Biological Trace Element Research
https://doi.org/10.1007/s12011-020-02348-9

Zinc Modulates the Response to Apoptosis in an In Vitro Model with High Glucose and Inflammatory Stimuli in C2C12 Cells

Mónica Andrews-Guzmán[1] · Manuel Ruz[2] · Miguel Arredondo-Olguín[1]

Received: 9 April 2020 / Accepted: 17 August 2020

Abstract

Apoptosis is programmed cell death and its alteration is related to cancer, neurologic, autoimmune, and chronic diseases. A number of factors can affect this process. The aim of this paper is to study the effect of supplemental zinc on apoptosis-related genes in C2C12 myoblast cells after being challenged with a series of stimuli, such as high glucose, insulin, and an inflammatory agent. C2C12 myoblast cells were cultured for 24 h with zinc (Zn) ($ZnSO_4$) 10 or 100 μM and/or glucose 10 or 30 mM. In addition to these stimuli, the cells were challenged with insulin 1 nM or interleukin-6 (IL-6) 5 nM. The mRNA expression of proapoptotic genes caspase 3 and Fas, the antiapoptotic genes, Xiap and Bcl-xL and the ratio of pro-/antiapoptotic genes Bax/Bcl-2, were determined by qRT-PCR. The expression of caspase-3 gene was significantly increased in the presence of the combination high Zn/high glucose with and without the presence of insulin and IL6 in the culture medium Fas expression instead, showed uneven responses. The expression of Bcl-xL and Xiap was increased in most conditions by having high Zn in the medium regardless of the presence of insulin or IL6. Bax/Bcl2 ratio was decreased in the presence of high Zn. Zn was able to stimulate the expression of antiapoptotic genes. This effect was specially noted in high-glucose conditions with and without the presence of insulin. This effect is partially overridden by the presence of an inflammatory agent such as IL-6.

Keywords Zinc · Apoptosis · Bax/Bcl2 ratio · Caspase-3 · Fas

Abbreviations

DMT1	Divalent metal transporter 1
ZnT	Zinc transporter
FBS	Fetal bovine serum
qRT-PCR	Quantitative reverse transcription–polymerase chain reaction
B2M	Beta-2 microglobulin
ANOVA	Analysis of variance
SEM	Standard error of the mean

Introduction

There is a series of conditions that can lead to inflammation and oxidative stress, such as high circulating glucose [1] and saturated fatty acids [2]. These, along with iron [3] or copper [4], could trigger a number of additional adverse effects. In addition, decreased insulin production in the pancreas and increased inflammation in liver and muscle are able to induce alterations in glucose uptake, glucose output, and increased apoptosis in these tissues [5]. Zinc (Zn) is an essential micronutrient that plays a role in processes like DNA/RNA synthesis, cell division, and apoptosis [6]. Zn deficiency has been related to chronic diseases such as cancer [7], liver disease [8], bowel disease [9], impaired function of the immune system [10], and type 2 diabetes [11, 12]. Zn is a trace element that has antiinflammatory activity [13], also decreases oxidative stress damage [14], and it has insulin mimetic activity [6].

Apoptosis process involves cell changes that are implicated in a number of pathological conditions [15]. Once apoptotic stimuli are sensed, the extrinsic and intrinsic pathways induce caspase activation and apoptosis is carried out. Caspase dependent apoptosis can also be activated via an intrinsic pathway. In this situation, the apoptosis stimuli induce cytochrome

✉ Miguel Arredondo-Olguín
marredon@inta.uchile.cl

[1] Micronutrient Laboratory, Institute of Nutrition and Food Technology, University of Chile, El Líbano 5524, Macul, Santiago, Chile

[2] Department of Nutrition, Faculty of Medicine, University of Chile, Avenida Independencia 1027, Independencia, Santiago, Chile

Published online: 25 August 2020

c release from mitochondria, activating major executioner caspases, including caspase 3 [16]. Bcl-2 family members such as the anti-apoptotic Bcl2/Bcl-xL and the proapoptotic Bax, can orchestrate the process of cell death. The ratio between pro and antiapoptotic molecules helps, in part, to determine the susceptibility to cells to a death signal [17, 18].

Increased blood glucose can trigger cell apoptosis [2]. Glucose can activate the pathways that end in cell death [19–21] and also decrease antiapoptotic factors [22]. In the apoptosis extrinsic pathway glucose activates Fas, once the cell death receptor is activated, recruit adaptor proteins, which in turn recruit procascase-8 into a proapoptotic complex. This cascade ends in the activation of caspase 3 and cell death [23].

