Can Vitamin D be a potential treatment for Type 2 diabetes mellitus

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Abstract

Diabetes has become the next most widespread epidemic after cancer and extensive research is being carried out to develop drugs for its therapeutics. Recent studies have found that deficiency of Vitamin D results in reduction in insulin secretion and thus in hyperglycemia. Both insulin secretion and sensitivity depends upon intracellular calcium concentration also and Vitamin D is one of the hormone which has been found to regulate calcium flux within the cells. In both observational and case-control studies, an inverse relationship has been reported with level of 25(OH)Vit D and degree of glycemic control. Therefore, in the present review, an attempt is being made to present scientific evidence of linkage of Type 2 diabetes with Vitamin D levels in order to explore the possibility of Vit D as an add-on therapy to the existing treatment to strive near normo-glycemia.

Keywords

Diabetes, Vitamin D; Calcium homeostasis

Figures and tables from this article:

![Figure 1: Mechanism of synthesis of Vitamin D3 in skin.](image-url)
Diabetes, pancreatic cancer and vitamin D. Is there a link?

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Abstract

The role of vitamin D is not merely limited to maintaining skeletal health but also extends to maintaining glucose homeostasis by preserving insulin secretion and sensitivity and thus deficiency of vitamin D plays an important role in aetiopathogenesis of T2 diabetes. In addition to its many other roles, vitamin D has recently been found to have growth inhibiting affects on pancreatic cancer cells. Ecological studies have shown that there exists an inverse correlation between sun exposure and death rates for pancreatic cancer. Since vitamin D has promising role in both type 2 diabetes mellitus and pancreatic cancer, its deficiency may be associated to any or both of these chronic diseases. The present review thus aims to find correlation between diabetes and pancreatic cancer and if vitamin D is a common link between the two.

Highlights

- Hyperglycemia or hyperinsulinemia can be a cause of both diabetes and pancreatic cancer.
- Vitamin D can have a role in treating diabetes, by maintaining required intracellular calcium concentration and thus proper insulin secretion/sensitivity.
- It can also influence growth factors that promote tumor growth in pancreatic cancer.
- Vitamin D can inhibit pancreatic cancer cell growth by activation of p21 and p27, which influence cell cycle progression and arrest cells in G0/G1.

Keywords

Type 2 diabetes; Vitamin D; Pancreatic cancer
Effect of 1, 25(OH)2 vitamin D3 on glucose homeostasis and DNA damage in type 2 diabetic mice

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ABSTRACT

Aims: The purpose of the study was to examine the effect of 1, 25(OH)2 VitaminD3 supplementation on type 2 diabetic (T2DM) mice.

Materials and Methods: A total of 24 mice were taken and divided into three groups of control; diabetic and diabetic + vitamin D supplemented ones. Serum calcium level, fasting blood glucose level (FBG), hexokinase activity, glucose-6-phosphatase and fructose 1,6 bisphosphatase activity were measured to establish a relevant correlation between vitamin D supplementation and hyperglycemia in T2DM.

Results: There occurred an increase in FBG levels (250 ± 0.41 mg/dl) and a significant decrease in serum calcium levels in the diabetic group (8.63 ± 0.40 mg/ml) both of which reached near control levels on vitamin D3 supplementation. The activity of the glucose metabolic enzymes was also assayed in diabetic group and was found to be deviated from control group; hexokinase (0.0241 ± 0.014 pg/mg/ml), FBPase (0.433 ± 0.002 Mg/mg/ml) and G6Pase (0.918 ± 0.02 ng/mg/ml). However, the activity of these enzymes returned to near control values with hexokinase activity reaching 0.717 ± 0.003 Hg/mg/ml on vitamin D3 supplementation. The FBPase and G6Pase activities were decreased to 0.2733 ± 0.008 ng/mg/ml and 0.71 ± 0.01 1g/mg/ml respectively. In addition to enzymatic analysis, the organs of all three groups of mice were subjected to comet assay. The diabetic group receiving vitamin D supplementation showed a marked recovery exhibiting shorter tail length both in liver (21.80 ± 2.40 pm) and pancreatic cells (19.25 ± 1.90 pm) as compared to the diabetic group exhibiting a tail length of 30.41 ± 2.50 pm and 32.45 ± 2.87 pm in liver and pancreatic cells respectively.

Conclusion: The present study shows that vitamin D3 supplementation is positively correlated with decrease in blood glucose level and serum calcium level in fasting condition. This suggests a positive influence of vitamin D on glucose homeostasis. Besides, the activity of various glucose metabolic enzymes (hexokinase, FBPase and G6Pase) as shown by our results and the remarkable shortening of DNA tail length in vitamin D3 supplemented diabetic group as compared to diabetic group without supplementation further support the idea that vitamin D supplementation might be an add-on therapy for patients with T2DM.

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1. Introduction

Globally, the number of people with diabetes is expected to rise from the current estimate of 150 million to 220 million in 2010 and 300 million in 2025. Type 2 diabetes is the predominant form of diabetes worldwide, accounting for 90% of cases globally. Evidence has accumulated from observational studies and randomized controlled trials, on the beneficial effects of good blood glucose control in the prevention of micro-vascular and macro-vascular damage in people with diabetes. The pathogenesis of T2DM is complex, therefore from a pathophysiological standpoint; persons with T2DM consistently demonstrate three cardinal abnormalities: resistance to the action of insulin in peripheral tissues, defective insulin secretion particularly in response to glucose and increased glucose production by liver.

