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Research Letter

TDF Promotes Glycolysis and Mitochondrial Dysfunction to Accelerate Lactate Accumulation by Downregulating PGC1α in Mice

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Tenofovir disoproxil fumarate (TDF), is a product of tenofovir and has been recommended for long-term use by guidelines[1] because of its favorable efficacy in the treatment of human immunodeficiency virus (HIV) and hepatitis B virus (HBV) infection. Hence, a better understanding of the safety profiles of long-term TDF use is extremely important. Lactic acidosis, as a rare but fatal adverse event of TDF, were reported both in HIV-infected patients,[2–4] and in CHB patients.[5–7] Hyperlactatemia occurred in 15.6% HIV-infected patients using TDF in a Cameroon cohort study[8] and was 3% in another South Africa cohort study.[9] Therefore, TDF increases the risk of abnormal serum lactate, but the mechanism is unclear.

In this study, we treated mice with TDF by oral gavage for 4 months to simulate long-term use in humans. Detailed methods are described in the Supplementary File 1. As shown in Supplementary Figure 1A, TDF treatment significantly increased the blood lactate levels in mice. The body weight and liver weight were similar between TDF-treated mice and control mice (Supplementary Fig. 1B–D).

To explore the underling mechanism by which TDF increased lactate levels, we first analyzed lactate generation in skeletal muscle, as lactate is the end-product of glycolysis and is primarily produced in muscle. TDF treatment resulted in a large increase of pyruvate levels in skeletal muscle (Fig. 1A). The activity of lactate dehydrogenase-A (LDHA) was also elevated in response to TDF (Fig. 1B). Additionally, there was no difference in pyruvate dehydrogenase (PDH) activity between the control and TDF-treated mice (Fig. 1C), but the activity of phosphofructokinase (PFK), a key enzyme in glycolysis, was enhanced in the skeletal muscle of TDF-treated mice (Fig. 1D). The mRNA expression of enzymes involved in glycolysis was unchanged by TDF, except for hexokinase 2 (HK2) (Fig. 1E). We also evaluated the effect of TDF on glucose uptake by measuring the expression of glucose transporter type 4 (GLUT4). As shown in Figure 1E, the GLUT4 mRNA levels were decreased in TDF-treated mice. It is worth noting that the glycogen content in skeletal muscle was much lower in TDF-treated mice than that in the controls (Fig. 1F). The data indicate that TDF accelerated glycolysis in skeletal muscle.

To assess whether the impact of TDF on lactate production was specific to skeletal muscle, we next examined glycolysis in the myocardium. Pyruvate levels were similar in the hearts of control mice and TDF-treated mice (Supplementary Fig. 2A). TDF also failed to affect LDHA, PDH and PFK activity in heart tissue (Supplementary Fig. 2B–D). Additionally, the expression of glycolysis-related enzymes and the glycogen content was unchanged in the hearts of TDF-treated mice (Supplementary Fig. 2F). These data suggest that TDF does not affect glycolysis in the heart.

After we confirmed that TDF increased lactate production specifically in skeletal muscle, we next investigated whether TDF affected lactate clearance. Lactate is primarily cleared by gluconeogenesis in the liver and kidney,[10,11] thus, we examined the expression of phosphoenolpyruvate carboxykinase (Pepck) and glucose 6-phosphatase (G6pase), two key enzymes in gluconeogenesis. We found that TDF did not influence the expression and distribution of either Pepck or G6pase in the liver and kidney (Supplementary Fig. 3A–D). Additionally, the glycogen content in the liver and kidney did no differ between the control and TDF-treated mice (Supplementary Fig. 3E). Moreover, the blood glucose levels re-

