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PKCδ regulates hepatic triglyceride accumulation and insulin signaling in Lepr$^{db/db}$ mice

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ABSTRACT

PKCδ has been linked to key pathophysiological features of non-alcoholic fatty liver disease (NAFLD). Yet, our knowledge of PKCδ's role in NAFLD development and progression in obese models is limited. PKCδ$^{-/-}$/Lepr$^{db/db}$ mice were generated to evaluate key pathophysiological features of NAFLD in mice. Hepatic histology, oxidative stress, apoptosis, gene expression, insulin signaling, and serum parameters were analyzed in Lepr$^{db/db}$ and PKCδ$^{-/-}$/Lepr$^{db/db}$ mice. The absence of PKCδ did not abrogate the development of obesity in Lepr$^{db/db}$ mice. In contrast, serum triglyceride levels and epididymal white adipose tissue weight normalized to body weight were reduced in PKCδ$^{-/-}$/Lepr$^{db/db}$ mice compared Lepr$^{db/db}$ mice. Analysis of insulin signaling in mice revealed that hepatic Akt and GSK3β phosphorylation were strongly stimulated by insulin in PKCδ$^{-/-}$/Lepr$^{db/db}$ compared Lepr$^{db/db}$ mice. PKCδ may be involved in the development of obesity-associated NAFLD by regulating hepatic lipid metabolism and insulin signaling.

1. Introduction

Non-alcoholic fatty liver disease (NAFLD) is believed to be initiated by the accumulation of lipids in the liver (steatosis) and is commonly associated with obesity, type 2 diabetes, and the metabolic syndrome [1]. Insulin resistance is believed to underlie the development of NAFLD. Up to 30% of the people who have NAFLD are thought to develop a more advanced form of liver disease termed non-alcoholic steatohepatitis (NASH) which can progress to cirrhosis [2].

The classical (α, β, and γ) and novel (δ, ε, and θ) protein kinase C (PKC) isoforms are intracellular signaling molecules activated acutely and chronically by diacylglycerol (DAG), a free fatty acid metabolite [3,4]. Experimental treatments such as high fat feeding, bolus lipid treatment, and genetic obesity which are known to promote DAG and fat (triglyceride) accumulation in the liver have been shown to activate the novel PKC isoforms, PKCδ and PKCε, and the classical PKC isoform, PKCβ [5–8]. Recent studies in genetically modified mice indicate that PKCβ, PKCδ, and PKCε independently regulate high fat induced triglyceride (TG) accumulation in the liver and hepatic lipogenic gene expression [6,7,9,10]. PKCβ, PKCδ, and PKCε null mice are also protected from high fat diet induced whole body and hepatic insulin resistance [6–10]. Alternatives to the high fat diet model of NAFLD also suggest a role for PKC isoforms in hepatic lipid metabolism. In a non-obese model of severe NAFLD, hepatic PKCδ gene and protein expression are upregulated, and PKCδ null mice were found to have reduced TG accumulation in the liver and altered hepatic lipogenic gene expression [11]. Further, a reduction in oxidative stress and apoptosis, key aspects of the pathophysiology of NAFLD progression, was also observed in PKCδ null mice [11]. PKCδ hepatic gene expression and activation has been detected in Lep$^{ob/ob}$ mice and Zucker Lepr$^{fa/fa}$ rats [6,8], genetic models of obesity in which hyperphagia leads to obesity and is accompanied with insulin resistance and glucose intolerance [12]. Of the three PKC isoforms implicated in NAFLD, only PKCβ has been studied in the background of a genetically obese animal. In Lep$^{ob/ob}$ mice lacking PKCβ, hepatic TG accumulation is reduced and insulin sensitivity is

Abbreviations: NAFLD, non-alcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; PKC, protein kinase C; MCD, methionine and choline-deficient; ALT, alanine aminotransferase; TBARS, thiobarbituric acid reactive substances; 4-HNE, 4-hydroxy-2-nonenal; TG, triglyceride; NEFA, non-esterified fatty acids.

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improved compared to Lep$^{ob/ob}$ mice containing PKCβ [9]. However, a reduction in body and adipose tissue weight in Lep$^{ob/ob}$ mice lacking PKCβ suggest that hepatic changes may be secondary to changes in obesity.

PKCδ appears to modulate hepatic TG accumulation in lean and high fat diet fed mice. Yet, whether PKCδ has a protective role in profound obesity induced NAFLD is unknown. Therefore, in the present study, we investigated the role of PKCδ in the regulation of hepatic lipid metabolism, oxidative stress, apoptosis, and insulin signaling, key pathophysiological features in NAFLD, using PKCδ$^{-/-}$/Lepr$^{db/db}$ mice.

