Exercise Protects against Diet-Induced Insulin Resistance through Downregulation of Protein Kinase Cβ in Mice

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Abstract

Physical exercise is an important and effective therapy for diabetes. However, its underlying mechanism is not fully understood. Protein kinase Cβ (PKCβ) has been suggested to be involved in the pathogenesis of obesity and insulin resistance, but the role of PKCβ in exercise-induced improvements in insulin resistance is completely unknown. In this study, we evaluated the involvement of PKCβ in exercise-attenuated insulin resistance in high-fat diet (HFD)-fed mice. PKCβ−/− and wild-type mice were fed a HFD with or without exercise training. PKC protein expression, body and tissue weight change, glucose and insulin tolerance, metabolic rate, mitochondria size and number, adipose inflammation, and AKT activation were determined to evaluate insulin sensitivity and metabolic changes after intervention. PKCβ expression decreased in both skeletal muscle and liver tissue after exercise. Exercise and PKCβ deficiency can alleviate HFD-induced insulin resistance, as evidenced by improved insulin tolerance. In addition, fat accumulation and mitochondrial dysfunction induced by HFD were also ameliorated by both exercise and PKCβ deficiency. On the other hand, exercise had little effect on PKCβ+/− mice. Further, our data indicated improved activation of AKT, the downstream signal molecule of insulin, in skeletal muscle and liver of exercised mice, whereas PKCβ deficiency blunted the difference between sedentary and exercised mice. These results suggest that downregulation of PKCβ contributes to exercise-induced improvement of insulin resistance in HFD-fed mice.

Introduction

Diabetes mellitus, especially type 2 diabetes, is one of the most common chronic diseases worldwide [1]. Diabetes is growing worldwide both in number and significance, due to an increase in economic development and urbanization. Diabetes was reported to affect 366 million people globally in 2011, and this number is expected to rise to 552 million by 2030 in both developed and developing countries [2].

Protein kinase C (PKC) is a family of protein kinases that phosphorylates other proteins at serine and threonine residues [3,4]. PKC family proteins are involved in multiple cellular processes, including metabolism, differentiation, and cell growth. They are classified into subfamilies, including conventional isoforms (α, β, and γ) that are dependent on both Ca2+ and diacylglycerol (DAG) for stimulation, novel isoforms (δ, ε, η, and ι) that are dependent on DAG only, and atypical isoforms (ζ and τ/λ) that are independent of Ca2+ and DAG [3,4]. Abnormal expression of PKC family proteins has been observed in skeletal muscles of patients and animals with diabetes [5,6,7]. Among these PKC isoforms, PKCβ protein content was significantly higher, whereas PKCθ and PKCη were significantly lower, in muscle of obese patients compared with muscle of lean control subjects, without a corresponding change in membrane-associated PKC activity [5]. The PKCβ isoform inhibitor ruboxistaurin, which is the most studied PKC inhibitor, has shown some positive effects on diabetes and diabetic complications in clinical trials [8,9,10]. Our previous studies demonstrated that PKCβ deficiency alleviated insulin resistance and obesity in mice [11,12]. Although PKCβ is important in both obesity and insulin resistance, its role in exercise-related changes in HFD-induced metabolic disorders has not yet been reported.

Numerous studies have shown that a high-fat diet (HFD) and sedentary behavior increase the risk of obesity and insulin resistance, whereas increased physical activity reduces this risk [13,14,15,16]. The potential mechanism by which exercise attenuates HFD-induced insulin resistance involves increasing insulin sensitivity and glucose transport into contracting skeletal muscles [17]. However, the underlying molecular mechanisms are not fully understood due to the complicated processes involved in exercise [17]. Given the important regulatory role of PKCβ in insulin resistance, we postulated that it might also play a role in
exercise-induced improvement of insulin resistance. We thereby used PKCβ knockout mice and a diet-induced obesity model to test this hypothesis. To our knowledge, this is the first study demonstrating the role of PKCβ in exercise-attenuated insulin resistance by using PKCβ deficiency mice.

