Review

Vitamin D – cellular Ca²⁺ link to obesity and diabetes

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Abstract

The vitamin D hormone 1,25-Dihydroxyvitamin D₃ (1,25(OH)₂D₃)-induced cellular Ca²⁺ signals regulate apoptosis in adipocytes and insulin secretion from pancreatic β-cells, and low vitamin D status is considered a risk factor for obesity and type 2 diabetes. The anti-obesity effects of 1,25(OH)₂D₃ in mature adipocytes are determined by its activity to generate, via multiple Ca²⁺ signaling pathways, a sustained increase in intracellular Ca²⁺ followed by activation of the Ca²⁺-dependent initiators and effectors of apoptosis. In pancreatic β-cells, 1,25(OH)₂D₃ induces synchronous Ca²⁺ oscillations, which pattern pulsatile insulin secretion from these cells. An increased intake of vitamin D₃ in a high fat diet-induced obesity mouse model is associated with a decreased weight of white adipose tissue due to induction of apoptosis and the improved blood markers related to adiposity, diabetes, and vitamin D status (plasma concentrations of glucose, insulin, adiponectin, 25-hydroxyvitamin D, and 1,25(OH)₂D₃). High vitamin D₃ intake is also effective in increasing the mineral content of growing bone in obese mice via regulatory effects mediated by 1,25(OH)₂D₃ – parathyroid hormone axis. The 1,25(OH)₂D₃-dependent cellular Ca²⁺ signaling can be important for maintaining the normal levels of apoptosis in adipose tissue and insulin secretion from pancreatic β-cells. An increased intake of vitamin D may contribute to the prevention of obesity, type 2 diabetes, and bone disorders associated with these diseases.

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Keywords:

1,25-dihydroxyvitamin D₃; Intracellular Ca²⁺; Apoptosis; Obesity; Type 2 diabetes; Vitamin D status

1. Introduction

The hormone 1,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃) functions via genomic and nongenomic mechanisms in a number of cell types [1-5]. Moreover, paracrine and autocrine modes of action of 1,25(OH)₂D₃ appear to be important in several cell types, including adipocytes and the secretory cells of the pancreas [1, 2, 4]. Vitamin D, a hormone precursor and a micronutrient, is increasingly often considered important for maintaining good health throughout the life and preventing diseases [4, 6]; however, the causality of these claims have not been mechanistically or probabilistically substantiated.

1,25(OH)₂D₃ plays an important role in regulation of cellular Ca²⁺ signaling, which is linked to a number of cellular responses and processes [7-9]. The sustained Ca²⁺ signals triggered by 1,25(OH)₂D₃ have been implicated in regulation of apoptosis, the process which can determine the cell fate in health and disease, including obesity and type 2 diabetes (T2D) [8, 10, 11]. Moreover, 1,25(OH)₂D₃-induced Ca²⁺ signals (Ca²⁺ oscillations) can regulate insulin secretion from pancreatic β-cells [12].

Vitamin D status has been linked to obesity and T2D in observational studies [13-18]. Vitamin D deficiency and insufficiency have been associated with the increased risk of obesity and T2D; however, causality of these associations remains unproven [3-5, 18]. A promising approach for the prevention and treatment of obesity is selective induction of apoptosis resulting in a reduction of the number of fat cells in adipose tissue [7-9, 13, 19]. In the secretory cells, vitamin D appears to be protective against apoptosis due to a transient and localized nature of the Ca²⁺ signals induced by 1,25(OH)₂D₃ [12, 20, 21]. Understanding the role of 1,25(OH)₂D₃ in regulation of cellular Ca²⁺ signaling in obesity and T2D may lead to discovery of the novel

therapeutic and preventive modalities for these diseases.

The purpose of this review is to discuss the role of $1,25(\text{OH})_2\text{D}_3$ in regulation of apoptosis in adipocytes and insulin secretion from pancreatic β -cells, with emphasis on signaling pathways that involve the vitamin D-dependent regulators, initiators and effectors activated via $1,25(\text{OH})_2\text{D}_3$ -induced cellular Ca^{2+} signaling. The potential links of vitamin D status to the prevention of obesity, T2D, and associated disorders are also briefly discussed.

