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Involvement of Calcium and Vitamin C in Type 2 Diabetes

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Running Head: Role of Calcium and Vitamin C in Type 2 diabetes

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The diabetes type 2 or diabetes mellitus (type 2DM) is an increasing at a frightening rate both national level and worldwide, with more than 3 million new cases per year diagnosed in the United States alone. Diabetes is the third leading cause of death in the United States, and it is also a major cause of significant morbidity. Although our current methods of treating type 2 DM and its complications have improved, prevention of the disease is preferable. Indeed, epidemiology suggest that 4 out of 5 cases of type 2 DM could be attributed to habits and forms of the modifiable behavior like obesity, sedentary lifestyle, unhealthy eating habits, family history and genetics, high blood pressure and high cholesterol. Although weight loss has been shown to be successful in delaying diabetes type 2, it is difficult to achieve and maintain for long term. Therefore, identification of environmental and easily modified risk factors is needed to prevent development of diabetes type 2. The major and most well-known function of vitamin C is to maintain calcium and potassium homeostasis and promote bone mineralization. However, recent evidence suggests that vitamin C and calcium homeostasis may also be important for a variety of non-skeletal outcomes and provide neutralizing oxygen free radicals in the body. Based on basic studies, vitamin C and calcium have also been suspected as modifiers of diabetes risk. More recently, there is accumulating evidence to suggest that altered vitamin C and calcium homeostasis may also play a role in the growth of diabetes type 2. The purpose of our systematic review was to examine: 1) the association between vitamin C & calcium status and risk of diabetes type 2. 2) The effect of vitamin C and calcium supplementation on glucose metabolism[1].

Over 99% of total body calcium is found in bones and teeth, where it functions as a key structural element. The remaining body calcium functions in metabolism, serving as a signal for vital physiological processes, including vascular contraction, blood clotting, muscle contraction and nerve transmission. Inadequate intakes of Calcium have been associated with increased risk[2] of osteoporosis, nephrolithiasis, insulin resistance and obesity. Most of these disorders have treatments but no cures. Calcium is unique among nutrients (WHO 2006). Insulin not only moves glucose into the cells, but it also escorts Vitamin C. Blood sugar hogs the seats on the bus in most diabetics, therefore reducing the amount of Vitamin C can absorbed[3].

Calcium: Ca^{2+} ion is a highly versatile intracellular signal that can regulate many different cellular functions[4, 5]. to achieve this versatility, the Ca^{2+} signaling system operates in many different ways to regulate cellular processes that functions over a wide dynamic range. At the synaptic junction, Ca^{2+} triggers exocytosis within microseconds, whereas at the other end of the scale Ca^{2+} has to operate over minutes to hours to drive events such as gene transcription and cell proliferation. One of the challenges is to understand how these widely different Ca^{2+} signaling systems can be set up to control so many divergent cellular processes.

Cytosolic free calcium concentration controlled by fluxes across the plasma membrane and from intracellular stores, regulates myriad cellular functions[6, 7] it has been established that elevated cytosolic Ca^{2+} concentration is the primary trigger for insulin release. However, reduced Ca^{2+} concentration in the lumen of acidic a compartment was also shown to inhibit exocytosis in the INS-1 ß-cell line. Indeed, insulin is released from pancreatic secretory ß-cells, both under basal condition and in response to glucose secretion is defective in type 2 diabetes[8, 9].

Ca2+ Signaling toolkit and signaling dynamics: Ca^{2+} is a universal signal transduction element in cells modulating cell growth and differentiation The calcium level outside cells are 10,000 times higher than free intracellular Ca^{2+}. However, free Ca^{2+} is the physiologically active form of calcium. The level of free intracellular calcium Ca^{2+} is regulated and maintained as low as (~100 nM) through the action of a number of binding proteins and ion exchange mechanisms. Each cell has a unique set of Ca^{2+} signals to control its function. Ca^{2+} signal transduction is based on rise in free cytosolic Ca^{2+} concentration. Ca^{2+} can flow from the extracellular space or be released from intracellular stores. The endoplasmic reticulum

