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Zinc deficiency exacerbates diabetic down-regulation of Akt expression and function in the testis: essential roles of PTEN, PTP1B and TRB3

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Abstract

Since zinc (Zn) plays an important role in the spermatogenesis and Zn deficiency exacerbated diabetes-induced testicular apoptosis, the present study investigated the effect of Zn deficiency on diabetes-induced testicular Akt-mediated glucose metabolism changes and inflammation. Zn deficiency was induced by chronic treatment of normal and diabetic mice with the Zn chelator N,N,N′,N′, tetrakis (2-pyridylmethyl) ethylenediaminepentaethylene (TPEN). After diabetes onset induced by streptozotocin, both diabetic and age-matched control mice were given TPEN intraperitoneally for 4 months. Western blotting assay revealed that Akt-mediated glucose metabolism signaling was down-regulated in the diabetic testis and was further decreased in diabetic mice with Zn deficiency, reflected by reduced phosphorylation of both Akt and GSK-3β and increased phosphorylation of glycogen synthase along with a disarrangement of fatty acid metabolism (increased expression of PPAR-α and decreased adenosine-monophosphate-activated protein kinase phosphorylation). Testicular expressions of plasminogen activator inhibitor-1 and intracellular adhesion molecule-1 as inflammatory factors were increased in the TPEN or diabetes-alone group, but not additive in the group of diabetes with Zn deficiency. A mechanistic study showed that Akt negative regulators phosphatase and tensin homology deleted on chromosome 10 (PTEN), protein tyrosine phosphatases 1B and Tribbles 3 all increased in diabetic testis and further increased in the testis of diabetic mice with Zn deficiency. These studies suggest that Zn deficiency significantly exacerbated diabetic down-regulation of Akt expression and function, most likely by up-regulation of Akt negative regulators. Therefore, prevention of Zn deficiency for diabetic patients is important in order to avoid the exacerbation of diabetic inhibition of glucose metabolism in the testis.
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Keywords: Zn chelator; Testicular AMPK; Testicular PGC-1α; Testicular Sirt1; Akt negative modulator; Insulin resistance

1. Introduction

Infertility is a common complication in diabetic men [1,2], mainly due to the loss of germ cells by apoptotic cell death [3–5]. However, the underlying mechanisms contributing to diabetic induction of testicular apoptosis have not been well described. Zinc (Zn) is known as an essential trace element required for the maintenance of germ cells, the progression of spermatogenesis and the regulation of sperm motility [6–8]. Zn is a cofactor for many enzymes and proteins involved in antioxidant defenses [9–11] and also involved in insulin-stimulated Akt signaling [12–16]. Akt signaling mainly involves stimulating glucose metabolisms that play an important role in maintaining cell survival [17]. In our previous study, we have demonstrated that Zn deficiency only induced testicular cell death and also significantly enhanced diabetes-induced testicular cell death [18]. Whether these effects are related to the effect of Zn deficiency on Akt-mediated metabolic changes needs to be further defined.

For glucose metabolism, insulin via its receptor (IR) initiates phosphorylation of IR intracellular substrates. IR substrate family members serve as docking proteins for downstream signaling molecules, one of which is phosphatidylinositol 3 (PI3) kinase. Activation of PI3 kinase and its downstream protein kinase B (Akt) is essential for insulin-induced glucose and fatty acid metabolism such as glucose uptake, glycogen synthesis and suppression of triglyceride synthesis [17]. Glycogen synthase kinase 3 (GSK-3) is a ubiquitously expressed serine/threonine kinase that has versatile biological functions in cells, including regulation of metabolism, cell growth/death, gene transcription and translation [17]. Although both GSK-3α and GSK-3β appear to be equally important in certain

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aspects, GSK-3α has a more critical role than GSK-3β in regulating hepatic glucose metabolism and insulin sensitivity, and GSK-3β is the predominant regulator of glycogen synthase (GS) in other tissues [17,19,20], including the testis [21]. Unlike most protein kinases, GSK-3 remains active in its dephosphorylated form and is inactivated upon phosphorylation by other protein kinases, such as Akt. In response to insulin, therefore, activated Akt inhibits the activity of GSK-3α or GSK-3β isoform by phosphorylating their N-terminal serine residues (Ser21 in GSK-3α and Ser9 in GSK-3β), releasing their inhibitory effects on GS. Dephosphorylated GS and phosphorylated GS (Ser641) are active and inactive forms, respectively, in glycogen synthesis [17]. In diabetes mellitus, GSK-3β was activated by decreasing its phosphorylation [22,23]. In contrast, inactivation of GSK-3β, either by increased Akt phosphorylation [22,23] or by GSK-3β inactivator [24], increased glucose utilization and lipid metabolism. These studies thus suggest that GSK-3β phosphorylation plays a critical role in glucose metabolism.

In addition, glucose and fatty acid metabolisms are also transcriptionally regulated by peroxisome proliferator-activated receptor family (PPAR-α, β/δ and γ). Both PPAR-α and -β play critical roles in the testis in regulating glucose and fatty acid metabolisms, with the PPAR-α as the predominant one for the fatty acid metabolism in certain organs. PPAR-α has been confirmed to be expressed in the rat neonatal and adult testes [25]. PPAR-γ coactivator 1α (PGC-1α) is essential for the maintenance of maximal and efficient mitochondrial fatty acid oxidation, ATP synthesis and lipid homeostasis, and also plays an important role in mitochondrial biogenesis [26]. Several other kinases and proteins are also involved in glucose and fatty acid metabolic regulation, among which are the 5′ adenosine-monophosphate-activated protein kinase (AMPK) and Sirt1. The AMPK is an enzyme that plays a role in cellular energy homeostasis and is expressed in a number of tissues, including the testis [27,28]. The net effect of AMPK activation is stimulation of hepatic and muscle fatty acid oxidation, and it may also play a similar important role in the testicular fatty acid oxidation [27,28]. The Sirt1, as one of the sirtuin family of proteins, functions as a NAD-dependent deacetylase to play several physiological roles, including the regulation of glucose metabolism, cell survival and mitochondrial respiration. Sirt1 deficiency was found to markedly attenuate spermatogenesis, confirming the important role of Sirt1 in the spermatogenesis and germ cell function [29].