2. $1,25(\text{OH})_2\text{D}_3$ -mediated apoptosis in adipocytes

$1,25(\text{OH})_2\text{D}_3$ regulates the fate of adipocytes via apoptosis, the “programmed” form of cell death [8, 9, 19, 22, 23]. The apoptotic effect of $1,25(\text{OH})_2\text{D}_3$ in adipocytes is determined by its role in regulation of Ca^{2+} signaling through Ca^{2+} channels, which appear to be linked to membrane vitamin D receptors (VDRs) [1, 8, 24, 25]. The possible production of $1,25(\text{OH})_2\text{D}_3$ by adipocytes and the surrounding cells should be also considered.

Cellular Ca^{2+} signaling is critical among the signaling pathways in adipocytes, including signaling pathways linking obesity and T2D [4, 5, 26]. Cellular Ca^{2+} signaling is the main target of the Ca^{2+} regulatory hormone $1,25(\text{OH})_2\text{D}_3$ [1, 8]. Intracellular Ca^{2+} signals have been implicated in triggering and regulating apoptosis, but Ca^{2+} -dependent mediators involved in apoptotic signaling have not been conclusively identified [9, 15].

The increases in concentration of intracellular Ca^{2+} ($[\text{Ca}^{2+}]_i$) occur in the early and late stages of apoptosis [7-11, 15, 16, 22, 25, 27-31]. The essential feature of the apoptotic Ca^{2+} signal is a sustained, prolong, and globalized increase in $[\text{Ca}^{2+}]_i$, reaching elevated, but not cytotoxic levels. The mechanism of intracellular Ca^{2+} in apoptotic pathways involves the activation of

Ca^{2+} -dependent effectors of apoptosis, the Ca^{2+} -dependent calpains and caspases [4, 7, 15, 32].

It has been shown [4, 7-9, 33-35] that a sustained, not reaching cytotoxic level, increase in $[\text{Ca}^{2+}]_i$ initiates apoptosis via activation of the Ca^{2+} -dependent protease μ -calpain followed by activation of the Ca^{2+} /calpain-dependent caspase-12 and, probably, other effector caspases. A low expression of the cytosolic Ca^{2+} binding proteins (e.g., vitamin D-dependent calbindins) decreases ability of the cell to buffer increases in $[\text{Ca}^{2+}]_i$ and, thus, facilitates induction of Ca^{2+} -mediated apoptosis [20, 36, 37]. An induced expression of the intracellular Ca^{2+} buffers (e.g., calbindins by $1,25(\text{OH})_2\text{D}_3$ in certain normal cell types in the intestine, kidney, pancreas, brain, mammary gland) or suppression of the apoptotic Ca^{2+} signal (e.g., by Ca^{2+} channel blockers) can protect against Ca^{2+} -mediated apoptosis [9, 15, 38]. Importantly, $1,25(\text{OH})_2\text{D}_3$ differentially activates the voltage-dependent and voltage-insensitive Ca^{2+} entry pathways and differentially triggers Ca^{2+} release from the endoplasmic reticulum (ER) stores through the inositol 1,4,5-trisphosphate receptor/ Ca^{2+} release channels (IP_3Rs) and ryanodine receptor/ Ca^{2+} release channels (RYRs) in several cell types, including pancreatic β -cells and mature adipocytes [8, 9, 15, 16, 19]. It appears that the induction of $1,25(\text{OH})_2\text{D}_3$ -dependent Ca^{2+} signals is linked to vitamin D receptors (VDRs) associated with the plasma and the ER membranes [7-9].

$1,25(\text{OH})_2\text{D}_3$ induces apoptosis in mature adipocytes via Ca^{2+} -mediated mechanism: activation of Ca^{2+} -dependent calpain and Ca^{2+} /calpain-dependent caspase-12 [19, 22, 25]. Treatment of mature adipocytes with $1,25(\text{OH})_2\text{D}_3$ triggered, in a concentration- and time-dependent fashion, a sustained, prolong increase in the basal concentration of intracellular Ca^{2+} . This increase in $[\text{Ca}^{2+}]_i$ was associated with induction of apoptosis and activation of μ -calpain and caspase-12. Susceptibility of mature adipocytes to the Ca^{2+} -elevating effect of $1,25(\text{OH})_2\text{D}_3$ appears to be determined by the reduced Ca^{2+} -buffering capacity of these cells. These findings

demonstrate that $1,25(\text{OH})_2\text{D}_3$ can induce Ca^{2+} -mediated apoptosis in mature, differentiated adipocytes and that the apoptotic targets activated by $1,25(\text{OH})_2\text{D}_3$ in these cells are Ca^{2+} -dependent calpain and caspase-12.