Vitamin D3's importance to the human body has been recognized for almost two centuries, although our understanding of its influence on the overall human health is relatively recent and still expanding. The discovery of the receptors for 1α, 25-dihydroxyvitamin D3 (1,25(OH)2D3), the activated form of vitamin D, in tissues with no direct role in calcium and bone metabolism (e.g. pancreatic beta cells) has broadened our view of the physiological role of this molecule (Holick, 2003; Haussler, Whitfield, & Haussler, 1998). Biologically active vitamin D in addition to its classical actions also acts as a "gene switch" to regulate the transcription of specific target genes.
genes within the human genome including the insulin gene where the presence of vitamin D response elements has recently been elucidated. This finding suggests that the functions of vitamin D might extend to prevent impaired insulin secretion and sensitivity (Boucher, Mantan, Noonan, Hales, & Evans, 1995). A significant decrease has been reported in the levels of 1, 25(OH)\(_2\) vitamin D\(_3\) in type 2 diabetics both in human (Bouillon, Verstruyf, Verlinden, Eelen, & Mathieu, 2003) and animal models. Studies from different parts of India have shown widespread vitamin D deficiency in all age groups (Isaia, Giorgino, & Adami, 2001) and that Women who consumed 800 IU or more of total vitamin D per day had a 23% lower risk for development of incident diabetes compared with women who consumed ≤200 IU/day and those who consumed ≤400 IU/day vitamin D from supplements compared with women who consumed ≤100 IU/day had a 13% lower risk of diabetes (Scragg, Sowers, & Bell, 2004). Therefore, these studies suggest that vitamin D might play a role in hyperglycemia. Vitamin D deficiency may, therefore, be involved in the pathogenesis of T2DM (Kadowaki & Norman, 1984), and a better understanding of the mechanisms involved could lead to the development of preventive strategies.

In this study we have estimated the changes in the fasting blood glucose and calcium levels in diabetic groups given vitamin D\(_3\) supplementation and those not given the supplementation. In order to be more precise, we also assayed the activity of hepatic glucose metabolic enzymes like hexokinase, G6Pase and FBPase. Since diabetes affects and damages almost all major organs of the body, we also observed the extent of nuclear DNA damage in hepatic and pancreatic tissues of diabetic group and its recovery in the organs of vitamin D\(_3\) supplemented group.

2. Materials and methods

2.1. Study population

The present study was conducted on female albino mice at the Department of Biochemistry, Aligarh Muslim University. The mice were divided in three groups of 8 mice each. The first group was kept as control (C) and no treatment was given to it. To the second group an intra-peritoneal injection of alloxan (200 mg/kg body weight) was given and their fasting blood glucose (FBG) was monitored till they became diabetic (D). The third group was subjected to the same administration of alloxan and after it was confirmed that mice had become diabetic (D). The diabetes state was induced by giving an intra-peritoneal injection of 300 mg/kg alloxan. The establishment of diabetic state was further carried out by adding 200 mg/kg of alloxan. The diabetic state was confirmed by giving the diabetes state to the mice by the method of Trinder (1969) using Ranbaxy Diagnostic kit. Briefly 10 μl of the fasting blood samples was mixed with 1 ml of working solution (containing 6.7 U/ml of glucose oxidase, 6.2 U/ml of horseradish peroxidase, 0.2 Mm of 4-aminoantipyrine, 8 M of phosphate buffer and 86 mM of phenol). The blank and standard solutions were also prepared simultaneously by adding 10 μl of distilled water and 10 μl of standard glucose (100 mg/dl) to 1 ml of working solution. All the tubes were mixed well and incubated at room temperature for 30 min. Blank solution was used to set the spectrophotometer and absorbance was read at 505 nm.

2.2. In vivo 1, 25(OH)\(_2\) vitamin D\(_3\) administration

One group of vitamin D deficient mice was made diabetic by administration of alloxan (Sigma-aldrich, US.). The diabetic state was induced by giving an intra-peritoneal injection of 300 μl containing 200 mg/kg of alloxan. The establishment of diabetic state was further confirmed by estimating fasting blood glucose level using a glucometer. This group was termed as diabetic group (D).

Another group of vitamin D deficient diabetic mice received 1,25(OH)\(_2\)D\(_3\) intra-peritoneally (Sigma-aldrich, US.) for 15 days after they had become diabetic. This treatment consisted of 7 ng/gm/day of 1, 25(OH)\(_2\)D\(_3\) dissolved in propylene glycol given intra-peritoneally for 15 days. This group was termed as treated group or (D + Vit D).

2.3. Sample collection

Overnight fasting mice were sacrificed by cervical dislocation at the end of 15 day period and their blood and tissue samples were collected for analysis. 2–3 ml blood was collected in heparinised centrifuge tubes which were immediately centrifuged at 1000 × g for 15 min. Furthermore, the serum parameters were analyzed on the same day while the tissues (liver and pancreas) were kept in Heps buffer at – 20 °C for further enzyme analysis.

2.4. Biochemical analysis

Fasting blood glucose and serum calcium levels were analyzed in the treated animals. Activity of glucose metabolic enzymes like Hexokinase, G6Pase and FBPase was also analyzed from the liver tissues collected.

2.5. Fasting blood glucose estimation

Glucose levels were estimated in collected serum samples by glucose oxidase–peroxidase method of Trinder (1969) using Ranbaxy Diagnostic kit. Briefly 10 μl of the fasting blood samples was mixed with 1 ml of working solution (containing 6.7 U/ml of glucose oxidase, 6.2 U/ml of horseradish peroxidase, 0.2 Mm of 4-aminoantipyrine, 8 M of phosphate buffer and 86 mM of phenol). The blank and standard solutions were also prepared simultaneously by adding 10 μl of distilled water and 10 μl of standard glucose (100 mg/dl) to 1 ml of working solution. All the tubes were mixed well and incubated at room temperature for 30 min. Blank solution was used to set the spectrophotometer and absorbance was read at 505 nm.

