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Zinc Supplementation and Strength Exercise in Rats with Type 2 Diabetes: Akt and PTP1B Phosphorylation in Nonalcoholic Fatty Liver

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Abstract

Type 2 diabetes mellitus (T2D) is a metabolic disorder caused by chronic hyperglycemia due to a deficiency in the secretion and/or action of insulin. Zinc (Zn) supplementation and strength exercise increases insulin signaling. We evaluate the effect of Zn supplementation and strength exercise on insulin resistance in the liver of rats with diet-induced T2D through the study of phosphorylation of Akt and protein tyrosine phosphatase 1B (PTP1B). Rats were fed with a high-fat diet (HFD) for 18 weeks to induce T2D and then assigned in four experimental groups: HFD, HFD-Zn (Zn), HFD-strength exercise (Ex), and HFD-Zn/strength exercise (ZnEx) and treated during 12 weeks. Serum Zn, lipid profile, transaminases, glucose, and insulin were measured. In the liver with/without insulin stimuli, total and phosphorylated Akt ($pAkt^{Ser473}$) and PTP1B ($pPTP1B^{Ser50}$) were determined by western blot. Hepatic steatosis was evaluated by histological staining with red oil and intrahepatic triglyceride (IHTG) content. There were no differences in biochemical and body-related variables. The ZnEx group showed a higher level of pAkt, both with/without insulin. The ZnEx group also showed higher levels of pPTP1B with respect to HFD and Zn groups. The ZnEx group had higher levels of pPTP1B than groups treated with insulin. Liver histology showed a better integrity and less IHTG in Ex and ZnEx with respect to the HFD group. The Ex and ZnEx groups had lower IHTG with respect to the HFD group. Our results showed that Zn supplementation and strength exercise together improved insulin signaling and attenuated nonalcoholic liver disease in a T2D rat model.

Keywords Zinc · Exercise · Type 2 diabetes · NAFLD · Akt and PTP1B

Introduction

Type 2 diabetes mellitus (T2D) is a group of metabolic disorders characterized by chronic hyperglycemia due to defective or lack of insulin secretion, alteration of its action, or both [1]. T2D is characterized by a loss of sensitivity to insulin, triggering a resistance to the hormone, resulting in a decrease in insulin secretion and long-term beta cell failure [2, 3]. T2D is a risk factor for the development of nonalcoholic fatty liver disease (NAFLD) [4]. Approximately 70% of those with T2D also have NAFLD [5]. Patients with NAFLD have higher levels of lipolysis and gluconeogenesis (GNG) [6] making insulin resistance (IR) one of the main markers of NAFLD [7] and its progression to nonalcoholic steatohepatitis (NASH) [8]. Under IR condition, action of insulin in the liver is characterized by its inability to suppress gluconeogenesis but promotes lipogenesis contributing to the development of NAFLD [9].

Zinc (Zn) is a transition metal and cofactor of more than 300 enzymes, mainly associated with intermediate metabolism [10]. In the pancreas, Zn is necessary for the correct storage and release of insulin by β cells [11], which is reflected in subjects with T2D, who have reduced Zn content in their β cells [12]. Stimuli such as prolonged hyperglycemia, EGF or insulin, increase free Zn concentration. Also, Zn is redox inert in biology and cannot change the redox state of cysteine. However, reactive oxygen species (ROS) release Zn from metallothionein and other proteins, increasing free Zn concentrations. The insulin-mimetic effects of Zn are associated with an increased tyrosine phosphorylation, and it has

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been suggested that these effects are due to a direct interaction of Zn ions with proteins tyrosine phosphatase (PTPs) inhibiting its activity [13]. Numerous protein tyrosine phosphatase 1B (PTP1B) substrates have been identified as a key components in metabolic signaling including insulin, leptin, ER stress, cell–cell communication, energy balance, and vesicle trafficking, among others [14]. Protein tyrosine phosphatase 1B (PTP1B) is regulated by oxide reduction, proteolysis, and phosphorylation changes [15]. Zn binds to the catalytic pocket of the phospho-intermediate form of PTP1B. The regulation of PTP1B by cellular Zn and the localization of both, PTP1B and Zip7 on the ER membrane, strongly suggests physiological significance [15]. The regulation of PTP1B activity can be modulated by either oxidation or Zn.

On the one hand, PTP1B is oxidized, independently of physiological stimulation and substrate binding. On the other, Zn modulates PTP1B after physiological stimulation and substrate binding [16]. PTP1B catalyzes tyrosine dephosphorylation in the insulin receptor substrate-1 (IRS1) [17–19] and in type 1 insulin-like growth factor (IGF1) [20], affecting insulin signaling in early stages [21]. PTP1B contains the amino acid sequence RYRDVS that can be recognized by Akt protein (RXRXXS/T). This suggests that Akt could phosphorylate PTP1B (serine 50) inhibiting PTP1B activity [22].