An increased intake of vitamin D in a high fat diet-induced obesity (DIO) mouse model of pre-T2D, characterized by obese phenotype, elevated blood glucose and impaired glucose tolerance, delayed the development of adiposity and T2D. Mice fed high fat diet with high vitamin D_3 content demonstrated a decreased weight of white adipose tissue depots and improved blood markers related to adiposity, T2D, and vitamin D status [39]. Specifically, fasting plasma glucose and insulin concentrations in DIO mice fed high vitamin D_3 diet were significantly decreased, approaching levels found in the normal-weight, non-obese control, whereas concentration of adiponectin (an insulin sensitizing adipokine) demonstrated an increasing trend. DIO was accompanied by low vitamin D status (a decreased level of 25-hydroxyvitamin D ($25(\text{OH})\text{D}$) and a decreased plasma concentration of $1,25(\text{OH})_2\text{D}_3$. High vitamin D_3 intake induced a significant increase in plasma concentrations of $25(\text{OH})\text{D}$ and $1,25(\text{OH})_2\text{D}_3$, indicating high vitamin D nutritional status and normal vitamin D hormonal status. High vitamin D_3 intake was also effective in normalization (an increase) of the mineral (Ca and P) content of growing bone in obese mice via regulatory effects mediated by $1,25(\text{OH})_2\text{D}_3$ - PTH axis [40]. These findings demonstrate that high vitamin D intake is effective in decreasing blood glucose and insulin in DIO and that the hormonal mechanism of this effect can involve $1,25(\text{OH})_2\text{D}_3$. The findings also imply that increased vitamin D intake may contribute to the prevention of obesity, T2D, and bone disorders associated with these diseases.

3. 1,25(OH)₂D₃, cellular Ca²⁺ signaling and insulin secretion from pancreatic β-cells

There is convincing evidence that 1,25(OH)₂D₃ can regulate insulin secretion from pancreatic β-cells [12, 20, 36]. The central signaling messenger that triggers insulin release is a rapid increase in [Ca²⁺]_i, which results from interactions between nutrient secretagogues with hormones and neurotransmitters [41, 42]. The central role of 1,25(OH)₂D₃ in insulin secretion derives from its effects on Ca²⁺ influx, Ca²⁺ mobilization, and Ca²⁺ buffering in pancreatic β-cells. 1,25(OH)₂D₃ induces rapid (within 5-10 s), synchronous, sinusoidal [Ca²⁺]_i oscillations in the pancreatic β-cells in vitro, which are independent on glucose [12]. The mechanism of these oscillations involves Ca²⁺ entry from the extracellular space and Ca²⁺ mobilization from the intracellular stores. Specifically, 1,25(OH)₂D₃ stimulates Ca²⁺ influx through both voltage-dependent Ca²⁺ channels (VDCCs) and voltage insensitive Ca²⁺ channels (VICCs) as well as Ca²⁺ mobilization from the ER stores through the RYRs, but not through the activation of IP₃Rs. The effects of 1,25(OH)₂D₃ on intracellular Ca²⁺ in pancreatic β-cells appear to be linked to the membrane VDRs associated with the plasma and the ER membranes [24, 38, 43].

Remarkably, 1,25(OH)₂D₃-evoked Ca²⁺ oscillations pattern oscillatory, pulsatile insulin release from the pancreatic β-cells and the amplitude and frequency of the Ca²⁺ oscillations and the insulin release oscillations are proportional to the 1,25(OH)₂D₃ concentration [12, 20, 36]. The physiological significance of the 1,25(OH)₂D₃ effects on Ca²⁺ in pancreatic β-cells (Ca²⁺ oscillations) may be in supporting insulin secretion at a steady-state glucose concentration in blood, e.g., during fasting; 1,25(OH)₂D₃ would then regulate or maintain insulin secretion relatively independently of glucose.

It is important to note that obesity and T2D can be considered as the interrelated diseases

[3, 4, 13]. Epidemiological studies have shown that obesity is a significant risk factor for T2D [6, 18]. Obesity and T2D can arise as a consequence of dysregulation of common endocrine and intracellular signaling pathways determining the fate and functioning of both adipocytes and pancreatic β -cells [4, 5, 13]. In this context, the effects of $1,25(\text{OH})_2\text{D}_3$ on the cellular Ca^{2+} signaling in adipocytes and pancreatic β -cell can be linked to regulation of adipose tissue mass and insulin secretion as well as imply a potential role for the hormone $1,25(\text{OH})_2\text{D}_3$ and vitamin D status in the prevention and treatment of obesity and T2D. On the other hand, it is important to take into consideration that risk factors for the development of obesity and T2D are also determinants of vitamin D status [3, 5, 44].