Methods

Animals and diet
Production of PKCβ−/− mice in C57BL/6J background and genotypic determination were performed as described previously [18]. At the age of four weeks, PKCβ−/− and wild-type (WT) C57BL/6J mice were fed a high-fat diet (HFD) containing 42% of calories from fat (TD88137, Harlan, Madison, WI). At the age of 12 weeks, both WT and PKCβ−/− mice were randomly assigned into sedentary (SED) or exercise (EX) group for 8 weeks (Figure S1). All mice were allowed to eat and drink ad libitum throughout the duration of the study. The mice were housed on a 12:12-hour light-dark cycle in a temperature and humidity controlled vivarium. National Institutes of Health guidelines for the care and use of laboratory animals were strictly followed, and all experiments were approved by the Animal Care and Use Committee at The Ohio State University.

Exercise intervention
Exercise intervention was performed as described previously [19]. Briefly, mice in exercise group were exercise-trained on a motorized treadmill (Columbus Instruments, Columbus, OH) at a speed of 15 m/min, 40 min/day, and 5 days/week for 8 weeks. Mice in SED group were put on the same treadmill without running for 40 min/day and 5 days/week for 8 weeks.

Body weight, tissue weight, food intake and water intake
Body weight, food intake, and water intake were recorded weekly during the exercise intervention. 24 hours after the end of the exercise intervention, all mice were fasted overnight and euthanized by CO2 inhalation overdose. Blood samples were obtained and plasma was collected and stored at −80°C immediately. Heart, liver, calf muscles (gastrocnemius and soleus), thigh muscle (quadriceps femoris and adductor magnus), epididymal fat, inguinal fat, together with brown adipose tissue from the interscapular depot were carefully excised. All the tissue samples were weighed and then immediately frozen in liquid nitrogen.

Magnetic resonance imaging (MRI)
Body fat mass (abdominal cavity) was evaluated by in vivo MRI, as described previously [19]. Briefly, 11.7 T small bore vertical NMR system (BioSpec, Bruker, Ettlingen, Germany) was used. First, mouse was anesthetized with isoflurane (1.5–2.0%) and placed in a 30-mm birdcage coil. After the mouse was positioned in the scanner, a coronal spin-echo localizing sequence was used to identify both kidneys. Finally, from the superior pole of the uppermost kidney to the caudal aspect of the mouse, thirty contiguous 1-mm thick axial slices were obtained using a spin-echo sequence with a 256×256 pixel size (30×30 mm). Data were analyzed by National Institutes of Health ImageJ software.

Glucose tolerance test (GTT) and insulin tolerance test (ITT)
After 8 weeks exercise intervention, a glucose tolerance test (overnight fasting) and insulin tolerance test (6 hours fasting) were performed on all mice as previously described [20]. Briefly, mice...
were weighed and then injected intraperitoneally with either glucose (2 mg/kg body weight) or insulin (0.5 U/kg body weight). Blood samples were collected through the tail vein and glucose concentrations were measured before and 30, 60, 90 and 120 min after the challenge on an Elite Glucometer (Bayer, Leverkusen, Germany). Area under the curve was calculated using GraphPad software.

Plasma insulin, leptin, adiponectin level and insulin resistance assessment

After overnight fasting, blood samples were collected into EDTA-coated tubes and plasma was collected after centrifugation at 2000×g for 15 min. Plasma insulin level was determined following a standard protocol of an ultrasensitive Mouse Insulin ELISA kit (Crystal Chem, Downers Grove, IL) [21]. Leptin level was determined following a standard protocol of the QuantiKine Mouse Leptin ELISA kit (R&D, Minneapolis, MN). Adiponectin level was measured according to the manufacturer’s instructions using the QuantiKine Mouse Adiponectin/Acrp30 ELISA kit (R&D, Minneapolis, MN). Insulin resistance (IR) was calculated using the homeostasis model assessment (HOMA) method based on the formula

\[
HOMA-IR = \frac{\text{Glucose (mg/dL)} \times \text{Insulin (µU/mL)}}{405}
\]

Oxygen consumption and CO₂ production measurements

Oxygen consumption and CO₂ production were measured simultaneously for each mouse using a computer-controlled, Comprehensive Lab Animal Monitoring (CLAMS) System (Columbus Instruments, Columbus, OH) [23]. Each mouse was measured individually in a resting state for 24 hours at 22°C in presence of food and water or measured individually when running on a treadmill at a speed of 15 m/min for 40 min.