Physical strength exercise (Ex) also has beneficial effects in the management of T2D, including increased translocation of GLUT-4 via AMPK [23], increased mitochondrial oxidation, improved antiinflammatory profile in adipose tissue of T2D [24] and increased Akt phosphorylation in serine 473 and threonine 308 [25, 26]. In liver, Ex attenuates IR through the inhibition of GNG enzymes [27], and partially tempering NAFLD in T2D [28, 29].

Our aim was to study the activity of phosphorylated PTP1B and AKT enzymes in the presence of Zn supplementation and/or strength exercise in rats with high-fat diet–induced diabetes.

Methods and Materials

Experimental Design

Fifty-two 14-week-old Wistar rats were used. Rats were fed with a high-fat diet (HFD) for 18 weeks to induce type 2 diabetes in animal models [30, 31]. Dietary intake by rats was evaluated by registering food was added daily and weighing remaining food. Since there were 4 rats/cage, amounts were divided by four to obtain intake/rat/day. Diabetes diagnosis was established with a blood glucose concentration of 250 mg/dL or above, after 120 min of intraperitoneal glucose administration as part of an intraperitoneal glucose tolerance test. Details are as follows: 2 g glucose (as 30% glucose solution)/kg body weight was infused intraperitoneally, and blood glucose was measured at 0 and 120 min from a blood drop of the tail using a glucometer (Abbott Diabetes Care Ltd., UK). Rats with blood glucose ≥ 250 mg/dL (13.9 mmol/L) at 120 min were considered diabetics, while those with blood glucose between 220 and 250 mg/dL were injected with a very low dose of streptozotocin (10 mg/kg body weight). After 4 days, rats injected with were streptozotocin subjected to another intraperitoneal glucose tolerance test. Only those with blood glucose ≥ 250 mg/dL were included in the next phase of the study: 32 of the 52 animals. Rats were distributed among all the experimental groups [32]. Rats were then randomly assigned to one of the following four groups: (a) high-fat diet (HFD); (b) HFD with supplemental zinc (Zn); (c) HFD with physical strength exercise (Ex), and (d) HFD with supplemental Zn and strength exercise (ZnEx). Treatments lasted 12 weeks.

The HFD was composed of 62.9% fat, 20.2% carbohydrates, and 16.9% protein; it contained 32.1 mg of Zn/kg. For the HFD plus Zn group, diet composition was similar except that the Zn concentration was 250 mg Zn/kg. The chemical form of Zn used for fortification was $ZnSO_4 * 7H_2O$,

Rats were subjected to a strength exercise protocol. This consisted of making the rats support their own weight on a rack with their rear and front legs. This procedure was carried out 3 times a week (Monday, Wednesday, and Friday) during 12 weeks. An initial evaluation was carried out prior to the start of treatment to determine the physical status of the rats, and was repeated at week 6 and week 12. As the training weeks progressed, extra weight was added to the rat's tails. This additional weight varied between 0 and 55% of body weight (first 2 weeks and last 2 weeks respectively). The strength exercise was performed under the following scheme: 3 or 4 sets (depending on the day of the week) of 2 repetitions each, in which each repetition lasted 45 seconds, with 30-s breaks between each repetition and 2 min between each series.

After 12 weeks of treatment with Zn and/or exercise, the rats were sacrificed, and a series of biochemical parameters were determined in plasma.

Determination of Biochemical Parameters in Plasma

After a 6-h fast, a cardiac puncture was performed for blood collection to measure glycated hemoglobin (HbA1c), lipid profile, transaminases, insulin, and glucose. For HbA1c quantification, a method of latex immune agglutination was used, and readings were made using Siemens D 2000+ equipment. For glucose, lipid profile and transaminases Promedar DiaLab kits were used (Wiener Neudorf, Austria): D08220 for glucose, D95116 for cholesterol, D00127 for HDL-cholesterol, D00389 for TG, D98624 for GPT, and D98616 for GOT. The measurement of insulin in blood was performed through an enzyme-linked immunosorbent assay (ELISA) (Alpco, Salem, NH, USA. 80-INSRTU-E01). HOMA IR index was calculated using

the following formula: ((glycemia (mg/dL) * Insulinemia (μUI/ml))/405). Serum zinc was determined by flame atomic absorption spectrometry in a Perkin-Elmer Analyst 100 spectrophotometer (Perkin-Elmer Corp., Waltham, MA)

After sacrifice, the liver was extracted and subdivided into 4 samples of similar size. Ex vivo insulin stimulation was performed with a Krebs-Ringer Buffer with 100 nM insulin for 10 min as described in other studies [33–36]. Then, protease and phosphatase inhibitors were added. The second sample was incubated directly with protease and phosphatase inhibitors. Both samples were frozen at − 80 ° C. The third sample was used for intrahepatic triglycerides (IHTG) quantification, and the fourth sample was treated with isopentane to staining neutral lipid with oil red.