It is known that Akt expression and function are upon many positive and negative stimuli. Currently, several Akt negative regulators have been identified [30]. Phosphatase and tensin homology deleted on chromosome 10 (PTEN), as a negative regulator of PI3K/Akt pathway, has been extensively reported in terms of its role not only in the heart [31,32] but also in the testis [33]. Protein-tyrosine phosphatase-1B (PTP1B) is another Akt negative regulator [34,35] and also exists in the germ cells [36]. The newly discovered TRB3, a mammalian homolog of *Drosophila* tribbles, is a negative modulator of Akt to inhibit insulin function *in vitro* and *in vivo* [37–39]. Expression of TRB3 was increased in the tissues of diabetic mice [40]. Therefore, up- or down-regulation of these Akt negative regulators in response to certain stress affects glucose metabolism and cell survival signaling [41,42]. Reportedly Zn is able to negatively modulate several Akt negative regulators, including PTEN and TPT1B, to stimulate Akt-mediated glucose metabolisms and cell survival signaling [12–16].

In our recent study, we have demonstrated that treatment of nondiabetic mice with TPEN at 5 mg/kg daily for 4 months decreased testicular Zn level. If the diabetic mice were treated with TPEN after hyperglycemia onset, the testicular Zn level was significantly lower than either TPEN-treated or diabetic mice [18]. In that study, we also found that diabetes induced significant increases in testicular oxidative damage and mitochondrial cell death, which were exacerbated by TPEN-induced Zn deficiency. To extend the previous studies and also explore the mechanisms responsible for the exacerbation of diabetic apoptotic effect by Zn deficiency, we used the same animal model to investigate the effect of Zn deficiency on diabetes-induced testicular Akt expression and function, as well as associated signaling changes.

2. Materials and methods

2.1. Animals

FVB mice were used for this study. All mice were housed in the University of Louisville Research Resources Center at 22°C with a 12-h light/dark cycle and were provided with free access to standard rodent chow and tap water. All animal procedures were approved by the Institutional Animal Care and Use Committee, which is certified by the American Association for Accreditation of Laboratory Animal Care.

Type 1 diabetes model induced by multiple low doses of streptozotocin (STZ) and Zn deficiency induced with chronic treatment with TPEN were described in detail previously [18]. At the time of sacrifice, two testes were harvested for the following histopathological and biochemical studies.

2.2. Western blotting

Western blots were performed as described in our previous studies [18,43]. Briefly, testicular tissues were homogenized and fractionated on 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis gels, and proteins were transferred to a nitrocellulose membrane. The membrane was incubated overnight at 4°C with the following antibodies: anti-phospho-Akt (Ser473), anti-Akt, anti-phospho-PTEN (Ser380/Thr382/383), anti-PTEN, anti-phospho-AMPKα (Thr172), anti-AMPKα, anti-phospho-GSK3β (Ser9), anti-GSK3β, anti-phospho-GS (Ser641), anti-GS (1:1000, Cell Signaling, Beverly, MA, USA), anti-ICAM and anti-HKII (1:500, Santa Cruz Biotechnology, Santa Cruz, CA, USA), anti-PTP1B and anti-PAI-1(1:2000, BD Biosciences, Rockville, MD, USA), anti-TRB3(1:1000, Calbiochem, La Jolla, CA, USA), anti-PPAR-α, anti-Sirt1 and anti-PGC-1α (1:1000, Abcam, Cambridge, MA, USA). The rest of the Western blotting and quantitative densitometry procedure has been described in detail in previous studies [18,43].

2.3. Testicular periodic acid-Schiff staining and double immunofluorescent staining for Akt and PTP1B

Testicular tissues were immersion fixed in 10% neutral-buffered formalin and tissue blocks and sectioned (5 μm). First sections were stained by the periodic acid-Schiff (PAS) with hematoxylin counterstaining (PAS-H). Histopathological damage was assessed by examination of 100 seminiferous tubule cross-sections per testis. Second, for localization of Akt, phosphorylated Akt and PTP1B in the testis, indirect double staining was used. The tissue sections on poly-L-lysine solution charged slides were deparaffinized, and the antigens were unmasked in Target Retrieval Solution (Dako, Carpinteria, CA, USA) for 10 min at 98°C. The sections were blocked by 5% normal donkey serum for 20 min and incubated in specific primary antibody combination of either Akt and phospho-Akt or phospho-Akt and PTP1B at 4°C overnight. The primary antibodies used included rabbit monoclonal anti-phospho-Akt and rabbit polyclonal anti-Akt (1:100, Cell Signaling), and mouse monoclonal anti-PTP1B antibody (1:100, BD Biosciences). The secondary antibodies CY3-conjugated Affinipure Donkey anti-rabbit IgG(H+L) (Cell Signaling), Goat pAb to rabbit IgG (FITC) and rabbit polyclonal anti-mouse IgG+IgM+IgA (FITC) (Abcam) were applied in a dilution 1:200 in phosphate-buffered saline for 1 h at room temperature. Slides were counterstained with DAPI (Sigma-Aldrich), covered with aqueous mounting medium (Sigma-Aldrich) and analyzed under fluorescent microscope (Nikon, Tokyo, Japan).

2.4. Statistical analysis

Data were collected from repeated experiments and were presented as mean ± S.D. One-way analysis of variance was used to determine if differences exist, and if so, a post hoc Tukey's test was used for analysis for the difference between groups with Origin 7.5 laboratory data analysis and graphing software. Statistical significance was considered as $P<.05$.

3. Results

3.1. Effects of Zn deficiency on diabetes-decreased glucose metabolism signaling pathway

We have confirmed that both diabetes and chronic treatment with TPEN for 4 months significantly reduced testicular Zn level, and diabetes with TPEN treatment showed a synergistic reduction of

testicular Zn level [18]. Using the same tissues from these animals used for the previous study [18], we demonstrated a significant decrease of testicular Akt phosphorylation in diabetic mice at 4 months after diabetes onset, examined by Western blotting (Fig. 1A), along with a significant decrease in GSK-3β phosphorylation (Fig. 1B) and an increase in GS phosphorylation (Fig. 1C). Zn deficiency induced by chronic treatment with TPEN did not affect Akt and GSK-3β phosphorylation (Fig. 1A, B), but did increase the GS phosphorylation (Fig. 1C) in nondiabetic mice. However, Zn deficiency significantly enhanced diabetic effects on the decreases in Akt and GSK-3β phosphorylation (Fig. 1A, B) and increase in GS phosphorylation (Fig. 1C).