2. Materials and methods

2.1. Antibodies

See the Supplementary Methods for details.

2.2. Animals

Heterozygous PKCδ$^{-/+}$ mice in a mixed 129SX1 × C57BL/6 background were backcrossed up to six times with C57BL/6 N mice from Harlan Laboratories (Somerville, NJ). PKCδ genotyping was performed as previously described [13]. Heterozygous PKCδ$^{-/+}$ mice in a C57BL/6N background were backcrossed two times with Lepr$^{db/+}$ C57BL/6J mice from Jackson Laboratories (Bar Harbor, MA) and then interbred to generate PKCδ$^{-/-}$/Lepr$^{db/db}$ mice and Lepr$^{db/db}$ littermates. Mice were placed on a low fat diet (MP Biomedical, Cat #96044) for 4 weeks. Mice were housed 2–4 per cage in Thoren units in the Bassett Research Institute, an AAALAC accredited animal facility, in light/dark (12L:12D), temperature 22 °C, and humidity controlled rooms. Mice were provided with standard laboratory chow and water ad libitum in accordance with an Institutional Animal Care and Use Committee approved protocol. The mean and standard error (SE) of the final body, liver, and fat weight was determined. No procedures were undertaken that caused more than minimal pain, distress, or discomfort.

2.3. Histological analysis and special staining of liver tissue

Paraffin embedded sections were stained with hematoxylin and eosin and examined in a blinded fashion by a board certified pathologist, grading for steatosis as previously described [14]. TG was extracted using the Bligh and Dyer method [15] and assayed using a kit from Thermo Scientific (Rockford, IL) and normalized to the protein content measured using the BCA protein assay reagent (Thermo Scientific, Rockford, IL).

2.4. Serum metabolic parameters

Alanine aminotransferase (ALT) and triglycerides were assayed as previously described [14]. Insulin was assayed using the Ultra Sensitive Mouse Insulin ELISA Kit from Crystal Chem Inc (Downers Grove, IL). NEFA was assayed using the kit from Zen-Bio, Inc. (Research Triangle Park, NC).

2.5. Liver oxidative stress analysis

Liver samples were flash frozen and ground in liquid nitrogen. Ground tissue (50–100 mg) was homogenized on ice in PBS pH 7.4 buffer. The homogenate was tested for thiobarbituric acid reactive substances (TBARS) (ZeptoMetrix, Buffalo, NY) following manufacturer's instructions. Protein content was determined using the Pierce BCA Protein assay (Thermo Scientific/Pierce, Rockford, IL). TBARS units (nmol/ml) were normalized to protein concentration. 4-Hydroxy-2-nonenal (4-HNE) staining was performed using a 4-HNE (HNE11-S) antibody (Alpha Diagnostics, San Antonio, TX). Five random fields per slide were scored and the results were determined from an average of those scores.

2.6. Apoptosis analysis

TUNEL positive cells were detected using the DeadEnd Fluorometric TUNEL system (Promega, Madison, WI) and the manufacturer's recommendations for controls. Propidium iodide (0.25 mg/ml) was used as the counterstain. An average score was generated based on a ratio of positive nuclei to total nuclei in 3 random fields.

2.7. Immunoblotting

See the Supplementary Methods for details.

2.8. RNA extraction and qRT-PCR

See the Supplementary Methods for details.

2.9. Insulin stimulation

Mice were fasted overnight, then anesthetized with an intraperitoneal injection of avertin (2,2,2-tribromoethanol) in PBS (0.5 mg/g), and the abdominal cavity opened. Insulin (12 mU/g) or sterile PBS was injected into the inferior vena cava and then the liver was harvested after 2 min and flash frozen in liquid N_2.

2.10. Statistical analysis

All data are presented as the mean ± 1 standard error (SE). Statistical significance was determined by Student's *t*-test ($\alpha = 0.05$) using the XLSTAT 2009 program (Addinsoft, New York, NY). Pairwise comparisons were made using Tukey's test ($\alpha = 0.05$).

3. Results

3.1. Body and organ weights and serum parameters

As expected, PKCδ protein expression was not detected in the liver of PKCδ$^{-/-}$/Lepr$^{db/db}$ mice (Supplemental Fig. 1). No differences in body weight, glucose, liver weight, NEFA, ALT, and insulin were observed in PKCδ$^{-/-}$/Lepr$^{db/db}$ compared to Lepr$^{db/db}$ mice. In contrast, a reduction in fat pad weight normalized to body weight and serum TG was observed in PKCδ$^{-/-}$/Lepr$^{db/db}$ compared to Lepr$^{db/db}$ mice (Table 1).

Table 1
Body, liver, and fat pad weights and serum metabolic parameters from male Lepr$^{db/db}$ and PKCδ$^{-/-}$/Lepr$^{db/db}$ mice.