Measurement of blood inflammatory biomarkers

At the end of the study, blood was collected and plasma was stored at −80°C for the analysis of cytokines. Plasma levels of TNF-α, IFN-γ, and monocyte chemoattractant protein 1 (MCP-1) were measured using Mouse Inflammation 6-Plex Kit from BD Bioscience (San Diego, CA), according to manufacturer’s instruction. The cytokine levels were then determined using a BD LSR II instrument and analyzed by the BD CBA software (BD Biosciences, San Jose, CA) [21].

Transmission electron microscopy

To investigate the mitochondrial changes in situ between groups, we examined the ultrastructure of mitochondria by transmission electron microscopy.
electron microscopy (TEM) as previously described [19]. Briefly, muscle tissue was excised into small pieces (around 1 mm³) and fixed in 2.5% gluteraldehyde (0.1 M phosphate buffer, pH 7.4) for 3 hours. Then each specimen was post-fixed in 1% osmium tetroxide for 1 hour and dehydrated through a graded ethanol series (50–100%). After embedded in eponate 12 resin, sections at a thickness of 80 nm were cut and stained by 2% aqueous uranyl acetate followed by lead citrate. The grids were loaded and observed in a Tecnai G2 Spirit transmission electron microscope (FEI, Hillsboro, OR). The images of mitochondria were captured at a magnification of 18,500×. For the morphometric analysis, five micrographs per tissue were counted. Mitochondrial size and number were analyzed by National Institutes of Health Image J software.

Immunoblotting

Mice tissues were collected after overnight fasting. Tissue lysates from the soleus muscle and liver were prepared in a lysis buffer containing 50 mM Tris-HCl (pH 8.0), 2 mM EGTA, 1% SDS, and protease inhibitors. After adjusting the protein concentration, the samples were loaded, and proteins were separated by 10% SDS/PAGE gel electrophoresis and transferred to PVDF membrane. The membranes were incubated with specific primary antibodies against PKCα, PKCβ (BD Transduction Laboratories, Lexington, KY), phospho-AKT, AKT (Cell Signaling Technology, Danvers, MA; 1:1000 dilution), PKCβ, PKCγ, PKCε, β-actin (Santa Cruz, Santa Cruz, CA; 1:200 dilution), and GAPDH (eBioscience, San Diego, CA; 1:1000 dilution), followed by visualization using horseradish peroxidase-conjugated specific secondary antibodies. All immunoreactive bands were detected by SuperSignal substrate kit (Thermo Fisher, Rockford, IL).

Data analyses

All data are expressed as means ± SEM unless otherwise specified. Difference between two groups was tested by student’s t test. Differences among groups were tested by two-way ANOVA and Boneferroni’s post hoc test using GraphPad Prism ver. 5 (GraphPad Software, La Jolla, CA). P values of <0.05 were considered statistically significant.

Results

Decreased expression of PKCβ in both skeletal muscle and liver after exercise

PKC proteins have been suggested to play a role in the insulin sensitivity of skeletal muscle [5,6,7]. To investigate the involve-
mice was lower than that of sedentary WT mice. H, Fasting serum adiponectin level.

Both exercise and PKCβ deficiency ameliorated HFD-induced obesity.

After eight weeks of exercise, exercised WT mice had a less body weight compared with WT mice (WT SED 41.2 ± 4.2 g vs. WT EX 38.6 ± 3.2 g after 8 weeks of exercise, p < 0.05). The body weights of both sedentary and exercised PKCβ−/− mice were lower than that of corresponding WT mice (WT SED 41.2 ± 4.2 g vs. PKCβ−/− SED 36.6 ± 3.4 g, p < 0.05; WT EX 38.6 ± 3.2 g vs. PKCβ−/− EX 35.6 ± 2.1 g, p < 0.05). Interestingly, we failed to detect a significant difference in body weight between exercise PKCβ−/− and sedentary PKCβ−/− mice (Figure 2A). The total weight gain of sedentary WT mice was significantly higher than in the other groups, while no significant difference of weight gain was observed between exercised WT and exercised PKCβ−/− mice (WT SED 16.3 ± 1.2 g vs. WT EX 11.9 ± 1.7 g vs. PKCβ−/− SED 12.4 ± 1.7 g vs. PKCβ−/− EX 8.2 ± 1.6 g, p < 0.05; Figure 2B).