2.6. Serum calcium estimation

Serum calcium levels were estimated by using alkaline murexide reagent (Sare, 1964). To 1.5 ml of color reagent (containing 50 ml of stock color reagent and 1.5 ml of 3.75 N KOH) 2.5 ml of distilled water and 0.05 ml of serum sample were added. The stock color reagent was prepared by dissolving 40 mg of ammonium purpurate in 5 ml of distilled water and then diluting it with 500 ml of propylene glycol. The blank and the standard solutions were also prepared simultaneously by adding 2.55 ml and 2.5 ml of distilled water respectively to 1.5 ml of color reagent. To make up the volume 0.05 ml of standard calcium (0.1 mg/ml) was added to standard solution. The tubes were capped with Parafilm and mixed thoroughly. The tubes were then allowed to stand for next 5 min and were finally read at 490 μm.

2.7. Hexokinase activity

The hexokinase activity was measured by the method of Crane and Sols (1953). The liver tissues were collected and homogenized in sodium phosphate buffer using table top homogenizer (REMI, U.S.A.). The reaction was carried out at 37 °C by adding 1 ml reaction mixture containing Tris Hcl (50 μm), MgCl\(_2\) (10 μm), ATP (5 μm), glucose (2 μm) is added to 1–1.5 mg of sample protein. The reaction was stopped after 1 h using 10% barium hydroxide and 10% zinc sulphate solutions. The samples were centrifuged at 2000 × g for 10 min and glucose was estimated in supernatant (free from phosphorylated derivatives). The glucose estimation was then done by the method of Nelson (1994), with standard glucose solution ranging between 5 and 45 μg.

2.8. FBPase activity

The liver FBPase activity was measured by the method of Freedland and Harper (1959). The reaction was carried out by adding
2.10. Comet assay

1.5 ml of reaction mixture containing Tris HCl (50 μM), MgCl₂ (10 μM), Cysteine HCl (12 μM), Fructose 1,6 diphosphate (10 μM) to sample containing 0.6-0.8 mg. The reaction mixture was stopped by adding 1 ml of 10% TCA after 60 min and the samples were centrifuged at 2000 x g for 10 min. The phosphate released was estimated by the method of Tausky and Shorr (1953).

2.9. G6Pase activity

It was assayed according to the method of Shull, Ashmore, and Mayer (1956). The reaction mixture in a total volume of 1.5 ml contained Tris-Hcl buffer (50 μM); MgCl₂ (10 μM); Glucose-6-Phosphate (10 μM) and 2–3 mg sample protein. The reaction was carried out at 37 °C and stopped with 1 ml of 10% TCA after 60 min. The samples were centrifuged at 2000 x g and phosphorous was estimated in the protein free supernatant by the method of Tausky and Shorr (1953).

2.11. Statistical analysis

Results are expressed in mean ± SEM for all continuous variables. Differences between the control, diabetic and diabetic + vitamin D supplemented groups are assessed using ANOVA followed by Student's t-test. For all the tests the p-value ≤ 0.05 was considered to be statistically significant. All the analysis was performed using SPSS and Origin 6.1.

3. Results

3.1. 1.25(OH)₂vitamin D₃ treatment causes a decrease in FBG and serum calcium levels in diabetic mice

Figs. 1 and 2 show the fasting blood glucose levels and serum calcium levels in all the three groups (C, D, D + VitD). The FBG is seen to be increased in the diabetic group (250 ± 0.41 mg/dl) marking the proper establishment of diabetic state as compared to the control group (160.97 ± 0.21 mg/dl). The diabetic group given vitamin D supplementation shows a significant decrease in the FBG levels (150 ± 0.37 mg/dl) thus suggesting its role in pathogenesis of hyperglycemia. Fig. 2 depicts the difference in the serum calcium levels of the three groups of mice. The increase in the serum calcium level in the vitamin D treated group (9.5 ± 0.40 mg/ml) in comparison to the decreased levels in diabetic group (7 ± 0.43 mg/ml) is symptomatic of function of vitamin D in maintaining calcium homeostasis.

3.2. Changes in the enzyme activities of glucose metabolic enzymes by 1,25(OH)₂vitamin D₃ supplementation

Figs. 3, 4A and 4B represent the variation in the activity of glucose metabolic enzymes i.e. hexokinase, G6Pase and FBPase respectively. The activity of hexokinase was found to decrease in the diabetic group (0.241 ± 0.014 μg/mg/ml) and further increased (0.717 ± 0.003 μg/mg/ml) on vitamin D supplementation. Further the activities of G6Pase and FBPase were analyzed in control group and were found to be 0.533 ± 0.02 μg/mg/ml and 0.10707 ± 0.002 μg/mg/ml respectively. However, there was a significant increase in their activities in diabetic group, the FBPase activity was estimated to be 0.433 ± 0.004 μg/mg/ml and G6Pase activity was found to be 0.918 ± 0.008 μg/mg/ml in diabetic group showing a rapid enhancement of glycolysis. Their activities again decreased FBPase (0.2733 ± 0.008 μg/mg/ml) and G6Pase (0.71 ± 0.01 μg/mg/ml) respectively on vitamin D supplementation.

3.3. 1.25(OH)₂ Vitamin D₃ treatment caused decrement in the DNA tail length in liver and pancreatic samples

Diabetes caused major DNA damage in liver and pancreas as evidenced by elongated tail length in diabetic group as compared to control group. Diabetic group showed an increase in the DNA tail length in liver and pancreatic cells with 30.41 ± 2.50 μm and 32.45 ± 2.87 μm respectively (Figs. 5B and 6B) as compared to control group with tail length 7.65 ± 2.20 μm and 6.66 ± 1.20 μm in liver and pancreatic cells respectively (Figs. 5A, 6A). However, vitamin D
supplemented group showed a significant decrease in liver and pancreatic DNA tail length with values of 21.80 ± 2.40 μm and 19.25 ± 1.90 μm respectively (Figs. 5C, 6C) (Table 1).

4. Discussion

Diabetes, a multifaceted disease characterized by high blood glucose level (Ceriello, 2005) has taken the form of an epidemic in 21st century (Amos, McCarty, & Zimmet, 2010). However, in recent decades there have been significant improvements in patient care but substantial numbers of patients are still not achieving the glycemic control levels specified in current guidelines (World Health Organization (WHO), 1999). Drugs that are presently being used to avert or diminish the onset of diabetes are not good enough, on the contrary, providing drugs is pricey and the side effects require cautious monitoring therefore vitamins offer a promising potential to help in combating diabetes and its complications without showing any side effects.

