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Redox-Mediated Activation of SIRT1 by Ozone Therapy Attenuates *Ucp2* Expression in Experimental Diabetes

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Abstract: Oxidative stress contributes to pancreatic β-cell dysfunction and mitochondrial dysregulation in diabetes. Although controlled ozone exposure may activate endogenous antioxidant responses, its relationship with the pancreatic SIRT1–Ucp2 axis remains unclear. This exploratory study investigated whether ozone therapy induces redox adaptation associated with SIRT1 restoration and Ucp2 suppression in streptozotocin (STZ)-induced experimental diabetes. Male Sprague–Dawley rats (n = 28) were randomly assigned to four groups: healthy control

(CT), STZ-induced diabetes (45 mg/kg) (STZ), STZ plus oxygen (STZ + O_2), and STZ plus ozone (150 μg/kg/day, intraperitoneally for 7 days) (STZ + O_3). Fasting glucose, insulin, and HOMA-IR were measured. Pancreatic SIRT1 levels were quantified by ELISA, Ucp2 expression was assessed by RT-qPCR, SOD and GPx activities were measured using activity assays, and MDA levels were determined by the TBARS assay. Ozone therapy significantly increased pancreatic SIRT1 levels and suppressed Ucp2 upregulation compared with untreated diabetic rats. These molecular changes were accompanied by increased SOD and GPx activities and reduced MDA levels. However, fasting glucose remained elevated, and HOMA-IR did not significantly improve compared with untreated diabetic rats. These findings suggest that short-term ozone therapy induces pancreatic redox adaptation and modulates the SIRT1–Ucp2 axis in STZ-induced experimental diabetes. Further studies are needed to determine whether longer treatment periods and models with preserved β-cell function can translate these molecular effects into systemic metabolic improvement.

Keywords: diabetes mellitus; insulin resistance; mitochondrial function; oxidative stress; ozone therapy; redox signaling; SIRT1; uncoupling protein 2.

臭氧疗法通过氧化还原介导的SIRT1激活减弱实验性糖尿病中的Ucp2表达

摘要：氧化应激会导致糖尿病中胰腺β细胞功能障碍和线粒体调控失衡。尽管受控臭氧暴露可能激活内源性抗氧化反应，但其与胰腺SIRT1-Ucp2轴之间的关系仍不明确。本探索性研究旨在探讨臭氧疗法是否能够在链脲佐菌素（STZ）诱导的实验性糖尿病中诱导与SIRT1恢复和Ucp2抑制相关的氧化还原适应。雄性Sprague-Dawley大鼠（n = 28）被随机分为四组：健康对照组（CT）、STZ诱导糖尿病组（45 mg/kg）（STZ）、STZ加氧气处理组（STZ + O_2）以及STZ加臭氧处理组（150 μg/kg/day，腹腔注射，连续7天）（STZ + O_3）。测定空腹血糖、胰岛素和HOMA-IR。采用ELISA检测胰腺SIRT1水平，采用RT-qPCR评估Ucp2表达，采用活性测定法检测SOD和GPx活性，并通过TBARS法测定MDA水平。与未治疗的糖尿病大鼠相比，臭氧疗法显著提高了胰腺SIRT1水平，并抑制了Ucp2的上调。这些分子变化伴随着SOD和GPx活性的增加以及MDA水平的降低。然而，空腹血糖仍然升高，HOMA-IR与未治疗的糖尿病大鼠相比未出现显著改善。这些结果表明，短期臭氧疗法可在STZ诱导的实验性糖尿病中诱导胰腺氧化还原适应，并调节SIRT1-Ucp2轴。未来仍需进一步研究，以确定更长治疗周期以及保留β细胞功能的模型是否能够将这些分子效应转化为全身性代谢改善。