4. Role of the vitamin D receptor in Ca^{2+} signaling in adipocytes and pancreatic β -cells

Inactivation of the vitamin D receptor (VDR) in VDR knockout mice or using siRNA for VDR has provided insights into the role of the vitamin D signaling pathways in health and disease, including obesity and T2D [45, 46]. VDR-deficient mice (VDR KO mice) develop abnormality similar to the type II rickets patients [47] and are resistant to the diet-induced weight gain [48], while overexpression of VDRs in adipose tissue appears to lead to increased adipose tissue mass [49]. Alterations in expression of Ca^{2+} channels, Ca^{2+} receptors, and Ca^{2+} binding proteins (e.g., in intestine and skin) due to VDR deficiency have been also reported [50]. Furthermore, VDR expression has been shown to be a determinant of insulin secretory capacity [51]. These findings imply the involvement of genomic vitamin D signaling mediated by VDRs in functioning of adipocytes and pancreatic β -cells as well as development of adiposity and T2D. At the same time, these observations underscore that rapid, nongenomic Ca^{2+} signaling (probably, mediated

by membrane VDRs) appears to be uncoupled from genomic vitamin D signaling mediated by nuclear VDRs and that possible interactions of these signaling systems needs to be elucidated. It is also worth mentioning that VDRs occupy over 2700 genomic positions and dramatically change expression of at least 229 human genes [52], and the massive genomic disturbance in VDR-deficient mice could manifest in impaired growth and body weight gain, generalized tissue atrophy, and dysregulation of overall energy balance.

5. Vitamin D status in obesity and diabetes

Epidemiologic evidence has emerged suggesting the role of vitamin D in obesity and T2D, including observational studies that demonstrated the association between vitamin D status, body composition, insulin secretion and resistance to T2D [3, 13, 53, 54]. In these studies, a statistically significant inverse correlation suggesting that a low vitamin D status (concentration of 25(OH)D in blood) is associated with a larger fat mass and a greater risk of weight gain over time as well as development of T2D has been reported. However, randomized controlled trials, including recently published studies, did not provide conclusive causal or mechanistic insights into whether or how vitamin D might regulate body weight, insulin production and secretion, or prevent T2D [55-60]. Confounding effects could explain the association between vitamin D status and obesity or T2D in a non-casual or reverse-casual way [3, 5, 13]. It is also necessary to note that, from a mechanistic point of view, the circulating concentration of the hormone 1,25(OH)₂D₃ is maintained at the precise, narrow level within a broad range of concentrations of 25(OH)D₃ to allow 1,25(OH)₂D₃ to mediate, via VDRs, the cellular responses (genomic and nongenomic) and perform its physiological functions in a fashion similar to that of other steroid

hormones [5]. For example, as discussed in Section 2, it has been demonstrated in a mouse model of diet-induced obesity that normalization (an increase) in concentration of $1,25(\text{OH})_2\text{D}_3$ can prevent an increase of body fat and normalize glucose, insulin, and adiponectin levels in blood [3, 39, 40]. The rational design of vitamin D analogs selectively interacting with membrane VDRs and capable of regulating Ca^{2+} signaling in adipocytes and pancreatic β -cells could lead to a more positive clinical outcome in obesity and T2D.

Conclusions

The studies reviewed here have identified the mechanisms of Ca^{2+} regulatory effects of the hormone $1,25(\text{OH})_2\text{D}_3$ in adipocytes and pancreatic β -cells. $1,25(\text{OH})_2\text{D}_3$ regulates Ca^{2+} -mediated apoptotic pathway in adipocytes: sustained increase in $[\text{Ca}^{2+}]_i \rightarrow \mu\text{-calpain activation} \rightarrow \text{caspase-12 activation} \rightarrow \text{apoptosis}$ (Fig. 1A). The $1,25(\text{OH})_2\text{D}_3$ -activated apoptotic molecular targets in adipocytes are Ca^{2+} -dependent calpain and Ca^{2+} /calpain-dependent caspase-12. Mature adipocytes are susceptible to $1,25(\text{OH})_2\text{D}_3$ -induced apoptosis, apparently, because of their low Ca^{2+} buffering capacity and/or high permeability of the Ca^{2+} entry and Ca^{2+} mobilization pathways.