Abbreviations: ATF2, activating transcription factor 2; CREB, cAMP response element–binding protein; FoxO1, forkhead box O1; G6pase, glucose 6-phosphatase; GLUT4, glucose transporter type 4; HBV, hepatitis B virus; HIV, human immunodeficiency virus; HK2, hexokinase 2; LDHA, lactate dehydrogenase-A; MEF2, myocyte enhancer factor 2; PAS, periodic acid-Schiff; PDH, pyruvate dehydrogenase; Pepck, phosphoenolpyruvate carboxykinase; PFK, phosphofructokinase; PGC1α, peroxisome proliferator-activated receptor-γ coactivator 1α; RNA-seq, RNA sequencing; TDF, Tenofovir disoproxil fumarate; TFAM, mitochondrial transcription factor A; TFB1M, mitochondrial transcription factor B1; TFB2M, mitochondrial transcription factor B2.

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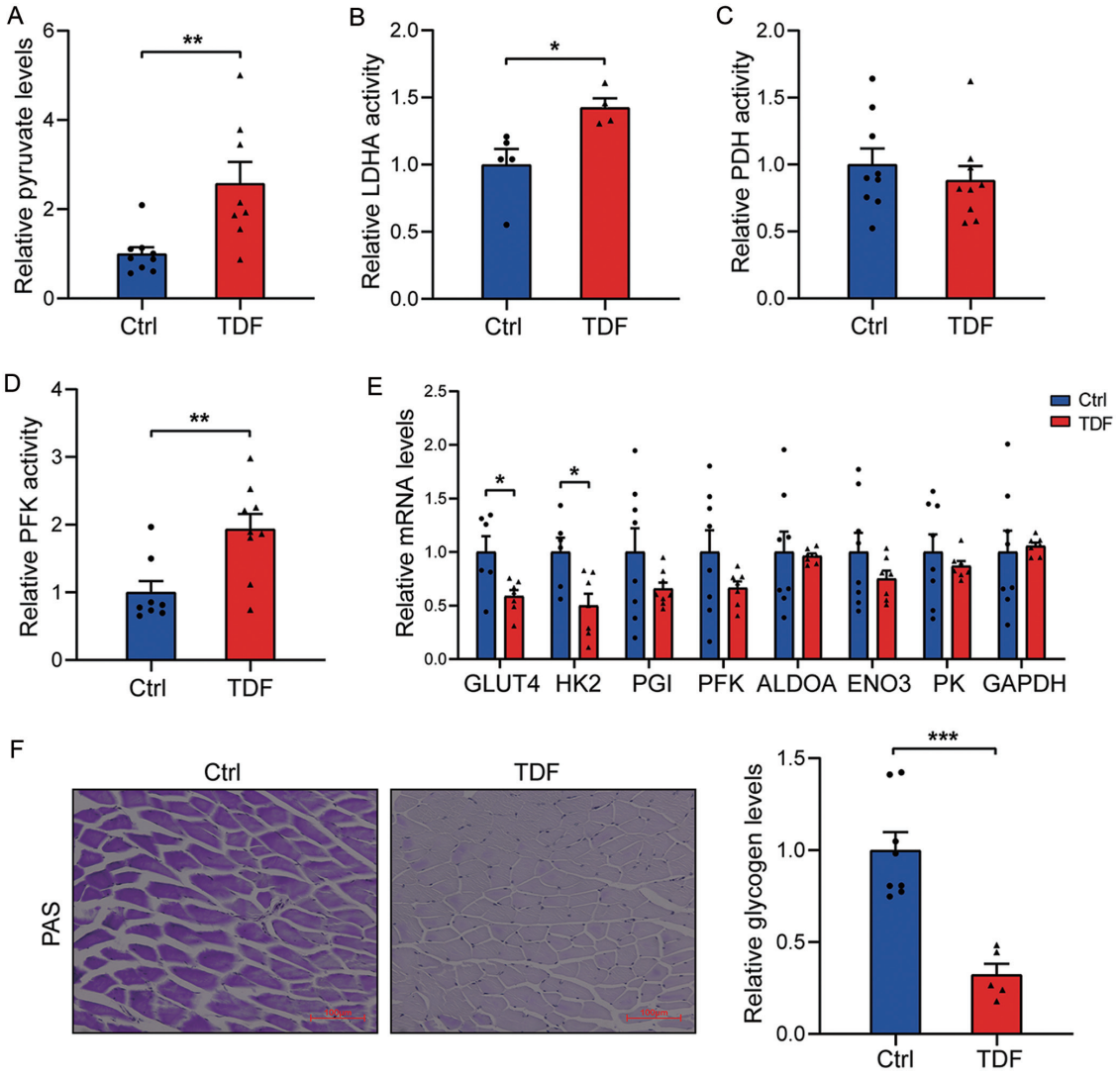