(ER) is a major site for sequestered Ca^{2+} ions. Ca^{2+} is accumulated in intracellular stores by means of Ca^{2+} pumps and released by inositol-1, 4, 5- trisphosphate (IP3) via IP_3 receptors (IP_3R) and by cyclic adenosine diphosphate ribose (cADPr) via ryanodine receptors (RyR). Store-operated calcium channels (SOCs) open in response to depletion of the (ER) Ca^{2+} stores. Calcium influx factor (CIF) has postulated to mediate the signal from IP3R to the plasma membrane store-operated calcium channels (SOCs). A connection has been demonstrated between the filling status of the intracellular calcium stores and the plasma membrane calcium channel activity. Extracellular Ca^{2+} enters the cell through various types of plasma-membrane Ca^{2+} channels. Soluble proteins, such as calmodulin, contribute to the buffering of cell Ca^{2+}, but membrane-intrinsic transporting proteins are more important. Ca^{2+} is transported across the plasma membrane (channel, pump, Na^+/Ca^{2+} exchanger) and across the membrane of organelles[10]. External signals arriving at the cell engage plasma membrane receptors to initiate cell signaling pathways.

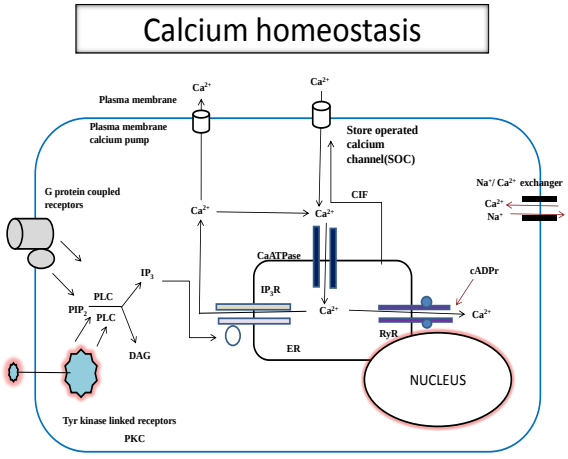

Figure 1 Representation of calcium homeostasis in a single cell.

External signals arriving at the cell engage plasma membrane receptor to initiate cell signaling pathways.One of the end results is the increased intracellular calcium concentration. On stimulation this level can rise globally to in excess of 1molar. This increase can be generated from sources both within and outside the cell. The formation of IP_3 is the focal point for two major pathways, one initiated by a family of G protein-linked receptors and the other by receptors linked by tyrosine kinases either directly or indirectly. These separate receptor mechanisms are coupled to energy-requiring transducing mechanisms which activate phospholipase C (PLC) to hydrolyse the lipid precursor phosphatidylinositol 4, 5-biphosphate to generate both DAG and IP_3. The latter then binds to an IP_3 receptor (IP_3R) to mobilize stored calcium and to promote an influx of external calcium[11].

Inositol-1, 4, 5-phosphate: The three inositol phosphates, I(1)P, $I(1,4)P_2$ and $I(1,4,5)P_3$, that potentially can be formed upon PI-PLC—activated cleavage of the phosphoinositides, $I(1,4,5)P_3$ is unique in its ability to mobilize Ca^{2+}. IP_3 receptor Ca^{2+} release channel in many mammalian cells, IP_3 (inositol-1, 4, 5-trisphosphate) triggers Ca^{2+} release from the endoplasmic reticulum. The "second messenger" IP_3 is produced, e.g., in response to hormonal signals, from the membrane lipid phosphatidylinositol. The IP_3 receptor is a ligand-gated Ca^{2+} -release channel embedded in endoplasmic reticulum membranes. It is distinct from but partly homologous to the ryanodine receptor channel. IP_3 binds to a cytosolic domain of the receptor, promoting channel opening. IP_3 may displace a regulatory phospho-protein IRBIT, which binds at the same site. Ca^{2+} also binds to the ligand-binding domain of the IP_3 receptor, and promotes channel opening. However, high cytosolic Ca^{2+} which develops after channel opening promotes channel closure. Thus both the IP_3-activated & ryanodine-sensitive channels are activated by low cytosolic Ca^{2+} and inhibited by high cytosolic Ca^{2+}. The feedback inhibition of $Ca^{2+\ released}$ by high cytosolic Ca^{2+}, along with activity of Ca^{2+}-ATPase pumps, contributes to signal turn off and makes possible observed oscillations in Ca^{2+} concentration. Pore structure of the IP_3 receptor has not yet been determined at atomic resolution. Structures of cytosolic domains of the IP_3 receptor, including the IP_3 binding site, have been solved, but the pore structure of the IP_3 receptor has not yet been determined at atomic resolution[12].

