Vitamin D and Insulin Response in Streptozotocin-Induced Diabetic Rats: An In Vivo Investigation

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Background: Type 2 diabetes mellitus (T2DM) is a widespread global health problem, defined by increasing insulin resistance and gradual deterioration of β-cell function. Vitamin D is posited to influence glucose metabolism, although the evidence about its impact on insulin dynamics is inconsistent. **Objective:** The aim of this study was to assess the effect of vitamin D supplementation on insulin levels in streptozotocin (STZ)-induced diabetic rats. **Methods:** An in vivo experimental design was executed involving 25 Sprague Dawley rats, which were randomly allocated into five groups: Group A (negative control), Group B (healthy rats receiving vitamin D at 415 IU/day), Group C (T2DM without supplementation), Group D (T2DM receiving vitamin D at 415 IU/day), and Group E (T2DM receiving vitamin D at 1,100 IU/day). Diabetes was established in Groups C-E using a high-fat diet for three weeks, followed by an intraperitoneal injection of STZ. Vitamin D supplementation was administered orally via gavage for a duration of 30 days. Plasma levels of insulin and vitamin D were quantified utilizing ELISA. Data were evaluated utilizing ANOVA accompanied by Bonferroni post hoc testing. **Results:** Insulin levels exhibited substantial variation among groups ($p=0.022$). Compared with Group C ($p = 0.004$) and Group D ($p = 0.005$), Group B exhibited a pronounced elevation in insulin concentrations. Group E had a propensity for elevated insulin levels relative to the diabetes control; nevertheless, the difference lacked statistical significance ($p=0.224$). No substantial association was identified between vitamin D and insulin levels ($p=0.415$, $r=0.171$). **Conclusion:** Vitamin D treatment markedly increased insulin levels in healthy rats, although did not stimulate insulin secretion in diabetic rats. These results imply that the stimulatory role of vitamin D is more evident in healthy conditions or initial stages of metabolic dysfunction, but less effective once β-cell damage becomes severe.

Keywords: Insulin, Rat model, Streptozotocin, Type 2 diabetes mellitus, Vitamin D, β-cell function

Diabetes mellitus (DM) is a complex chronic metabolic condition that involves various organ systems and persists over time. It is primarily defined by prolonged hyperglycemia arising from insufficient insulin production, reduced insulin effectiveness, or a combination of these mechanisms [1,2]. Among its subtypes, type 2 diabetes mellitus (T2DM) is the most prevalent, accounting for about 95% of cases worldwide [1,3]. This form of diabetes is marked by progressive insulin resistance, declining insulin secretion, and, in some cases, relative insulin deficiency. The disease typically develops gradually and often presents without obvious early symptoms, making its diagnosis and management more challenging [2].

The worldwide prevalence of T2DM is on the increase. The NCD Risk Factor Collaboration (2022) estimates that 828 million individuals are currently diagnosed with DM, with projections indicating a significant increase by 2050 [1,3]. It is important to highlight that the occurrence of T2DM is not limited to older adults anymore; it has also escalated among younger individuals (under 40 years), showing a two- to three-fold rise in recent decades [1]. The main mechanisms of pathophysiology associated with T2DM include dysfunction of β-cells, which results in inadequate insulin secretion, as well as insulin resistance observed in peripheral tissues like muscle, adipose tissue, and liver. These disturbances ultimately interfere with glucose homeostasis and lead to chronic hyperglycemia [1].

Beyond persistent hyperglycemia, T2DM is associated with severe complications including cardiovascular disorders, renal failure, visual impairment, cognitive decline, and limb amputations, all of which substantially contribute to increased morbidity and mortality [3]. These complications underscore the critical need to investigate innovative preventive and therapeutic approaches for T2DM.

Growing evidence supports the idea that vitamin D influences glucose regulation. Traditionally valued for its role in calcium and bone physiology, it is now increasingly recognized for possible anti-diabetic properties. These include enhancing insulin secretion and sensitivity, supporting pancreatic β-cell function, and exerting anti-inflammatory as well as immunomodulatory effects [4,5].

Epidemiological evidence indicates that inadequate vitamin D status increases the likelihood of developing T2DM, whereas optimal levels appear to support insulin responsiveness and β-cell activity. However, findings from intervention studies on vitamin D supplementation remain inconsistent. Several trials have reported that supplementation, even at varying doses, does not significantly reduce the risk of developing T2DM [4]. Nonetheless, additional studies evaluating indicators associated with T2DM, instead of the incidence itself, have yielded varying results. Mitri et al. (2011) showed that taking cholecalciferol at a dosage of 2,000 IU/day for 16 weeks led to enhancements in insulin secretion [6]. In a similar vein, Lemieux et al. (2019) discovered that a daily supplementation of 5,000 IU

improved peripheral insulin sensitivity and the function of pancreatic β-cells [7]. Evidence from animal studies further supports this association, indicating that supplementation with vitamin D at varying doses contributes to reduced blood glucose levels in rats with induced T2DM [8].