The major players of T2DM are pancreatic β-cell and insulin but the pathogenic mechanisms by which hyperglycemia arises differ significantly. The characteristic features of T2DM are insulin resistance and decreased insulin secretion (Bell & Polonsky, 2001) which together are dependent on intracelular calcium concentration (Wollheim & Sharp, 1981). Since vitamin D plays a role in maintaining calcium homeostasis it may also be involved in T2DM. Therefore, we have tried to see the role of vitamin D in treatment of diabetes.

Vitamin D has been associated with numerous non-skeletal diseases including T2DM over a decade (Alvarez & Ashraf, 2010). Compared to healthy controls subjects with T2DM have been observed to have significantly lower circulating 25(OH)D concentrations (Scragg et al., 1995). However, from a pathophysiological standpoint, both vitamin D deficiency and T2DM share the same risk factors including increased adiposity, age and physical inactivity (which may translate to decreased time spent outdoors or reduced sun exposure) (Norman, Frankel, Heldt, & Grodsky, 1980). It has also been found that there occurs a seasonal variation in glucose and insulin concentration (De Souza & Meier, 1987) which may correlate to the seasonal variation of vitamin D exposure. In spite of all these findings the mechanism which relates vitamin D deficiency and T2DM remains elusive (Chertow et al., 1983). Our present work is an attempt to shed some light on mechanism of action of vitamin D in T2DM systems.

In the present work we demonstrate the effect 1, 25(OH)2D on glucose metabolism and calcium homeostasis in addition to its involvement in alteration of glucose metabolic pathway. Here, we report that the treatment of 1,25(OH)2D produced significant changes in blood glucose and calcium concentration as well as in the activities of enzymes of glucose metabolic pathway as compared to diabetic group without supplementation. These changes are in accordance with the studies showing the lowering of serum glucose and an increase in serum calcium on vitamin D supplementation (Pittas, Lau, Hu, & Dawson-Hughes, 2007). Vitamin D deficiency causes an increase in PTH (Baynes, Boucher, Feskens, & Kromhout, 1997) thereby leading to an increase in intracellular calcium (De Boland & Norman, 1990) thus lowering its level in plasma, however vitamin D supplementation re-establishes calcium homeostasis.

The explanation for reduced levels of FBG and increased serum calcium in vitamin D treated groups as compared to diabetic can be given on the basis of its molecular action as well as its role in maintaining calcium homeostasis. Since, calcium is essential for proper insulin secretion and sensitivity (Bjorklund, Lansner, & Grill, 2000), sustained elevations of intracellular calcium may inhibit insulin secretion and the ability of insulin-target cells from sensing the brisk intracellular calcium fluxes necessary for insulin action, such as glucose transport (Worrall & Olefsky, 2002) resulting in peripheral insulin resistance and thus increased plasma blood glucose levels. Vitamin D binds to its receptor (VDR) on the surface of pancreatic β-cells (Vidal, Ramana, & Dusso, 2002). The ligand bound receptor then enters the cell and forms a heterodimer with retinoid X receptor (RXR) (Lin & White, 2003). This complex may then bind to the vitamin D response elements (VDRE) present in insulin gene promoter (Christakos, Barletta, & Huening, 2003; Maestro, Davila, Carranza, & Calle, 2003) thereby increasing its transcription and
finally increasing the levels of insulin in plasma. This increased insulin secretion can further be a cause of decreased FBG levels in vitamin D treated group.

Another mechanism by which 1,25(OH)$_2$D$_3$ might act in lowering FBG is suggested by its role in maintaining intracellular calcium concentration for adequate insulin secretion and sensitivity both of which are influenced by intracellular calcium concentration (Zemel, 1998). Therefore, the increase in serum calcium levels in vitamin D$_3$ supplemented group is suggestive of the indirect effects of Vitamin D$_3$ on insulin secretion and sensitivity (Bourlon, Faure-Dussert, & Billaudel, 1997), which may be mediated via its important and well-recognized role in regulating extracellular calcium and calcium flux through the β-cell or by its genomic actions.

Our estimations of the enzyme activities of hexokinase, G6Pase and FBPase showed a distinct pattern. The decreased hexokinase activity in diabetic group is an evidence of the fact that there is insufficient amount of glucose reaching the liver even in the state of hyperglycemia (Glinsman, Hem, & Lynch, 1959), whereas increased activities of G6Pase and FBPase showed an almost twofold increase in gluconeogenic pathway which contributes to increased hepatic glucose production and thus hyperglycemia (Magnusson, Rothman, Katz, Shulman, & Shulman, 1992). However, the activities of these enzymes were found to be different in vitamin D$_3$ treated group showing an increase in hexokinase and a decrease in G6Pase and FBPase activities, thereby providing an insight on the effect of vitamin D$_3$ on glucose metabolism which eventually leads to maintenance of glucose homeostasis (Consoli, Nurjhan, Capani, & Gerich, 1989). These results confirm the anti-hyperglycemic properties of 1,25(OH)$_2$D$_3$.

In the present study we report that the supplementation of vitamin D$_3$ caused significant recovery from the diabetes induced tissue damage (liver and pancreas) as shown by vitamin D$_3$ treated diabetic group when compared to diabetic group with no supplementation. We demonstrated it by comet assay of the tissues (pancreas and liver) from the vitamin D treated diabetic animals which exhibited the shortening of nuclear DNA tail length as compared to diabetic group without supplementation. The most valued explanation of vitamin D$_3$ induced recovery shown by the supplemented group can be given on the basis of immune-modulatory and thus anti-apoptotic actions of vitamin D$_3$ (Riachy, Vandewalle, Conte, & Merman, 2002; D’Herbomez, Lefebvre, & Patrou, 2001).