Inflammation is a strong stimulus to apoptosis. TNF-α, IL-6, and IL-1β are inflammatory molecules and are primarily responsible for insulin resistance during obesity and their increase is the main risk factor for type 2 diabetes development [22]. The activation of NF-κB and JNK induces oxidative stress through NADPH oxidase, which activates the intrinsic pathway of apoptosis [24].

Zn could be protective to apoptosis [25], is able to suppress caspase-3 activity and decreased apoptosis in vivo has been reported [26]. The antiapoptotic mechanisms of Zn are related to its role in oxidative stress. Zn can limit the damage induced by oxyradicals and thereby can suppress the signaling pathways leading to caspase activation and, in turn, apoptosis [25]. Zn is a cellular regulator of caspase-3 activation and is colocalized with the zymogen form of caspase-3 in the apical cytoplasm of sheep and human airway epithelial cells [27]. This study aims to show that Zn modulates the response to apoptosis in myoblast challenged with high-glucose concentration and/or zinc and inflammatory stimulus with IL-6 for 24 h.

Material and Methods

The cell line C2C12 (ATCC CRL 1772, Virginia, USA) was used in all experiments. Cells were cultivated in Dulbecco's Modified Eagle Medium (DMEM) (Gibco, ThermoFisher, Massachusetts, USA), 10% fetal bovine serum (FBS) and 10 IU/mL penicillin and streptomycin (Invitrogen, ThermoFisher, Massachusetts, USA). Basal glucose and zinc content were 25 mM and 5 μM, respectively. Cells were cultured with low or high-glucose concentration, normal or high Zn concentration, and presence or absence of inflammatory stimuli. Cells were maintained at standard temperature and CO_2. C2C12 cells were challenged for 24 h with: Zinc (Zn) ($ZnSO_4$) 10 or 100 μM and/or glucose 10 or 30 mM. In addition to these stimuli, the cells were challenged with insulin 1 nM or interleukin-6 (IL-6) 5 nM. The mRNA relative abundance of Bax, Bcl-2, Caspase 3, Xiap, Bcl-xL, and Fas were determined by qRT-PCR. The experiments were made in triplicate, and they were repeated at least three times.

qRT-PCR of Gene Related to Apoptosis and Inflammation

RNA from C2C12 cells was extracted using Trizol reagent according to product protocol (Invitrogen, ThermoFisher, Massachusetts, USA). The extracted total RNA was treated with RNase-Free DNase Set (Qiagen, Düsseldorf, Germany) according to manufacturer's instructions. Total RNA concentration was measured by absorption at 260 nm. RNA purity and concentration were checked by determining the OD ratio at 260/280 nm using a Biowave II Spectrophotometer. Reverse transcription of RNA (1.5 μg) was done using an Affinity ScripT cDNA Synthesis Kit (Stratagene, Darmstadt, Germany). Real time PCR was performed using Brilliant II SYBR Green QPCR Master Mix (Stratagene, Darmstadt, Germany) on a Step One equipment (Applied Biosystems, ThermoFisher, Massachusetts, USA). Beta-2-microglobulin (B2M) was used as housekeeping gene.

The primers were (5′-3′, respectively) were B2M: CCGCCTCACATTGAAATCCA and CTGCAGGC GTATGTATCAGT (NM_009735.3); Bax: ACTAAAGT GCCCGAGCTGAT and ATGGTCACTGTCTGCCATGT (NM_007527.3); Bcl2: GTGGATGACTGAGTACCTGA and AAACAGAGGTCGCATGCT (NM_009741.5); Caspase 3: TCAAAGGACGGGTCGTGGTT and TGCCACCTTCCTGTTAACGC (NC_000074.6); Fas: GCATGACAGCATCCAAGACA and GTTCTGCG ACATTCGGCTTT (DQ846748.1); Bcl-xL: TGGTGAGT CGGATTGCAAGT and TGCTGCATTGTTCCCGTAGA (L35049.1); Xiap: TGGCCGGACTATGCTCATTT and TGTTCTGACCAGGCACGAT (NM_001301641.1). Each set of primers was used in the PCR reaction to amplify their corresponding gene, and the products were confirmed using agarose gel electrophoresis. The number of mRNA copies of target and housekeeping genes was calculated according to the standard curve method. PCR amplification efficiency of each primer pair was calculated from the slope of the standard curve. Melting curve analysis was constructed to verify the presence of gene-specific amplification and for absence of primer dimers. Agarose gel electrophoresis was performed to test amplicon specificity.

Statistical Analysis

The cells maintained with DMEM and 10% FBS and normal zinc were used as a control in all assays (Cn). The experiments were performed in triplicate and repeated at least three times. Statistical analyses were performed with Graph-Pad Prism 5.0 software (San Diego, CA, USA). The data were first analyzed to test normality of results distribution. Since distribution was not normal, results are presented as median and interquartile interval. Statistical comparisons were conducted by using the

Kruskal–Wallis test (Dunn's multiple comparisons post hoc). Statistical significance was considered when $p < 0.05$.