Ca2+ Release via the ryanodine receptors: The second mechanism by which Ca^{2+} may be mobilized from the SER involves a Ca^{2+} channel of the organelle membrane commonly known as the ryanodine receptor (RyR), because of its high

affinity for ryanodine, a plant alkaloid extracted from *Ryana speciosa*. Thus ryanodine is largely used as a ligand for the identification, purification, cloning and functional characterization of the RyR family (Masumiya, 2001).

Structure and Molecular diversity of Ryanodine receptors: Three RyR isoforms have been identified to date (RyR1, 2 and 3). They show ~ 70% sequence homology and hyper variability within their C-termini. The type 1 RyR presents two variants (ASI and ASII), the expression of which is developmentally regulated and tissue dependent (Shoshan-Barmatz, Ashley, 1998). The type 3 isoform undergoes extensive splicing, which would be expected to have a physiological functional significance. At least 3 variants of RyR3 are known as I, II and III of which the expression is tissue-dependent (Miyatake, 1996). The RyR1 is expressed mainly in skeletal muscle (Takeshima, 1989), but also in some smooth muscles (Neylon, 1995) and in some brain areas such as the cerebellar Purkinje cells (Furuichi, 1994). The RyR2 is highly expressed in cardiac muscle (Nakai, 1990) and the most distributed isoform in the brain but with low expression (Giannini, 1995). A weak expression of RyR2 in smooth muscle has also been reported (Neylon, 1995). As for RyR3, it is highly expressed within specific regions of the (hippocampus, thalamus and striatum) and in smooth muscle (Giannini, 1992), and also weakly in skeletal and cardiac muscle (Giannini, 1995), and in some non-excitable cells such as T-lymphocytes (Hakamata1994). Many cell types express more than one RyR isoform, but the physiological significance of this co-expression has not been established[13].

The RyR is an intracellular Ca^{2+} channel structurally resembling the IP_3R (Grunwald; Meissner, 1995) but possesses distinct biophysical and pharmacological characteristics. The primary domain of the receptor is present upon the cytosolic face of the membrane.The transmembrane segments containing the channel pore are located within the C-terminal region, and both the N and C termini are cytosolic[14].

Calcium signaling toolkit: The Ca^{2+} signaling has very large toolkit of signaling components that can be mixed and matched to create a diverse array of signaling units that can deliver Ca^{2+} signals with very different spatial and temporal properties (Table 1). The following list is by no means inclusive but it summarizes some of the main toolkit components in mammalian cells.

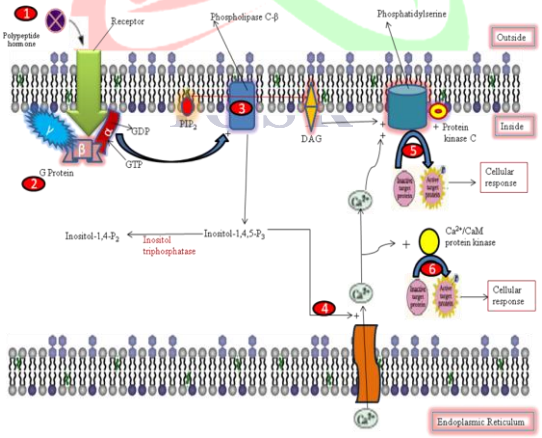

Fig: 2. IP_3-mediated signal transduction pathways. Increased Ca^{2+} activates protein kinases, which phosphorylate target proteins. Ca^{2+} /CaM represents calcium-calmodulin Ca^{2+} complexed with the regulatory protein calmodulin).

A. Biophysical characteristics of Ryanodine receptors: The cationic selectivity of the RyR is low. The channel pore does not or only weakly discriminate between Ca^{2+} and Ba^{2+} (Shoshan-Barmatz; Ashley, 1998). All the RyR isoforms show multiple conductance states, the most frequent of which is 100 to 150pS. They are characterized by a high unity conductance, both for monovalent (~ 750 pS for K^+) and for divalent cations (~ 150 pS for Ca^{2+}) (Meissner, 1994). The permeability of the RyR to anions is negligible.