Recent studies have provided conclusive evidence that pancreatic β cells mass is reduced in T2DM and a probable reason for this reduction can be β cell apoptosis (Sempoux, Guiot, Dubois, Moulin, & Rahier, 2001). The mechanism of this β-cell apoptosis is mediated via hyperglycemia. In diabetes, hyperglycemic excursion can elicit β cell production of IL-1β (Maedler et al., 2002), followed by Fas upregulation. In the presence of the caspase-8 inhibitor FLICE inhibitory protein (FLIP), Fas engagement is directed to proliferation. However, excessive glucose stimulation (hyperglycemia) will decrease FLIP, switching this adaptive pathway toward deleterious signals and eventually to diabetes (Maedler et al., 2002). This is the site where vitamin D$_3$ might exert its effect and thereby stop apoptosis. Previous studies have shown that the inhibition of beta cell function (insulin synthesis and insulin secretion) induced by IL-1β or IFN-γ in vitro is prevented by 1,25(OH)$_2$D$_3$(50). Therefore, our results of comet assay are in accordance with the studies showing anti-apoptotic effect of vitamin D$_3$ on pancreatic β cells (Sandler, Buschard, & Bendtzen, 1994).

The explanation for shortening of nuclear DNA tail length in hepatocytes of the vitamin D treated group can be given by the fact that patients with type 2 diabetes have increased circulating levels of
free fatty acids, leptin and TNF-α (Van Steenbergen & Lanckmans, 1995) which are also found to be involved in the pathogenesis of non-alcoholic steatohepatitis (NASH), (Shimabukuro, Zhou, Levi, & Unger, 1998; Hotamisligil & Spiegelman, 1994), further leading to cirrhosis (liver damage) via fibrosis. However, vitamin D supplementation causes a decrease in the cytokine majorly involved in NASH. Vitamin D causes a decrease in the expression of TNF-α (Cantorna, Woodward, Hayes, & De Luca, 1998), and may also help in reducing FFA and leptin by improving glucose homeostasis thereby reducing liver cirrhosis as shown by the shortening of nuclear DNA tail length in liver cells taken from vitamin D supplemented group as compared to diabetic group receiving no vitamin D.

Hence, from the studies carried out, we conclude that the supplementation of 1.25(OH)2 vitamin D to patients with type 2 diabetes will in all probability help to restore glucose homeostasis (normoglycemia) and will thereby reduce the pancreatic and liver damage.

References


Pharmacology of signaling pathways: In type 2 diabetes

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1. Introduction

In Type 2 diabetes mellitus, insulin is present at normal levels but the tissues do not respond to the hormone. This is caused by decreased sensitivity of target tissues to the metabolic effect of insulin [1]. In type 2 or non-insulin-dependent diabetes mellitus, muscle and fat cells become 'resistant' to the actions of insulin and compensatory mechanisms that are activated in the β-cell to secrete more insulin are not sufficient to maintain normal glucose levels within a normal physiological range [2]. Patients with T2DM consistently demonstrate three cardinal abnormalities: defective insulin secretion, particularly in response to a glucose stimulus (β-cell dysfunction); resistance to action of insulin in the peripheral tissues, particularly muscle and fat but also liver (insulin resistance); and increased glucose production by the liver (hyperglycemia). The precise way these factors interact, finally lead to the onset of T2DM [3].

From a pathophysiological standpoint, it is actually the inability of the pancreatic beta cell to adapt to the reductions in insulin sensitivity that occurs over the lifetime of human subjects that precipitates the onset of T2DM [4]. The most common factors that place an increased secretory burden on the beta cell are puberty, pregnancy, a sedentary lifestyle, and an over-eating leading to weight gain.

2. β-Cell dysfunction

The endocrine pancreas contains β cells that secrete insulin in response to elevated glucose levels. Once secreted, insulin performs the function of maintaining normal glucose levels in
the body by interacting with the plasma membrane receptors of a number of different cell types. It is the inability of the β cells to secrete required amounts of insulin in response to circulating levels of glucose, that paves the way for T2DM. The exact mechanism by which glucose stimulates insulin production by β cells is mediated by facilitated diffusion of glucose inside pancreatic β cells via glucose transporter GLUT2 present on the cell surface of pancreatic β cells. The numbers of these transporters are however found to be reduced on the cells of T2 diabetic patients. Glucose after entering β cells is converted to glucose-6-phosphate, by the one of the glycolytic rate determining enzyme, glucokinase [5, 6]. Glucokinase acts as a glucose sensor of the β cells and leads to increased insulin secretion. The entry of glucose to β cells and insulin releases follows a cause and effect pathway. This dose-response relationship leads to more insulin secretion as more and more glucose enters the pancreatic β-cell. The pathway by which glycolysis mediates insulin secretion depends upon the opening and closing of ATP dependent K⁺ channel present inside the cell. The step involving the oxidation of glyceraldehyde-3-phosphate generates NADH which eventually generates ATP. Increase in ATP concentration in β cells leads to blockade of ATP-dependent K⁺ channel causing membrane depolarization followed by increased cytosolic Ca²⁺ concentration which leads to fusion of preformed insulin granules with the plasma membrane and thus insulin secretion [7].

On the other hand, in case of obesity (an underlying cause of T2DM) there occurs β cell compensation such that there is increased insulin secretion even in response to normal glucose levels. This abnormality can only be possible if there is increased β-cell sensitivity in response to glucose. The root cause of this defect can be: (a) an increase in β cell mass and thus presence of more GLUT2 receptors as found in obese individuals [8] or (b) increased expression of hexokinase as compared to glucokinase leading to increased insulin secretion in response to wide range of glucose concentrations, thus resulting in hyperinsulinemia [9].

3. Insulin resistance

Insulin resistance indicates the presence of an impaired biologic response to either exogenously administered or endogenously secreted insulin. It is manifested by decreased insulin stimulated glucose transport and metabolism in adipocytes and skeletal muscles and by impaired suppression of hepatic glucose output [10].