We investigated the localization of Akt and phosphorylated Akt in the testis by double immunofluorescent stains with a nuclear staining using DAPI. As shown in Fig. 2A, both Akt and phosphorylated Akt expressions are mainly in the Leydig cells and less in the spermatogonia. Akt phosphorylation level in the group of diabetic mice with Zn deficiency was significantly decreased as compared to controls.

As shown by PAS staining (Fig. 2B), testicular sections from control mice contained an array of developing germ cells from spermatogonia at the basement of the seminiferous tubules to spermatids that are adjacent to the lumen of the seminiferous epithelium, but testicular sections from the diabetes with Zn deficiency (DM/TPEN) mainly contained many seminiferous tubules with significant or partial depletion of spermatogenic cells, along with several multinucleated giant cells. PAS staining was predominantly in Leydig cells and very less in the spermatogonia, and the interstitial PAS-positive materials become predominant in diabetes and diabetes with Zn deficiency (DM/TPEN, Fig. 2).

3.2. Effects of diabetes and Zn deficiency on fatty acid metabolism-related mediators

Decreased glucose metabolism often is accompanied with increased fatty acid metabolism. We first examined the testicular expression of PPAR-α, which is an important mediator for fatty acid utilization [25]. Western blotting assay indicated that diabetes significantly increased the testicular expression of PPAR-α (Fig. 3A), suggesting the increase in lipid metabolism in the diabetic testis. Zn deficiency did not affect the testicular expression of PPAR-α either in nondiabetic mice or diabetic mice.

AMPK, as another important mediator to stimulate fatty acid oxidation, was examined. Results showed that diabetes significantly decreased testicular activation of AMPK (Fig. 3B). Zn deficiency did not significantly affect the testicular AMPK activation in either nondiabetic mice or diabetic mice (Fig. 3B).

3.3. Effects of Zn deficiency on diabetes-induced inflammatory response

Since imbalanced glucose/fatty acid metabolism often leads to inflammation [24], we next examined the effect of Zn deficiency on diabetic inflammatory response. Western blotting revealed that diabetes induced significant increases in ICAM (Fig. 4A) and PAI-1 (Fig. 4B) expression, indicating the induction of testicular inflammation. Zn deficiency increased ICAM expression, but not PAI-1 expression, in the testis of nondiabetic mice. Unexpectedly, there was no synergistic effect of Zn deficiency on diabetes-induced testicular inflammation (Fig. 4), suggesting that the exacerbation of diabetic down-regulation of Akt-mediated glucose metabolisms by Zn deficiency in the testis is directly related to testicular inflammation.

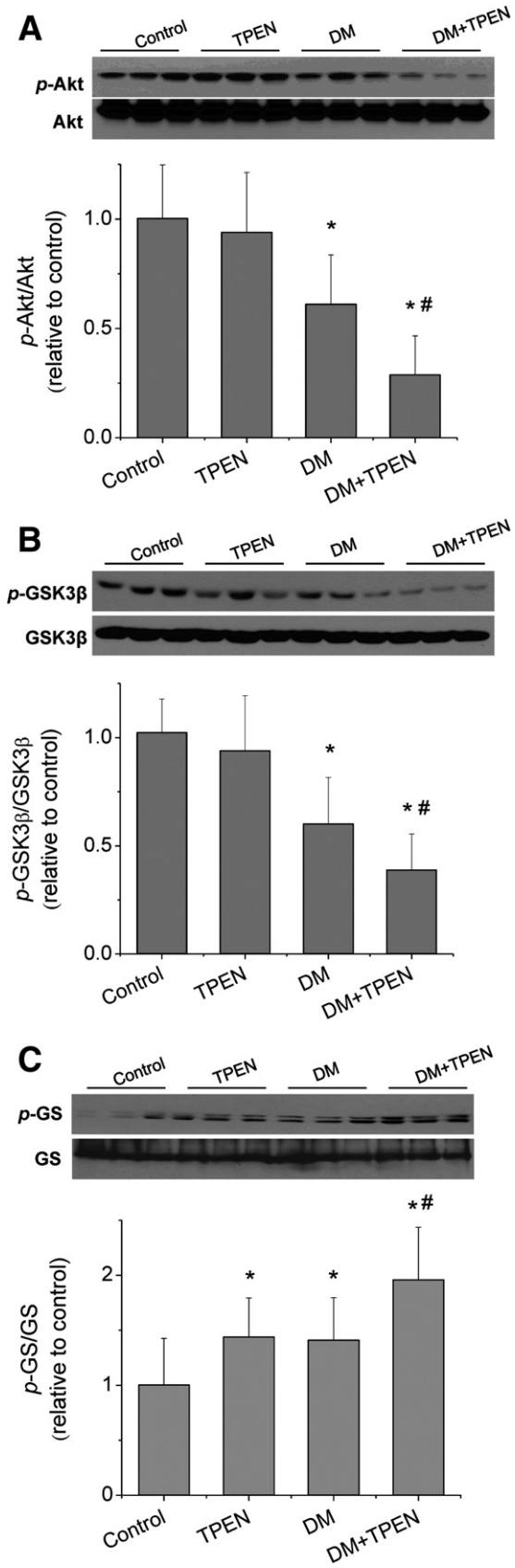


Fig. 1. Effects of diabetes and TPEN on Akt, GSK-3β and GS phosphorylation. Diabetes was induced with MLD-STZ, and both diabetic and age-matched control mice were treated with and without TPEN at 5 mg/kg daily for 4 months. Testicular expressions of total and phosphorylated Akt (A), GSK-3β (B) and SG (C) were measured by Western blotting assay. Data are presented as mean ± S.D. (n=6 at least in each group). DM: diabetes. *P<.05 vs. control group; #P<.05 vs. DM group.

3.4. Effects of diabetes and Zn deficiency on testicular Sirt1 and PGC-1a expressions

Growing evidence suggests that Sirt1 regulates glucose or lipid metabolism through its deacetylase activity for many substrates [44] and also has anti-inflammatory effect [45]. Therefore, we examined whether the testicular inflammation was related to the change of Sirt1 expression by Western blotting assay (Fig. 5A). Diabetes significantly decreased testicular Sirt1 expression. Although Zn deficiency did not significantly affect the testicular Sirt1 expression in nondiabetic mice, it significantly decreased its expression in diabetic mice as compared to that in diabetic mice (Fig. 5A).