关键词：糖尿病；胰岛素抵抗；线粒体功能；氧化应激；臭氧疗法；氧化还原信号传导；SIRT1；解偶联蛋白2。

1. Introduction

Diabetes mellitus (DM) has become one of the most consequential public health crises of the modern era. Affecting more than 537 million adults worldwide, DM is projected to claim nearly 780 million individuals by 2045 [1], a trajectory that places pressure on healthcare infrastructure and aging populations alike. Beyond its high prevalence, DM is a foremost driver of premature mortality and disability, mainly through its chronic macrovascular and microvascular complications such as cardiovascular disease, nephropathy, neuropathy, and retinopathy [2]. These sequelae arise from sustained and progressive metabolic dysregulation. Despite remarkable advances in pharmacotherapy, available treatments remain largely centered on glycemic control and fall critically short of addressing the underlying molecular mechanisms that drive disease progression and end-organ injury [3].

Central to the pathophysiology of DM is a state of chronic oxidative stress that disrupts cellular homeostasis [4], impairing both β-cell function and peripheral insulin sensitivity [5]. The excessive generation of reactive oxygen species (ROS) compromises mitochondrial integrity, impedes ATP synthesis, and interferes with key nodes of the insulin signaling cascade, creating a vicious cycle of metabolic deterioration [6]. At the intersection of redox regulation and energy metabolism, an NAD^+-dependent deacetylase Sirtuin 1 (SIRT1) has emerged as a main regulator of metabolic homeostasis. SIRT1 orchestrates improvements in insulin sensitivity by deacetylating substrates such as PGC-1α, FOXO1, and p53 [7]. Crucially, SIRT1 represses transcription of Uncoupling Protein 2 (*Ucp2*), a mitochondrial inner-membrane proton transporter whose overexpression dissipates the electrochemical gradient, attenuating ATP production, impairing glucose-stimulated insulin secretion, and amplifying ROS generation [8]. The dysregulation of the SIRT1–*Ucp2* axis is therefore increasingly recognized as a central node linking oxidative stress to the metabolic dysfunction that characterizes both type 1 and type 2 diabetes.

Therapeutic strategies that target redox signaling offer considerable promise [9]. Ozone therapy, for instance, could activate endogenous antioxidant defense systems when delivered at precisely controlled, sub-toxic doses. This adaptive response is mediated by the activation of redox-sensitive transcription factors such as Nrf2 [10]. Emerging evidence supports the metabolic and cytoprotective benefits of ozone therapy in a range of conditions [11], yet its capacity to directly modulate SIRT1-dependent mitochondrial signaling in the diabetic milieu remains poorly defined. Mechanistic evidence bridging redox adaptation to the regulation of mitochondrial uncoupling and insulin resistance is absent from the literature.

Previous studies have shown that controlled ozone exposure can attenuate pancreatic injury and activate endogenous antioxidant responses in experimental diabetes [12]. Mild ozonization has also been linked to Keap1/Nrf2-dependent cytoprotective signaling [20,21]. However, the molecular link between ozone-induced redox adaptation and mitochondrial metabolic regulation remains incompletely defined. In particular, whether ozone therapy modulates the pancreatic SIRT1–*Ucp2* axis has not been directly investigated. The present study addresses this gap by evaluating the effects of short-term ozone therapy on pancreatic SIRT1 expression, *Ucp2* regulation, antioxidant-enzyme activities, lipid peroxidation, and systemic metabolic parameters in STZ-induced diabetic rats. We hypothesized that controlled ozone exposure would induce a redox-adaptive response associated with restoration of SIRT1 expression and suppression of *Ucp2* upregulation.

2. Materials and Methods

2.1. Animal Care

Adult male Sprague–Dawley rats (12–14 weeks; 180 ± 10 g) were obtained from a certified commercial breeder and housed under room temperature (22–25°C), 12-hour light/dark cycle), for a 7-day acclimatization period. Animals had unrestricted access to standard chow and water throughout the study.