Fig. 1. TDF accelerates glycolysis in skeletal muscle. (A) Pyruvate levels in the skeletal muscle of the control and TDF-treated mice. (B-D) Activity of LDHA (B), PDH (C) and PFK (D) in the skeletal muscle of control mice and TDF-treated mice. (E) mRNA levels of genes related to glycolysis in TDF-treated skeletal muscle. (F) Representative images (left) and quantification (right) of PAS staining in skeletal muscle sections from the control and TDF-treated mice. Scale bar, 100μm. n=4–9. *p<0.05, **p<0.01, ***p<0.001. LDHA, lactate dehydrogenase-A; PDH, pyruvate dehydrogenase; PFK, phosphofructokinase; GLUT4, glucose transporter type 4; HK2, hexokinase 2; PGI, phosphoglucose isomerase; PFK, phosphofructokinase; ALDOA, fructose diphosphate aldease A; ENO3, enolase 3; PK, pyruvate kinase; GAPDH, glyceraldehyde-phosphate dehydrogenase; PAS, periodic acid-Schiff.

mained unchanged in TDF-treated mice (Supplementary Fig. 3F). Collectively, the data suggest that TDF had no effect on gluconeogenesis in the liver and kidney.

It is well known that mitochondria play crucial roles in the regulation of lactate metabolism. Previous studies have demonstrated that mitochondrial dysfunction without abnormal glycolysis results in high lactate levels.[12] Meanwhile, NAs were reported to be associated with mitochondrial toxicity.[13] As such, we evaluated whether TDF influenced mitochondrial function in muscle, liver, and kidney. As shown in Figure 2A, TDF treatment produced no significant changes in the activity of mitochondrial complex II in the skeletal muscle, heart, liver, and kidney (Fig. 2A). The activity of complex III and IV was markedly inhibited in the skeletal muscle of TDF-treated mice, but was maintained in the heart, liver, and kidney (Fig. 2A). A similar trend was observed in ATP production (Fig. 2B). We next measured the mitochondrial DNA (mtDNA) copy number in the mice. As shown in Figure 2C, TDF reduced the mtDNA copy number in skeletal muscle, but not in other tissues (Fig. 2C). Alterations of the mtDNA copy number are related to mtDNA transcription, so we examined the expression of mitochondrial transcription factors. We found that TDF did not influence the expression of mitochondrial transcription factor A (TFAM) and mitochondrial transcription factor B2 (TFB2M), but did result in a decrease expression of mitochondrial transcription factor B1 (TFB1M) in skeletal muscle (Fig. 2D, E and Supplementary Fig. 4A, B). However, the changes were not observed in the heart, liver, and kidney (Fig. 2D and Supplementary Fig. 4A, B). Together, the data indicate that TDF impaired mitochondrial function in skeletal muscle, but not in the heart, liver, and kidney.