Results

Figure 1 shows the expression of two proapoptotic genes, Fas, and caspase-3, under three conditions: (a) low glucose (10 mM)/ high glucose (30 mM) and low Zn (10 μM/high Zn (100 μM); (b) same previous condition but in the presence of 1 nM insulin; and (c) in the presence of 5 nM IL6. Caspase-3 expression was significantly increased in the presence of the combination high Zn/high glucose with and without the presence of insulin in the culture medium ($p < 0.01$). Fas expression instead showed uneven responses, for instance high Zn induced greater expression of this gen only under low glucose conditions ($p < 0.05$). The opposite trend, that was high expression in high glucose and low Zn medium, was observed when cells were cultured in the presence of IL6 ($p < 0.001$).

Under similar conditions as described above, Fig. 2 shows the expression of two antiapoptotic genes, Bcl-xL and Xiap. It is noteworthy that in most conditions of high glucose, having high Zn in the medium, expression of these genes was increased ($p < 0.001$),

Figure 3 presented the results of the ratio of a proapoptotic gene (Bax) and an antiapoptotic gene (Bcl-2). There was a significant reduction of this ratio mainly in the presence of high Zn content in the medium. This is seen in low and high

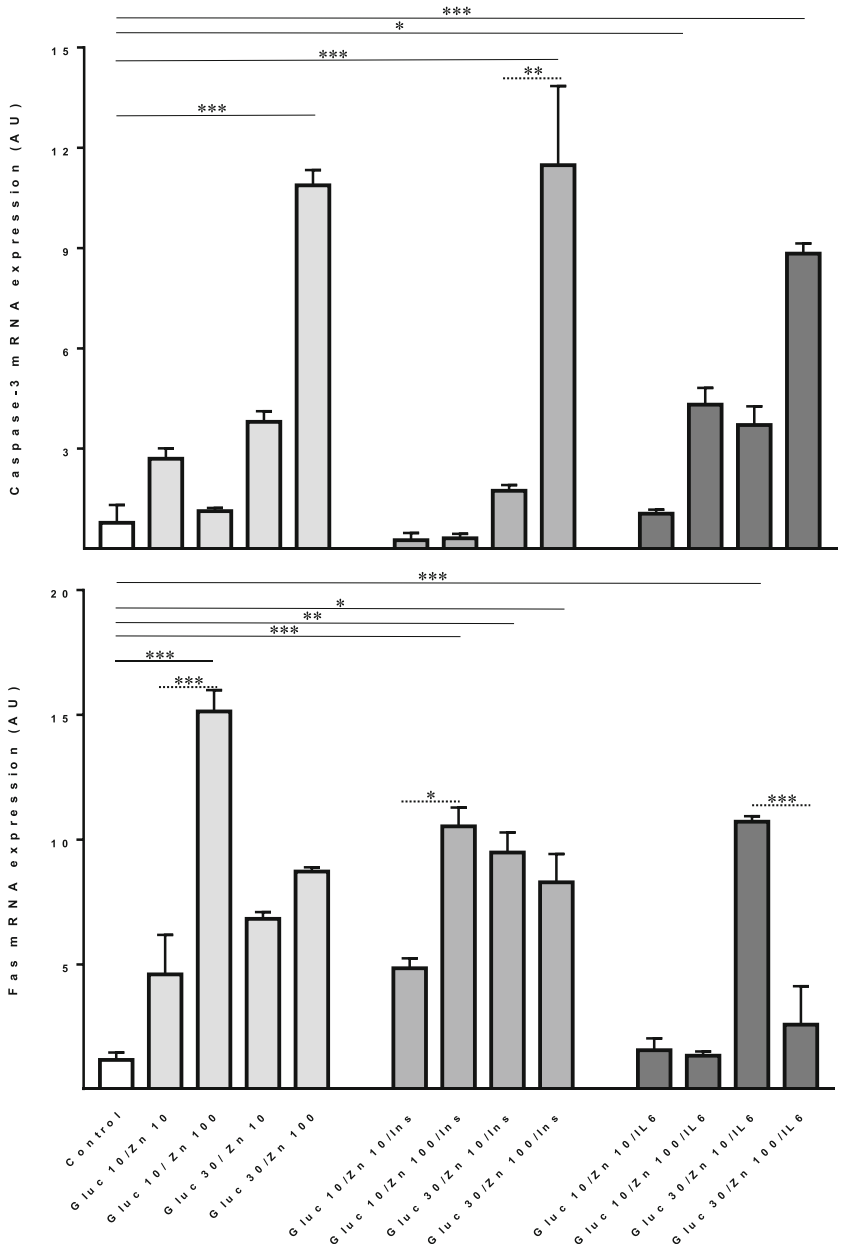

Fig. 1 mRNA expression of pro-apoptotic genes caspase-3 and Fas in myoblasts cultured in low/high glucose, low/high zinc and in the presence of insulin and interleukin 6. C2C12 cells cultured for 24 h with glucose 10 or 30 mM; zinc 10 or 100 μM; insulin 1 nM; interleukin 6 (IL6) 5 nM. Treatments are as follows: Control group: Gluc 10/Zn10 = glucose 10 mM and zinc 10 μM; Gluc 10/Zn100 = glucose 10 mM and zinc 100 μM; Gluc 30/Zn10 = glucose 30 mM and zinc 10 μM; Gluc 30/Zn 100 = glucose 30 mM and zinc 100 μM. Insulin group: Gluc 10/Zn10/Ins = glucose 10 mM and zinc 10 μM and insulin 1 nM; Gluc 10/Zn100/Ins = glucose 10 mM and zinc 100 μM and insulin 1 nM; Gluc 30/Zn10/Ins = glucose 30 mM and zinc 10 μM and insulin 1 nM; Gluc 30/Zn 100/Ins = glucose 30 mM and zinc 100 μM and insulin 1 nM; and Interleukin 6 group: Gluc 10/Zn10/IL6 = glucose 10 mM and zinc 10 μM and IL6 5 nM; Gluc 10/Zn100/IL6 = glucose 10 mM and zinc 100 μM and IL6 5 nM; Gluc 30/Zn10/IL6 = glucose 30 mM and zinc 10 μM and IL6 5 nM; Gluc 30/Zn 100/IL6 = glucose 30 mM and zinc 100 μM and IL6 5 nM. Results are presented as median ± interquartile range. Kruskal-Wallis test, Dunn's multiple comparisons post hoc (*$p < 0.05$; **$p < 0.01$; *** $p < 0.001$). Differences against the control group (—); Difference between zinc concentrations (——)