2.2. Experimental Groups

This study was designed as an exploratory mechanistic animal experiment. No formal a priori power calculation was performed. The minimum sample size was estimated using the Federer resource-equation approach for experimental studies: [(t-1)(n-1) ≥ 15], where (t) represents the number of experimental groups and (n) represents the number of animals per group. With four experimental groups, the calculation was: [(4-1)(n-1) ≥ 15], resulting in a minimum requirement of six animals per group. One additional animal was included in each group to account for potential attrition during the experimental period. Therefore, seven animals were allocated to each group, yielding a total of 28 rats. Because this approach does not incorporate an expected effect size or variance estimate, it should be interpreted as a resource-equation-based sample-size justification rather than a formal a priori power analysis.

A total of 28 rats were allocated randomly to four groups: (1) healthy control (CT), (2) untreated diabetic (STZ), (3) diabetic + oxygen vehicle control (STZ+O_2), and (4) diabetic + ozone therapy (STZ+O_3) using a computer-generated random-number sequence. Outcome assessment was performed without blinding. The absence of a formal power calculation and blinded outcome assessment is acknowledged as a limitation.

Diabetes was induced by a single intraperitoneal injection of streptozotocin (STZ; 45 mg/kg body weight; Sigma-Aldrich, St. Louis, MO, USA), freshly dissolved in 0.1 M citrate buffer (pH 4.5). Body weight and general health status were monitored daily. At 72 hours post-injection, fasting blood glucose was assessed via tail-vein sampling using a calibrated glucometer (Accu-Chek; Roche Diagnostics, Mannheim, Germany). Animals with fasting blood glucose ≥ 250 mg/dL were confirmed diabetic and enrolled in the study; non-diabetic animals were excluded.

Animals in the STZ+O_2 group received daily intraperitoneal injections of medical-grade oxygen (0.5 mL/day), while those in the STZ+O_3 group received ozone at a dose of 150 µg/kg/day (0.5 mL, intraperitoneal) for seven consecutive days. The ozone dose of 150 µg/kg/day and the 7-day intervention period were selected as a short-term mechanistic protocol based on previous preclinical evidence demonstrating pancreatic protective effects of intraperitoneal oxygen–ozone administration in STZ-induced diabetic rats [12]. The intraperitoneal route was chosen to provide controlled and reproducible systemic exposure in the experimental model. This route was used for preclinical proof-of-concept purposes and should not be considered directly equivalent to clinical ozone-delivery methods, such as major autohemotherapy. Ozone was generated using a calibrated medical-grade ozone generator (EXT120, Canada Longevity) and administered within 5 minutes of preparation to ensure concentration accuracy and minimize spontaneous decomposition. Ozone concentration was verified prior to each administration using a UV photometric analyzer. Healthy and untreated diabetic control groups received no intervention.

2.3. Metabolic Assessment

Fasting blood glucose was measured via tail-vein sampling using a glucometer. Animals were then anesthetized with ketamine (50 mg/kg, i.p.) and xylazine (10 mg/kg, i.p.) cocktail, and blood was collected by cardiac puncture into EDTA-containing tubes. Plasma was separated by centrifugation (1,500 × g, 15 min, 4°C) and stored at −80°C until analysis.

Fasting plasma insulin was quantified using a validated ELISA kit (RE3153R, Reed Biotech Ltd), according to the manufacturer's instructions. Insulin resistance was calculated as: HOMA-IR = [fasting glucose (mmol/L) × fasting insulin (µIU/mL)] / 22.5.

2.4. Tissue Collection and Processing

Immediately after cardiac puncture, the pancreas was rapidly excised, rinsed in ice-cold phosphate-buffered saline (PBS), blotted dry, and weighed. Tissue aliquots designated for protein and RNA analyses were snap-frozen in liquid nitrogen and stored at −80°C. Separate aliquots were preserved for biochemical enzyme assays.

2.5. SIRT1 Protein Quantification

Pancreatic SIRT1 protein levels were quantified using a commercially available sandwich ELISA kit (RE1902R, Reed Biotech Ltd) following the manufacturer's protocol. Tissue was homogenized in ice-cold PBS. Absorbance was measured at 450 nm using a microplate reader, and SIRT1 concentrations were interpolated from a four-parameter logistic standard curve. SIRT1 levels were normalized to total protein concentration determined using standard Biuret assay.