Calcium Signaling Toolkit		
Sl. No.	**Name**	**Types**
1.	Receptors, Transporters & Channels	Adra1a, Adrb3, Aqp2, Ccr2, Cd28, Ceacam1, Ctla4, Gcgr, Glp1r, Icam1, Il4ra, Nsf, Rab4a, Sell (LECAM-1), Slc2a4 (GLUT4), Slc14a2, Snap23, Snap25, Stx4a, Stxbp1, Stxbp4, Tnfrsf1a, Tnfrsf1b, Vamp2, Vamp3, Vapa.
2.	Nuclear Receptors	Pparα, Pparγ
3.	Metabolic Enzymes	Ace, Acly, Dpp4, Enpp1, Fbp1, G6pc, G6pd2, Gpd1, Gsk3b, Hmox1, Ide, Nos3, Parp1 (Adprt1), Pck1, Pfkfb3, Pygl, Sod2. Secreted Factors: Agt, Ccl5 (Rantes), Gcg, Ifng, Il6, Il10, Il12b, Ins1, Retn, Tgfb1, Tnf, Vegfa.
4.	Signal Transduction	Akt2, Dusp4, Igfbp5, Ikbkb (IKKbeta), Inppl1 (SHIP2), Irs1, Mapk8 (JNK1), Mapk14 (p38 MAPK), Pik3cd, Pik3r1, Ptpn1 (PTP-1B), Trib3 (Skip3). Transcription Factors: Cebpa, Foxc2, Foxg1, Foxp3, Hnf4a, Pdx1 (Ipf1), Neurod1, Nfkb1, Nrf1, Pax4, Ppargc1a, Srebf1, Tcf2 (HNF1b).
5.	Second-messenger-operated channels (SMOCs)	cyclic nucleotide gated channels (CNGA 1–4, CNGB 1, CNGB 3) | arachidonate-regulated Ca2+ channel (IARC)
6.	Transient receptor potential (TRP) ion-channel family	TRPC1–7 | TRPV1–6 | TRPM1–8 | TRPML (mucolipidin 1, 2) | TRPNI (ANKTM1) Inositol-1,4,5-trisphophate receptors (Ins(1,4,5)P3Rs): Ins(1,4,5)P3R1–3
7.	Ryanodine receptors (RYRs)	RYR1–3
8.	Polycystins	PC-1 | PC-2
9.	Channel regulators	triadin | junctin | sorcin | FKBP12 | FKBP12.6 | phospholamban | IRAG | IRBIT
10.	Calcium buffers	ER/SR buffers and chaperones: calnexin | calreticulin | calsequestrin | GRP 78 | GRP 94 Calcium effectors Ca^{2+} binding proteins: calmodulin | troponin C | synaptotagmin | S100A1–12, S100B, S100C, S100P | annexin I–X | neuronal Ca2+ sensor family (NCS-1) | visinin-like proteins (VILIP-1, VILIP-2, VILIP-3) | hippocalcin | recoverin | Kv-channel-interacting proteins (KchIP1–3) | guanylate-cyclase-activating proteins (GCAP1–3) Calcium-sensitive enzymes and processes
11.	Ca2+ regulated enzymes	Ca^{2+} /calmodulin-dependent protein kinases (CaMKI–IV) | myosin light chain kinase (MLCK) | phosphorylase kinase | Ins(1,4,5)P3 3-kinase | PYK2 | protein kinase C (PKC-α, PKC-βI,PKC-βII,PKC-γ) | cyclic AMP phosphodiesterase (PDE1A–C) | adenylyl cyclase (AC-1,AC-III,AC-VIII,AC-V,AC-VI) | nitric oxide synthase (endothelial NOS, eNOS; neuronal NOS, nNOS) | calcineurin | Ca^{2+} -activated proteases (calpain I and II)
12.	Transcription factors	nuclear factor of activated T cells (NFATc1–4) | cyclic AMP response element-binding protein (CREB) | downstream regulatory element modulator (DREAM) | CREB-binding protein (CBP)
13.	Ca2+ sensitive ion channels	Ca^{2+} activated potassium channels (SK, small conductance Ca^{2+} -sensitive channel; IK, intermediate conductance Ca^{2+} -sensitive channel; BK, large conductance Ca^{2+}-sensitive channel) | Human Cl– channel, Ca^{2+}-activated (HCLCA1)
14.	Calcium pumps and exchangers	K^+/Ca^{2+} exchangers (NCXs): NCX1–3
15.	Endoplasmic reticulum channels and exchangers	permeability transition pore | K^+/Ca^{2+} exchanger | Ca2+ uniporter | H+/Ca2+ exchanger Plasma membrane Ca^{2+} ATPases (PMCAs): PMCA1–4 Sarco (endo) plasmic reticulum Ca^{2+} -ATPases (SERCAs): SERCA1–3
16.	Golgi pumps	SPCA1, SPCA2
17.	Others	Serpine1 (PAI-1), Ucp2

B. Modulation of RyR activity: The RyR activity is modulated by a variety of intracellular second messengers and many drugs. In this section only modulators of physiological importance are considered, along with the most widely used pharmacological agents. More detailed discussions about RyR pharmacology may be found in other sources.