Immunodetection of Insulin Signaling Proteins by Western Blot

The antibodies used were total PTP1B (R&D systems RD, catalog No. AF13661-SP), $pPTP1B^{Ser50}$ (Abbexa, catalog No. abx011885), total Akt (Cell signaling, catalog No. 4691S), and $pAkt^{Ser473}$ (Cell signaling, catalog No. 927S). For protein extraction, the tissue was homogenized, treated with RIPA lysis buffer and phosphatase inhibitors (Sigma-Aldrich, catalog No. 4906837001) and proteases inhibitors (Sigma-Aldrich, catalog No. 4693159001). The mixture was diluted with lysis buffer and centrifuged for 15 min at 12,000×*g* at 4 ° C. Protein concentration was determined by the Lowry method [37]. For western blot [38], 10% acrylamide gels were used to perform the SDS-PAGE. Protein extract (50 mg) was loaded in each lane. Electrophoresis was performed with a power source at 90 mV for 2.5 h and then transfer the proteins to a nitrocellulose membrane (Bio-rad Catalog 1620112). Then, the membrane was incubated with 3% bovine serum albumin for 1 h (membrane blocking) and incubated with the primary antibody overnight at 4 ° C. Then, the membrane was incubated with secondary antibody for 1 h at room temperature. Chemiluminescence reaction (ThermoFisher catalog 34095) was used for detection. Detection and quantification of bands were performed in C-DiGit® Blot Scanner and Image Studio™ Lite (LI-COR Biosciences) respectively.

Oil Red Staining for Intrahepatic Triglycerides

After the liver was removed, they were immersed for 15 s in isopentane (2-methylbutane, Sigma-Aldrich, catalog No. 10537370) to avoid crystal formation during tissue freezing and thus maintain their structure. Subsequently, the samples were adhered to cork paper with optimal cutting temperature (Fisher HealthCare™) to be cut in a Cryostat (Leica, Biosystems, Nussloch, Germany) using a thickness of 5 μm at a temperature between − 20 and − 30 °C for control and fatty livers, respectively. The tissue cuts in the histological plate were hydrated with distilled water and treated with 60% isopropanol, and then subjected to Oil Red staining (Merck, Darmstadt, Germany, catalog No. 105230) for 1 min. Later, the tissue was washed with 60% isopropanol and distilled water, and the cell nuclei were stained with Mayer's hematoxylin for 1 min and then treated with a high ion solution (running water) for 2 min. The images were observed in a light microscope (× 40, CX22 Olympus, Tokyo, Japan), photographed and integrated with a Toup View software (ToupTek Photonics, Zhejiang, China). Image analyses were performed using Image J software version 1.51s (Java, National Institutes of Health).

Intrahepatic Triglycerides Quantification

To quantify triglycerides in liver tissue, 100 mg of liver was mechanically homogenized with 1 mL of chloroform-methanol solution (2:1). The solution was centrifuged at 1300×*g* for 10 min at room temperature, and the organic phase was isolated. Intrahepatic triglycerides were quantified with a TG Color GPO/PAP AA kit (Wiener Lab, catalog No. 1780105), following manufacturer instructions. Results were expressed as mg of TG/g of tissue.

Statistical Analysis

To calculate the sample size, we considered a level of significance equal to 0.05 ($\alpha = 0.05$), a power of 80%, a SD of 5, and a difference of 12% in the phosphorylation of PTP1B enzyme between the HFD group and Zn group. A number of 5 was obtained, although the final sample size considered one or two additional rats per group, to account for potential dropout. To verify the assumption of normality, the Shapiro-Wilk test was used, and for the assumption of variances homogeneity, the Bartlett test was applied. Values with normal distributions were expressed as mean ± SD, and those with a nonnormal distribution were expressed as median and interquartile range. For statistical analysis between groups, one-way ANOVA with Dunnett's post hoc test was used if the distribution of the response variable was normal, and Kruskal–Wallis with Dunn's post hoc test if the distribution of the response variable was not normal. A significant difference was considered with a p value of less than 0.05. GraphPad Prism 6.0 for Windows (GraphPad Software, Inc.) was used for the statistical analysis.

Results

Evolution of Body Weight During the Study

Body weight (g) at the start of treatment with HFD (14 weeks old) and at the start (32 weeks old) and end (44 weeks old) of

protocol (Zn supplementation and/or exercise) was greater than at the beginning of the experiment in all groups (Fig. 1a). Body weights (g) at end of protocol (sacrifice) were HFD = 680 ± 135, Zn = 654 ± 97, Ex = 629 ± 211, and ZnEx = 652 ± 200 (one-way ANOVA, NS). Liver weights (g) at sacrifice were HFD = 16.1 ± 4.9, Zn = 15.4 ± 2.2, Ex = 17.3 ± 7.3, and ZnEx = 17.1 ± 5.1 (1-way ANOVA, NS) (Fig. 1b). Food intake (g/day) in HFD group was 31.3 ± 18.6, Zn group 31.6 ± 20.9, Ex group 32.1 ± 7.1, and ZnEx group 32.6 ± 36.6. No significant differences between groups were observed (Fig. 1c).

Biochemical Parameters at the End of the Study

Levels of biochemical parameters at the end of the study are shown in Table 1. No differences were observed in any of these parameters. Intraperitoneal glucose tolerance test (mg/dL): at minute 60 and 120 were higher than at minute 0 in all groups ($p < 0.0001$), without differences between groups (two-way ANOVA; $p = 0.526$), data not shown.