To investigate the underlying mechanism by which TDF affects glycolysis and mitochondrial function in skeletal muscle, we performed RNA sequencing (RNA-seq) analysis in

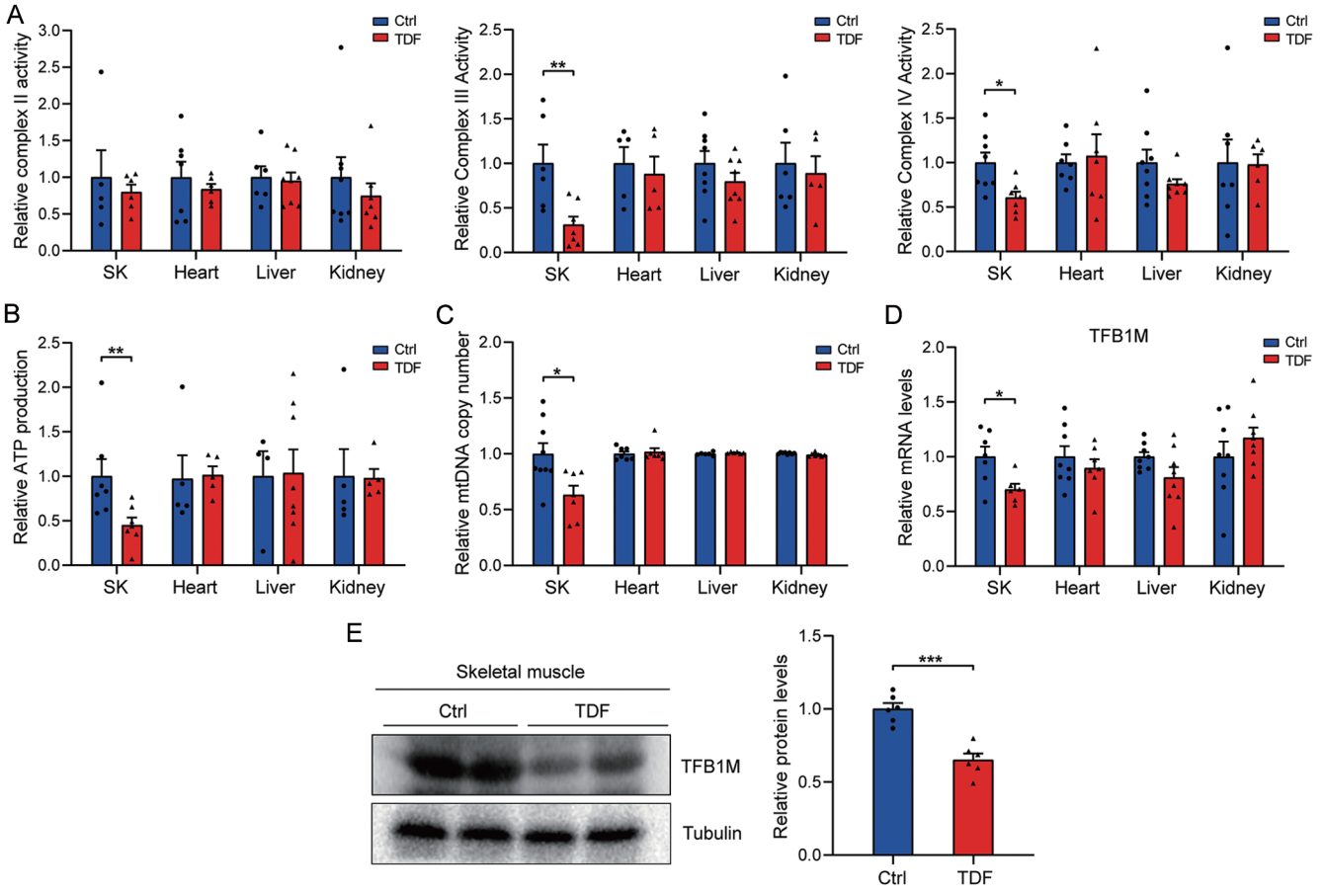

Fig. 2. TDF impairs mitochondrial function in skeletal muscle. (A) The activity of mitochondrial complex II (left), III (middle) and IV (right) of the skeletal muscle, heart, liver and kidney from the control and TDF-treated mice. (B) ATP production of TDF-treated skeletal muscle, heart, liver, and kidney. (C) Mitochondrial DNA copy number in TDF-treated skeletal muscle, heart, liver, and kidney. (D) TFB1M mRNA levels in the indicated tissues. (E) Protein levels (left) and quantification (right) of TFB1M in the skeletal muscle of the control and TDF-treated mice. n=4–8. *p<0.05, **p<0.01, ***p<0.001. TFB1M, mitochondrial transcription factor B1; SK, skeletal muscle; ATP, adenosine triphosphate; mtDNA, mitochondrial DNA.

