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GRK5 ablation contributes to insulin resistance

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ABSTRACT

The G-protein-coupled receptor kinase 5 (GRK5) is an important member of the threonine/serine kinase family that phosphorylates and regulates the G-protein-coupled receptor (GPCR) signaling pathway. GRK5 is highly expressed in adipose tissue and may act as an adipogenetic factor under high-fat load [1]. Insulin resistance is associated with the pathogenesis of metabolic disorders such as type 2 diabetes and obesity; however, the potential role of GRK5 in insulin resistance is unknown. We characterized the biochemical and molecular alterations related to metabolic complications observed in GRK5$^{-/-}$ mice. These mice, which are partially resistant to obesity induced by a high-fat diet, had impaired glucose tolerance and insulin sensitivity, as well as disruption of AKT signaling transduction compared with their wild-type littermates. Further study showed that the decreased insulin sensitivity was not attributable to alterations in inflammatory status such as the NF-κB signaling pathway or inflammatory gene expression. Instead, hepatic steatosis and changes of mRNA in genes involved in hepatic glucose and lipid homeostasis were found. Overall, our data identified GRK5 as a positive regulator of insulin sensitivity. Our results showed that this protein is a potential therapeutic target in the treatment of insulin resistance and related disorders.

1. Introduction

Insulin resistance, a condition in which cells fail to respond to the action of insulin, is a key feature in the pathogenesis of metabolic disorders such as type 2 diabetes and obesity [2]. Adipocytes are insulin-sensitive cells that take up glucose and store energy in the form of triglycerides. In addition to their storage function, adipocytes have recently been shown to be dynamic endocrine cells that produce and secrete various adipocytokines, such as TNF-α, IL-6, leptin, resistin, and adiponectin [3]. The insulin resistance that accompanies obesity is attributable, at least in part, to changes in adipokine secretion. Excess accumulation of adipose tissue is detrimental to many systems, and involved in the pathologies including insulin resistance, type 2 diabetes (T2D) and fatty infiltration of the liver [4,5]. Lipodystrophy, the lack of adipose tissue, is also associated with insulin resistance and abnormal lipid metabolism [6]. However, the precise mechanisms regulating insulin resistance in physiopathological conditions are not fully understood.

G-protein-coupled receptor (GPCR) kinase 5 (GRK5) is an important member of the threonine/serine kinase family that phosphorylates GPCRs as part of feedback inhibition of GPCRs in signal transduction [7,8]. The *in vivo* physiological functions of GRK5 have been ascribed to its kinase activity, phosphorylating and desensitizing specific GPCRs [9], as well as its kinase independent function and targeting to non-GPCR substrates. GRK5 interacts with IκBα and inhibits NFκB-mediated transcriptional responses [10]. GRK5 also phosphorylates p53 and regulates p53-mediated apoptosis in response to DNA damage [11]. Chen et al. recently identified GRK5 as a critical mediator of dendritic development that coordinates the actin cytoskeleton and membrane remodeling [12]. These results suggest that GRK5 might regulate many aspects of physiology by multiple molecular mechanisms. Our previous studies found that GRK5-deficient mice fed on a high-fat diet (HFD) show reduced gain in body weight and white adipose tissue (WAT). This is not due to changes in food consumption and energy expenditure induced by GRK5 ablation; instead, GRK5 deficiency decreased the transcription of adipogenic genes and inhibited adipocyte differentiation [1]. A recently performed genome-wide association study has found that the rs10886471, a T2D risk-increasing allele, was associated with higher *GRK5* mRNA expression level, higher fasting insulin, but not with higher fasting

Abbreviations: ANOVA, analysis of variance; GPCR, G-protein-coupled receptor; GRK, GPCR kinase; KO, knockout; SD, standard diet; HFD, high-fat diet; WAT, white adipose tissue; PBS, phosphate buffered saline; EDTA, ethylene diamine tetraacetic acid; TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