$1,25(\text{OH})_2\text{D}_3$ regulates insulin secretion from the pancreatic β -cells by inducing Ca^{2+} oscillations that pattern insulin release. The mechanism of the Ca^{2+} oscillations involves Ca^{2+} influx through VDCCs and VICCs as well as Ca^{2+} release from the ER stores through RYRs (Fig. 1B).

The role of $1,25(\text{OH})_2\text{D}_3$ in Ca^{2+} signaling in adipocytes and pancreatic β -cells can be exploited in the discovery and development of vitamin D analogs effective in induction of Ca^{2+} -

mediated apoptosis and regulation of Ca^{2+} -mediated insulin secretion; those analogs may also promote reduction of adipose tissue mass, which would be beneficial for the prevention of T2D. Moreover, low vitamin D status in obesity and T2D may indicate a role of vitamin D in these diseases. Animal and pre-clinical studies will be necessary to confirm the validity of the $1,25(\text{OH})_2\text{D}_3/\text{Ca}^{2+}$ -dependent mechanisms and molecular targets in the apoptotic pathways in obesity and the insulin secretory pathways in T2D.

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References

1. Sergeev IN, Rhoten WB, Spirichev VB. Vitamin D and intracellular calcium. *Subcell Biochem* 1998; 30:271-297.
2. Norman AW, Nemere I, Zhou J, Bishop JE, Lowe KE, Maiyar AC, Collins ED, Taoka T, Sergeev I, Farach-Carson MC. 1,25(OH)₂-Vitamin D₃, a steroid hormone that produces biological effects via genomic and nongenomic pathways. *J Steroid Biochem Mol Biol* 1992; 41:231-240.
3. Christakos S, Hewison M, Gardner DG, Wagner CL, Sergeev IN, Rutten E, Pittas AG, Boland R, Ferrucci L, Bilke DD. Vitamin D: beyond bone. *Ann NY Acad Sci* 2013; 1287:45-58.
4. Sergeev IN. Regulation of apoptosis in adipocytes and breast cancer cells by 1,25(OH)₂-vitamin D₃: a link between metabolic disorders and breast cancer. *Horm Mol Biol Clin Invest* 2013; 14:99-106.
5. Sergeev, I.N. Vitamin D-mediated apoptosis in cancer and obesity. *Horm. Mol. Biol. Clin. Invest* 2014; 20:43-49.
6. Norman AW, Bouillon R. Vitamin D nutritional policy needs a vision for the future. *Exp Biol Med* 2010; 235:1034-1045.
7. Sergeev IN. Vitamin D and cellular Ca²⁺ signaling in breast cancer. *Anticancer Res* 2012; 32:299-302.
8. Sergeev IN. Calcium signaling in cancer and vitamin D. *J Steroid Biochem Mol Biol* 2005; 97:145-151.
9. Sergeev IN. Calcium as a mediator of 1,25-Dihydroxyvitamin D₃-induced apoptosis. *J Steroid Biochem Mol Biol* 2004; 89-90:419-425.

10. Berridge MJ, Bootman MD, Lipp P. Calcium: a life and death signal. *Nature* 1988; 395:645-648.
11. Orrenius S, Zhivotovsky B, Nicotera P. Regulation of cell death: the calcium-apoptosis link. *Nat Rev Mol Cell Biol* 2003; 4:552-565.
12. Sergeev IN, Rhoten WB. 1,25-Dihydroxyvitamin D₃ evokes oscillations of intracellular calcium in a pancreatic β -cell line. *Endocrinology* 1995; 136:2852-2861.
13. Song Q, Sergeev IN. Calcium and vitamin D in obesity. *Nutr Res Rev* 2012; 25:130-141.
14. Vucenik I, Stains JP. Obesity and cancer risk: evidence, mechanisms, and recommendations. *Ann NY Acad Sci* 2012; 1271:37-43.
15. Mathiasen IS, Sergeev IN, Bastholm L, Elling F, Norman AW, Jaattela M. Calcium and calpain as key mediators of apoptosis-like death induced by vitamin D compounds in breast cancer cells. *J Biol Chem* 2002; 277:30738-30745.
16. Sergeev IN, Rhoten WB. Regulation of intracellular calcium in breast cancer cells. *Endocrine* 1998; 9:321-327.
17. Li M, Chen P, Li J, Chu R, Xie D, Wang H. The impacts of circulating 25-hydroxyvitamin D levels on cancer patient outcomes: a systematic review and meta-analysis. *J Clin Endocrinol Metab* 2014; 99:2327-2336.
18. Seida JC, Mitri J, Colmers IN, Majumdar SR, Davidson MB, Edwards AL, Hanley DA, Pittas AG, Tjosvold L, Johnson JA. Clinical review: Effect of vitamin D₃ supplementation on improving glucose homeostasis and preventing diabetes: a systematic review and meta-analysis. *J Clin Endocrinol Metab* 2014; 99:3551-3560.
19. Sergeev IN. 1,25-Dihydroxyvitamin D₃ induces Ca²⁺-mediated apoptosis in adipocytes via activation of calpain and caspase-12. *Biochem Biophys Res Commun* 2009; 384:18-21.