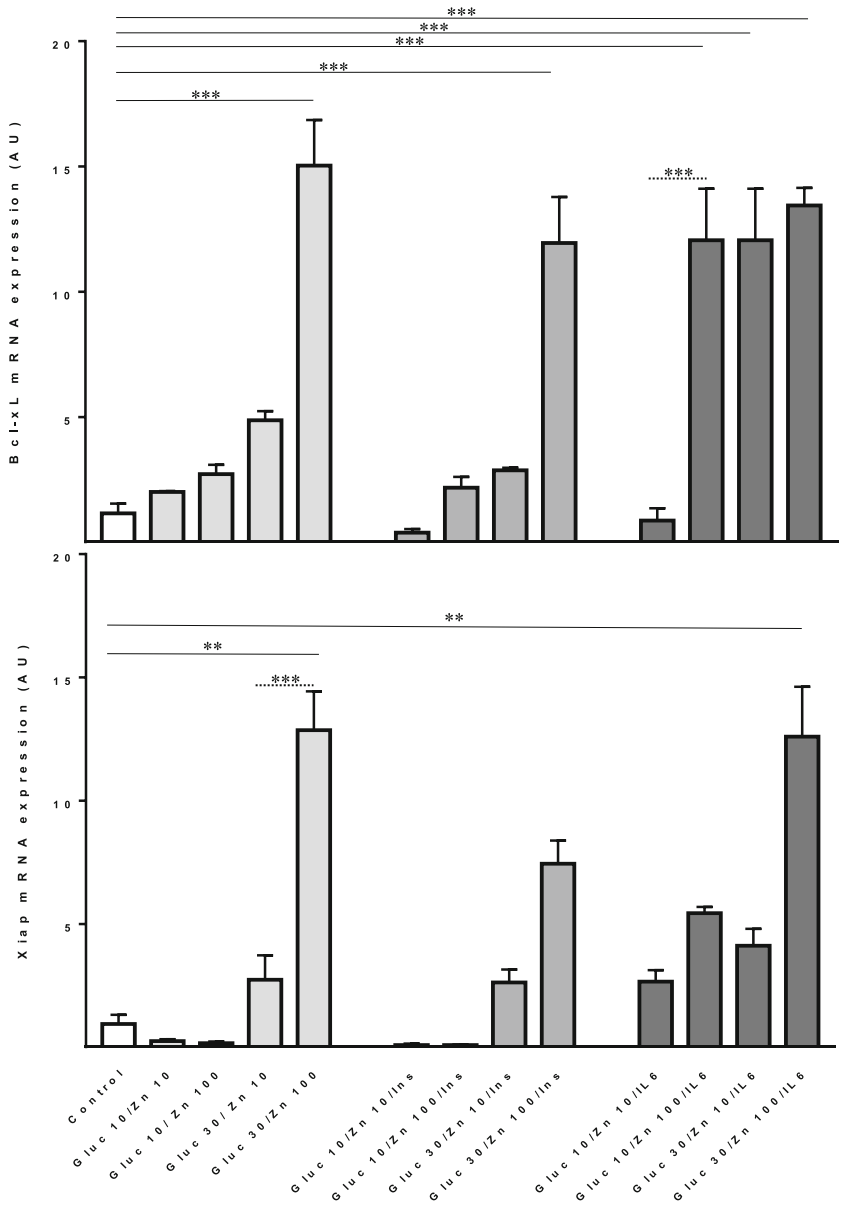

Fig. 2 mRNA expression of anti-apoptotic genes Bcl-xL and Xiap in myoblasts cultured in low/high glucose, low/high zinc, and in the presence of insulin and interleukin 6. C2C12 cells cultured for 24 h with glucose 10 or 30 mM; zinc 10 or 100 μM; insulin 1 nM; interleukin 6 (IL6) 5 nM. Treatments are as follows: Control group: Gluc 10/Zn10 = glucose 10 mM and zinc 10 μM; Gluc 10/Zn100 = glucose 10 mM and zinc 100 μM; Gluc 30/Zn10 = glucose 30 mM and zinc 10 μM; Gluc 30/Zn 100 = glucose 30 mM and zinc 100 μM. Insulin group: Gluc 10/Zn10/Ins = glucose 10 mM and zinc 10 μM and insulin 1 nM; Gluc 10/Zn100/Ins = glucose 10 mM and zinc 100 μM and insulin 1 nM; Gluc 30/Zn10/Ins = glucose 30 mM and zinc 10 μM and insulin 1 nM; Gluc 30/Zn 100/Ins = glucose 30 mM and zinc 100 μM and insulin 1 nM; and Interleukin 6 group: Gluc 10/Zn10/IL6 = glucose 10 mM and zinc 10 μM and IL6 5 nM; Gluc 10/Zn100/IL6 = glucose 10 mM and zinc 100 μM and IL6 5 nM; Gluc 30/Zn10/IL6 = glucose 30 mM and zinc 10 μM and IL6 5 nM; Gluc 30/Zn 100/IL6 = glucose 30 mM and zinc 100 μM and IL6 5 nM. Results are presented as median ± interquartile range. Kruskal-Wallis test, Dunn's multiple comparisons post hoc (*$p < 0.05$; **$p < 0.01$; *** $p < 0.001$). Differences against the control group (—); Difference between zinc concentrations (——)

glucose with no insulin and high glucose with insulin ($p < 0.001$). The response is attenuated by IL6.

Discussion

In some pathologies like diabetes, among the host of mechanisms involved in the loss of glycemic control is apoptosis of pancreatic beta cells contributing to impairments of the function of these cells and leading to a dramatic decrease of insulin secretion. Muscle cells are also a major player in glycemic control by its participation in glucose uptake. Functional alterations of these cells as result of direct oxidative stress damage or by any other mechanism, such as apoptosis, could trigger serious adverse consequences on glycemic control. In this study, myoblasts were challenged with high concentrations of glucose (30 mM) and/or IL-6 as an inflammatory stimulus and also with insulin; under these conditions, the cells also were exposed to normal (10 μM) and high zinc (100 μM) concentrations in the medium, and expression of genes related with apoptosis, either pro- or antiapoptotic were studied.

There are two major apoptotic signaling pathways: the extrinsic that involves cell death receptors and the intrinsic that is mitochondria mediated. The extrinsic pathway is activated by death ligands, such as Fas ligand (FasL) or TNFα, which bind to receptors at plasma membrane Fas or TNFR [15]. Numerous studies suggest that Fas, rather than TNFR, is the major mechanism underlying the activation of extrinsic apoptosis.