2.6. Gene Expression Analysis

Total RNA was extracted from pancreatic tissue using SV Total RNA Isolation System (Z3100, Promega) according to the manufacturer's instructions. RNA integrity and purity were confirmed using a NanoDrop spectrophotometer (Thermo Fisher Scientific). Quantitative PCR was performed in triplicate using GoTaq Probe one-step RT-qPCR kit (A6121; Promega). Amplification conditions were initial denaturation at 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 60 s. *Ucp2* mRNA expression was normalized to the reference gene *Gapdh*. Relative quantification was performed using the 2^−ΔΔCt method.

2.7. Oxidative Stress Assays

Total superoxide dismutase (SOD) (E-BC-K020-M, Elabscience) and glutathione peroxidase (GPx) (E-BC-K096-S, Elabscience) activities were determined using colorimetric assay kits according to manufacturer’s instruction. Lipid peroxidation was assessed by measuring malondialdehyde (MDA) as a thiobarbituric acid reactive substance (TBARS), using a standardized colorimetric assay. All assays were performed in duplicate and normalized to total protein concentration.

2.8. Statistical Analysis

All data are expressed as mean ± standard deviation (SD). Between-group differences were evaluated by one-way analysis of variance (ANOVA), with Tukey's Honest Significant Difference post hoc tests. The p-value < 0.05 was considered statistically significant. All analyses were performed using GraphPad Prism version 11.0.0 (93).

3. Results

3.1. Ozone Does Not Restore Glycemic Parameters Within the Treatment Window

In the present study, STZ induction successfully produced hyperglycemia in all diabetic groups, with blood glucose levels ≥250 mg/dL observed 72 hours after induction (Fig. 1A). The diabetic rats were then subjected to ozone treatment for 7 days. Fasting blood glucose differed significantly across experimental groups. The healthy control (CT) group showed the lowest glucose levels, whereas the STZ+O3 group exhibited the highest levels. Post hoc analysis demonstrated a significant difference between CT and STZ+O3 groups, while differences among diabetic groups were not statistically significant (Fig. 1B).

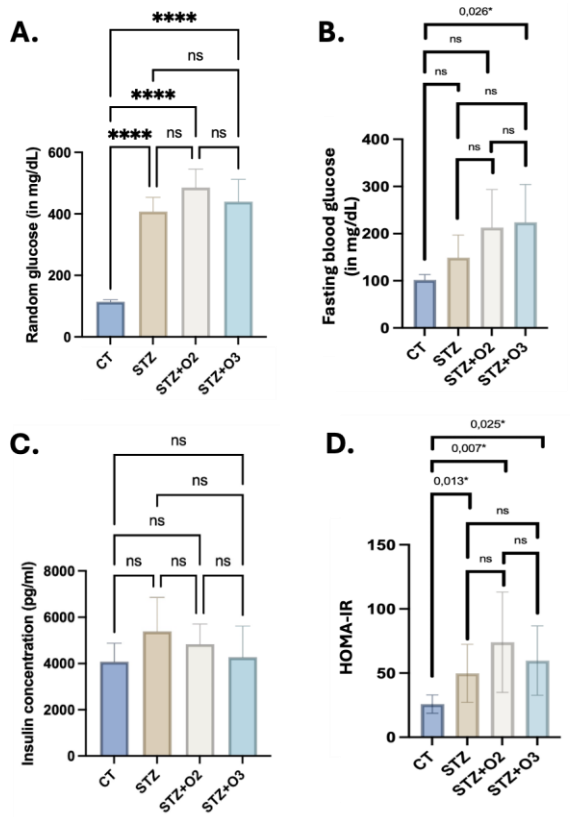

Figure 1. Effect of ozone therapy on systemic glycemic parameters. (A) STZ induction produced marked hyperglycemia 72 h post-injection. (B) Fasting blood glucose remained elevated after 7 days of ozone treatment. (C) Fasting insulin were similar in all groups. (D) HOMA-IR was significantly increased in all diabetic groups relative to healthy controls, without improvement following ozone therapy. Data are presented as mean ± SD. *p < 0.05; ****p < 0.0001; ns, not significant.