20. Rhoten WB, Sergeev IN. Calbindin-D_{28k} appears to buffer intracellular Ca²⁺ in butyrate-treated rat insulinoma cells. *Endocrine* 1994; 2:989-995.
21. Satin LS, Butler PC, Ha J, Sherman AS. Pulsatile insulin secretion, impaired glucose tolerance and type 2 diabetes. *Mol Aspects Med.* 2015; 42:61-77.
22. Sergeev IN. Vitamin D/calcium-mediated apoptosis in cancer cells and adipocytes. *FASEB J* 2010; 24:917.16.
23. Sergeev, I.N. Vitamin D/Ca²⁺-dependent calpain and caspases: novel molecular targets for obesity-related breast cancer drug discovery. *FASEB J* 2014; 28:261.5.
24. Nemere I, Hintze K. Novel hormone "receptors". *J Cell Biochem* 2008;103:401-407.
25. Sergeev IN. Vitamin D regulates apoptosis in adipocytes via Ca²⁺ signaling. *FASEB J* 2012; 26:386.2.
26. Sergeev IN. Genistein induces Ca²⁺-mediated, calpain/caspase-12-dependent apoptosis in breast cancer cells. *Biochem Biophys Res Commun* 2004; 321:462-467.
27. Sergeev IN, Norman AW. 1,25-Dihydroxyvitamin D₃ and calcium signaling. In: *Vitamin D Endocrine System: Structural, Biological, Genetic and Clinical Aspects*. Riverside: Univ. California, 2000:715-718.
28. Sergeev IN, Rhoten WB, Norman AW. 1,25-Dihydroxyvitamin D₃, intracellular Ca²⁺ and apoptosis in breast cancer cells. In: *Vitamin D: Chemistry, Biology and Clinical Applications of the Steroid Hormone*. Riverside: Univ. California, 1997:473-474.
29. Sergeev IN, Colby J, Norman AW. 1,25-Dihydroxyvitamin D₃ triggers calcium-mediated apoptosis in breast cancer cells. In: *Vitamin D Endocrine System: Structural, Biological, Genetic and Clinical Aspects*. Riverside: Univ. California, 2000:399-402.
30. Carafoli E, Santella L, Branca D, Brini M. Generation, control, and processing of cellular

- calcium signals. *Crit Rev Biochem Mol Biol* 2001; 36:107-260.
31. McConkey DL, Orrenius S. The role of calcium in regulation of apoptosis. *Biochem Biophys Res Commun* 1997; 239:357-366.
 32. Carafoli E, Molinari M. Calpain: a protease in search of a function? *Biochem Biophys Res Commun* 1998; 247:193-203.
 33. Sergeev IN, Li S, Colby J, Ho C-T, Dushenkov S. Polymethoxylated flavones induce Ca^{2+} -mediated apoptosis in breast cancer cells. *Life Sci* 2006; 80:245-253.
 34. Sergeev IN, Ho C-T, Lee S, Colby J, Dushenkov S. Apoptosis-inducing activity of hydroxylated polymethoxyflavones and polymethoxyflavones from orange peel in human breast cancer cells. *Mol Nutr Food Res* 2007; 51:1478-1484.
 35. Sergeev IN, Li S, Ho C-T, Rawson NE, Dushenkov S. Polymethoxyflavones activate Ca^{2+} -dependent apoptotic targets in adipocytes. *J Agric Food Chem* 2009; 57:5771-5776.
 36. Sergeev IN, Rhoten WB, Carney MD. Calbindins decreased after space flight. *Endocrine* 1996; 5:335-340, Cover illustration.
 37. Sergeev IN, Norman AW. Calcium as a mediator of apoptosis in bovine oocytes and preimplantation embryos. *Endocrine* 2003; 22:169-175.
 38. Farach-Carson MC, Sergeev IN, Norman AW. Nongenomic actions of 1,25-dihydroxyvitamin D_3 in rat osteosarcoma cells: structure-function studies using ligand analogs. *Endocrinology* 1991;129: 1876-1884.
 39. Sergeev IN, Song, Q. High vitamin D and calcium intakes reduce diet-induced obesity in mice by increasing adipose tissue apoptosis. *Mol Nutr Food Res* 2014; 58:1342-1348.
 40. Song, Q., Sergeev, I.N. High vitamin D and calcium intakes increase bone mineral (Ca and P) content in high-fat diet-induced obese mice. *Nutr Res* 2015; 35:146-154.