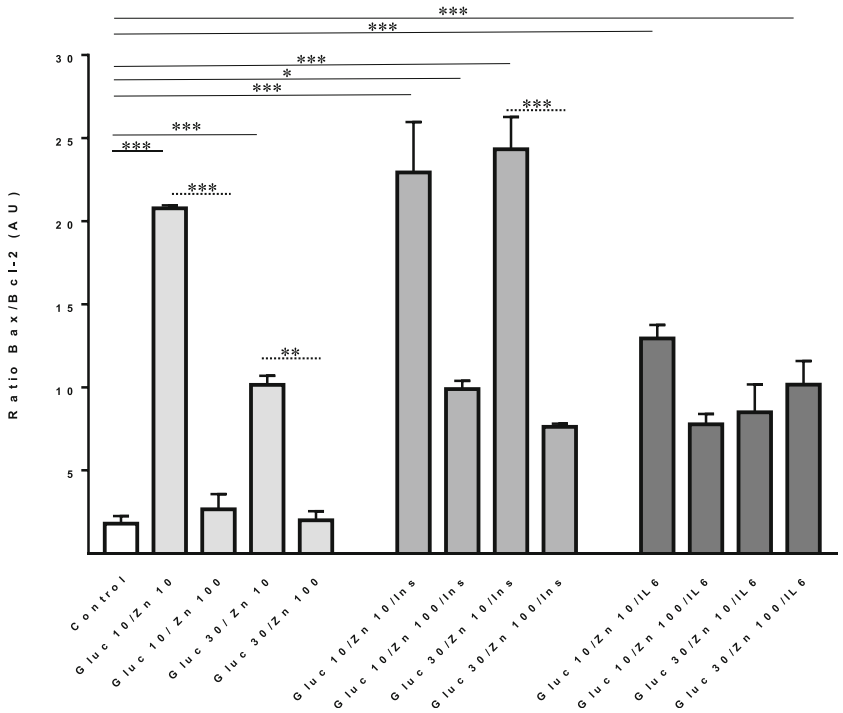

Fig. 3 Bax/Bcl2 mRNA expression ratio in myoblasts cultured in low/high glucose, low/high zinc and in the presence of insulin and interleukin 6. C2C12 cells cultured for 24 h with glucose 10 or 30 mM; zinc 10 or 100 μM; insulin 1 nM; interleukin 6 (IL6) 5 nM. Treatments are as follows: Control group: Gluc 10/Zn10 = glucose 10 mM and zinc 10 μM; Gluc 10/Zn100 = glucose 10 mM and zinc 100 μM; Gluc 30/Zn10 = glucose 30 mM and zinc 10 μM; Gluc 30/Zn 100 = glucose 30 mM and zinc 100 μM. Insulin group: Gluc 10/Zn10/Ins = glucose 10 mM and zinc 10 μM and insulin 1 nM; Gluc 10/Zn100/Ins = glucose 10 mM and zinc 100 μM and insulin 1 nM; Gluc 30/Zn10/Ins = glucose 30 mM and zinc 10 μM and insulin 1 nM; Gluc 30/Zn 100/Ins = glucose 30 mM and zinc 100 μM and insulin 1 nM; and Interleukin 6 group: Gluc 10/Zn10/IL6 = glucose 10 mM and zinc 10 μM and IL6 5 nM; Gluc 10/Zn100/IL6 = glucose 10 mM and zinc 100 μM and IL6 5 nM; Gluc 30/Zn10/IL6 = glucose 30 mM and zinc 10 μM and IL6 5 nM; Gluc 30/Zn 100/IL6 = glucose 30 mM and zinc 100 μM and IL6 5 nM. Results are presented as median ± interquartile range. Kruskal-Wallis test, Dunn's multiple comparisons post hoc (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$). Differences against the control group (—); Difference between zinc concentrations (——)

There is close correlation between hyperglycemia and changes in the cellular function and morphology. High glucose has been shown to disturb cell cycle, increase DNA damage, delay endothelial cell replication, and cause excessive cell death [20]. In cultured endothelial cells, hyperglycemia selectively triggers apoptosis [20]; also high glucose induces an accelerated programmed cell death of retinal microvascular cells in situ in human and experimental diabetic retinopathy [28]. In our model, we found that high glucose was associated with increased expression of caspase-3; furthermore, high Zn induced increased expression. However, regarding Fas, the combinations of low glucose/high Zn and high glucose/low Zn showed marked increased expressions.

Short-term hyperglycemia in rats triggers oxidative stress and nonoxidative glucose pathways, i.e., advanced glycated end species, polyol, and PKCβ2 pathways, and in parallel, increased myocardial apoptosis [29]. Moreover, in a prospective cohort study in patients with acute myocardial infarction, high blood glucose was the most important factor to determine mortality. In addition, glucose was correlated with Fas levels [30]. Studies in human melanoma cells and in neurons have shown that Zn could be toxic when it is increased in the cytoplasm; excess Zn results in the induction of ROS production by the mitochondria and also promotes ROS formation outside the mitochondria by interaction with the enzymes NADPH oxidase and 12-lipoxoygenase [31, 32]. Thus, Zn can be protective at physiological levels but toxic when it is in excess.

