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Fenofibrate improves high-fat diet-induced and palmitate-induced endoplasmic reticulum stress and inflammation in skeletal muscle

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ABSTRACT

Aims: Fenofibrate (FF) is commonly used clinically as a lipid-lowering drug, but whether it participates in endoplasmic reticulum (ER) stress and decreases inflammation in skeletal muscle is still unknown. The aim of this study is to determine whether FF treatment reduces insulin resistance (IR) by alleviating ER stress and downstream inflammation in skeletal muscle tissues and cells.

Main methods: Female SD rats were randomly divided into groups receiving the standard chow diet (SCD), a high-fat diet (HFD), or HFD plus FF (HFD + FF). The rats in the latter two groups were subjected to a standard HFD for 20 weeks, then the HFD + FF rats were administered FF (30 mg/kg once daily via gavage) for another 8 weeks. Whole-body IR, expression of peroxisome proliferator-activated receptor α (PPARα), ER stress-related genes, and inflammatory genes in the soleus muscle were assessed. The differentiated C2C12 myotubes were treated with palmitic acid or pretreated with fenofibric acid or 4-phenylbutyric acid (4-PBA), etomoxir, and the expression of ER stress, beta-oxidation-related genes, inflammatory genes, Toll-like receptor 4 (TLR4), and insulin-signaling-related molecules were determined.

Key findings: Eight weeks of FF treatment attenuated HFD-induced IR by decreased tribbles 3 (TRB3) expression, ER stress and inflammation in skeletal muscle. FA pretreatment markedly inverted the PA-induced expression of TLR4 and downstream inflammatory genes, activated ER stress, improved β-oxidation and insulin signaling in differentiated myotube cells.

Significance: FF treatment significantly improved HFD-induced IR in skeletal muscle and PA-induced IR in myotube cells, which may be related to reduced ER stress-induced inflammation.

1. Introduction

Obesity is a major risk factor for insulin resistance (IR) and metabolic syndrome [1]. Since skeletal muscle accounts for more than 80% of insulin-mediated glucose uptake and fatty acid β-oxidation, lipid-induced mitochondrial stress and incomplete fatty acid oxidation results in skeletal muscle IR and is fundamental to the development of IR in the whole body [2]. These processes are collectively termed lipotoxicity, and a recent study showed that lipotoxicity is also related to endoplasmic reticulum (ER) expansion and stress in the skeletal muscle [3].

The ER is a site of secretory and membrane-protein synthesis and modification. A specialized form of ER, known as sarcoplasmic reticulum (SR), is found in skeletal muscle cells. Disruption of either protein-folding or modification within the ER/SR activates the unfolded protein response (UPR) [4,5]. The UPR usually activates three ER transmembrane proteins: inositol-requiring enzyme 1α (IRE1α), ER-

Abbreviations: Akt, v-akt murine thymoma viral oncogene homologue 1; Acox1, Acyl-CoA oxidase; ATF6, Activating transcription factor 6; CHOP, CCAAT/enhancer binding protein (C/EBP) homologous protein; eIF2α, Eukaryotic initiation factor 2α; ER, Endoplasmic reticulum; ETO, Extomoxir; FA, Fenofibric acid; FF, Fenofibrate; GRP78, Glucose-regulated protein 78; HFD, high-fat diets; HFD + FF, HFD plus FF treatment group; IR, Insulin resistance; IRE1α, Inositol-requiring kinase 1α; JNK, c-jun NH2-terminal kinase; NFκB, Nuclear factor κB; PA, Palmitic acid; PERK, ER-resident PKR-like eIF2α kinase; PPARα, Peroxisome proliferator-activated receptor α; SCD, Standard chow diets; SR, Sarcoplasmic reticulum; TLR4, Toll-like receptor 4; TRB3, Tribbles 3; UPR, Unfolded protein response; XBP1, X-box binding protein 1.

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resident PKR-like eIF2α kinase (PERK) and activated transcription factor (ATF6). Recently, there had been report about tribbles 3 (TRB3) mediated ER stress-induced insulin resistance in skeletal muscle [6].

ER stress is also sited at the crossroad between inflammation and IR in skeletal muscle [7]. The saturated fatty acid palmitate (C16:0) activates Toll-like receptor 4 (TLR4) and the downstream nuclear factor kappa B (NFκB) pathway, which results in the production of the proinflammatory cytokines interleukin-6 (IL-6) and tumor necrosis factor-α (TNFα) [8]. But there still was controversial about the relationship between ER stress and inflammation.

Peroxisomal proliferator-activated receptor alpha (PPARα), a member of the subfamily of nuclear transcription receptors, is widely expressed in muscle and contributes greatly to the regulation of lipid metabolism and transport. PPARα also controls inflammatory responses by inhibiting the inflammatory gene expression induced by NFκB in endotoxin-induced uveitis [9]. Fenofibrate (FF) is a typical agonist of PPARα and is a commonly used clinical drug with blood lipid-modifying actions. Recent studies reported that FF influenced ER stress in the liver and in hepatomas [10,11], but there has been no study on its effects on ER stress and whether it influences TRB3 expression in skeletal muscles. In the meantime, whether the beneficial effects of FF on ER stress and inflammatory markers are dependent on increased fatty acid β-oxidation is unknown.