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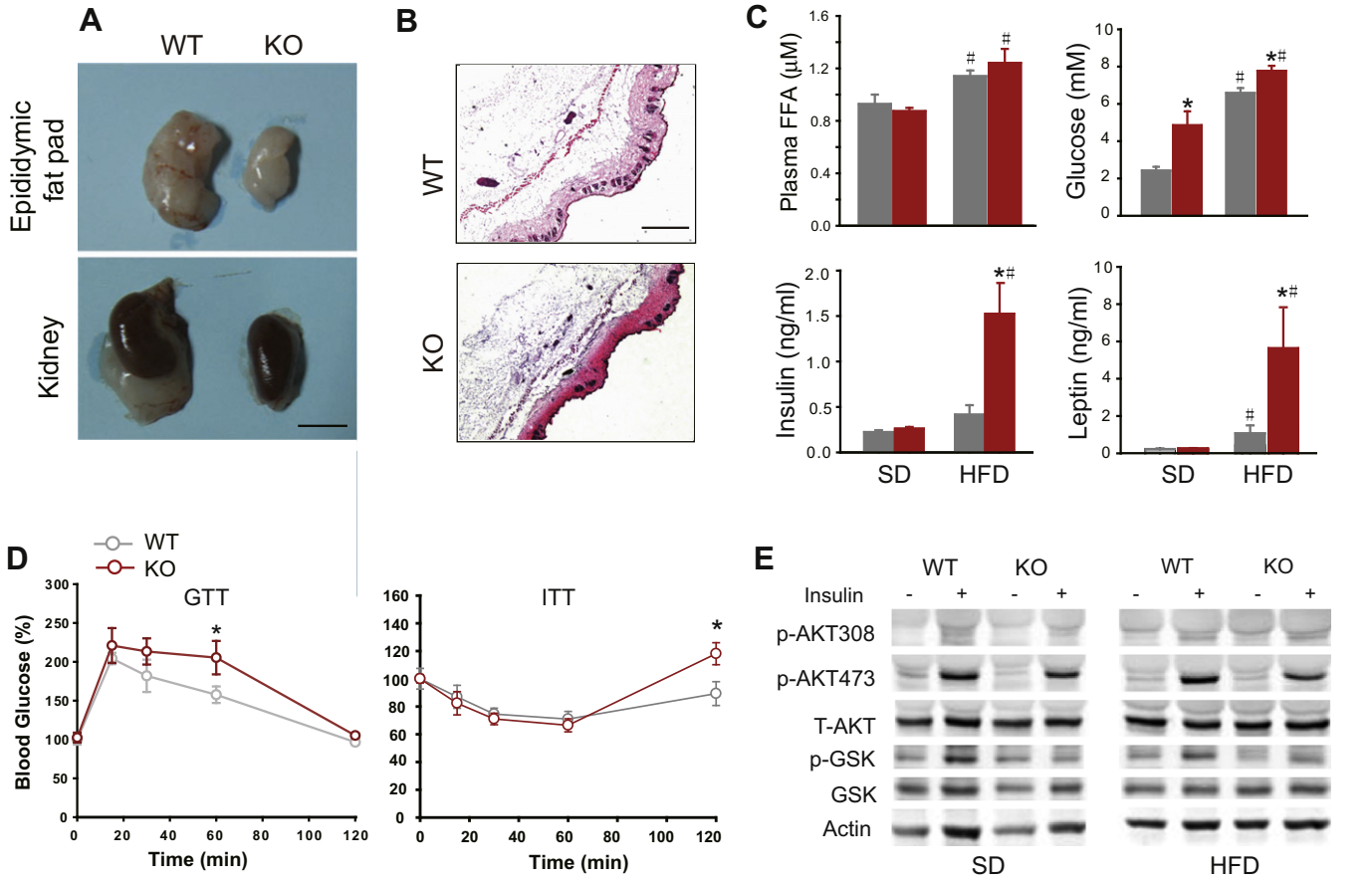