41. Henquin JC. Triggering and amplifying pathways of regulation of insulin secretion by glucose. *Diabetes* 2000; 49:1751-1760.
42. Bergsten P. Slow and fast oscillations of cytoplasmic Ca^{2+} in pancreatic islets correspond to pulsatile insulin release. *Am J Physiol* 1995; 268(2 Pt 1):E282-287.
43. Norman AW, Sergeev IN, Bishop JE, Okamura WH. Selective biological response by target organs (intestine, kidney and bone) to 1,25-dihydroxyvitamin D₃ and two analogs. *Cancer Res* 1993; 53:3935-3942.
44. Spirichev VB, Sergeev IN. Vitamin D: experimental research and its practical application. *World Rev Nutr Diet* 1988; 56:173-216.
45. Bouillon R, Lieben L, Mathieu C, Verstuyf A, Carmeliet G. Vitamin D action: lessons from VDR and Cyp27b1 null mice. *Pediatr Endocrinol Rev* 2013; Suppl 2:354-366.
46. Demay, MB. Physiological insights from the vitamin D receptor knockout mouse. *Calcif Tissue Int* 2013; 92:99-105.
47. Kato S, Takeyama K, Kitanaka S, Murayama A, Sekine K, Yoshizawa T. In vivo function of VDR in gene expression-VDR knock-out mice. *J Steroid Biochem Mol Biol* 1999; 69:247-251.
48. Narvaez CJ, Matthews D, Broun E, Chan M, Welsh JE. Lean phenotype and resistance to diet-induced obesity in vitamin D receptor knockout mice correlates with induction of uncoupling protein-1 in white adipose tissue. *Endocrinology* 2009; 150: 651-661.
49. Mutt SJ, Hyppönen E, Saarnio J, Järvelin MR, Herzig KH. Vitamin D and adipose tissue – more than storage. *Front Physiol* 2014; 5:228.
50. Bikle DD, Oda Y, Tu CL, Jiang Y. Novel mechanisms for the vitamin D receptor (VDR) in the skin and in skin cancer. *J Steroid Biochem Mol Biol* 2015; 148:47-51.

51. Ogunkolade BW, Boucher BJ, Prah J, Bustin SA, Burrin JM, Noonan K, North BV, Mannan N, McDermott MF, DeLuca HF, Hitman GA. Vitamin D receptor (VDR) mRNA and VDR protein levels in relation to vitamin D status, insulin secretory capacity, and VDR genotype in Bangladeshi Asians. *Diabetes* 2002; 51:2294-2300.
52. Ramagopalan SV, Heger A, Berlanga AJ, Maugeri NJ, et al. A ChIP-seq defined genome-wide map of vitamin D receptor binding: associations with disease and evolution. *Genome Res* 2010; 20:1352-1360.
53. Mitri J, Pittas AG. Vitamin D and diabetes. *Endocrinol Metab Clin North Am*. 2014; 43:205-232.
54. Mitri J, Muraru MD, Pittas AG. Vitamin D and type 2 diabetes: a systematic review. *Eur J Clin Nutr*. 2011; 65:1005-1015.
55. Seida JC, Mitri J, Colmers IN, Majumdar SR, Davidson MB, Edwards AL, Hanley DA, Pittas AG, Tjosvold L, Johnson JA. Clinical review: Effect of vitamin D₃ supplementation on improving glucose homeostasis and preventing diabetes: a systematic review and meta-analysis. *J Clin Endocrinol Metab* 2014; 99:3551-3560.
56. Tabesh M1, Azadbakht L, Faghihimani E, Tabesh M, Esmailzadeh A. Effects of calcium-vitamin D co-supplementation on metabolic profiles in vitamin D insufficient people with type 2 diabetes: a randomised controlled clinical trial. *Diabetologia* 2014; 57:2038-2047.
57. Oosterwerff MM, Eekhoff EM, Van Schoor NM, Boeke AJ, Nanayakkara P, Meijnen R, Knol DL, Kramer MH, Lips P. Effect of moderate-dose vitamin D supplementation on insulin sensitivity in vitamin D-deficient non-western immigrants in the Netherlands: A randomized placebo-controlled trial. *Am J Clin Nutr* 2014; 100:152-160.