Exercise reduced fat mass and increased skeletal muscle weight in WT mice but not PKCβ−/− mice. Compared to WT controls, PKCβ−/− mice (both exercised and sedentary) had slightly increased tissue weights of skeletal muscles and significantly reduced tissue weights of inguinal fat, epididymal fat, and interscapular brown fat. The liver weight of sedentary WT mice was also higher than that of sedentary PKCβ−/− mice, while no significant difference of liver weight was observed among other groups (Figure 3A). Similar results of tissue weight were obtained when normalized to body weight (Figure 3B). Consistently, MRI scans also suggested that both the visceral and subcutaneous fat of PKCβ−/− mice was lower than that of sedentary WT mice.

Figure 4. Effect of exercise and PKCβ deficiency on insulin resistance. A & B. Intraperitoneal glucose tolerance test (IPGTT) in WT and PKCβ−/− mice with or without exercise. A, Blood glucose responses; B, Area under the curve (AUC) of IPGTT. C & D. Intraperitoneal insulin tolerance test (IPITT) in WT and PKCβ−/− mice with or without exercise. C, Blood glucose curve; D, Area under the curve (AUC) of IPITT. E, Homeostasis model assessment-estimated insulin resistance (HOMA-IR) in WT and PKCβ−/− mice with or without exercise. HOMA-IR was calculated using the formula HOMA-IR = fasting glucose (mg/dl) x fasting insulin (µU/mL)/405. F, Fasting serum insulin level. After 16 h of fasting, serum was collected for ELISA detection of insulin. Exercise decreased fasting insulin level in WT but not in PKCβ−/− mice. Insulin level in sedentary PKCβ−/− mice was lower than that of sedentary WT mice. G, Fasting serum leptin level. After 16 h of fasting, serum was collected for ELISA detection of leptin. Exercise decreased fasting leptin level in WT but not in PKCβ−/− mice. Leptin level in sedentary PKCβ−/− mice was lower than that of sedentary WT mice. H, Fasting serum adiponectin level. After 16 h of fasting, serum was collected for ELISA detection of adiponectin. No significant difference of adiponectin was detected among the 4 groups.
Production in WT mice, while it had no production: WT SED mice was detected (Insulin: WT SED 6.22 mL/kg/min vs. PKC\(^{-/-}\) SED 4.47 mL/kg/min). CO\(_2\) production of WT and PKC\(^{-/-}\) mice with or without exercise was measured in a resting state for 24 hours in presence of food and water using CLAMS. D, Average resting CO\(_2\) production in WT and PKC\(^{-/-}\) mice with or without exercise. E, Average exercise CO\(_2\) production of WT and PKC\(^{-/-}\) mice with or without exercise was measured. F, Average exercise O\(_2\) consumption in WT and PKC\(^{-/-}\) mice with or without exercise. G, CO\(_2\) production with exercise intervention. O\(_2\) consumption of WT and PKC\(^{-/-}\) mice with or without exercise was measured. H, Average exercise CO\(_2\) production in WT and PKC\(^{-/-}\) mice with or without exercise. WT, wild-type; EX, exercise; SED, sedentary; Data are expressed as mean ± SEM; n = 5, *, P<0.05.

Exercise increased metabolic rate via decrease in PKC\(_\beta\)