	Lepr$^{db/db}$	PKCδ$^{-/-}$/Lepr$^{db/db}$
Body weight (g)	48.9 ± 1.2[b]	53.1 ± 1.4
Glucose (mg/dL)	305 ± 43	251 ± 28
Liver weight (g)	3.58 ± 0.30	4.15 ± 0.17
Fat pad weight (g)	3.83 ± 0.28	3.43 ± 0.10
Liver-body weight (%)	7.30 ± 0.54	7.79 ± 0.18
Fat pad-body weight (%)	7.80 ± 0.43	6.47 ± 0.17**[c]
Triglyceride (mg/dL)	115.3 ± 23.7	75.9 ± 3.8*
NEFA[a] (mM)	1.83 ± 0.18	1.63 ± 0.14
ALT[a] (U/L)	10.34 ± 1.99	17.62 ± 2.49
Insulin (ng/ml)	12.54 ± 3.00	10.77 ± 1.95

[a] NEFA, non-esterified fatty acids; ALT, alanine aminotransferase.
[b] Values represent the means ± SEM for $n = 4–5$.
[c] Compared to Lepr$^{db/db}$ *$P < 0.05$, **$P < 0.01$.

3.2. Steatosis and lipid metabolism gene expression

Histological examination of livers from Lepr$^{db/db}$ and PKCδ$^{-/-}$/Lepr$^{db/db}$ mice showed marked steatosis in the animals (Fig. 1A). A significant reduction (~57%) in hepatic TG was observed in the PKCδ$^{-/-}$/Lepr$^{db/db}$ mice compared to the Lepr$^{db/db}$ mice (Fig. 1B). To further explore hepatic lipid metabolism in mice, we investigated the expression of lipid metabolism genes (Fig. 1C). No significant differences were found in key lipogenic genes.

3.3. Inflammation

Hepatic inflammation was assessed by determining the frequency of the histological inflammation scores from Lepr$^{db/db}$ and PKCδ$^{-/-}$/Lepr$^{db/db}$ liver sections. This qualitative analysis revealed that mild inflammation was induced in the PKCδ$^{-/-}$/Lepr$^{db/db}$ mice (Fig. 1D). In contrast, inflammation was absent in Lepr$^{db/db}$. Inflammation was assessed further by examining the expression of key inflammatory genes in the liver. A significant increase in the expression of interleukin-1 beta (IL-1β), a pro-inflammatory gene, and interleukin-10 (IL-10), an anti-inflammatory gene was observed in PKCδ$^{-/-}$/Lepr$^{db/db}$ mice (Fig. 1E). The phosphorylation state of IκB kinase (IKK) β was assessed to further characterize inflammation in the mice. Surprisingly, a reduction in phospho-IKKβ in PKCδ$^{-/-}$/Lepr$^{db/db}$ mice was observed (Fig. 1F).

3.4. Oxidative stress

To assess oxidative stress in the liver, we analyzed 4-HNE staining in liver sections from the mice. 4-HNE is a highly reactive aldehyde generated by the exposure of polyunsaturated fatty acids to peroxides and reactive oxygen species. A non-significant ($\alpha = 0.05$) reduction was found in 4-HNE staining in liver sections from PKCδ$^{-/-}$/Lepr$^{db/db}$ mice (Fig. 2A). Consistent with this result, a non-significant ($\alpha = 0.05$) reduction was also observed in TBARS levels (Fig. 2B). To further investigate hepatic oxidative stress, we analyzed expression of genes regulating oxidative stress (Fig. 2C). No change in the expression of Apolipoprotein E (ApoE), heme oxygenase-1 (Hmox1), Catalase (CAT), superoxide dismutase 1 (SOD1), nitric oxide synthase 2 (Nos2) and NADPH oxidase homolog (NOX4) was observed. Surprisingly, expression of components of the NOX2 containing NADPH oxidase complex: Cybα (p22^{phox}),