Understanding of the mechanism and signaling of insulin allows the division of the mechanism of insulin resistance into three groups: prereceptor, receptor and postreceptor insulin resistance. Type A insulin resistance is due to a decrease in the number of insulin receptors, whereas patients with type B insulin resistance have circulating auto-antibodies against some portion of their insulin receptors, and this results in impaired binding of insulin to target cells [11]. The majority of insulin resistance is of the postreceptor type due to the extraordinary complexity of the intracellular pathways of insulin action.

A large number of epidemiologic studies showed that insulin resistance arises as the body fat content increases from the very lean to very obese: implying that the absolute amount of body fat has an effect on insulin sensitivity [12].

4. From obesity to insulin resistance

The signaling pathway by which adiposity is linked to insulin resistance is however rather complex. Adipocytes release non-esterified fatty acids (NEFA), tumor necrosis factor α (TNF-α) and hormones like leptin and adiponectin which modulate insulin action in the peripheral tissues thus becoming a major player of insulin resistance [13, 14]. Studies have found out certain agents that may be helpful in nullifying the diseased conditions caused due to imbalance of these factors (TNF, leptin, adiponectin). Vaspin (visceral adipose tissue derived serpin) is an adipocyte serine protease inhibitor that improved insulin sensitivity and glucose homeostasis when administered to obese, insulin-resistant rodents [15]. An acute effect was seen within 1–2 h, and chronic administration was accompanied by normalized gene expression for leptin, resistin, TNF-α and adiponectin.

5. Peripheral insulin resistance

5.1. Skeletal muscle insulin resistance

5.1.1. Non esterified fatty acids

NEFA's being the most critical of all; compete with glucose for substrate oxidation in isolated muscle [16]. The increase in fatty acid metabolism leads to an increase in intramitochondrial acetyl coenzyme A (CoA)/CoA and reduced adenine dinucleotide (NADH)/NAD⁺ ratios, with subsequent inhibition of pyruvate dehydrogenase. The resulting increased intracellular mitochondrial and cytosolic citrate concentrations result in allosteric inhibition of phosphofructokinase, the key regulating enzyme of glycolysis. Subsequent accumulation of glucose 6 phosphate would then inhibit hexokinase II activity, resulting in an increased intracellular glucose concentration and decreased glucose uptake thus resulting in insulin resistance [17]. Increased NEFA concentration inside the cells can also trigger a serine/threonine signaling cascade via fatty acid metabolites like diacylglycerol (DAG) and fatty acyl coenzyme A (fatty acyl CoA). This pathway once activated causes phosphorylation of insulin receptor substrate 1 (IRS1) and insulin receptor substrate 2 (IRS2) thereby decreasing their ability to bind phosphatidylinositol-3-OH kinase (PI(3)K), this interaction is however necessary for glucose transporter 4 (GLUT4) translocation and thus insulin mediated glucose uptake [18].

5.1.2. Triglyceride content

Glucose uptake and intramuscular triglyceride content has been found to follow an inverse relationship. The increase in the triglyceride content occurs due to imbalance of free fatty acid uptake and oxidation, as in case of obese individuals. The major reason for this can be the presence abdominal adiposity as abdominal fat is lipolytically more active thus leading to increased circulating levels of NEFA's, followed by muscular uptake of free fatty acids than more than it can oxidize. The mechanism involved in this mismatch of uptake and oxidation is mediated by malonyl CoA [19]. The increased insulin levels in circulation due to β cell dysfunctions mediate initial increase in glucose uptake by skeletal muscles. This glucose entering the glycolytic pathway causes an increase in citrate levels by activating tricarboxylic acid (TCA) cycle and providing an alternate substrate for acetyl CoA carboxylase (ACC). ACC after activation generates malonyl CoA thus inhibiting carnitine palmitoyltransferase-1 (CPT-1) thus blocking the transfer of fatty acyl CoA into mitochondria for oxidation [20–22]. This accumulation of triglycerides like long chain fatty acyl CoA and diacylglycerol after activation sets several protein kinases into action, leading to serine/threonine phosphorylation of insulin receptor and resulting in decreased IR kinase activity finally causing insulin resistance [23].

5.2. Adipocyte insulin resistance

Adipocyte insulin resistance is actually adipocyte mediated insulin resistance. Adipocytes secrete hormones which regulate and mediate processes like lipid metabolism, lipolysis, and insulin mediated glucose uptake and utilization. The abnormal decrease in
the levels of these hormones adds to the already existing conditions for T2DM. In obesity, the levels of adipocytokine hormones like leptin and adiponectin are decreased while the levels of TNF-a and resistin are found to be increased thus making obesity a cause for T2DM.

5.2.1. Tumor necrosis factor α

TNF, synthesized by adipocytes, is a 26 kD prohormone which undergoes cleavage to give 17 kD soluble form [24-27]. It binds to its receptor p60 and p80 present in most cell surfaces and mediates its actions. Several studies have demonstrated that TNF causes insulin resistance in 3T3-L1 adipocytes and L6 myoblasts in cell cultures [28-31]. This resistance can be mediated by several pathways, like downregulation of GLUT4, inhibition of insulin mediated IRS-1 phosphorylation, inhibition of tyrosine kinase activation or by inactivating insulin receptor. Studies suggest that one of the mechanism by which TNF plays its role is by downregulating GLUT4 in adipocytes both by destabilizing GLUT4 mRNA and by decreasing GLUT4 transcription [32-36]. Isoferric acid, another potential anti-diabetic drug, has been found to increase muscle glucose transport with increased expression of GLUT4 in insulin-resistant rats. It also inhibits gluconeogenesis by reducing phosphoenolpyruvate carboxykinase [PEPCK] [37]. However, it has been found that the mechanism whereby TNF stimulates serine phosphorylation of IRS-1 plays a central role in conferring insulin resistance.