Due to the variability in existing results, mo investigation is warranted to determine the role of vitamin D supplementation in insulin regulation. In this study, we examine whether vitamin D administration alters insulin levels in rats with T2DM.

This investigation was conducted through in vivo experimental methods. The experimental procedures adhered to the established protocol from our prior study[8], with variations solely in the measured variable.

The subjects of the experiment consisted of white rats (Rattus norvegicus) from the Sprague Dawley strain, aged between 8 to 12 weeks and weighing between 150 to 200 grams. The animals were kept in laboratory cages with regulated environmental parameters (temperature, light, and humidity) and were given a diet based on their group assignment, along with access to drinking water at all times.

A total of 25 rats were used and randomly assigned into five groups as follows:

- Group A : Negative control
- Group B : Non diabetic rats + vitamin D supplementation (415 IU/day)
- Group C : Diabetic rats without vitamin D supplementation
- Group D : Diabetic rats + vitamin D supplementation (415 IU/day)
- Group E : Diabetic rats + vitamin D supplementation (1100 IU/day)

The induction of T2DM in Groups C, D, and E was commenced by administering a high-fat diet for a duration of three weeks. Subsequently, rats in the designated groups received a single intraperitoneal injection of streptozotocin (STZ) at 50 mg/kg BW. Ten days later, blood glucose was measured, and animals with levels above 200 mg/dL were considered successfully induced. Group A and B rats were administered standard feed and treatments specific to their designated groups.

Vitamin D supplementation was provided following the successful induction of T2DM. Supplementation was administered through oral gavage over a period of 30 consecutive days. The dosage selection was informed by a prior study involving rats, which showed that supplementation with vitamin D at 12.5-25 µg/kg BW for 14 days resulted in an increase in GLUT 4 expression compared to the control group [9].

The doses used were adjusted according to experimental requirements in animals using the animal Equivalent Dose (AED) conversion formula from human to rat as follows:

AED (mg/kgBW) = human dose (mg/kgBW) x Km ratio

with a Km ratio of 6.2 for rats. According to these formulas, 415 IU/day in rats translates to 4,000 IU/day in humans, whereas 1,100 IU/day in rats equates to 10,000 IU/day in humans. fter 30 days of vitamin D supplementation, the rats were euthanized, and blood samples were collected to measure plasma insulin and vitamin D levels.

Blood samples were centrifuged at 300 rpm for 10 minutes. Quantitative analysis of plasma vitamin D and insulin levels was performed using ELISA kits (Cat. No. E-EL-0014, Elabscience®, USA; Cat. No. E-EL-3034, Elabscience®, USA) following the manufacturer's instructions.

Data analysis was carried out using SPSS version 26.0. The Shapiro–Wilk test was used to assess normality, while Levene's test evaluated homogeneity of variance. For normally distributed and homogeneous data, comparisons among groups were performed using one-way ANOVA followed by post hoc testing.

The findings revealed a notable variation in insulin levels across the groups (p=0.022). Further analysis using the Bonferroni post hoc test revealed that insulin concentrations in Group B (healthy rats given 415 IU/day vitamin D) were significantly greater than those observed in Group C (diabetic rats without vitamin D, p = 0.004) and Group D (diabetic rats supplemented with 415 IU/day vitamin D, p = 0.005). While the comparison between Group C and Group E (diabetic rats + vitamin D supplementation at 1,100 IU/day) did not achieve statistical significance (p=0.224), a trend towards elevated insulin levels in Group E was observed. In summary, Group E exhibited elevated insulin levels in comparison to Groups C and D, yet this variation failed to achieve statistical significance (Figure 1).

The analysis showed no significant correlation between vitamin D and insulin levels (p = 0.415), although a weak positive relationship was observed (r = 0.171) (Figure 2). Consequently, while there

appeared to be a trend indicating that elevated vitamin D levels correlated with higher insulin levels, the association was not robust enough to achieve statistical significance according to our analysis.

This study demonstrated that non-diabetic rats supplemented with vitamin D (Group B) exhibited a significant increase in insulin levels. The observed outcome can be attributed to vitamin D's direct action on pancreatic β-cells. Specifically, 1,25-dihydroxyvitamin D3 has been demonstrated to improve glucose-stimulated insulin secretion (GSIS) through regulation of voltage-gated calcium channels, a process mediated by the vitamin D receptor (VDR) found in human and rat β-cells. [10,11]. VDR activation promotes calcium influx via R-type VGCCs, a key step in the insulin release process (Lilja et al., 2018). Pancreatic β-cells also have the capacity to produce active vitamin D locally, as they express the enzyme 1α-hydroxylase (CYP28B1), which transforms 25-hydroxyvitamin D into its active form, 1,25-dihydroxyvitamin D. Through autocrine and paracrine mechanisms, this process enhances vitamin D's influence on β-cell regulation[11–13].