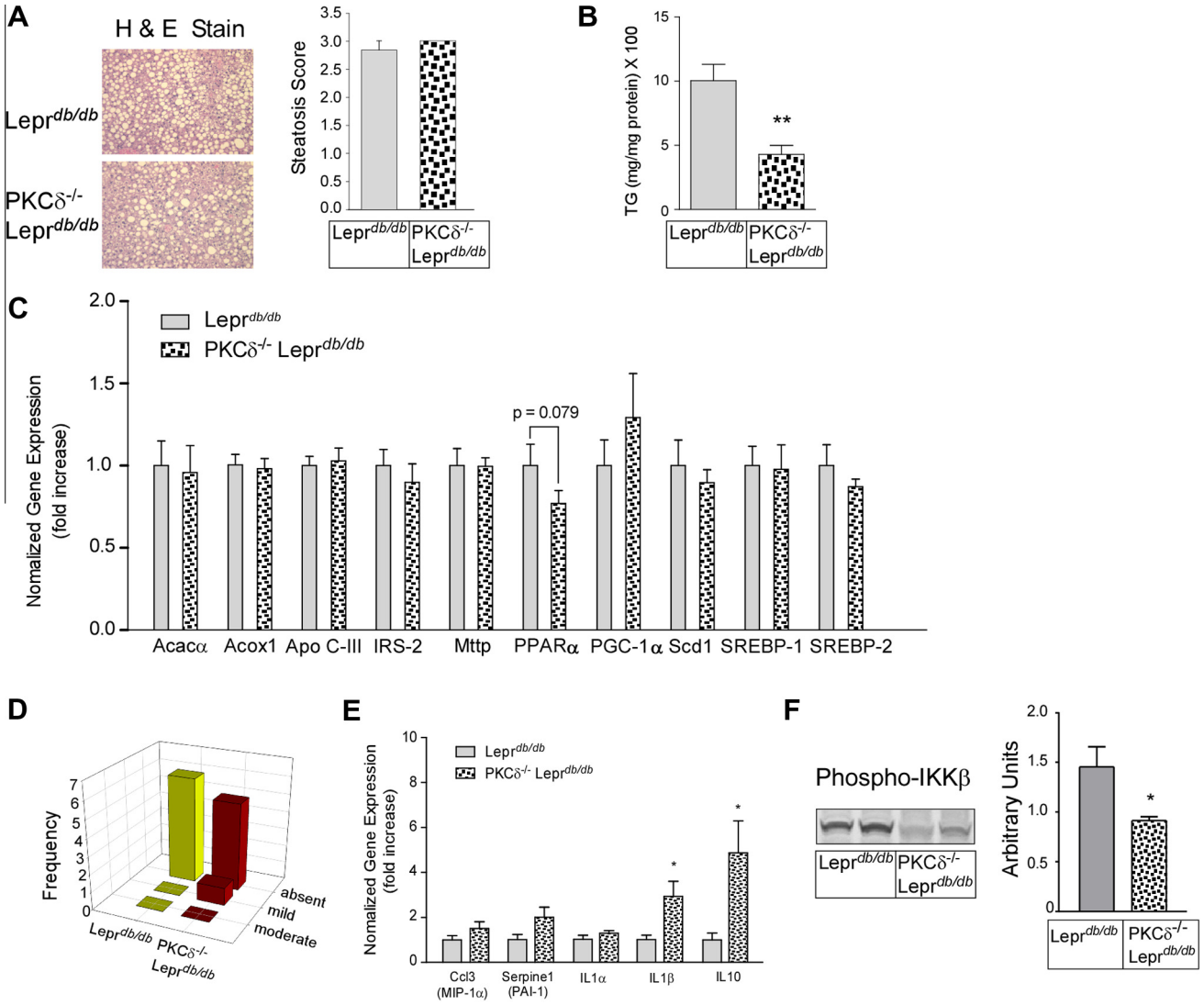

Fig. 1. Hepatic steatosis and inflammation. (A) Hematoxylin and eosin stained liver sections and quantitation shown as the means ± SE. (B) Quantitation of hepatic triglyceride content is shown as the means ± SE. (C) Hepatic lipid metabolism normalized gene expression (means ± SE). (D) Inflammation score frequency of liver sections (means ± SE). (E) Normalized inflammation gene expression and fold change (means ± SE). (F) Hepatic protein expression of phospho-IKKβ (means ± SE). ($^{*}P < 0.05$; $^{**}P < 0.01$ versus Lepr$^{db/db}$ mice.)