Dynamic Imaging of Calcium: Live-cell imaging offers the power of capturing the dynamics of biological action in live cells and in real time, something not previously available with biochemical approaches. Entry of calcium ions Ca^{2+} across the plasma membrane can replenish intracellular stores and is activated following the receptor-mediated release of calcium from the endoplasmic reticulum. This store-operated calcium entry pathway is a well-established mechanism for replenishing internal calcium stores in many cell types .Calcium conveyed via this pathway is often referred to as the calcium-release activated current and is mediated by plasma membrane localized CRAC or SOCE channels . The CRAC channel is the best-characterized store-operated Calcium influx channel and is essential to the immune response, where sustained activity of CRAC channels is required for gene expression and proliferation. Intracellular calcium along with two different fluorescent proteins are viewed in the same cell using live cell Imaging. Here they describe how a combination of advanced wide-field fluorescence imaging and total internal reflection fluorescence microscopy (TIRFM) has been used for live-cell microscopy. This fluorescence imaging approach allows the rapid multi-dimensional analysis of fluorescently labeled cell[15].

Ca^{2+} entry mechanism: Calcium Ca^{2+} is a common second messenger that regulates many processes in the cell (e.g., contraction, secretion, synaptic transmission, fertilization, nuclear pore regulation, transcription). In cardiac myocytes and muscle cells, Ca^{2+} concentrations alternate between high levels during contraction and low levels during relaxation[16]. Regulation of Ca^{2+} concentration in the cell is coupled with both, transmembrane channel and storage/release of organelles. Ca^{2+} entry across the surface membrane is realized via Calcium channels [Ca^{2+} (II) channels] and leads to elevated Ca^{2+} cytosol levels, providing Ca^{2+} trigger signals for a large number of physiological processes, including muscle contraction[17, 18].

However, most cells have developed an additional pathway to generate localized and fast Ca^{2+} signaling triggers deep inside the cell, which involves specialized intracellular Ca^{2+} storage/release organelles. Primary such intracellular Ca^{2+} storage/release organelle in most cells is endoplasmic reticulum (ER). In striated muscles, it is sarcoplasmic reticulum (SR). ER and SR contain specialized Ca^{2+} release channels: families of Ryanodine receptor and Inositol-1, 4, 5-triphosphate receptor (IP_3 receptor) [1]. Muscle relaxation is regulated by the subsequent return of Ca^{2+} to the lumen of the sarcoplasmic reticulum through the action of Ca^{2+} pumps, referred to as ATPase Ca^{2+} transporting (Ca-ATPase). Ca-ATPase molecules are 110-kDa transmembrane proteins that transport Ca^{2+} ions from the sarcoplasm to the lumen of the membrane system at the expense of ATP hydrolysis[19]. Activity of all sarcoplasmic reticulum channels is thoroughly regulated. And all three families of channels are regulated by Ca^{2+}[20] In addition; their activities are regulated by specific proteins. Phospholamban is an integral membrane protein highly expressed in cardiac and slow-twitch skeletal muscle fibers. It interacts with and regulates activity of Ca-ATPase2. Effects of Phospholamban on Ca-ATPase2 depend on the phosphorylation state of Phospholamban.