58. Krul-Poel YH, Westra S, ten Boekel E, ter Wee MM, van Schoor NM, van Wijland H, Stam F, Lips PT, Simsek S. Effect of Vitamin D supplementation on glycemic control in patients with type 2 diabetes (SUNNY Trial): A randomized placebo-controlled trial. *Diabetes Care* 2015; 38:1420-1426.
59. Chandler PD, Wang L, Zhang X, Sesso HD, Moorthy MV, Obi O2 Lewis J, Prince RL, Danik JS, Manson JE, LeBoff MS, Song Y. Effect of vitamin D supplementation alone or with calcium on adiposity measures: a systematic review and meta-analysis of randomized controlled trials. *Nutr Rev* 2015; 73:577-593.
60. Ceglia L, Nelson J, Ware J, Alysandratos KD, Bray GA, Garganta C, Nathan DM, Hu FB, Dawson-Hughes B, Pittas AG. Association between body weight and composition and plasma 25-hydroxyvitamin D level in the Diabetes Prevention Program. *Eur J Nutr* 2015 Nov 2. [Epub ahead of print]

Figure 1. 1,25(OH)₂D₃ regulates intracellular Ca²⁺, apoptosis and insulin secretion.

This cartoon provides a schematic representation of the mechanisms and sites of action of 1,25(OH)₂D₃ in induction of cellular Ca²⁺ signals and apoptosis in mature adipocytes and Ca²⁺-mediated insulin secretion from the pancreatic β -cells. 1,25(OH)₂D₃ regulates Ca²⁺ entry from the extracellular space, Ca²⁺ mobilization from the intracellular stores, and intracellular Ca²⁺ buffering. **(A)** In mature adipocytes, 1,25(OH)₂D₃ induces Ca²⁺ influx and Ca²⁺ mobilization from the endoplasmic reticulum (ER) stores via the high permeability voltage-insensitive Ca²⁺ channels (VICCs) and IP₃ receptors/Ca²⁺ release channels (IP₃Rs), respectively. 1,25(OH)₂D₃ induces the apoptotic Ca²⁺ signal – a sustained increase in [Ca²⁺]_i reaching elevating (200-500 nM), but not cytotoxic (>1 μ M) levels. This increase in [Ca²⁺]_i leads to activation of the calpain/caspase-dependent apoptotic pathway. μ -Calpain activation by an increase in [Ca²⁺]_i is followed by activation (i.e., Ca²⁺-dependent translocation from the ER membrane to cytosol and calpain-dependent processing/cleavage) of the Ca²⁺/calpain-dependent caspase-12. Activation of these proteases is sufficient for execution of apoptosis, as evaluated by hallmarks of this process: apoptotic changes of the plasma membrane (phosphatidylserine translocation from the inner to outer surface of the plasma membrane), DNA fragmentation/nuclear condensation, and formation of apoptotic bodies. **(B)** In pancreatic cells, Ca²⁺ enters through both the low permeability VICCs and voltage-dependent Ca²⁺ channels (VDCCs). 1,25(OH)₂D₃ rapidly triggers synchronous Ca²⁺ oscillations via activation of VDCCs and Ca²⁺ release via ryanodine receptors/ Ca²⁺ release channels (RYRs). Ca²⁺ oscillations induce pulses of insulin secretion by exocytosis. Vitamin D receptors (VDRs) are expressed in adipocytes and pancreatic β -cells, and they can be found associated with the cell membranes as well as in the nuclear and cytosolic

compartments. Pancreatic β -cells resist induction of apoptosis with $1,25(\text{OH})_2\text{D}_3$ due to their high cytosolic Ca^{2+} buffering capacity, nonsustained nature of the $1,25(\text{OH})_2\text{D}_3$ -induced increase in $[\text{Ca}^{2+}]_i$ (Ca^{2+} oscillations) and, probably, a partial lack of the Ca^{2+} -dependent apoptotic machinery (caspase-12).

Figure