Effect of Zn and/or Exercise on the Metabolic Signaling of Insulin: Akt Phosphorylation

Akt phosphorylation in the liver of rats with no insulin treatment (ratio between pAkt/total Akt) (Fig. 2b) was 0.01 ± 0.28 in the HFD group, 2.29 ± 1.49 in the Zn group, 1.94 ± 1.28 in the Ex group, and 8.40 ± 1.96 in the ZnEx group. pAkt was higher in the ZnEx group compared with other groups and was significantly different than the HDF group (Kruskal–Wallis test, Dunn's post hoc test, $p < 0.001$). Akt phosphorylation in liver with insulin-treatment was 1.06 ± 0.67 in the HFD + ins group; 3.63 ± 1.36 in the Zn + ins group; 2.05 ± 1.26 in the Ex+ins group; and 15.76 ± 1.48 in the ZnEx+ins group. Also, the ZnEx+ins group had higher values of pAkt compared with all other groups and was significantly different than the HDF + ins group (Kruskal–Wallis test, Dunn's post hoc test, $p < 0{,}001$) (Fig. 2b).

Effect of Zn and Exercise on the Metabolic Signaling of Insulin: PTP1B Phosphorylation

PTP1B phosphorylation with no insulin stimuli (ratio between p-PTB1B/total PTP1B) (Fig. 3a) was 1.06 ± 0.62 in the HFD group; 2.72 ± 0.61 in the Zn group; 1.47 ± 0.80 in the Ex group and 2.33 ± 0.46 in the ZnEx group. The ZnEx group had the highest levels of pPTP1B compared with all the other groups in liver without insulin treatment. There was a significant difference between the HDF group with Zn and the ZnEx group (one-way ANOVA, $p < 0.001$). pPTP1B in livers treated with insulin was 0.61 ± 0.55 in the HFD + ins group; 2.12 ± 0.79 in the Zn + ins group; 1.50 ± 1.27 in the Ex+ins group and 3.46 ± 0.51 in the ZnEx+ins group. The ZnEx+ins group had the highest levels of pPTP1B compared with all other groups

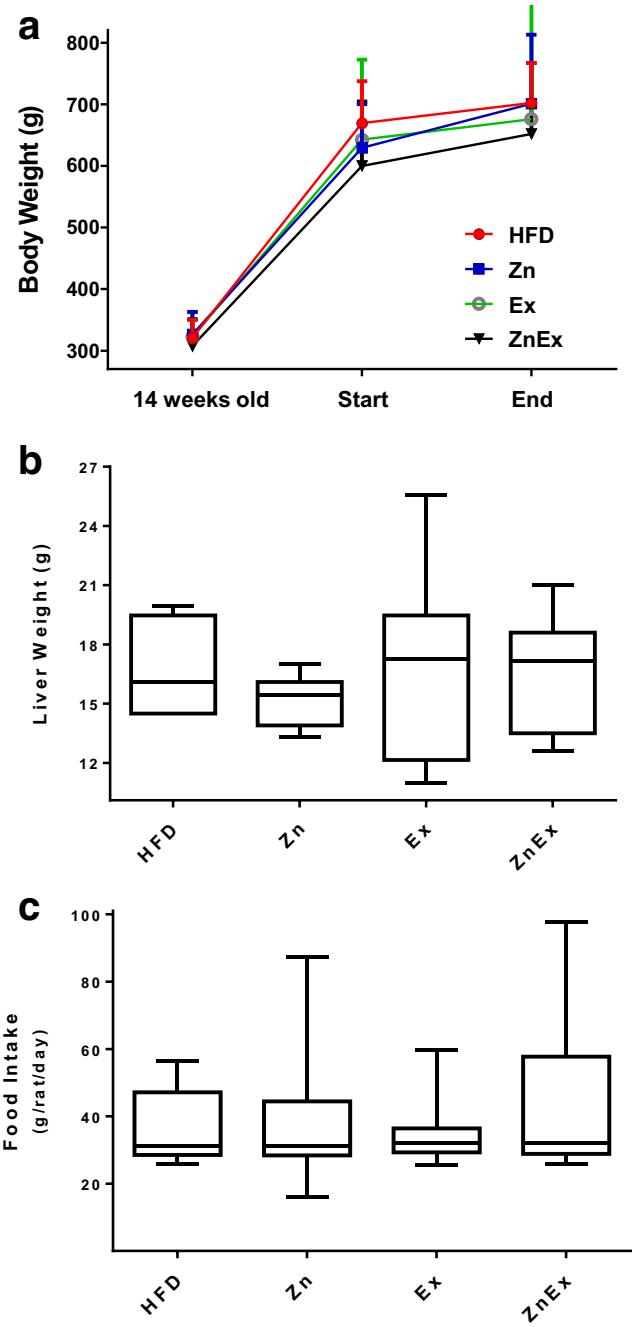

Fig. 1 Body and liver weight and food intake. **a** Body weight was measured at the beginning of treatment with HFD and at the start (Zn supplementation and/or exercise) and end of the study. There was a significant effect of time (**** $p < 0.001$), no effect of diet ($p < 0.526$), or interaction ($p < 0.288$) (2-way ANOVA). Values are expressed as mean ± SD ($n = 8$ per group). **b** Liver weight at sacrifice. There was no significant difference between groups (Kruskal–Wallis test; $p = 0.5480$). Values expressed as median ± interquartile range ($n = 8$ per group). **c** Food intake per day at sacrifice. There was no significant difference between groups (Kruskal–Wallis, Dunn's post hoc test test; $p = 0.526$). Values expressed as median ± interquartile range ($n = 8$ per group)