An earlier study showed that resveratrol, as one of the Sirt1 activators, protects cell apoptotic death and inflammation because resveratrol stimulates the Sirt1-mediated deacetylation of the transcriptional PGC-1α [46]. Since PGC-1α has several important physiologies, including mitochondrial biogenesis and maintenance of a maximal and efficient mitochondrial fatty acid oxidation, its testicular expression was examined and found to be significantly decreased in diabetic mice and further decreased in diabetic mice with Zn deficiency (Fig. 5B), although Zn deficiency did not affect its expression in nondiabetic mice.

3.5. Possible mechanisms for the exacerbating effect of Zn deficiency on diabetic down-regulation of Akt function

It is appreciated that Akt, as a pivotal mediator in insulin-mediated glucose metabolism signaling, is regulated by several negative regulators [16,30]. Testicular expressions of PTP1B and TRB3 were thus examined by Western blotting (Fig. 6A, B). Diabetes significantly increased the expression of PTP1B and TRB3. Zn deficiency did not increase PTP1B (Fig. 6A) but increased TRB3 (Fig. 6B) expressions. However, Zn deficiency further enhanced diabetic up-regulation of both PTP1B and TRB3 expressions.

Testicular PTEN expression and activation were also examined by Western blotting of the total and phosphorylated PTEN (Fig. 6C). Total PTEN expression was not changed among the groups, but its phosphorylation level was significantly increased in testis of diabetic or TPEN-treated nondiabetic mice. Zn deficiency further enhanced the diabetic activation of testicular PTEN (Fig. 6C).

To see whether the increased expression of Akt negative regulator PTP1B and decreased Akt phosphorylation are colocalized in the same cells, testicular tissues were double-stained by immunofluorescence. As shown in Fig. 7, PTP1B expression that is mainly in the Leydig cells was significantly increased in the groups of diabetes and diabetes/TPEN. The increased TPT1B expression is accompanied with a significant decrease in Akt phosphorylation at the same cells in the groups of diabetes and diabetes/TPEN (Fig. 7).

4. Discussion

The number of young patients with either type 1 or type 2 diabetes is increasing dramatically [47,48], and infertility of these young diabetic patients has become a concern [1,2]. As we reported previously, the significant induction of testicular apoptotic cell death may be the major cause of the infertility of men with diabetes [4,18]. It is known that diabetes is accompanied with impairment of Akt-mediated glucose metabolism in most of the peripheral tissues, reflected by decreased activation of Akt [24]. Akt is also an important cell survival mediator [17]; therefore, diabetic impairment of Akt-mediated glucose metabolism may be the major reason for diabetic induction of germ cell death, and Zn-deficiency-enhanced testicular apoptotic cell death may be related to its further down-regulating Akt function.

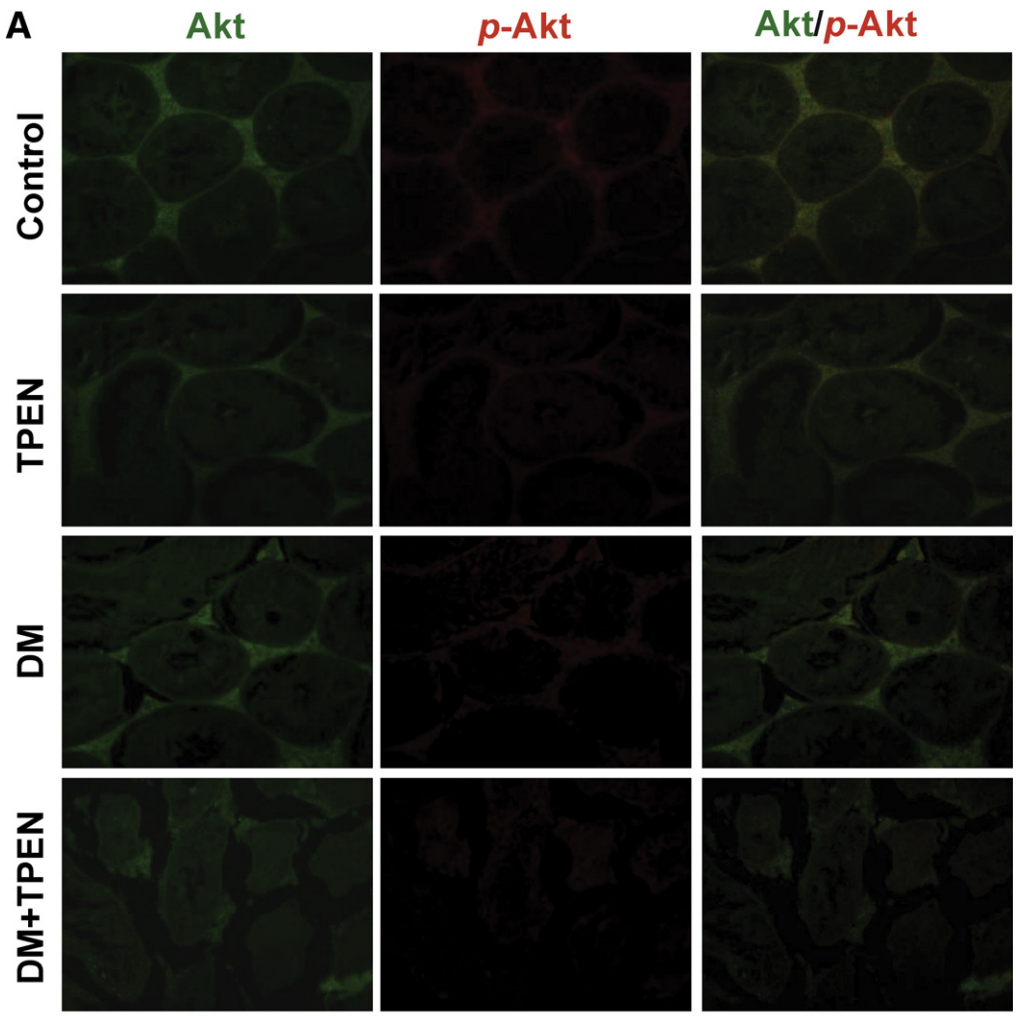
In the present study, we have demonstrated for the first time that diabetes significantly inhibited testicular Akt activation, along with a significant down-regulation of its downstream glucose-metabolism-related key mediators, GSK-3β and GS (Fig. 1). We have demonstrated the significant down-regulation of cardiac Akt function along with a decrease in GSK-3β phosphorylation and increases in GS phosphorylation, oxidative damage and cell death in STZ-induced diabetic mice that is the same as those used in the present study [24,49]. Akt plays an important role in the spermatogenesis and germ cell survival [21]. Reportedly, Akt protected testicular apoptotic cell death against radiation and chemical changes [50,51], and Akt1$^{-/-}$ mice significantly increased spontaneous apoptosis in the testis and attenuation of spermatogenesis [52]. To date, there has been no study to investigate its changes in the testis under diabetic conditions. We showed the significant down-regulation of Akt function along with decreases in Akt's downstream glucose-metabolism-related key mediators, GSK-3β phosphorylation and increased GS phosphorylation (Fig. 1). Zn deficiency can exacerbate diabetic effect on Akt-mediated signaling changes, which is in line with a recent study showing that chronic maternal Zn restriction altered the body composition and impaired the glucose-induced insulin secretion in the offspring [53]. Therefore, Zn deficiency has significantly exacerbated effect on diabetic induction of testicular cell death [18].