As shown in Figures 5A–D, exercise increased resting O\(_2\) consumption and CO\(_2\) production in WT mice, while it had no significant impact on metabolic rate of PKC\(^{-/-}\) mice. PKC\(^{-/-}\) mice had a higher O\(_2\) consumption and CO\(_2\) production than WT mice, regardless of exercise or sedentary (O\(_2\) consumption: WT SED 45.77±0.87 mL/kg/min vs. WT EX 49.54±0.81 mL/kg/min vs. PKC\(^{-/-}\) SED 53.60±1.61 mL/kg/min vs. PKC\(^{-/-}\) EX 56.22±1.92 mL/kg/min, p<0.05; CO\(_2\) production: WT SED 36.62±0.67 mL/kg/min vs. WT EX 35.92±0.70 mL/kg/min vs. PKC\(^{-/-}\) SED 43.23±0.03 mL/kg/min vs PKC\(^{-/-}\) EX 47.36±2.18 mL/kg/min, p<0.05). Consistently, exercised WT mice had a higher metabolic rate than sedentary WT mice when mice run on treadmill. Comparable O\(_2\) consumption and CO\(_2\) production were detected in exercised and sedentary PKC\(^{-/-}\) mice when mice run on treadmill (O\(_2\) consumption: WT SED 85.07±0.46 mL/kg/min vs. WT EX 95.97±3.73 mL/kg/min vs. PKC\(^{-/-}\) SED 96.39±4.74 mL/kg/min vs. PKC\(^{-/-}\) EX 98.72±3.23 mL/kg/min, p<0.05; CO\(_2\) production: WT SED 73.43±2.11 mL/kg/min vs. WT EX 81.92±2.49 mL/kg/min vs. PKC\(^{-/-}\) SED 85.16±3.22 mL/kg/min vs PKC\(^{-/-}\) EX 86.02±6.22 mL/kg/min, p<0.05; Figures 5E–H). These results indicated that exercise increases metabolic rate possibly through decreasing PKC\(_\beta\).

Both exercise and PKC\(_\beta\) deficiency reduced HFD-induced mitochondrial defects in the skeletal muscle

As shown in Figure 6A, mitochondria from the muscle in exercised WT mice had a more clearly defined internal membrane...
structure, including wider cristae, than those from sedentary WT mice. Mitochondrial number in the skeletal muscle of exercised WT mice was also increased compared with sedentary WT mice. However, similar mitochondrial numbers were found in exercised and sedentary PKC\(_b\)-/- mice (WT SED 16.56 \pm 1.55 vs. WT EX 20.85 \pm 1.57 vs. PKC\(_b\)-/- SED 19.00 \pm 3.40 vs. PKC\(_b\)-/- EX 20.50 \pm 1.33, p < 0.05; Figure 6B). Furthermore, the mitochondria from skeletal muscle of sedentary WT mice were found to be enlarged and disordered in comparison to that of exercised WT mice, while exercised and sedentary PKC\(_b\)-/- mice had mitochondria with similar size to exercised WT mice (WT SED 0.39 \pm 0.05 \(\mu\)m\(^2\) vs. WT EX 0.27 \pm 0.04 \(\mu\)m\(^2\) vs. PKC\(_b\)-/- SED 0.33 \pm 0.06 \(\mu\)m\(^2\) vs. PKC\(_b\)-/- EX 0.29 \pm 0.03 \(\mu\)m\(^2\), p < 0.05; Figures 6A & 6C).

Eight weeks of exercise did not have significant impact on adipose tissue inflammation

Increased ratio of M1 (classically activated macrophages) versus M2 (alternatively activated macrophages) is suggested to be an important feature of adipose inflammation and insulin resistance [24]. To investigate the significance of exercise on adipose tissue inflammation, we detected the macrophage percentage and M1/M2 ratio (CD11b\(^+\) CD11c\(^-\) cell/CD11b\(^+\) CD204\(^+\) cell) in stromal vascular fraction (SVF) of epididymal fat. As shown in Figures 7A & 7B, exercise slightly decreased macrophage numbers in epididymal SVF from WT mice although not to a statistically significant level. Deficiency of PKC\(_b\) reduced macrophage infiltration in epididymal fat (WT SED 40.13 \pm 4.29\% vs. WT EX 36.45 \pm 3.75\% vs. PKC\(_b\)-/- SED 26.36 \pm 1.85\% vs. PKC\(_b\)-/- EX 22.08 \pm 2.91\%, p < 0.05). Mice from all the four groups had similar levels of classical and alternative macrophage activation (Figures 7C & 7D). In addition, the plasma levels of cytokines, including IL-6, IL-10, and MCP-1, were comparable among all the four groups (Figures 7E–G).