(Fig. 3b). There was a significant difference between the HDF group compared with the Zn, Ex, and ZnEx groups (one-way ANOVA, $p < 0.001$; < 0.05; < 0.001, respectively).

Table 1 Biochemical parameters in the studied groups

	HFD	HFD-Zn	HFD-Ex	HFD-Zn-Ex	*p* value
Glycemia (mg/dl)	279 ± 65	347 ± 76	315 ± 61	325 ± 49	0.176*
Insulin (ng/mL)	0.14 (0.09–0.17)	0.11 (0.09–0.17)	0.14 (0.01–0.18)	0.11 (0.09–0.14)	0.582
HOMA-IR	2.54 (1.55–4.32)	2.79 (2.37–3.74)	2.84 (2.13–4.07)	2.55 (2.07–2.99)	0.758
HbA1C (%)	4.01 (3.90–8.21)	4.05 (3.75–4.38)	3.95 (3.70–4.35)	3.85 (3.78–3.90)	0.373
Cholesterol (mg/dL)	51.8 ± 4.5	59.7 ± 11.3	59.3 ± 7.3	60.6 ± 5.2	0.257*
HDL-cholesterol (mg/dL)	16.1 ± 0.9	17.3 ± 3.7	19.9 ± 2.9	17.9 ± 3.5	0.193*
LDL-cholesterol (mg/dL)	27.5 ± 6.2	27.9 ± 4.7	28.3 ± 4.0	30.5 ± 1.2	0.579*
Triglycerides (mg/dL)	77.2 ± 17.2	94.0 ± 46.7	82.4 ± 25.4	105.6 ± 41.0	0.469*
GOT (IU/L)	76.6 ± 21.1	74.0 ± 13.5	97.4 ± 26.9	83.9 ± 25.6	0.356*
GPT (IU/L)	28.4 ± 11.8	23.3 ± 3.8	39.0 ± 12.9	40.7 ± 12.9	0.059*
Zinc (μg/dL)	128.8 ± 13.1	130.2 ± 17.5	131.9 ± 15.3	146.6 ± 23.4	0.243*

GPT glutamic pyruvate transaminase, *GOT* glutamic oxaloacetic transaminase

Values are expressed as mean ± SD or median ± interquartile range. *p* value: *1-way ANOVA, with Tukey's post hoc test or Kruskal–Wallis test, with Dunn's post hoc test

Effect of Zn and Exercise in Nonalcoholic Fatty Liver Disease

Liver morphology at sacrifice showed that liver samples from the HFD group were larger than samples obtained for comparative purposes from a rat fed a regular diet, identified as DN (Fig. 4a). A more pronounced paleness in the livers of the HFD group was observed with respect to the Ex and ZnEx groups (Fig. 4a). Neutral lipid staining with Oil Red in liver showed that the HFD group had a higher lipid staining and tissue damage compared with the Ex and ZnEx group (Fig. 4b). The quantification of IHTG (g/L per g of tissue) was 18.6 ± 5.5 in the HFD group; 7.9 ± 5.1 in the Zn group; 5.8 ± 2.3 in the Ex group; and 6.7 ± 4.3 in the ZnEx group. The Ex and