Impairment of glucose metabolism is often paralleled with an increased fatty acid metabolism as we have observed in the heart of diabetes [24]. Although a few early studies have demonstrated the increased metabolism of fatty acid in the testis of diabetes, there was no signaling components in these studies [54–56]. In the present study, we showed a significant up-regulation of fatty acid metabolism-related mediator PPAR-α in diabetic testis (Fig. 3A). Although AMPK also acts as a fatty acid oxidation stimulator, it was significantly decreased in the diabetic testis (Fig. 3B). In addition, we found that PGC-1α, which is an essential factor for the mitochondrial biogenesis and also a key mediator to maintain mitochondrial maximal and efficient fatty acid oxidation, was also significantly decreased in diabetic testis (Fig. 5B). These findings suggest that although testicular use of fatty acid is increased in the diabetic testis with an impairment of glucose metabolism, the efficacy of fatty acid oxidation is significantly decreased so that the incomplete fatty acid metabolisms lead to the accumulation of fatty acid metabolic intermediates that cause the testicular oxidative damage and cell death [18].

Zn deficiency did not significantly affect diabetic increase in PPAR-α expression (Fig. 2A) and decrease in AMPK activation (Fig. 3B), but significantly exacerbated diabetic down-regulation of PGC-1α expression (Fig. 5B). The latter, but not the former, is similar to the change profile of Akt function (Fig. 1A). This discrepancy of the profiles between PPAR-α and AMPK expressions suggests that changes of testicular PPAR-α expression and AMPK activation may not directly be mediated by Akt-mediated signaling, rather than secondary to the impairment of glucose metabolism, while PGC-1α expression may be directly related to Akt changes, as reported by others [57,58].

Consistent with the profile of PGC-1α, Sirt1, a member of the sirtuin family of proteins, was also significantly decreased in diabetic testis and further decreased in the testis of diabetic mice with Zn deficiency (Fig. 5A). Sirt1 has been extensively proven as one Akt down-stream signaling mediator that plays important physiological roles, including the regulation of glucose metabolism, cell survival and mitochondrial respiration [59,60]. Sirt1 deficiency markedly attenuates spermatogenesis [29]; therefore, Zn deficiency significantly exacerbated diabetic induction of testicular cells death since it exacerbated diabetic down-regulation of Akt function (Fig. 1A) and Sirt1 expression (Fig. 5A).

Regarding the mechanisms responsible for Zn down-regulation of Akt function, the present study provides the novel evidence that Zn deficiency significantly up-regulated Akt negative regulators, PTP1B and TRB3 expression and PTEN phosphorylation (Fig. 6). Previous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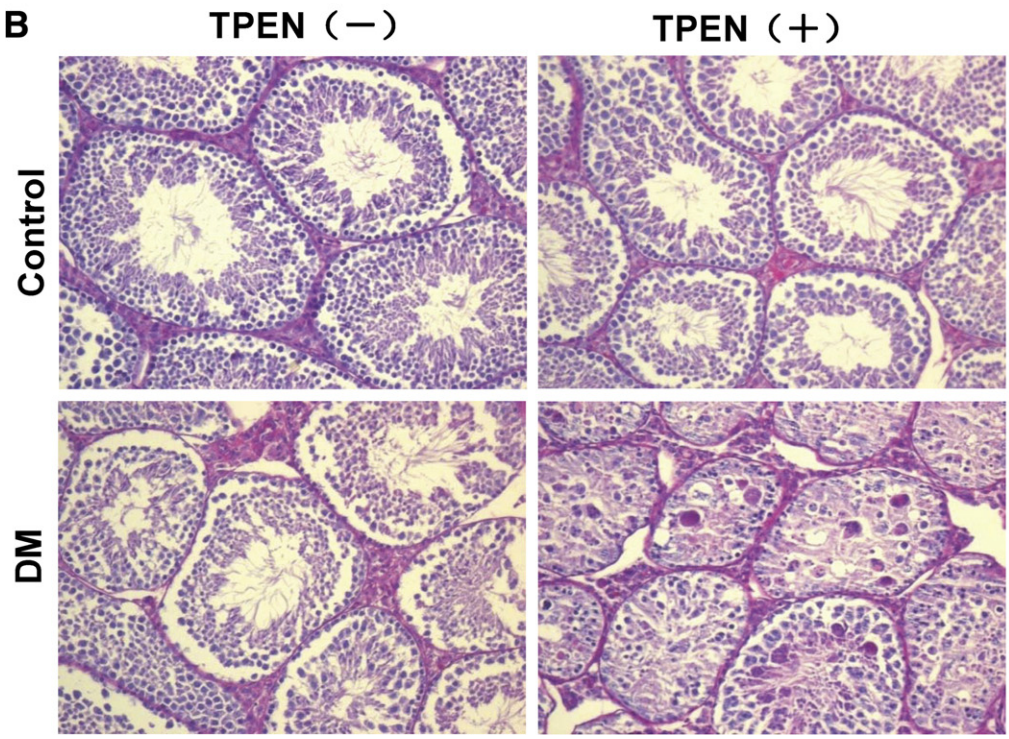
Akt
p-Akt
Akt/p-Akt
Control
TPEN
DM
DM+TPEN