Both exercise and PKC\(_b\) deficiency enhanced insulin signaling in peripheral tissues

As depicted in Figure 8A, higher activation of AKT was observed in the liver of exercised WT mice, exercised PKC\(_b\)-/- mice, and sedentary PKC\(_b\)-/- mice when compared with that of sedentary WT mice. Similar results were found in the skeletal muscle (Figure 8B).

Discussion

Obesity and diabetes are increasing worldwide at an alarming rate largely due to increased prosperity and sedentary life styles. Appropriate diets and exercise are two important interventions for both type 1 and type 2 diabetes. Characterizing the beneficial effects of exercise on insulin sensitivity has been the focus of the
research. Despite important advancements in recent years, the mechanistic basis behind how exercise improves insulin signaling is still poorly understood. In the current study, we discovered a potential mechanism by which exercise improved insulin sensitivity in obese subjects.

Expression levels of several isoforms of PKC were altered in skeletal muscle of obese or diabetic patients [5,6,7]. Furthermore, forced expression of PKC\(_{\beta}\) in skeletal muscle caused a decrease in activation of IRS1 and glucose uptake [25]. However, the involvement of PKC proteins in exercise and exercise-mediated metabolic changes is unknown. By detecting different isoforms of PKC, we found that PKC\(_{\beta}\) levels were significantly decreased in both skeletal muscle and liver, suggesting that PKC\(_{\beta}\) might be involved in exercise-mediated improvements in insulin sensitivity. Our study further demonstrated that PKC\(_{\beta}\) deficiency, like exercise, could increase insulin sensitivity, as evidenced by improved ITT and HOMA-IR indices. Similarly, the activation of insulin downstream molecule AKT was enhanced by exercise and PKC\(_{\beta}\) deficiency. Moreover, no significant differences in insulin sensitivity were observed between exercised and sedentary PKC\(_{\beta}\)-/- mice, indicating that exercise possibly attenuates insulin resistance via the reduction of PKC\(_{\beta}\) levels. However, the response of exercised WT mice to glucose challenge in the IPGTT assay was not as significant as that in the ITT, although the blood glucose levels at 0 and 120 min were lower than those of sedentary WT

Figure 7. Effect of exercise and PKC\(_{\beta}\) deficiency on adipose tissue inflammation and plasma cytokines. A–D, Adipose inflammation in WT and PKC\(_{\beta}\)-/- mice with or without exercise. Exercise slightly reduced the infiltration of macrophages although not statistically significant. Macrophage percentage in SVF was significantly lower in PKC\(_{\beta}\)-/- mice. Macrophages with M1 or M2 phenotype were not affected by exercise or PKC\(_{\beta}\) deficiency. A, Representative flow cytometric plots of adipose tissue macrophages; B, flow statistical analyses of adipose tissue macrophages; C, Percentage of classically activated macrophages (M1, CD11b\(^+\) CD11c\(^+\)) in adipose tissue macrophages; D, Percentage of alternatively activated macrophages (M2, CD11b\(^+\) CD204\(^+\)) in adipose tissue macrophages. E–G, Plasma cytokine levels in WT and PKC\(_{\beta}\)-/- mice with or without exercise. Plasma isolated from exercise or sedentary mice was collected for inflammatory cytokine detection using BD\textsuperscript{TM} Cytometric Bead Array Mouse Inflammation Kit. Exercise and PKC\(_{\beta}\) deficiency do not affect the plasma level of IL-6, IL-10, and MCP-1. E, Plasma IL-6 level; F, Plasma IL-10 Level; G, Plasma MCP-1 Level. WT, wild-type; EX, exercise; SED, sedentary; Data are presented as mean ± SEM; n = 5, *, P<0.05.

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mice. This result is not surprising given the dependence of the IPGTT response on a multitude of factors and this is likely caused by the compensation of insulin secretion, as plasma insulin levels of sedentary WT mice are significantly higher than those of exercised mice.