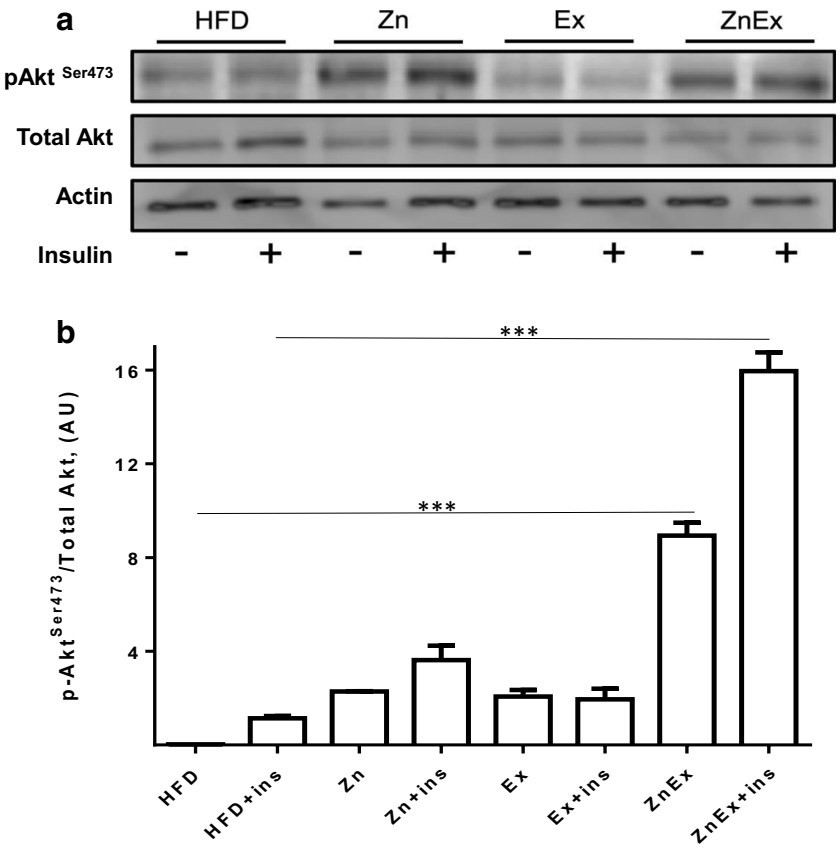

Fig. 2 Insulin signaling phosphorylation assay. **a** Representative image of Akt phosphorylation: pAkt^{Ser473} and total Akt with or without insulin. **b** Quantification of data showed in **a**. Kruskal–Wallis test, Dunn's post hoc test. *** $p < 0.001$. Values expressed as median ± interquartile range ($n = 4–6$)

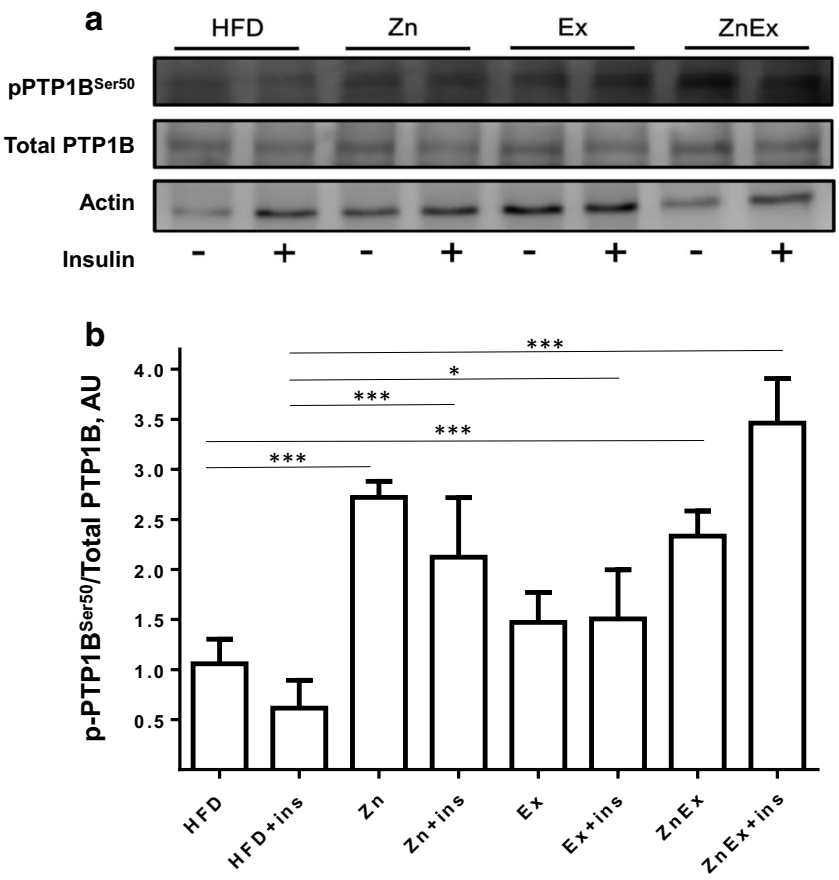

Fig. 3 Insulin signaling phosphorylation assay. **a** Representative image of PTP1B phosphorylation. $pPTP1B^{Ser50}$ and total PTP1B with or without insulin. **b** Quantification of data showed in **a**. Kruskal–Wallis test, Dunn's post hoc test. * $p < 0.05$; *** $p < 0.001$. Values expressed as median ± interquartile range ($n = 4–6$)

ZnEx groups had lower levels of intrahepatic triglycerides compared with the HFD group (one-way ANOVA, $p < 0.001$) (Fig. 4c).

Discussion

Since it has been suggested that both Zn and exercise could have effects on insulin signaling in target tissues and also in liver metabolism, we proposed that both Zn and physical strength exercise would increase the phosphorylation of Akt protein in its activating residue, serine 473 [26, 39], contributing to the metabolic activity of insulin in very early stages [23]. Since PP2A modulation is prior to Akt activation [40], PP2A could inhibit Akt activation [41]. This suggests that zinc, by being able to bind and inhibit PP2A [42], could indirectly increase Akt activity. In addition, strength exercise would improve the anti-inflammatory profile as well as the insulin signaling cascade [24], specifically contributing to the increase of Akt phosphorylation in serine 473 and threonine 308 [11, 25] residues, leading to an increase in Akt activity post exercise as has been observed by others [43]. This, in addition to the effect of lower hepatic glucose synthesis by inhibition of key gluconeogenesis (GNG) enzymes in a state of IR [28], contributes, in part, to the reduction of NAFLD in these patients [29, 30]. We proposed that combined the effect would be synergic.