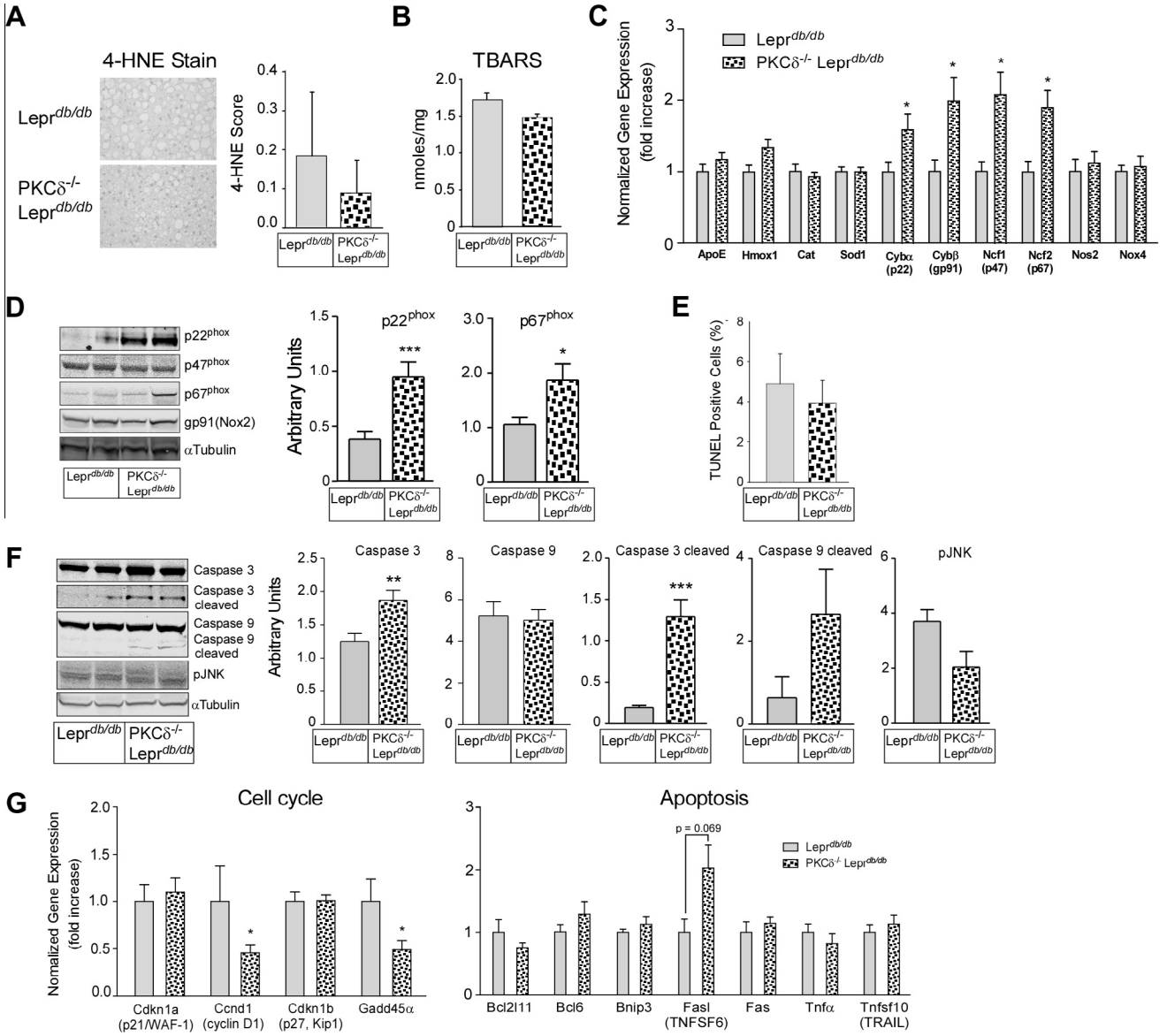

Fig. 2. Hepatic oxidative stress and apoptosis. (A) 4-HNE stained liver sections (*left panel*) and quantitation (means ± SE) (*right panel*). (B) Quantitation of hepatic TBARS (means ± SE). (C) Normalized oxidative stress gene expression and fold change (means ± SE) relative to Lepr$^{db/db}$ mice. (D) Hepatic expression of oxidative stress proteins. Representative immunoblots (*left panel*) and quantitation (means ± SE) of protein level of p22^{phox} and p67^{phox} (*right panel*). (E) TUNEL staining of liver sections (means ± SE). (F) Hepatic apoptosis protein expression (means ± SE). (F) Representative immunoblots (*left panel*) and quantitation (means ± SE) of apoptosis proteins (*right panel*). (G) Normalized gene expression and fold change (means ± SE) of cell cycle (*left panel*) and apoptosis (*right panel*) genes relative to Lepr$^{db/db}$ mice. ($^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ versus Lepr$^{db/db}$ mice.)

Cybβ (gp91^{phox}), Ncf1 (p47^{phox}), and Ncf2 (p67^{phox}) were significantly elevated in PKCδ$^{-/-}$/Lepr$^{db/db}$ mice. To verify our NADPH oxidase complex gene expression results, we determined the protein levels of p22^{phox}, gp91^{phox}, p47^{phox}, and p67^{phox}. Consistent with the gene expression, the protein levels of p22^{phox} and p67^{phox} were significantly elevated PKCδ$^{-/-}$/Lepr$^{db/db}$ mice (Fig. 2D). However, no change was found in the protein levels of p47^{phox} and gp91^{phox} (data not shown).