5.2.2. Leptin

Leptin is also known as an obesity hormone due to its primary physiological function of preventing obesity by regulating food intake and energy balance. Leptin plays a pivotal role in energy homeostasis by decreasing food intake and increasing energy expenditure via hypothalamic centers, affecting feeding behavior and activating the sympathetic nervous system [38]. Leptin is also a signal for adaptation to fasting [39]. Leptin mediates energy homeostasis, body weight, appetite, fat stores or glucose metabolism and the action of insulin [40,41]. However, the presence of central abdominal fat in case of obese individuals causes a decrease in leptin levels and thus paves the way for insulin resistance.

5.2.3. Adiponectin

Adiponectin, another hormone secreted from adipocytes, has insulin sensitizing effects, as it enhances inhibition of hepatic glucose output as well as increase glucose uptake and utilization in fat and muscle. The expression of adiponectin is decreased in obese humans and mice [42]. In humans, adiponectin levels relate with insulin sensitivity and thus their decrease in obesity increases the probability of insulin resistance [43,44]. Drugs like rimonabant are being used to reduce adiposity and improve insulin sensitivity in overweight and obese individuals with and without diabetes by increasing adiponectin production [45].

5.2.4. Resistin

Resistin is another adipocytokine hormone which has inverse relationship with insulin sensitivity. In obese individuals resistin levels are found to be increased thus accounting for decreased insulin sensitivity and high insulin resistance [46].

6. Hepatic glucose production: It's role in insulin resistance

Liver is the main site for glucose synthesis and storage in the form of glycogen which can later be utilized to give energy to carry out various cellular processes. This process of conversion of glycogen into glucose and glucose synthesis by other precursors is strictly controlled by the pancreatic hormone, insulin [47,48].

Insulin can inhibit both glycogenolysis and gluconeogenesis by activating and de-activating enzymes involved in these pathways. Insulin suppresses glycogenolysis by increasing phosphodiesterase activating and by changing the assembly of protein phosphatase complexes [49,50]. Gluconeogenesis is controlled by insulin mediated inhibition of phosphoenolpyruvate carboxykinase transcriptional activation via phosphorylation of forkhead transcription factor (FoxO1 and FoxO2) [51-54].

An alternate pathway of insulin mediated hepatic glucose production inhibition is mediated via glucagon and free fatty acids. Insulin causes a decrease in glucagon secretion from pancreatic α cells thereby inactivating and downregulating both glycogenolysis and gluconeogenesis [55]. Insulin also shows its anti-lipolytic effect by decreasing the activity of triacylglycerol lipase and other enzymes in adipocytes. This leads to decreased amount of fatty acids in circulation and thus uptake by liver eventually resulting in reduction in gluconeogenesis and hence glucose production.

In case of obese individuals, insulin mediated inhibition of glucose production is impaired due to the presence of abdominal fat deposits. These deposits are resistant to the anti-lipolytic actions of insulin and thus insulin in unable to inhibit lipoprotein lipase activity resulting in increased levels of free fatty acids in circulation, making them fully available to act as precursors for glucose synthesis in hepatocytes [56-57].

7. Role of central nervous system in T2DM

Central nervous system plays an important role in insulin secretion and glucose homeostasis. Insulin has its receptors not only on peripheral tissues but also on the surface of brain cells where it maintains energy balance [58] and deletion of these receptors from neurons have been found to induce obesity [59] thereby highlighting their probable importance in pathogenesis of T2DM. Any alteration in the number of brain insulin receptors causes an imbalance in the glucose homeostasis, not leaving behind the liver glucose production [60]. These findings thus, clearly suggest that T2DM can also result due to underlying defects in central nervous system [61-64].

8. Insulin signaling abnormalities in insulin resistance

8.1. Insulin signaling

Insulin signaling is initiated through the binding to and activation of its cell surface receptor and initiates a cascade of phosphorylation events, second messenger generation, and protein-protein interactions that result in the diverse metabolic events in nearly every tissue. The insulin receptor consists of two insulin binding α subunits and two catalytically β subunits that are disulfide linked into an α2β2 heterotetrameric complex [65]. Insulin binds to the extracellular α subunits, activating the intracellular tyrosine kinase domain of β subunit. Once receptor β subunit phosphorylates its partner on specific tyrosine residues that may have distinct functions such as stimulation of intramolecular association of signaling molecules such as Shc and Grb, members of the insulin receptor substrate family (IRS1,2,3,4), Shc adaptor protein isoforms, and SIRP (signal regulatory protein) family members, stimulation of mitogenesis and receptor internalization [66]. The insulin receptor β subunit has also been shown to undergo serine/threonine phosphorylation, which might decrease the ability of receptor to autophosphorylate. Intensity however reduces the activation of insulin receptor. The NEFA’s which enter the peripheral tissues change into lipid metabolites like fatty acyl CoA’s and diacylglycerol(DAG), DAG once formed activates novel PKC isoforms that catalyze the serine/threonine phosphorylation...
of the β subunit and thus attenuates insulin signaling, making way for insulin resistance. The activities of a number of protein kinase C (PKC) isoforms that catalyze the serine or threonine phosphorylation of the insulin receptor are found to be elevated in animal models of insulin resistance and in insulin resistant humans [67].

9. Downstream events following insulin receptor phosphorylation

The insulin receptor substrates (IRS's) act as multifactorial docking proteins activated by tyrosine phosphorylation. The IRS proteins have multiple functional domains including Pleckstrin homology (PH) and phosphotyrosine binding (PTB) and Shc domains that interact with other proteins to mediate insulin signaling events. Serine phosphorylation of IRS proteins can reduce the ability of these proteins to attract PI3-kinase, thereby minimizing its activation [68], and also can lead to an accelerated degradation of IRS-1 protein. Based on in vitro studies serine phosphorylation may lead to dissociation between insulin receptors/IRS-1 and/or PI3-kinase activation [69] and thus impaired insulin action. The causes of IRS-1 serine phosphorylation are given in the table below (Table 1) It has also been found that disruption of IRS1 in mice resulted in mild insulin resistance and growth retardation, whereas disruption of IRS2 resulted in β cell failure and secondary insulin resistance Table 2.