Although the plasma concentration of zinc is the most widely used biomarker, it is normally a biomarker that is used after supplementation of individuals with prior metal deficiency. It is a biomarker with limitations, since they are affected during inflammation and feeding, and its diurnal variation can range around 20%. Hypoalbuminemia decreases plasma zinc concentration and hemolysis of samples can increase plasma zinc concentration (erythrocytes contain 10 times more zinc than plasma) [44]; therefore, the observed values may not reflect the actual nutritional zinc status in these rats. Therefore, to approximate the nutritional status of zinc, it is suggested to complement the evaluation by ideally measuring the rapidly exchangeable zinc pool or zinc content in metallothioneins [45].

Zinc and exercise also have an antidiabetic effect, through the improvement of glycemic control due to increased muscle mass (increased glycogen storage) and translocation of Glut 4 via AMPK, as well as, increased mitochondrial oxidation in muscle in T2D [23, 24]. Thus, these treatments have also been associated with increased AMPK enzyme activity [26, 46]. Regarding insulin cell signaling assays in the liver, our results showed that Zn, along with strength exercise, increased the phosphorylation of Akt and this effect was exacerbated with

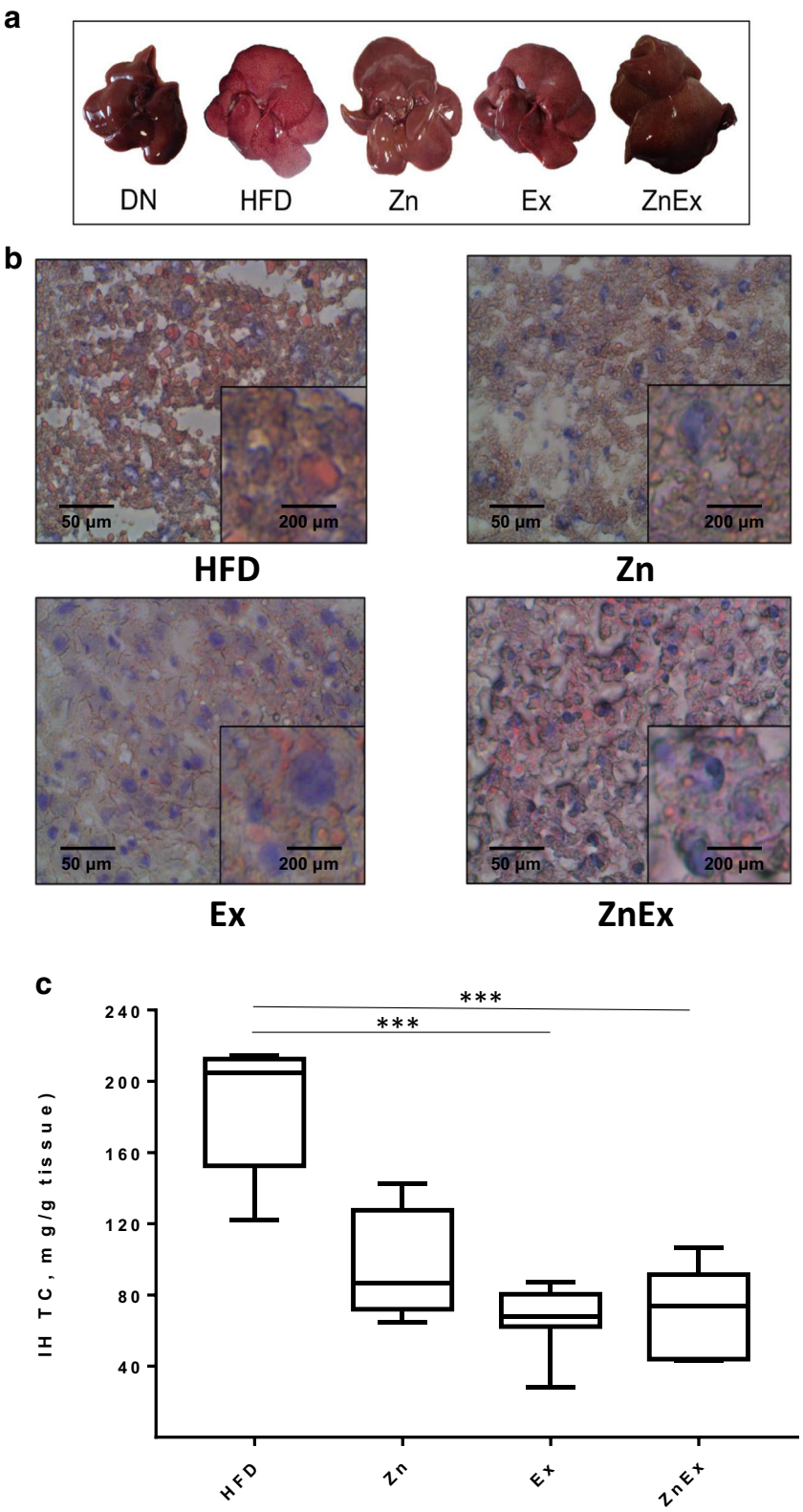

Fig. 4 Nonalcoholic fatty liver disease. **a** Representative images of the livers by group. **b** Histological staining of liver lipids with Oil Red and × 40 hematoxylin (representative images by group). **c** Intrahepatic triglycerides. Kruskal–Wallis test, Dunn's post hoc test. *** $p < 0.001$. Values expressed as median ± interquartile range ($n = 5–8$)