Experimental evidence supports this mechanism. A study by Bornstedt (2019) demonstrated that vitamin D deficiency in rats reduced glucose-stimulated insulin secretion, which was restored upon vitamin D administration. This finding highlights that vitamin D is not only essential for normal β-cell function but also plays a correlative role in deficiency states. Thus, the increase in insulin levels observed in healthy rats supplemented with vitamin D in this study is most likely derived from a combination of VDR activation that enhances insulin release (including through regulation of VGCC and related gene transcription) and the pancreas's ability to generate active vitamin D locally. These findings are consistent with existing literature emphasizing the crucial role of vitamin D in maintaining β-cell function and glucose homeostasis[14].

In diabetic rats supplemented with vitamin D (both moderate and high doses), a trend toward increased insulin levels was observed compared with the non-supplemented diabetic group (C). However, these results did not reach statistical significance. This can be explained by the pathophysiology of T2DM, which involves both β-cell dysfunction and insulin resistance. Under such conditions, metabolically compromised β-cells exposed to oxidative stress may not respond optimally to vitamin D stimulation. Nevertheless, studies in prediabetic animal models have shown relevant mechanistic effects of vitamin D. Supplementation with vitamin D_3 (100 and 1000 IU/kg BW) in prediabetic rats significantly reduced insulin resistance, improved glycemic control parameters (FBG, OGTT, HOMA-IR), and preserved the integrity of pancreatic islets [15]. Proposed mechanisms include modulation of insulin signaling pathways such as reduced IRS1 (Ser307) phosphorylation, increased PPARγ expression, decreased NF-κB activity, and improved pancreatic islet structure [15,16].

Moreover, vitamin D contributes to glucose homeostasis by promoting insulin secretion through various molecular mechanisms, including the regulation of intracellular calcium dynamics and modulation of insulin signaling pathways [6,17]. Type 2 diabetes is defined by peripheral insulin resistance accompanied by a gradual decline in pancreatic β-cell function. At advanced stages of T2DM, recovery of insulin secretion becomes increasingly difficult due to β-cell damage accompanied by oxidative stress, chronic inflammation, and apoptosis. Although vitamin D possesses anti-inflammatory and antioxidant properties and may support cellular regeneration via mechanisms such as enhanced autophagy and protection of β-cells from apoptosis [18]. These effects suggest that vitamin D interventions may be more beneficial as preventive strategies ("early-stage intervention") rather than as monotherapy in advanced diabetes.

The results of this study suggest that vitamin D levels are not significantly associated with insulin concentrations ($p = 0.415$), accompanied by a very weak correlation coefficient ($r = 0.171$). While a positive trend was noted, suggesting that increased vitamin D levels were somewhat linked to elevated insulin levels, the correlation lacked sufficient strength to achieve statistical significance in this scenario. Similar findings were reported by Haidari et al. (2010), who also observed no significant association between serum vitamin D and insulin levels ($r = 0.05$, $p > 0.05$) [19].

Vitamin D is essential for insulin secretion, acting through several molecular pathways. Its active form, 1,25-dihydroxyvitamin D, regulates intracellular calcium levels in pancreatic β-cells, a process critical for insulin exocytosis. This involves the activation of protein kinase A (PKA) and phospholipase C (PLC), which stimulate calcium release from the endoplasmic reticulum and open voltage-dependent L-type calcium channels, thereby

promoting the release of insulin into the bloodstream [15,17]. Nevertheless, empirical evidence from clinical and epidemiological studies has shown inconsistent associations between vitamin D and insulin or insulin resistance. For instance, a meta-analysis showed that vitamin D is typically weakly and negatively correlated with insulin and HOMA-IR, with correlation coefficients between –0.08 and –0.06 [20]. In patients with T2DM, one study reported a significant positive association between vitamin D levels and β-cell function (HOMA-B), along with a negative correlation with insulin resistance (HOMA-IR) [21]. Even so, findings from other research highlight that although vitamin D tends to be inversely related to insulin resistance, its supplementation shows mixed and uncertain effects on glycemic control and insulin sensitivity in human populations [20,22].

In summary, the weak and non-significant correlation ($r = 0.171$) identified in this study is consistent with wider findings in the field. This suggests that while vitamin D may theoretically impact insulin secretion and sensitivity, its measurable effects are probably modest and significantly influenced by physiological state, metabolic status, vitamin D dosage, and individual variability. Furthermore, in instances where vitamin D or insulin levels are within normal or close to normal ranges, the impact of supplementation might be too subtle to achieve statistical significance.

The results showed that vitamin D supplementation led to a marked increase in insulin levels among healthy rats, supporting its stimulatory influence on β-cell activity under physiological conditions. However, in rats with STZ-induced diabetes, vitamin D supplementation at either 415 IU or 1100 IU/day failed to yield meaningful differences in insulin concentrations relative to diabetic controls. Additionally, analysis revealed no significant association between vitamin D status and insulin levels overall ($p = 0.415$; $r = 0.171$). The results indicate that while vitamin D may serve as an immunomodulatory and metabolic agent, its influence on insulin secretion appears to be more significant in scenarios where there is no severe β-cell damage or in the initial phases of metabolic dysfunction.

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