skeletal muscle from the control and TDF-treated mice, focusing on genes related to glucose/energy metabolism. We detected 24 upregulated and four downregulated genes in this pathway (Fig. 3A). Subsequently, we analyzed the expression of these genes by quantitative PCR, and we found that the mRNA levels of PGC1α, which is a critical molecule in the regulation of lactate homeostasis and mitochondrial biosynthesis,[14,15] were most affected by TDF in skeletal muscle (Fig. 3B). The results of western blotting further confirmed that TDF treatment led to a marked decrease in PGC1α expression in skeletal muscle (Fig. 3C). Next, we screened the upstream molecules of PGC1α and found that cAMP response element–binding protein (CREB) was strongly downregulated by TDF (Fig. 3C, D). We did not observe any significant changes in the expression of other upstream transcription factors of PGC1α, such as myocyte enhancer factor 2 (MEF2), forkhead box O1 (FoxO1) and activating transcription factor 2 (ATF2), in TDF-treated skeletal muscle (Supplementary Fig. 5A). Additionally, the phosphorylation levels of CREB were also decreased after TDF treatment (Fig. 3C). However, the effects of TDF were not observed in the heart (Fig. 3D, E and Supplementary Fig. 5B). Collectively, the data suggest that TDF downregulated PGC1α expression in skeletal muscle.

In conclusion, TDF elevated lactate levels by accelerating glycolysis and disturbing mitochondrial function in skeletal muscle, which was caused, at least in part, by TDF-mediated downregulation of PGC1α (Fig. 3F). Therefore, we should pay attention to blood lactate levels in patients during clinical use of TDF.

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Conflict of interest

HR has been an editor-in-chief of *Journal of Clinical and Translational Hepatology* since 2013. PH has been an associate editor of *Journal of Clinical and Translational Hepatology*