B
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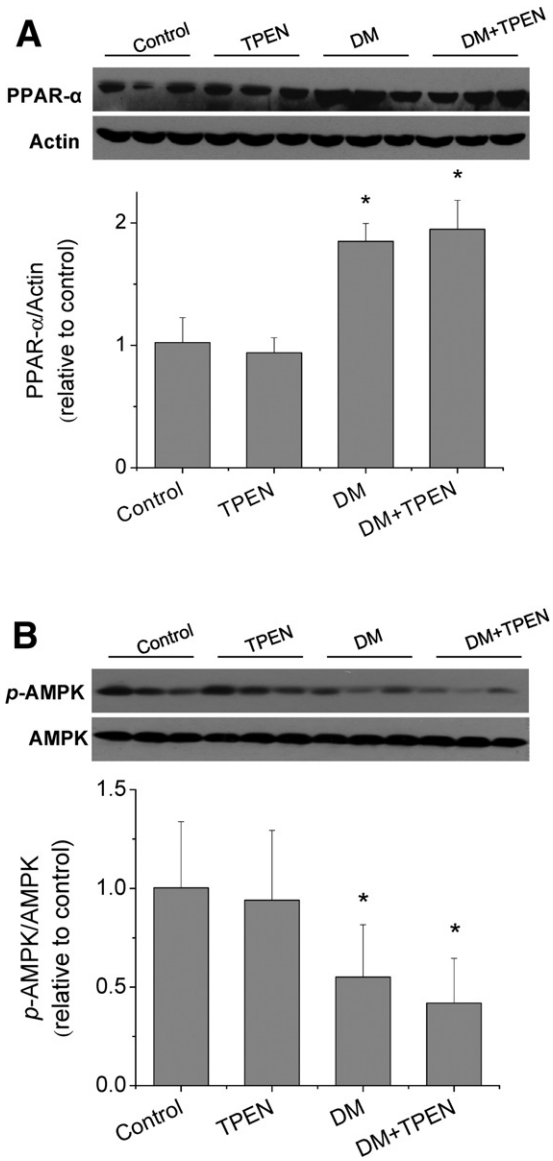

Fig. 3. Effects of diabetes and TPEN on PPARα expression and AMPK phosphorylation. Animal model and treatment are the same as described in Fig. 1. Testicular expressions of PPARα (A) and total or phosphorylated AMPK (t-AMPK or p-AMPK) were examined by Western blotting. Data are presented as mean ± S.D. (n=6 at least in each group). DM: diabetes. *P<.05 vs. control group; #P<.05 vs. DM group.

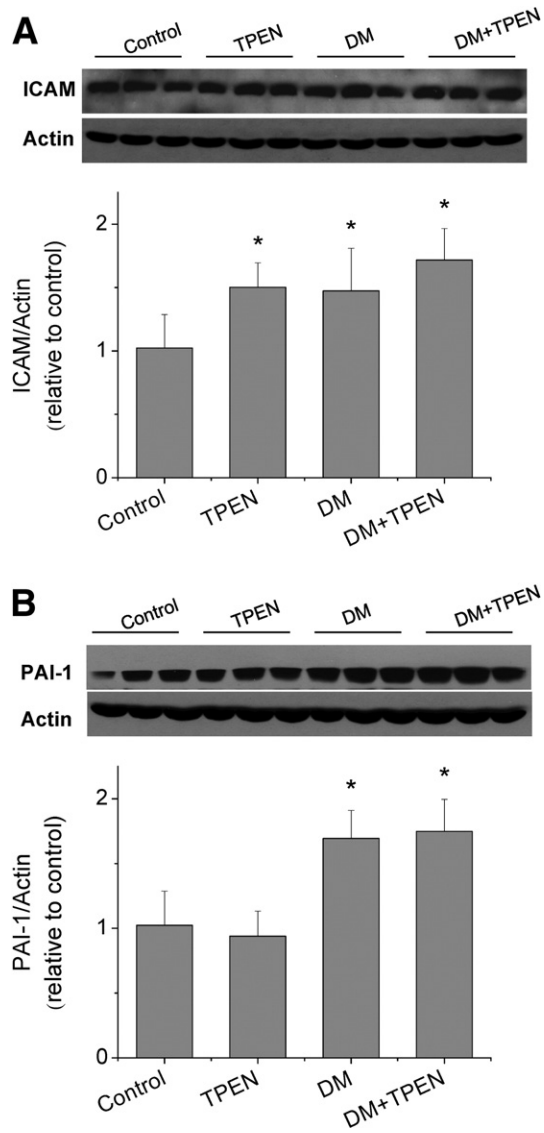

Fig. 4. Effects of diabetes and TPEN on testicular inflammation. Animal model and treatment are the same as described in Fig. 1. Testicular expressions of PAI-1 (A) and ICAM (B) were examined by Western blotting. Data are presented as mean ± S.D. (n=6 at least in each group). DM: diabetes. *P<.05 vs. control group; #P<.05 vs. DM group.

studies have clearly shown that up-regulation or down-regulation of these Akt negative regulators in response to certain stresses was able to cause either insulin resistance or insulin sensitization [41,42]. For instance, SIRT1 can improve insulin sensitivity under insulin-resistant conditions by repressing PTP1B [42]. In the present study, the effects of diabetes and Zn deficiency on Akt activation (Fig. 1A) are oppositely related to these regulators (Fig. 6), suggesting the potential role of Zn deficiency in up-regulation of these Akt negative regulators that cause further down-regulation of Akt function in diabetic mice. That the diabetic up-regulation of PTEN and PTP1B was further enhanced by Zn deficiency is most likely because Zn is a negative regulator of both PTEN and PTP1B [12–14,16]. For instance, Wu et al. have reported that exposure to Zn induces Akt activation since treatment with Zn resulted in a significant reduction in levels of PTEN protein in a dose- and time-dependent fashion. They also found that pretreatment of the cells with a proteasome inhibitor significantly blocked Zn-induced reduction of PTEN protein as well as the increase in Akt phosphorylation because Zn is able to induce ubiquitination of PTEN protein [13].