insulin stimulation. Despite the fact that this was an expected finding considering the mimetic insulin-like effect of Zn [40] and Ex [25], as observed previously in aerobic exercise in skeletal muscle and adipose tissue, we demonstrated that strength exercise and Zn increased Akt phosphorylation in liver tissue (Fig. 2).

In our protocol, PTP1B showed increased phosphorylation in its inactivating residue in the presence of Zn and Ex

treatments, suggesting a potential effect of these treatments in decreasing the activity of PTP1B. Bellomo et al. [15], using an optimized enzymatic assay and three dimensional analysis, showed an inhibition of PTP1B activity as a function of Zn concentration, specifically structural changes associated with kinetic alteration of the enzyme, without describing changes in the phosphorylation of the protein (Fig. 3). A decreased activity of the PTP1B enzyme attenuates IR of liver and also decreases the severity of NAFLD. This effect would be mediated in part, by an inactivation of the enzyme PTP1B, as a result of phosphorylation in the serine 50. This result suggests that an inhibition in PTP1B activity along with an increase in Akt activity, due to the effect of Zn levels and/or strength exercise, may improve IR. Although zinc has previously been described to stimulate both glucose uptake through GLUT4 translocation to the membrane surface, and downstream insulin signaling, specifically increasing the activity of Akt, GSK3beta, and S6K1 [47], and that zinc would negatively regulate the activity of PTP1B through its binding in all conformations of the enzyme: open, closed, and covalent [48], the interest of this work was to investigate the possible inhibition of PTP1B via phosphorylation of Akt (negative feedback) considering that PTP1B contains an amino acid sequence recognized by Akt [22] and, therefore, elucidate a possible phosphorylation-mediated inhibition in an in vivo model of type II diabetes.

IHTG levels were significantly lower in the Ex and ZnEx groups (Fig. 4). Exercise alone or in conjunction with Zn attenuated NAFLD development, lowering levels of IHTG [49, 50], which is consistent with the results of Hashida et al. [29] and van der Windt et al. [51]. Our findings were independent of weight loss as also reported by Hallsworth et al. [52]. It should be noted that strength exercise, unlike aerobic exercise, ameliorates NAFLD with lower energy consumption. Therefore, this type of exercise would be more beneficial in patients with less cardiorespiratory capacity [29], as is the case with T2D, whose incidence is closely associated with a deteriorated cardiorespiratory capacity [53]. The studied groups did not present NASH (normal transaminase levels, Table 1). The evolution from NAFLD to NASH involves two stages. The accumulation of liver fat, which makes the liver more susceptible to damage and the production of oxidative stress that induces damage and inflammation in the liver [54] In our study, the 14-week high saturated fat diet in Wistar rats generated increased peripheral fat but no development of NAFLD or NASH [55].

$pPTP1B^{Ser50}$ levels were negatively associated with IHTG levels. This result is consistent with the multiple roles of this phosphatase in the modulation of the proliferation and survival of hepatocytes, liver lipogenesis, and liver diseases (e.g., NAFLD, drug-mediated disorders, and hepatocellular carcinoma) [56].

In sum, our results showed that although plasma and body-related variables did not differ among groups, liver insulin signaling activity increased and NAFLD improved in T2D rats. This is related to, in part, the increased phosphorylation of Akt and an apparent inactivation of PTP1B. Thus, our findings demonstrate a benefit of Zn and strength exercise treatments for liver tissue, reflected in IR attenuation.

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Authors' Contributions AV, MRu, and MAO conceptualized and designed the research. AV, KM, AE, JC, JI, and KV performed the experiments. AV, MRi, and MAO analyzed the data. AV, MRi, MRu, and MAO interpreted the results of experiments. AV, MRi, and MAO prepared the figures. AV and MA drafted the manuscript. MRu and MAO edited and revised the manuscript. MAO and MRu approved the final version of manuscript.

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Compliance with Ethical Standards

Conflict of Interest Manuel Ruz, Jorge Inostroza, Diego García, and Miguel Arredondo received payment from the research project FONDECYT 1160792 that funded this study. The rest of authors declare that they have no conflicts of interest.

Ethical Approval Animal experiments were performed in accordance with animal protection regulations of the Faculty of Medicine, University of Chile. The protocol was approved by the Bioethics Committee, Faculty of Medicine, University of Chile.

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