3.5. Apoptosis

To detect the apoptotic effect, a TUNEL assay was performed. A low level of TUNEL positive hepatocytes was detected in the liver of both Lepr$^{db/db}$ and PKCδ$^{-/-}$/Lepr$^{db/db}$ mice (Fig. 2E). To further investigate apoptosis, we examined hepatic total and cleaved caspase 3 and 9 protein levels. As shown in Fig. 2F, total and cleaved caspase 3 protein levels were significantly increased in PKCδ$^{-/-}$/Lepr$^{db/db}$ mice compared to Lepr$^{db/db}$ mice. No differences in total caspase 9 protein levels were observed. A non-significant ($\alpha = 0.05$) reduction was observed in hepatic phospho-c-Jun N-terminal kinase (JNK). To further investigate apoptosis, cell cycle gene expression in the liver was analyzed. We were able to detect a significant decrease in Cyclin D1 (ccnd1) and Gadd45 gene expression in PKCδ$^{-/-}$/Lepr$^{db/db}$ mice (Fig. 2G, *left panel*). Further analysis of expression for genes regulating apoptosis revealed no significant changes (Fig. 2G, *right panel*).

3.6. Fibrosis gene expression

To investigate the fibrotic effect in the liver, expression of fibrosis regulatory genes were analyzed from Lepr$^{db/db}$ or PKCδ$^{-/-}$/Lepr$^{db/db}$ mouse livers. Collagen, type III, alpha 1(Col3α1) and Alpha

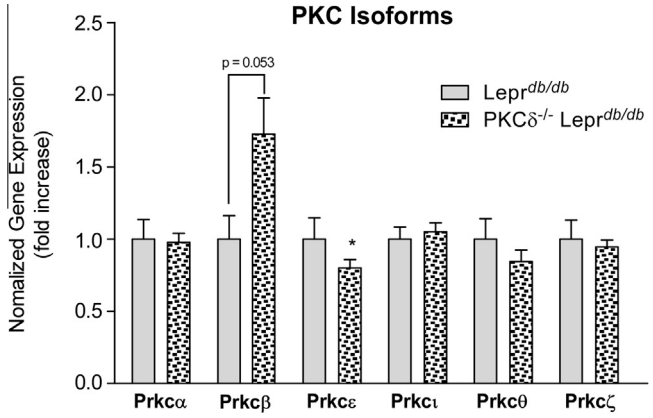

Fig. 3. PKC isoform gene expression. PKCα, PKCβII, PKCε, PKCι, PKCζ and PKCθ gene expression (means ± SE). (*$P < 0.05$ versus Lepr$^{db/db}$ mice.)

smooth muscle actin (α-SMA, acta2) gene expression were significantly elevated only in PKCδ$^{-/-}$/Lepr$^{db/db}$ mice (Suppl. Fig. 2).

3.7. PKC isoform gene expression

To determine whether hepatic gene expression of PKC isoforms was affected in PKCδ$^{-/-}$/Lepr$^{db/db}$ mice, PKCα, PKCβII, PKCε, PKCι, PKCζ and PKCθ expression was analyzed (Fig. 3). A 1.7-fold increase ($P = 0.053$) in PKCβ expression and a significant decrease in PKCε gene expression were observed in PKCδ$^{-/-}$/Lepr$^{db/db}$ mice. Otherwise, no significant genotype-related changes were observed in PKC isoform gene expression.

3.8. Hepatic insulin signaling

To explore insulin signaling in the liver, we investigated the phosphorylation state of insulin-stimulated hepatic Akt, GSK3β, MAPK and FOXO1α in Lepr$^{db/db}$ and PKCδ$^{-/-}$/Lepr$^{db/db}$ mice. Hepatic Akt, GSK3β and FOXO1α phosphorylation were not significantly increased upon insulin stimulation in Lepr$^{db/db}$ mice (Fig. 4). In contrast, insulin-stimulated hepatic phospho-Akt and phospho-GSK3β were elevated by 8.9-fold and 3.5-fold respectively in PKCδ$^{-/-}$/Lepr$^{db/db}$ mice compared to Lepr$^{db/db}$ mice (Fig. 4B and C). However, an increase in insulin-stimulated FOXO1α phopshorylation was not observed in Lepr$^{db/db}$ mice and an elevation in insulin-stimulated MAPK phosphorylation was found in Lepr$^{db/db}$ mice but not in PKCδ$^{-/-}$/Lepr$^{db/db}$ mice (Fig. 4D and E).