TRB3, a mammalian homolog of *Drosophila* tribbles, functions as a negative modulator of Akt. It is known that insulin promotes glucose uptake in muscle and suppresses glucose production in liver when glucose is available from food, for which Akt plays a pivotal role. TRB3

Fig. 2. Double immunofluorescent staining for Akt and phosphorylated Akt and pathological changes examined by PAS staining. (A) Akt was shown by green fluorescence with goat anti-rabbit IgG secondary antibody, and phospho-Akt was shown by red fluorescence with CY3-conjugated Affinipure Donkey anti-rabbit IgG(H+L). Nuclei were shown by blue fluorescence with DAPI. Both Akt and phosphorylated Akt were predominantly in Leydig cells and less in the spermatogonia (×200). (B) Animal model and treatment are the same as described in Fig. 1. The tissue sections processes for PAS-H staining and representative images from each group are shown (×200).

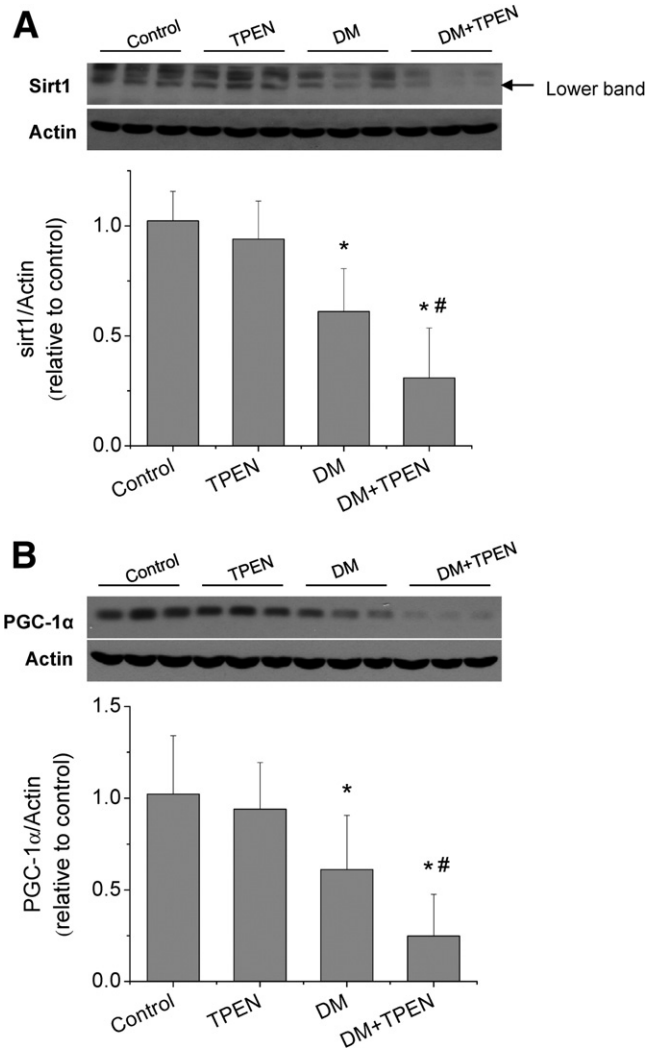

Fig. 5. Effects of diabetes and TPEN on Sirt1 and PGC-1α. Animal model and treatment are the same as described in Fig. 1. Testicular expressions of Sirt (A) and PGC-1α (B) were examined by Western blotting. Data are presented as mean ± S.D. (n=6 at least in each group). DM: diabetes. *P<.05 vs. control group; #P<.05 vs. DM group.

expression is induced in liver under fasting conditions and disrupts insulin signaling by binding directly to Akt for inhibiting its activation to allow hepatic generation of glucose [37–40]. Liu et al. reported that (1) skeletal muscle TRB3 protein levels are significantly elevated in type 2 diabetic patients; (2) skeletal muscle TRB3 protein levels are increased in STZ-diabetic rats, db/db mice and Zucker fatty rats; (3) TRB3 messenger RNA and protein levels are increased by high glucose concentrations, as well as by glucose deprivation in muscle cells; and (4) stable TRB3 hyperexpression in muscle cells blocks insulin-stimulated glucose transport and glucose transporter 4 translocation and impairs phosphorylation of Akt. These data identify TRB3 induction as a novel molecular mechanism in human insulin resistance and diabetes. TRB3 acts as a nutrient sensor and could mediate the component of insulin resistance attributable to hyperglycemia (i.e., glucose toxicity) in diabetes [39]. Knockdown of the gene encoding TRB3 improves insulin sensitivity in a rat model of insulin resistance [61]. Based on current knowledge, however, we do not know how Zn deficiency up-regulates TRB3 expression, which is a very interesting topic to be further investigated in future studies.

In summary, since Zn plays an important role in maintaining normal spermatogenesis, we have investigated the effect of Zn deficiency on diabetic changes of the testicular glucose and fatty acid