Fasting plasma insulin concentrations did not differ significantly among groups (Fig. 1C). HOMA-IR values were elevated in the diabetic groups relative to CT, indicating altered glucose–insulin homeostasis following STZ induction. However, no significant differences in HOMA-IR were detected between ozone-treated and untreated diabetic animals (Fig. 1D). Because the STZ model predominantly reflects β-cell injury and insulin deficiency, HOMA-IR should be interpreted cautiously as an exploratory metabolic index in this setting.

3.2. Ozone Restores Pancreatic SIRT1 Protein Expression in Diabetic Rats

Pancreatic SIRT1 levels showed marked intergroup differences. STZ induction significantly reduced SIRT1 expression compared with CT. Ozone therapy significantly increased SIRT1 levels relative to untreated diabetic rats, restoring SIRT1 expression to levels comparable with the CT group. Oxygen administration alone did not produce a significant improvement (Fig 2A).

3.3. Ozone Suppresses *Ucp2* Upregulation in Diabetic Rats

Expression of *Ucp2* differed significantly among groups. Diabetic animals exhibited a striking 17.2-fold upregulation of *Ucp2* relative to CT, consistent with mitochondrial uncoupling as a compensatory response to increased ROS burden. Ozone therapy significantly suppressed *Ucp2* expression compared with both STZ and STZ+O2 groups, reducing expression levels close to those observed in healthy controls. Oxygen administration alone failed to attenuate *Ucp2* upregulation, reinforcing the specificity of the ozone-mediated effect and excluding a non-specific oxygen-driven mechanism (Fig. 2B).

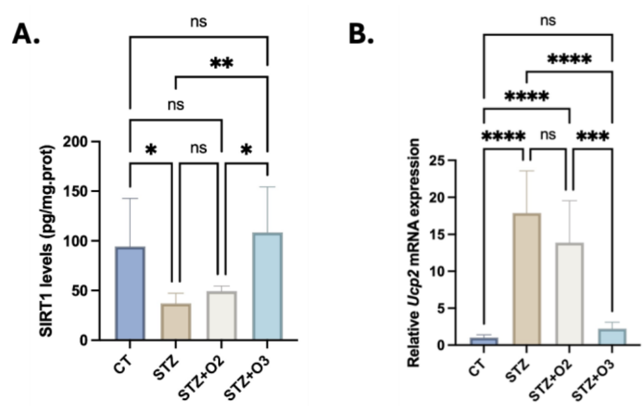

Figure 2. Ozone therapy restores pancreatic SIRT1 and *Ucp2*. (A) STZ reduced pancreatic SIRT1 levels, whereas ozone therapy restored it to near-control levels. (B) Diabetic rats exhibited marked *Ucp2* upregulation, which was significantly suppressed by ozone therapy. Data are presented as mean ± SD. *p < 0.05; ns, not significant.

3.4. Ozone Enhances Antioxidant Defense and Attenuates Lipid Peroxidation

Ozone therapy produced a consistent and coordinated improvement in pancreatic redox homeostasis. Relative to untreated diabetic animals, the STZ+O_3 group demonstrated significantly elevated SOD (Fig. 3A) and GPx (Fig. 3B) activities, alongside a significant reduction in MDA concentrations (Fig. 3C), reflecting attenuation of lipid peroxidation. These findings are consistent with ozone-induced oxidative preconditioning, whereby a controlled, sub-toxic oxidative stimulus upregulates endogenous antioxidant enzyme systems. Vehicle oxygen administration did not significantly alter any redox parameter relative to the untreated STZ group.