4. Discussion

Unlike Lep$^{ob/ob}$ mice lacking PKCβ [16], Lepr$^{db/db}$ mice lacking PKCδ are not protected from profound obesity and hyperinsulinemia. However, we did observe a reduction in eWAT weight relative to body weight in PKCδ$^{-/-}$/Lepr$^{db/db}$ mice compared to Lepr$^{db/db}$ mice. This result is consistent with our observations in lean mice [11] and suggests that PKCδ may have an adipose tissue specific function. In agreement with this conclusion, PKCδ has been reported to be activated in adipocytes isolated from high fat fed mice [17,18]. Further, a splice variant of PKCδ is differentially regulated during adipogenesis and promotes adipocyte cell survival [19,20].

Hepatic TG accumulation is a defining feature of NAFLD. Profound TG accumulation in Lepr$^{db/db}$ mice is consistent with the association of obesity with NAFLD. In the present study,

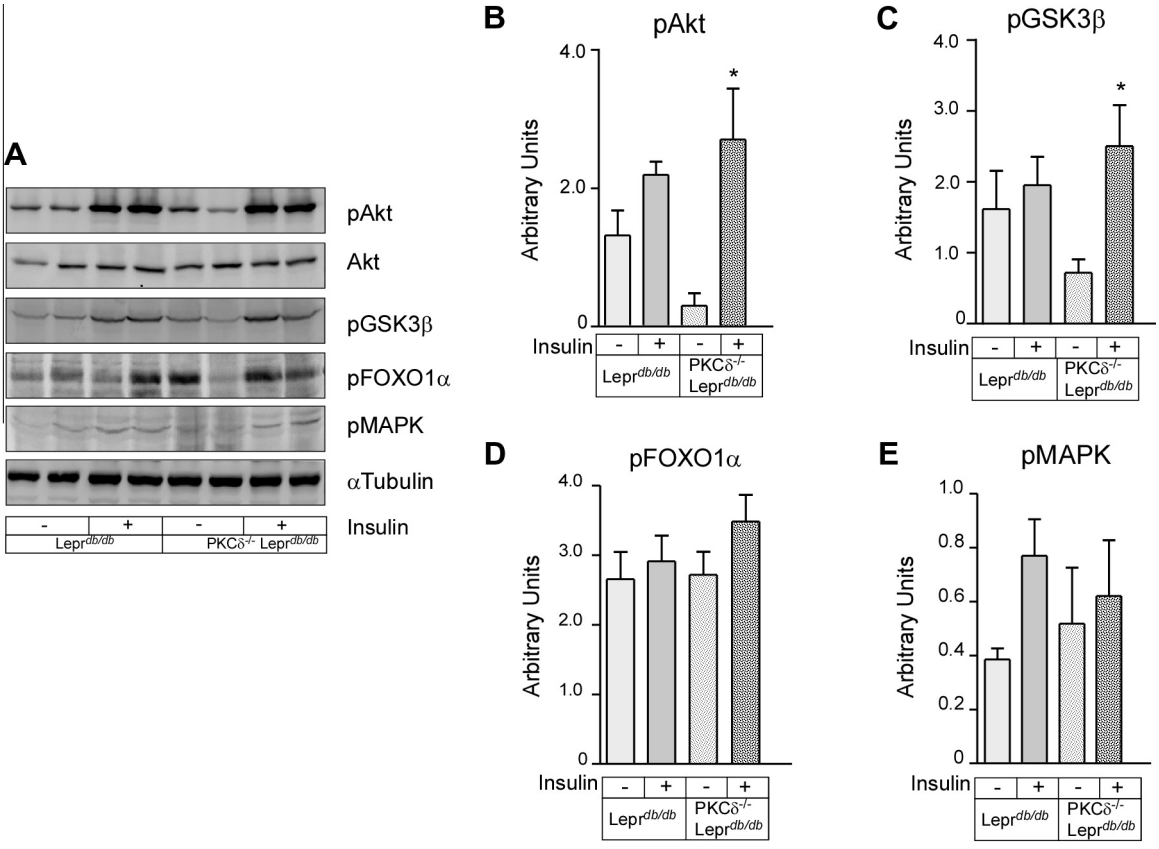

Fig. 4. Hepatic insulin signaling. Shown are insulin stimulated Akt, GSK3β, FOXO1α, and MAPK phosphorylation, total Akt, and α-tubulin representative blots (A) and the means ± SE (B) to (E). (*$P < 0.05$ versus Lepr$^{db/db}$ mice.)

histological scoring of liver sections indicated marked steatosis (>60% of liver parenchyma containing lipid vacuoles) in PKCδ$^{-/-}$/Lepr$^{db/db}$ and Lepr$^{db/db}$ mice. However, using a more quantitative assessment of hepatic TG levels, we were able to discern a significant difference in TG accumulation between PKCδ$^{-/-}$/Lepr$^{db/db}$ and Lepr$^{db/db}$ mice. A reduction in PPARα gene expression, albeit non-significant, is consistent with reduced hepatic TG levels in PKCδ$^{-/-}$/Lepr$^{db/db}$ mice. A significant reduction in serum TG in PKCδ$^{-/-}$/Lepr$^{db/db}$ mice further suggests that PKCδ may play a role in hepatic lipid metabolism.