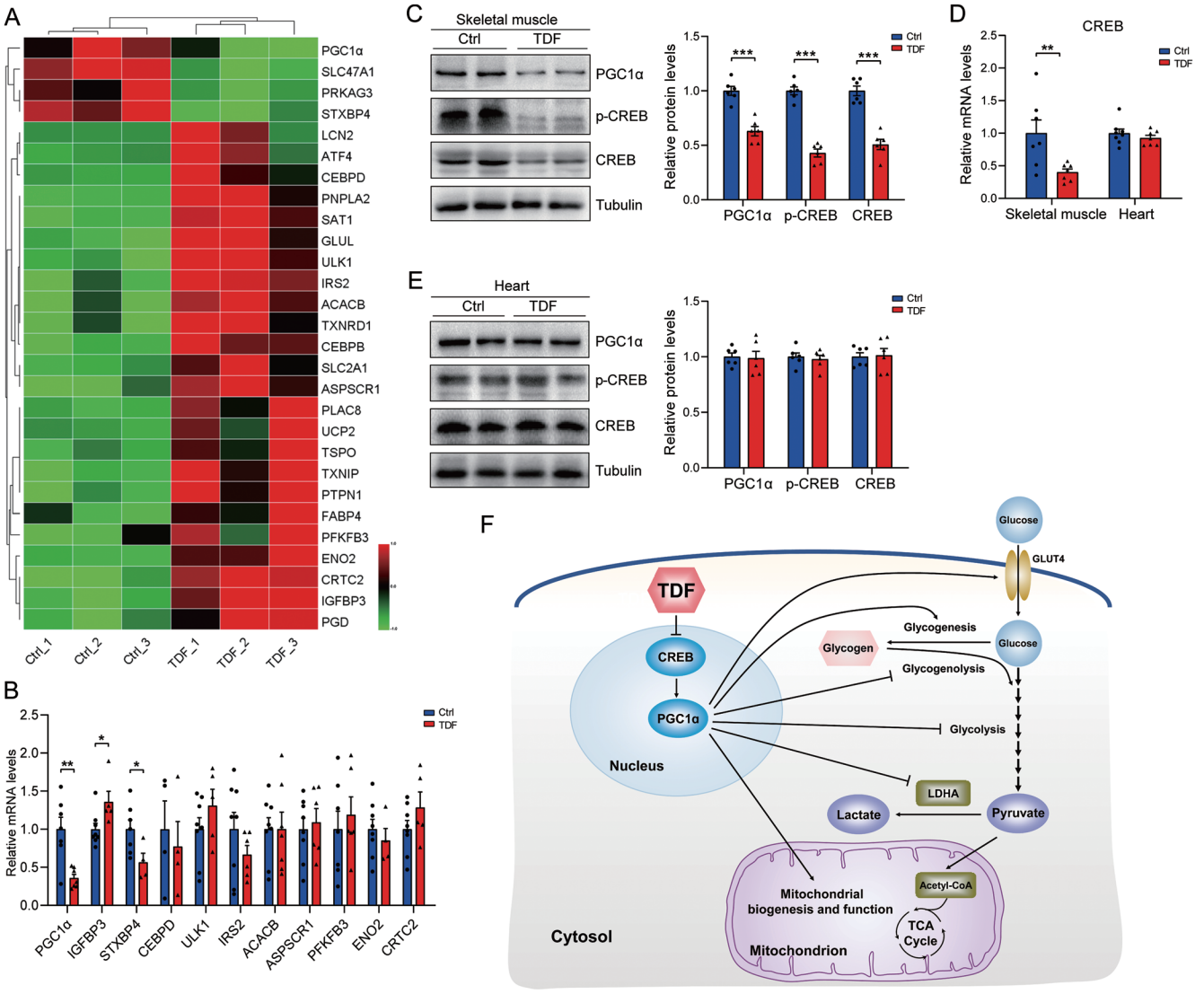

Fig. 3. TDF down-regulates the expression of PGC1α in skeletal muscle. (A) Heat map of differentially expressed genes related to the glucose and energy metabolism pathway in the skeletal muscle of the control and TDF-treated mice. Bright green, upregulation; black, no change; bright red, downregulation. (B) Gene expression in the skeletal muscle of control mice and TDF-treated mice was analyzed by quantitative PCR. (C) Representative western blotting showing the levels of PGC1α, p-CREB and CREB in the skeletal muscle of the control and TDF-treated mice. Quantification data is shown in the right panel. (D) CREB mRNA levels in the skeletal muscle and hearts. (E) Representative western blotting for PGC1α, p-CREB and CREB in the hearts from control mice and mice following TDF administration. Quantification data is shown in the right panel. (F) Schematic diagram showing that TDF promotes glycolysis and impairs mitochondrial function by downregulating PGC1α in skeletal muscle, and thus leading to an increase in serum lactate levels. n=4–8. *p<0.05, **p<0.01, ***p<0.001. TCA cycle, tricarboxylic acid cycle.

since 2022. The other authors have no conflict of interests related to this publication.

Author contributions

Study concept and design (PH, HR), acquisition of data (YL, ZC, ZL, AL), analysis and interpretation of data (YL, ZC, ZL, YZ), drafting of the manuscript (YL, ZC, ZL), critical revision of the manuscript for important intellectual content (YL, ZC, MP, MC, PH, HR). All authors have made a significant contribution to this study and have approved the final manuscript.

Ethical statement

The animal studies were approved by the Animal Ethics Committee of Second Affiliated Hospital of Chongqing Medical University.

Data sharing statement

The datasets used and analyzed during the current study are available from the corresponding author upon reasonable request.

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