10. PI3-kinase activation

The next step in the insulin signaling cascade after IRS phosphorylation requires the involvement of PI3-kinase. Binding of IRS to the regulatory subunit of this enzyme at Src homology 2 domain results in the activation of PI3-kinase which is necessary for insulin action on glucose transport [70], glycogen synthesis [71], protein synthesis [72], antilipolysis, and gene expression and inhibition of the enzyme by fungal inhibitor wortmannin, inhibited all these functions [73-76]. PI3K activation is also necessary for the stimulation of the glucose transporter GLUT4 mediated increase in glucose uptake in insulin sensitive tissues however in case of obesity and similar underlying conditions IRS's fail to bind and activate this second player of the cascade thereby causing insulin resistance and sustained increase in circulating glucose levels in spite of increased insulin secretion from pancreas [77-79]. Not only this, PI3 kinase also activates phosphatidylinositol 3,4,5 triphosphate (PIP3) dependent serine/threonine kinases such as PI dependent protein kinases 1 and 2 (PDK1 and PDK2) which activates the next participant of the pathway Akt and protein kinase c (PKC), therefore an alternative for this important participant of the insulin signaling pathway may be provided by using vanadium salts. These vanadium salts may prolong the TKA of insulin receptors and possibly some post-receptor kinases by inhibition of protein tyrosine phosphatases, especially PTP-1B [80]. They can also enhance glucose transport in skeletal muscle when PI3K signaling is blocked, suggesting an additional, more distal, and effect along the insulin signaling pathway.

11. Akt/PKB as a mediator of insulin action

The phosphoinositides generated by PI3 kinase cause the activation of Akt. These phosphoinositides bind directly to the PH domains of Akt/PKB and through a combination of events uncover the phosphorylation sites coupled with activation of PI-dependent serine/threonine kinases (PKD1, PKD2) which then phosphorylate Akt thereby activating it. Phosphorylation on two regulatory residues, S473 and T308 for Akt1, is critical for complete Akt/PKB activation. PKD1 is not regulated by PI3-kinase directly, but rather is thought to phosphorylate Akt/PKB after polyphosphoinositide binding increases T308 accessibility. Insulin activates Akt/PKB via PI3-kinase dependent phosphorylation in skeletal muscle, [81] adipose tissue, [82] and numerous tissue culture systems. Once activated Akt phosphorylates and activates proteins involved in glucose uptake, GLUT4 translocation, lipogenesis, and protein synthesis, [83] as well as providing protection from apoptosis. Disruption of Akt resulted in insulin resistance and diabetes in mice [84]. Studies have also shown a decrease in IRS-associated PI3-kinase and Akt activity in insulin resistant skeletal muscle [85]. However, omentin: another peptide from adipocytes has the ability to increase insulin-stimulated glucose in adipocytes and Akt phosphorylation in the absence and presence of insulin [86].

12. Signaling events downstream of Akt

Akt phosphorylates and inactivates glycogen synthase kinase 3 (GSK3) [87] thereby activating glycogen synthase leading to insulin mediated glycogen synthesis thus lowering the circulating glucose levels and maintaining normoglycemia. In addition to this, Akt also phosphorylates several proteins resident in GLUT4 containing vesicles [88] which may act as a mechanism of Akt mediated GLUT4 translocation to the cell surface and increased insulin mediated glucose uptake by the peripheral tissues [89,90]. The primary effect of insulin is to stimulate translocation of the glucose transporter GLUT4 from an intracellular pool to the surface of the cells, primarily in skeletal muscles and adipose
tissues. The normal signaling pathway results in the translocation via Akt but in obese individuals this pathway may be blocked at different steps by different biomolecules like NEFA’s, TNF-α, and adipokines. However, all the blockages converge to result in insulin resistance.

13. Incretins

The incretin effect is the process of increasing oral glucose stimulatory effect on insulin secretion as compared to intravenous glucose. These incretins also inhibit hyperglucagonemia and are found to have β-cell anti-apoptotic effects. These functions are however mediated by GLP-1 and GIP in case of humans. Nowadays many biomolecules are being synthesized which have incretin like Q2 effect [91]. Exenatide, a synthetic incretin is similar to naturally occurring incretin (exendin-4) found in the saliva of the Gila monster, is now being used for the treatment of T2DM. Once inside the system exenatide produces GLP-1 like effects and also resistant to degradation by enzyme dipeptidyl peptidase IV (DPP-IV) thus having an increased half-life 2h to carry out the required functions.

Vildagliptin and Sitagliptin are the two DPP-IV inhibitors, administration of which increases the half-life of incretin like molecules and are also found to moderately increase GLP-1 and GIP levels along with reductions in HbA1c levels. Liraglutide, another incretin-like molecule, is a DPP-IV resistant analog of GLP-1 and is shown to cause better lowering of fasting glucose as compared to other incretins. In addition to its beneficial actions another advantage of using liraglutide is the less number of side-effects this biomolecules has, like nausea etc. [91].

14. Conclusion

Insulin resistance, which is a major cause of T2DM can be used as a potential target for developing drugs to possibly treat this metabolic disorder. However, the only problem is the complexity of the insulin signaling pathway which limits the approach to this aspect of treatment. The pharmacology of T2DM has until now depended only on the use of drugs like metformin and thiazolidinediones, but now new areas should be explored using drugs that effect the phosphorylation/dephirosphorylation of insulin receptors, insulin receptor substrates and the factors involved in the downstream signaling pathway of insulin. Molecules improve the glucose/insulin dose-response relationship (incretins) should be made use of, by taking into consideration their side-effects Q3 as well. Development of such drugs will not only provide multiple targeted treatments but also might help in faster recovery of Type 2 diabetes.

References