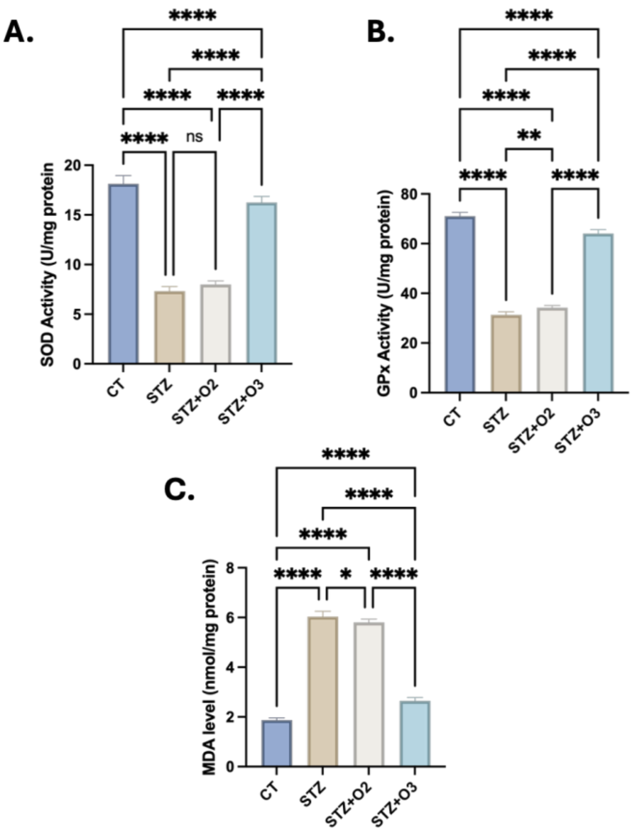

Figure 3. Ozone therapy improves redox status in pancreas of STZ-induced diabetic rats. (A) SOD activity, (B) GPx activity, and (C) MDA of ozone treated diabetic rats. Data are presented as mean ± SD. *p < 0.05; ** p < 0.01; *** p < 0.001; **** p < 0.0001, ns, not significant.

4. Discussion

This exploratory study demonstrates that short-term ozone therapy induces pancreatic redox adaptation in STZ-induced experimental diabetes. Ozone treatment increased SIRT1 protein levels, suppressed Ucp2 upregulation, enhanced SOD and GPx activities, and reduced MDA levels. Importantly, these molecular and biochemical changes occurred without significant restoration of fasting glucose or HOMA-IR. The findings therefore support a redox-modulating effect of ozone therapy but do not establish an acute improvement in systemic glucose metabolism.

Oxidative stress is a major driver of diabetic progression. Consistent with previous reports, STZ-induced diabetic rats exhibited elevated MDA levels together with reduced SOD and GPx activities, reflecting severe oxidative imbalance [13–15]. The profound suppression of pancreatic SIRT1 observed in STZ-induced diabetic animals is consistent with the well-established vulnerability of NAD^+-dependent deacetylase activity to chronic ROS-mediated NAD^+ depletion and proteasomal degradation [16]. The reciprocal upregulation of *Ucp2* in diabetic animals follows directly from the loss of SIRT1-mediated transcriptional repression at the *Ucp2* promoter, in agreement with the findings of Bordone and colleagues, who demonstrated that SIRT1-dependent *Ucp2* suppression is requisite for normal glucose-stimulated insulin secretion [17]. The magnitude of *Ucp2* upregulation observed here likely reflects the severity of oxidative β-cell injury induced by the 45 mg/kg STZ dose, which produces near-total insulinopenia rather than partial β-cell dysfunction. Study showed that *Ucp2* overexpression in β-cells resulted in a loss of glucose-stimulated hyper-polarization of the mitochondrial membrane potential and insulin secretion [18].

Ozone therapy reversed these alterations, supporting the concept of oxidative preconditioning or hormesis, in which controlled low-dose oxidative stimulation activates endogenous antioxidant pathways rather than causing oxidative injury [19]. Previous studies have shown that ozone activates NRF2-dependent antioxidant signaling [20,21], and the present findings extend this mechanism to regulation of the SIRT1–*Ucp2* pathway. The coordinated increase in SOD and GPx activities alongside reduced MDA levels in the STZ+O_3 group is consistent with this Nrf2-mediated adaptive response, corroborating prior findings by Martínez-Sánchez and colleagues in comparable STZ models [22]. Importantly, the fact that ozone-mediated SIRT1 activation and *Ucp2* suppression occurred alongside improved antioxidant enzyme status rather than worsened oxidative injury argues that, once SIRT1 is restored and endogenous antioxidant defenses are upregulated, the protective rationale for *Ucp2* overexpression is effectively superseded.