Consistent with the results reported by other groups [26,27], exercise lowered HFD-induced weight gain in WT mice. Exercised WT mice and PKCβ^{-/-} mice had a lower fat mass than sedentary WT mice. Of note, no significant effects of exercise on weight gain and visceral fat mass were observed in PKCβ^{-/-} mice. Compared to WT mice, PKCβ^{-/-} mice had lower weight gain in response to HFD feeding, despite comparable food and water intake. This result suggested the PKCβ may affect energy usage. Metabolic measurements indicated that both exercise and PKCβ deficiency enhanced metabolic rate, whereas no effects of exercise on metabolic rate were found in PKCβ^{-/-} mice. Compared to WT mice, PKCβ^{-/-} mice had lower weight gain in response to HFD feeding, despite comparable food and water intake. This result suggested the PKCβ may affect energy usage. Metabolic measurements indicated that both exercise and PKCβ deficiency enhanced metabolic rate, whereas no effects of exercise on metabolic rate were found in PKCβ^{-/-} mice. Despite less adiposity in PKCβ^{-/-} mice, we did not observe a significant difference in the level of plasma adiponectin between WT and PKCβ^{-/-} mice. This is probably caused by the fact that PKCβ might have a suppressive effect on adiponectin expression. PKCβ has been shown to induce the activation of JNK and subsequently suppress PPARγ, a transcription factor promoting the expression of adiponectin [28,29].

In addition to insulin sensitivity and metabolism, exercise has beneficial effects on HFD-induced mitochondrial dysfunction, which has been observed in skeletal muscle of both obese rodent and human subjects [30,31,32]. Both an HFD and a high-sucrose diet can induce reactive oxygen species production in skeletal muscle, which results in mitochondrial dysfunction [30]. In this study, exercise reduced HFD-induced mitochondrial defects in WT mice. We also observed an ameliorated mitochondrial abnormality induced by HFD (including a more clearly defined internal membrane structure, and appropriate size and number) in both exercised and sedentary PKCβ^{-/-} mice. It has been reported that adipose tissue inflammation contributes to the development of insulin resistance [33,34]. Recent studies suggest that long-term exercise reduces adipose inflammation via suppression of macrophage infiltration and a switch from M1 to M2 [35,36]. We detected a slight decrease in macrophage infiltration in exercised WT mice, although no statistical significance was found. We also failed to detect a switch from M1 to M2 in exercised mice, which was probably caused by a shorter exercise period and lower fat content (42%) than studied in previous reports [35,36]. However,
PKCβ deficiency significantly decreased macrophage infiltration in adipose tissue. Considering the fact that an exercise-induced decrease in PKCβ levels in skeletal muscle does not have a significant effect on adipose tissue macrophage infiltration, the decrease in adipose tissue macrophages in PKCβ−/− mice likely resulted from a defect of PKCβ in either adipose tissue or macrophages. In addition, exercise and PKCβ deficiency did not affect the plasma levels of cytokines, including IL-6, IL-10, and MCP-1. These results suggested that 8 weeks of exercise may not have a significant effect on adipose inflammation and that exercise-induced improvements in insulin resistance may be unrelated to changes in adipose tissue macrophage content or function. Circulating level of IL-6 has been reported to be elevated unrelated to changes in adipose tissue macrophage content or function. By 8 weeks of exercise training on a motorized treadmill at a speed of 15 m/min, 40 min/day, 5 days/week for 8 weeks. Mice in SED group were put in the treadmill without running 40 min/day, 5 days/week for 8 weeks. (TIFF)

Taken together, our results suggested that exercise decreased the expression of PKCβ in both skeletal muscle and liver. By reducing PKCβ expression, exercise improved HF-induced metabolic dysfunction, including insulin resistance, fat accumulation, and mitochondrial dysfunction. Moreover, our findings that PKCβ−/− mice have an increased basal metabolic rate suggest PKCβ could be a potential target for treating obesity and insulin resistance. However, the involvement of entire PKC family in exercise and insulin resistance might be complex due to the diversity of PKC isoforms and potential compensation among different isoforms. It requires further studies to investigate whether the other isoforms of PKC are involved in exercise-mediated improvement of insulin resistance.

**Author Contributions**

Conceived and designed the experiments: KM QS. Performed the experiments: XR JZ XX RJ SM ZB TYW. Analyzed the data: WH SA. Wrote the paper: XR. contributed discussion: MP SR.

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