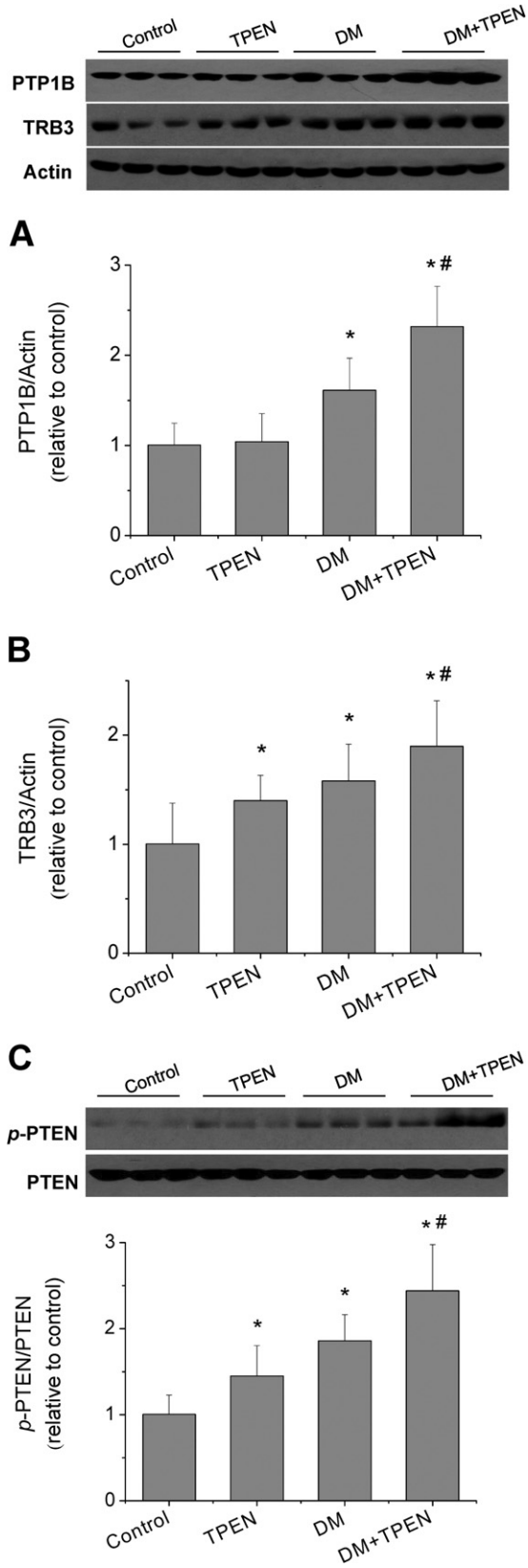

Fig. 6. Effects of diabetes and TPEN on Sirt1 and PGC-1α. Animal model and treatment are the same as described in Fig. 1. Testicular expressions of PTP1B (A) and TRB3 (B), and total and phosphorylated PTEN (t-PTEN, p-PTEN) were examined by Western blotting. Data are presented as mean ± S.D. (n=6 at least in each group). DM: diabetes. *P<.05 vs. control group; #P<.05 vs. DM group.

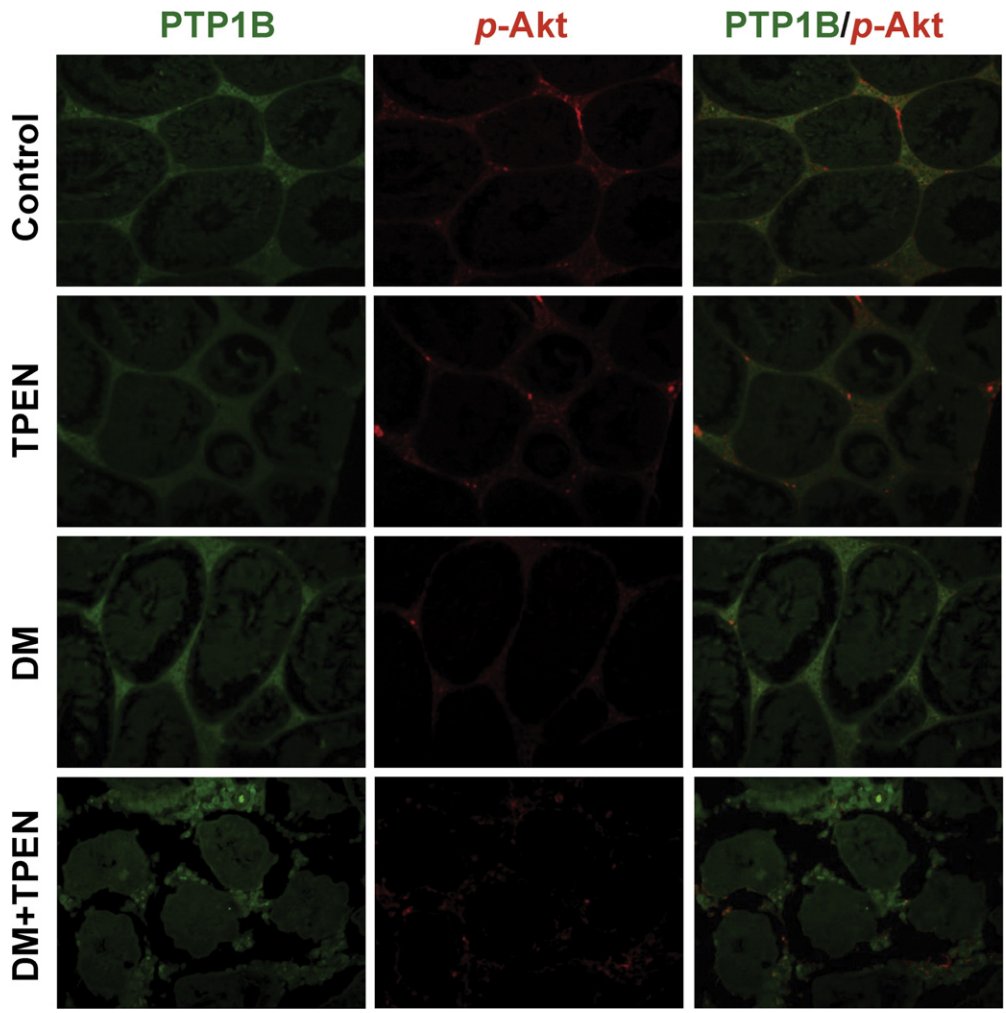

Fig. 7. Double immunofluorescent staining for PTP1B and phosphorylated Akt. PTP1B was probed with rabbit polyclonal anti-mouse IgG+IgM+IgA (FITC) secondary antibody (green fluorescence), and phosphorylated Akt was probed with CY3-conjugated Affinipure Donkey anti-rabbit IgG(H+L) (red fluorescence). Both PTP1B and phosphorylated Akt were mainly expressed in the Leydig cells (×200).

metabolism signalings in the present study. We demonstrated for the first time that diabetes down-regulates testicular Akt glucose metabolism signaling along with an increased, but inefficient, fatty acid metabolism (increased PPAR-α expression with decreased PGC-1α expression and AMPK function). This disarrangement of energy metabolisms may be the cause of diabetic induction of the testicular inflammation and apoptotic cell death. Zn deficiency exacerbated diabetic effects on Akt-mediated glucose metabolism, but not on testicular inflammation. For the possible mechanisms of the exacerbation of diabetic down-regulation of Akt glucose metabolism signaling by Zn deficiency, the increased expression of PTP1B and TRB3, and the phosphorylation of PTEN are most likely responsible since Zn deficiency may make the release of Akt negative regulators from their inhibitory status by Zn, leading to an increased inhibition of Akt function by these Akt negative regulators. This study highlights the importance of proper Zn intake for diabetic patients since it may prevent or delay diabetic complications, including reproductive system damage.

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