Consistent with a previous study in Lepr$^{db/db}$ mice [21], mild inflammation was observed in the livers of PKCδ$^{-/-}$/Lepr$^{db/db}$ and Lepr$^{db/db}$ mice. Congruent with this observation, we observed elevated hepatic pro- and anti-inflammatory gene expression (IL-1β and IL-10, respectively) in PKCδ$^{-/-}$/Lepr$^{db/db}$ mice.

A striking upregulation in the expression hepatic NADPH oxidase subunit (p22^{phox}, gp91^{phox}, p47^{phox}, and p67^{phox}) gene expression that was confirmed at the protein level for p22^{phox} and p67^{phox} was observed in PKCδ$^{-/-}$/Lepr$^{db/db}$ mice. This result is consistent with our prior observation that hepatic NADPH oxidase subunit (p22^{phox}, gp91^{phox}, p47^{phox}, and p67^{phox}) gene expression is upregulated in lean PKCδ null mice [11]. PKCδ regulation of NADPH oxidase also occurs post-transcriptionally as has been reported in adipocytes, neutrophils, and liver tissue [11,17,22]. Inhibition of p47^{phox} phosphorylation and membrane translocation are potential post-transcriptional mechanisms by which PKCδ modulates NADPH oxidase [11,22,23]. In the present study, we did not observe differences in hepatic 4-HNE staining and TBARS levels in PKCδ$^{-/-}$/Lepr$^{db/db}$ mice compared to Lepr$^{db/db}$ mice. However, the 4-HNE score detected (∼0.2 vs. 2.0) and TBARS levels (∼1.5 vs. ∼5.0 nmol/mg) are considerably reduced compared to that observed in livers with more severe form of NAFLD [11,14]. These results are consistent with Lepr$^{db/db}$ mice having a less severe form of NAFLD.

Even though Lepr$^{db/db}$ mice have a less serve form of NAFLD, we investigated hepatic apoptosis and fibrosis to examine whether Lepr$^{db/db}$ mice lacking PKCδ may be less susceptible to NAFLD progression. Our results demonstrate that activated caspase 3 was significantly increased and protein expression of IKKβ was significantly reduced in PKCδ$^{-/-}$/Lepr$^{db/db}$ mice. These results are consistent with the observation that hepatic apoptosis and activated caspase 3 are elevated in mice with a liver-specific deletion in IKKβ [24]. Further, we observed a significant increase in hepatic gene expression of type III collagen and α-SMA in PKCδ$^{-/-}$/Lepr$^{db/db}$ mice. Taken together, our data does not support a role for PKCδ in limiting NAFLD progression. Whether other PKC isoforms compensate for the absence of PKCδ is not known. One candidate is PKCβ which was upregulated 1.7-fold at the gene expression level in the liver of PKCδ$^{-/-}$/Lepr$^{db/db}$ mice. Further experimentation is required to delineate the role of PKCβ in NAFLD progression.

Finally, we determined whether the absence of PKCδ affords any improvement in hepatic insulin signaling in Lepr$^{db/db}$ mice which exhibit whole body and hepatic insulin resistance [25–27]. We and others have demonstrated that PKCδ plays a negative regulatory role in insulin signaling [28–30] and can modulate insulin resistance in high fat diet-fed mice [6–8]. Consistent with this role for PKCδ, we found that Akt and GSK3β phosphorylation was strongly stimulated by insulin in PKCδ$^{-/-}$/Lepr$^{db/db}$ compared to Lepr$^{db/db}$ mice. However, we did not observe a difference in serum insulin levels between Lepr$^{db/db}$ and PKCδ$^{-/-}$/Lepr$^{db/db}$ mice which indicates that obesity-associated hyperinsulinemia was not modulated by PKCδ. However, it has been demonstrated in mice fed diets high in polyunsaturated fat that PKCε is the novel PKC isoform mediating diet-induced hepatic and whole body insulin resistance [10,31]. Thus, it is possible that additional novel PKC isoforms can compensate for the absence of PKCδ in Lepr$^{db/db}$ mice.

In conclusion, the data presented here indicate that PKCδ does not ameliorate profound obesity in Lepr$^{db/db}$ mice. However, PKCδ may be involved in the development of obesity-associated NAFLD by regulating lipid metabolism and oxidative stress. Our data also indicate that PKCδ regulates insulin signaling in the liver of obese mice.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2014.07.048.

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