Fig. 1. GRK5$^{-/-}$ mice had decreased glucose tolerance and insulin sensitivity. (A) Representative photographs of epididymic fat pads and kidneys of 19-week-old male WT and GRK5$^{-/-}$ mice fed on a HFD. Scale bar, 1 cm. (B) Representative HE-staining photographs of cross-sections of skin from WT and GRK5$^{-/-}$ mice fed a HFD. Scale bar, 100 μm. (C) Plasma parameters from 6–12 fasted animals. $^{*}P < 0.05$ vs. WT mice of the corresponding diet group; $^{\#}P < 0.05$ vs. SD group of the corresponding genotype. (D) GTTs were performed on animals fasted for 24 h and given an injection of glucose. ITTs were performed on animals fasted for 4 h that received insulin. Glucose concentration was determined in tail vein blood samples. Results are means ± SEM for each group of 6–8 animals fed a HFD. (E) WT and GRK5$^{-/-}$ male mice were untreated or treated with insulin (5 mU/g body weight) for 15 min, and WAT was removed. Lysates were subjected to western blot with the indicated antibodies. Representative immunoblots of four independent experiments are shown.

glucose [13]. These results indicated GRK5 might act as a regulator in diet-induced obesity and T2D. Given the emerging role of signal transduction and adipocyte development in insulin resistance and metabolic disorders, we investigated the possibility that GRK5 might act as a modulator of metabolic complications such as insulin resistance.

2. Materials and methods

2.1. Mouse maintenance

GRK5 heterozygous C57BL/6 mice were provided by R.J. Lefkowitz and R.T. Premont (Duke University Medical Center, Durham, NC, USA). GRK5 knockout (GRK5$^{-/-}$) mice and their wild-type (WT) littermates were obtained by crossing GRK5 heterozygous mice. Genotyping was carried out by PCR amplification using tail tip DNA as described previously [14]. Mice were housed in groups and maintained on a 12 h light/dark cycle with food and water available *ad libitum*. Mice were fed on a standard diet (SD, 10% fat, 70% carbohydrates, and 20% proteins) or a high fat diet (HFD; 45% fat, 35% carbohydrates, and 20% proteins), for 12–16 weeks. All animal treatments were strictly in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All studies received approval from the University of Fudan Animal Care and Use Committee.

2.2. Signaling pathway analysis

Epididymal fat, liver and skeletal muscle tissues collected from GRK5$^{-/-}$ and WT mice were suspended in lysis buffer (50 mM Tris, pH 7.5, 5 mM EDTA, 250 mM sucrose, 0.5% Triton, 2 mM DTT, 1 mM sodium vanadate, 100 mM NaF) with freshly added protease inhibitor cocktail tablet (Roche Molecular Biochemicals, Basel, Switzerland). Crude lysates were centrifuged at 10,000*g* for 10 min and the protein concentration was determined using Pierce BCA Reagents (Pierce Biotechnology, Rockford, IL. USA) Protein samples of 40 μg were resolved by 10% SDS–polyacrylamide gel electrophoresis and transferred to nitrocellulose membrane. Rabbit anti-phospho-IκBα, phospho-NFκB, phospho-AKT (473), phospho-AKT (308), phospho-GSK3β, AKT, GSK3β, NFκB, or mouse anti-IκBα antibodies (Cell Signaling Technology, Beverly, MA, USA) were used to detect proteins. For Western analysis, blots were incubated with IRDye 800CW-conjugated or 700CW-conjugated antibody (Rockland Biosciences, Gilbertsville, PA, USA). Infrared fluorescence images were obtained with the Odyssey infrared imaging system (Li-Cor Bioscience, Lincoln, NE, USA).

2.3. Immunohistochemistry, oil red O staining and confocal microscopy

Tissues fixed in 4% paraformaldehyde were processed and subjected to dehydration in increasing sucrose solutions (20–30%).