In terms of antiapoptotic gene expressions, we found a protective role of additional Zn as result of the significant increase of Xiap and Bcl-xL, especially in the high-glucose condition. Interesting, this enhancing effect is observed in the presence of insulin. Xiap but not Bcl-XL expression is also increased by Zn in the presence of the inflammatory IL6 stimulus. We also observed that Bax (proapoptotic)/Bcl2 (antiapoptotic) ratio is marked decreased with high Zn in the medium in both low and high glucose. This effect is diminished by the presence of an inflammatory agent.

The main characteristic of the most chronic diseases is inflammation. This condition triggers a series of intracellular events; the primary outputs are activation of NF-κB, the mitogen-activated protein kinases (MAPKs) c-Jun amino-terminal kinase (JNK), and p38 [33]. Among further effects is apoptosis. The most recognized cytokine that induces apoptosis is TNFα by the activation of NF-κB and the amplification

of the inflammatory response. We studied the effect of IL6 in the myoblast model. This cytokine plays an important role in muscle function especially during exercise [34], but the effect of this cytokine during hyperglycemia in this tissue has been barely studied. We provide additional evidence regarding the relevance of inflammation as a modulating agent of the effects induced by varying concentrations of glucose and Zn in an insulin target cell. It must be noted however, that the study of apoptotic events in muscle cells in a diabetes-like environment is far from being complete.

In conclusion, we found that high Zn together with high-glucose concentration may induce the expression of some but not all proapoptotic genes. However, additional Zn strongly stimulated the expression of anti-apoptotic genes. This effect was especially noted in high-glucose conditions with and without the presence of insulin. This effect is partially overridden by the presence of an inflammatory agent.

Authors' Contributions MAG, MR, and MAO conception and design of research. MAG performed experiments. MAG and MAO analyzed data. MAG, MR, and MAO interpreted results of experiments. MAG and MAO prepared figures. MAG drafted manuscript. MAG, MR, and MAO edited and revised manuscript. MAO and MR approved final version of the manuscript.

Funding Information This research was funded by the National Fund for Development of Science and Technology (FONDECYT), research project 1120323.

Compliance with Ethical Standards

Conflicts of Interest Manuel Ruz and Miguel Arredondo received payment from the research project FONDECYT 1120323. The rest of authors declare that they have no conflicts of interest.