Due to sporadic reports of rhabdomyolysis [12,13], there have been few reports about the effects of FF on lipid metabolism in skeletal muscle. Therefore, we hypothesized that FF-mediated activation of PPARα attenuates ER stress and then inflammatory pathways, such as the TLR4/NFκB pathway, and ultimately improves IR in muscle cells. Because palmitic acid (PA) is the most abundant saturated fatty acid in the serum of HFD-fed rats, and fenofibric acid (FA) is the main metabolic form of fenofibrate *in vivo* [14], PA was selected as the representative saturated fatty acid and FA was selected as the drug in our cell experiment. The aim of our study was to confirm whether PPARα activation by FF in HFD-fed skeletal muscles, or PA-induced IR in muscle cells, was related to ER stress, fatty acid β-oxidation, TRB3 expression, and the downstream inflammation pathway. To our knowledge, this is the first study in the literature to discuss the relationships between FF, ER stress, fatty acid β-oxidation, and TRB3 expression in skeletal muscle.

2. Materials and methods

2.1. Materials

Fenofibrate (F6020), fenofibric acid (CDS003219), palmitic acid (P5585), tunicamycin (93755), etomoxir (E1905), 4-phenylbutyric acid (4-PBA, P21005), 2-deoxyglucose (D8375), and bovine serum albumin (BSA, essential fatty acid-free, A7030) were purchased from Sigma Chemical Company (St. Louis, MO, USA). Antibodies to TLR4 (ADI-CSA-801-E), NFκB p50 (SC-8414), glucose-regulated protein 78 (GRP78) (ab21685), c-Jun NH_2-terminal kinase (JNK, BS1544), phosphorylated JNK (p-JNK, BS4763), protein kinase B (AKT, 9272S), phosphorylated AKT (p-AKT, 4060S), and GAPDH mouse monoclonal antibody (CW100A) were purchased from Enzo Life Sciences Inc., Santa Cruz biotechnology Inc.(Santa Cruz, CA), Abcam Inc. (Cambridge, UK), Bioworld Technology Co., Ltd. (China), Cell Signaling Technology, Inc. (Danvers, MA), and Beijing ComVin Biotech Co., Ltd. (China), respectively. Goat-anti-mouse and anti-rabbit secondary antibody conjugated to horseradish peroxidase was purchased from Beijing Zhong Shan Golden Bridge Biological Technology, Co., Ltd. (China). TRIzol reagent and ReverseAid First Strand cDNA Synthesis Kit were purchased from Takara (Dalian, China) and Fermentas Life Sciences (MBI, America), respectively. 2-Deoxyglucose (2DG) uptake measurement kit was purchased from Cosmo Bio Co., Ltd. (Tokyo, Japan). All primers (Tables 1 and 2) were designed with Primer 5 software and synthesized by Shanghai Sangon Biotechnology Company (Shanghai, China). Other reagent-grade chemicals were obtained from commercial sources.

2.2. Experimental animals and protocols

All animal experiments were performed according to the principles approved by the Animal Ethics Committee of Anhui Medical University. Twenty-four female Sprague–Dawley rats (specific pathogen-free), aged 8 weeks, were obtained from the Experimental Animal Center of Anhui Medical University and subjected to a standard chow diet (SCD, D12450B, 10% kcal% fat from lard, n = 8) or a high-fat diet (HFD, D12451, 45% kcal% fat from lard, n = 16) for 20 weeks. The latter group was then randomly divided into the HFD group (n = 8) and the FF-treated HFD group (HFD + FF, 30 mg/kg/d, n = 8) for an additional 8 weeks. Body weight and food intake were measured weekly.

2.3. Glucose and insulin tolerance tests

The glucose tolerance test (GTT) and the insulin tolerance test (ITT) were performed to evaluate insulin sensitivity at week 28 [15]. Briefly, after overnight fasting, the GTT was started with an intraperitoneal injection of 2 g of glucose/kg body weight. The ITT assay was conducted with an intraperitoneal injection of insulin at 1 U/kg body weight after fasting for 4–6 h. Blood glucose concentrations were measured from nicked tail veins at 0, 30, 60, 90, and 120 min, respectively, with a OneTouch Ultra glucometer. The data were plotted as blood glucose concentrations over time.

Table 1
Real-time RT-PCR primers used for gene expression in soleus muscle of female SD rats.

Genes	Reference sequence	Primers	PCR product length (bp)
GRP78	NM_013083.2	Forward: 5′-TCAGCCCACCGTAACAATCAAG-3′ Reverse: 5′-TCCAGTCAGATCAAATGTACCCAGA-3′	88
CHOP	NM_001109986.1	Forward: 5′-TGGAAGCCTGGTATGAGGATCTG-3′ Reverse: 5′-GAGGTGCTTGTGACCTCTGCTG-3′	175
IRE1α	NM_001191926.1	Forward: 5′-ACGTCATTGCTCGTGAGTTGATAG-3′ Reverse: 5′-TGGGCTCGTGCCAGTAGTAGGT-3′	492
PPARα	NM_013196.1	Forward: 5′-TCCACAAGTGCCTGTCCGTC-3′ Reverse: 5′-TCCGAATCTTTCAGATCGTGTTC-3′	124
eIF2α	NM_019356.1	Forward: 5′-GGTCGAAGATGTAGTGATGGTGAA-3′ Reverse: 5′-GAACGGATACGTCGTCTGGATAAT-3′	212
TNFα	NM_012675.3	Forward: 5′-CGTAGCAAACCACCAAGCG-3′ Reverse:5′-CAGAGCAATGACTCCAAAGTAGACC-3′	443
IL-6	NM_012589.2	Forward: 5′-AAGGAGTGGCTAAGGACCAAGAC-3′ Reverse: 5′-GTTTGCCGAGTAGACCTCATAGTG-3′	87
GAPDH	NM_017008	Forward: 5′-GGCACAGTCAAGGCTGAGAATG-3′ Reverse: 5′-ATGGTGGTGAAGACGCCAGTA-3′	143