Despite these molecular effects, ozone therapy failed to significantly improve fasting glucose and HOMA-IR. This apparent discrepancy may reflect the relatively short intervention period, since molecular adaptation often occurs earlier than physiological recovery. In addition, the STZ model primarily induces β-cell destruction and insulin deficiency rather than classical obesity-associated insulin resistance [23], which may limit metabolic reversibility despite improvement in mitochondrial signaling. Moreover, the floor effect on circulating insulin inherent to this model reduces the statistical sensitivity of HOMA-IR as a functional readout. The present data therefore likely capture the molecular ignition of a therapeutic process whose downstream functional output requires extended treatment duration and residual β-cell mass to become measurable.

Several limitations should be acknowledged. No formal a priori power calculation was performed, and the study should therefore be considered exploratory. The intervention duration was relatively short, upstream signaling pathways such as NRF2 and AMPK were not evaluated, and causal confirmation of SIRT1 involvement was not performed. Furthermore, the STZ model may not fully represent the metabolic complexity of type 2 diabetes. The study was confined to pancreatic tissue, and whether ozone therapy produces analogous SIRT1–*Ucp2* modulation in skeletal muscle, liver, and adipose tissue, tissues that collectively govern peripheral insulin sensitivity, remains unaddressed. Finally, intraperitoneal ozone administration provides reproducible exposure in rodents but is not directly equivalent to clinical ozone-delivery methods.

From a translational perspective, The present study supports further investigation of ozone therapy as a redox-modulating adjunct rather than an acute glucose-lowering treatment. Clinical ozone delivery produces systemic redox-adaptive responses, and the identification of SIRT1 and *Ucp2* as pharmacodynamic endpoints creates a tractable biomarker framework for clinical trial design. The mechanistic convergence between ozone-mediated NAD^+ restoration and the actions of established SIRT1-activating strategies (including caloric restriction, nicotinamide riboside supplementation, and metformin) further suggests that combination approaches may yield synergistic metabolic benefits warranting systematic preclinical evaluation.

5. Conclusion

Ozone therapy induces pancreatic redox adaptation in STZ-induced experimental diabetes, characterized by increased SIRT1 expression, suppression of *Ucp2* upregulation, enhanced antioxidant defenses, and reduced lipid peroxidation. These molecular changes were not accompanied by restoration of fasting glucose or HOMA-IR within the 7-day treatment period. The findings support further investigation of ozone therapy as a redox-modulating strategy rather than an acute glucose-lowering intervention.

Declarations

Author Contributions

Conceptualization, T.D. and F.M.S.; methodology, T.D., I.R.B., and F.M.S.; validation, T.D. and A.L.E.; formal analysis, E.S.; investigation, E.S., T.D. and F.M.S.; resources, T.D. and I.R.B.; data curation, E.S. and F.M.S.; writing—original draft preparation, E.S. and F.M.S; writing—review and editing, T.D., A.L.E., and I.R.B.; visualization, F.M.S.; supervision, T.D., A.L.E., I.R.B., F.M.S.; project administration, T.D. All authors have read and agreed to the published version of the manuscript.

Data Availability Statement

Data is contained within the article.

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Institutional Review Board Statement

The animal study protocol was approved by the Ethic Committee of School of Medicine and Health Sciences, Atma Jaya Catholic University of Indonesia (Approval no. 02/10/KEP-FKIKUAJ/2025, approval date Oct 6th, 2025).

Informed Consent Statement

Not Applicable

Conflicts of Interest

The author declares that there is no conflict of interests regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancies have been completely observed by the authors.

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