References

1. Leibowitz G, Bachar E, Shaked M, Sinai A, Ketzinel-Gilad M, Cerasi ME, Kaiser N (2010) Glucose regulation of β-cell stress in type 2 diabetes. Diab Obesity Metab 12:66–75
2. Poitout V, Amyot J, Semache M, Zarrouki B, Hagman D, Fontés G (2010) Glucolipotoxicity of the pancreatic beta cell. Biochim Biophys Acta 1801:289–298
3. Puntarulo S (2005) Iron, oxidative stress and human health. Mol Asp Med 26:299–312
4. Dusek P, Roos PM, Litwin T, Schneider SA, Flaten TP, Aaseth J (2015) The neurotoxicity of iron, copper and manganese in Parkinson's and Wilson's diseases. J Trace Elem Med Biol 31: 193–203
5. Shoelson S, Lee J, Goldfine AB (2016) Inflammation and insulin resistance. J Clin Invest 116:1793–1801
6. Jansen J, Karges W, Rink L (2009) Zinc and diabetes clinical links and molecular mechanisms. J Nutr Biochem 20:399–417
7. Costello LC, Feng P, Milon B, Tan M, Franklin RB (2004) Role of zinc in the pathogenesis and treatment of prostate cancer: critical issues to resolve. Prostate Cancer Prostatic Dis 7:111–117
8. Costello LC, Franklin RB (2014) The status of zinc in the development of hepatocellular cancer: an important, but neglected, clinically established relationship. Cancer Biol Ther 15:353–360
9. El-Tawil AM (2012) Zinc supplementation tightens leaky gut in Crohn's disease. Inflamm Bowel Dis 18:E399. https://doi.org/10.1002/ibd.21926
10. Wong CP, Ho E (2012) Zinc and its role in age-related inflammation and immune dysfunction. Mol Nutr Food Res 56:77–87
11. Foster M, Chu A, Petocz P, Samman S (2014) Zinc transporter gene expression and glycemic control in post-menopausal women with type 2 diabetes mellitus. J Trace Elem Med Biol 28:448–452
12. Pearson E (2014) Zinc transport and diabetes risk. Nat Genet 46: 323–324
13. Prasad B, Beck B, Fitzgerald J, Snell D, Bao G, Singh T, Cardozo L (2010) Zinc decreases C-reactive protein, lipid peroxidation, and inflammatory cytokines in elderly subjects: a potential implication of zinc as an atheroprotective agent. Am J Clin Nutr 91:1634–1641
14. Foster M, Samman S (2010) Zinc redox signaling: perturbations associated with cardiovascular disease and diabetes mellitus. Antioxid Redox Signal 13:1549–1573
15. Taylor RC, Cullen SP, Martin SJ (2008) Apoptosis: controlled demolition at cellular level. Nat Rev Mol Cell Biol 9:231–241
16. Wu CC, Bratton SB (2013) Regulation of the intrinsic apoptosis pathway by reactive oxygen species. Antioxid Redox Signal 19: 546–558
17. Jiang X, Jiang H, Shen Z, Wang X (2014) Activation of mitochondrial protease OMA1 by Bax and Bak promotes cytochrome c release during apoptosis. Proc Natl Acad Sci U S A 11:14782–14787
18. Korsmeyer SJ, Wel MC, Saito M, Weller S, Oh KJ, Schlesinger PH (2000) Pro-apoptotic cascade activates BID, which oligomerizes BAK or BAX into pores that result in the release of cytochrome c. Cell Death Differ 7:1166–1173
