvers, MA, USA) or anti-G6pase (Sigma-Aldrich, St. Louis, MO, USA). Next, the samples were incubated with biotinylated secondary antibodies overnight. Detection of the proteins was based on the formation of an avidin-biotin-HRP complex (Thermo Fisher Scientific, Fremont, CA), and di-aminobenzidine was utilized as the chromogen. Nuclei were counterstained with hematoxylin. The sums of the integrated optical density, that is, the area sum of glycogen, Pepck and G6pase, were quantified using Image-Pro Plus software version 7.0 (Media Cybernetics, Rockville, MD, USA), and the mean density was calculated based upon the integrated optical density sum/area sum.

**RNA sequencing**

Total RNA from TDF-treated and control skeletal muscle tissue was isolated using TRIzol (Invitrogen, Fremont, CA), and then, the mRNA was subjected to whole transcriptome sequencing, as previously described.4 Briefly, differential gene expression was analyzed with EdgeR. The adjusted P values were computed using the Benjamini–Hochburg method. The threshold we used to screen for upregulated or downregulated mRNAs was a fold change > 2 and padj < 0.05. All sequencing was performed by the Beijing Genomic Institute at Shenzhen (BGI-Shenzhen, Shenzhen, China).

Glucose and energy metabolism pathways were identified in the PathCards pathway database (http://pathcards.genecards.org). The differentially expressed genes (DEGs) in this pathway are presented in a heat map that was generated using Heml version 1.0.3.7 software.

**Statistics**

Results were expressed as means ± standard error of the mean. Normality was analyzed using the Shapiro-Wilk test. If the data were shown to be normally distributed, Student’s *t*-test was used to analyze the statistical significance; otherwise, we used the non-parametric test. The level of statistical significance was set at *p* < 0.05 for all comparisons. All statistical analyses were performed using IBM SPSS, version 20.0 (IBM Corp., Armonk, NY, USA).

**References**

[1] Reagan-Shaw S, Nihal M, Ahmad N. Dose translation from animal to human studies revisited. Faseb j 2008;22:659-661. doi 10.1096/fj.07-9574LSF.

[2] Eran H, Chao W, He H, Manisha S, Eleen D, Mohammed S*, et al.* Transmission of chimeric HIV by mating in conventional mice: prevention by pre-exposure antiretroviral therapy and reduced susceptibility during estrus. Disease Models & Mechanisms 2013;6:1292-1298.

[3] Wang Z, Wang D, Han S, Wang N, Mo F, Loo TY*, et al.* Bioactivity-guided identification and cell signaling technology to delineate the lactate dehydrogenase A inhibition effects of Spatholobus suberectus on breast cancer. PLoS One 2013;8:e56631. doi 10.1371/journal.pone.0056631.

[4] Ding K, Wu S, Ying W, Pan Q, Li X, Zhao D*, et al.* Leveraging a Multi-Omics Strategy for Prioritizing Personalized Candidate Mutation-Driver Genes: A Proof-of-Concept Study. Scientific Reports 2015;5:17564.

**Supplementary Table 1. Primers used in this study for real-time PCR**

|  |  |  |
| --- | --- | --- |
| **Primer name** | **Forward primer (5′–3′)** | **Reverse primer (5′–3′)** |
| GLUT4 | GTGACTGGAACACTGGTCCTA | CCAGCCACGTTGCATTGTAG |
| HK2 | TGATCGCCTGCTTATTCACGG | AACCGCCTAGAAATCTCCAGA |
| PGI | TCAAGCTGCGCGAACTTTTTG | GGTTCTTGGAGTAGTCCACCAG |
| PFK | TGTGGTCCGAGTTGGTATCT | GCACTTCCAATCACTGTGCC |
| ALDOA | CGTGTGAATCCCTGCATTGG | CAGCCCCTGGGTAGTTGTC |
| ENO3 | CACAGCCAAGGGTCGATTCC | CCCAGGTATCGTGCTTTGTCT |
| PK | GCCGCCTGGACATTGACTC | CCATGAGAGAAATTCAGCCGAG |
| GAPDH | AATGGATTTGGACGCATTGGT | TTTGCACTGGTACGTGTTGAT |
| TFAM | ATTCCGAAGTGTTTTTCCAGCA | TCTGAAAGTTTTGCATCTGGGT |
| TFB1M | CGGGAGATCATTAAGTTGTTCGG | GCCCAGGACCCACTTCATAAA |
| TFB2M | GGCCCATCTTGCATTCTAGGG | CAGGCAACGGCTCTATATTGAAG |
| PGC1α | TATGGAGTGACATAGAGTGTGCT | GTCGCTACACCACTTCAATCC |
| CREB | AGCAGCTCATGCAACATCATC | AGTCCTTACAGGAAGACTGAACT |
| 18S | AAACGGCTACCACATCCAAG | TTGCCCTCCAATGGATCCT |
| 16S-copy | CCGCAAGGGAAAGATGAAAGAC | TCGTTTGGTTTCGGGGTTTC |
| 18S copy | TGTGTTAGGGGACTGGTGGACA | CATCACCCACTTACCCCCAAAA |
| ACACB | CGCTCACCAACAGTAAGGTGG | GCTTGGCAGGGAGTTCCTC |
| PFKFB3 | CCCAGAGCCGGGTACAGAA | GGGGAGTTGGTCAGCTTCG |
| ULK1 | AAGTTCGAGTTCTCTCGCAAG | CGATGTTTTCGTGCTTTAGTTCC |
| ASPSCR1 | TTCAACCCCAGTGAATACGACC | GCGATGCGAACTATGTTCTCAG |
| CRTC2 | ATGAACCCTAACCCCCAAGAC | CGTTCTCCTCAATAGCAGGGA |
| ENO2 | GTCCCTGGCCGTGTGTAAG | CATCCCGAAAGCTCTCAGC |
| CEBPD | CGACTTCAGCGCCTACATTGA | CTAGCGACAGACCCCACAC |
| IGFBP3 | CCAGGAAACATCAGTGAGTCC | GGATGGAACTTGGAATCGGTCA |
| STXBP4 | ACTGCTTACCTTACGGGTGG | CTCTGGGACAGTCCTCTTCAC |
| IRS2 | CTGCGTCCTCTCCCAAAGTG | GGGGTCATGGGCATGTAGC |
| MEF2 | CAGGTGGTGGCAGTCTTGG | TGCTTATCCTTTGGGCATTCAA |
| ATF2 | CCGTTGCTATTCCTGCATCAA | TTGCTTCTGACTGGACTGGTT |
| FoxO1 | CCCAGGCCGGAGTTTAACC | GTTGCTCATAAAGTCGGTGCT |