19. Busik JV, Mohr S, Grant MB (2008) Hyperglycemia-induced reactive oxygen species toxicity to endothelial cells is dependent on paracrine mediators. Diabetes 57:952–1965
20. Kageyama S, Yokoo H, Tomita K, Kageyama-Yahara N, Uchimido R, Matsuda N, Yamamoto S, Hattori Y (2011) High glucose-induced apoptosis in human coronary artery endothelial cells involves up-regulation of death receptors. Cardiovasc Diabetol Aug 4:10–73. https://doi.org/10.1186/1475-2840-10-73
21. Tian XF, Cui MX, Yang SW, Zhou YJ, Hu DY (2013) Cell death, dysglycemia and myocardial infarction. Biomed Rep 1:341–346
22. Green DR, Kroemer G (2004) The pathophysiology of mitochondrial cell death. Science 305:626–629
23. Lavrik I, Golks A, Krammer PH (2005) Death receptor signaling. J Cell Sci 118:265–267
24. Anuradha R, Saraswati M, Kumar KG, Rani SH (2014) Apoptosis of beta cells in diabetes mellitus, DNA. Cell Biol 33:743–748
25. Chimienti F, Seve M, Richard S, Mathieu J, Favier A (2001) Role of cellular zinc in programmed cell death: temporal relationship between zinc depletion, activation of caspases, and cleavage of Sp family transcription factors. Biochem Pharmacol 62:51–62
26. Kown MH, Van Der Steenhoven T, Blankenberg FG, Hoyt G, Berry GJ, Fait TJ et al (2000) Zinc-mediated reduction of apoptosis in cardiac allografts. Circulation 102(19 Suppl 3):III228–III232
27. Truong-Tran AQ, Ruffin RE, Zalewski PD (2001) The role of zinc in caspase activation n and apoptotic cell death. Biometal 14:315–330
28. Behl Y, Krothapalli P, Desta T, Roy DT (2009) Graves, FOXO1 plays an important role in enhanced microvascular cell apoptosis and microvascular cell loss in type 1 and type 2 diabetic rats. Diabetes 58:917–925
29. Mapanga RF, Joseph D, Symington B, Garson KL, Kimar C, Kelly-Laubscher R, Essop MF (2014) Detrimental effects of acute hyperglycemia on the rat heart. Acta Physiol (Oxford) 210:546–564

30. Chang J, Zhang G, Zhang L, Hou YP, Liu XL, Zhang L (2013) High admission glucose levels increase Fas apoptosis and mortality in patients with acute ST-elevation myocardial infarction: a prospective cohort study. Cardiovasc Diabetol 15:171. https://doi.org/10.1186/1475-2840-12-171
31. Provinciali P, Pierpaoli E, Bartozzi B, Bernardini G (2016) Zinc induces apoptosis of human melanoma cells, increasing reactive oxygen species, p53 and FAS ligand. Anticancer Res 35:5309–5316
32. Stork CJ, Li YV (2016) Elevated cytoplasmic free zinc and increased reactive oxygen species generation in the context of brain injury. Acta Neurochir Suppl 121:347–353
33. Gerald W, Dorn II (2010) Mechanisms of non-apoptotic programmed cell death in diabetes and heart failure. Cell Cycle 9: 3442–3448
34. Peake JM, Della-Gatta P, Suzuki K, Nieman DC (2015) Cytokine expression and secretion by skeletal muscle cells: regulatory mechanisms and exercise effects. Exerc Immunol Rev 21:8–25