When phosphorylated by Calcium/calmodulin-dependent protein kinase II (CaMKII) or Protein kinase A (PKA), Phospholamban binds to Ca^{2+}ATPase2 and increases the affinity of the SR Ca^{2+} pump for Ca^{2+}. Dephosphorylated Phospholamban binds and inhibits Ca-ATPase2 stabilizing enzyme in inactive conformation[21]. Ryanodine receptor 1 on the surface of SR is the major Ca^{2+} release channel required for skeletal muscle excitation-contraction coupling. Ryanodine receptor 1 function is modulated by proteins that bind to its large cytoplasmic scaffold domain, including the FK506 binding protein (FKBP12) and PKA[22]. PKA phosphorylation of Ryanodine receptor 1 activates the channel. FKBP12 modulates of the Ryanodine receptor 1 channel, but specific mechanisms involved are still being investigated. It was proposed that FKBP12 can stabilize Ryanodine receptor[23]. The IP_3 receptor channels require the presence of Inositol-1, 4, 5-trisphosphate (IP_3) for their activity[24]. And all three families of channels are regulated by Ca^{2+}.To prevents overloading of intracellular stores, the Ca^{2+} that entered through sarcolemma must be extruded from the cell. The Sodium/Calcium exchanger like solute carrier family 8 member 1 (NCX1) is the primary mechanism by which the Ca^{2+} is extruded from the cell during relaxation. NCX1 is an integral membrane protein that is expressed in many tissues. It was proposed that NCX1 is part of a macromolecular complex which also includes protein kinase[a catalytic and regulatory subunits (PKA-cat and PKA-reg)], protein kinase C (PKC), a kinase anchoring proteins (AKAP6) and phosphatases(PP1 and PP2A). Kinases and phosphatases are possibly linked by protein AKAP6[25].

Cytoplasmic Ca^{2+} influences on the activity of numerous proteins. Several PKC (conventional PKC-alpha, PKC-beta and PKC-gamma) are allosterically activated by Ca^{2+} .The other target for Ca^{2+} is a protein Ca^{2+} calmodulin. Calcium-bound

calmodulin associates with and activates serine/threonine phosphatase Calcineurin. Calcineurin dephosphorylates NF-AT family of transcription factors leading to theirs translocation to the nucleus[26]. Calcium-bound calmodulin also activates calcium/calmodulin-dependent protein kinases CaMKI, CaMKII, and CaMKIV, as well as calcium/calmodulin-dependent protein kinase kinase (CaMKK). CaMKII and CaMKIV regulate transcription via phosphorylation of several transcription factors, including cAMP responsive element binding protein (CREB) [27.] Another pathway of Ca^{2+}-mediated transcription regulation is phosphorylation of Histone deacetylases (HDAC4, HDAC5, and HDAC7) by CaMKI and CaMKIV with subsequent inhibitory effects on Myelin expression factor 2 (MEF2) transcriptional activity[28]. Membrane-spanning proteins CD44 can regulate Ca^{2+} efflux from intracellular stores by activation of IP_3 receptor. CD44 binds ERM family of proteins [VIL2 (ezrin), RDX (radixin), MSN (moesin)]. VIL2 (ezrin) action results in the release of Ras homolog gene family, member A (RhoA) from Rho GDP dissociation inhibitor (GDI) alpha (RhoGDI) and its translocation to membrane, where it activates Rho-associated coiled-coil containing protein kinases (ROCK) (ROCK1 and ROCK2). ROCK in turn phosphorylates and activates IP_3 receptors[29].

Ca^{2+} signaling in the β-cell: Ca^{2+} is a ubiquitous second messenger in cells, involved in a vast array of cellular processes including growth, gene regulation, proliferation, metabolism, exocytosis, and apoptosis[27, 30]. Persistently elevated Ca^{2+} is toxic to cells, and thus its levels must be carefully regulated. Cytosolic baseline levels of Ca^{2+} are typically in the 100 nM range; about 20,000 times lower than the extracellular environment[31]. The ER serves as a major intracellular Ca^{2+} store, storing micromolar amounts of the molecule[32]. Ca^{2+} signal themselves, whether initiated extrinsically or intrinsically, are coded in a variety of ways transient rise in Ca^{2+} can vary in amplitude, frequency, and spatial localization; they can rise locally at a mouth of a channel; be confined to one area of a cell, or propagate in a[33] Global wave that can then spread between cells[34]. These Ca^{2+} signals can originate from channels on the ER, the IP_3R- and RyR-gated channels, from the various other channels and pumps on the plasma membrane, or from other organelles such as the golgi apparatus and mitochondria. Additionally, there are many channels and pumps that buffer Ca^{2+}, to turn signals off and return the cytosol to baseline levels, including the ER Ca^{2+} uptake pump, SERCA. Together, these mechanisms serve as the cellular Ca^{2+} signaling toolkit[35].creating a complex code of signals that can control a vast array of cellular processes. Well-studied examples of Ca^{2+} signalling occur in secretory cells, such as the pancreatic β-cells. The combination of cellular Ca^{2+} channels and pumps creates cytosolic Ca^{2+} oscillations that drive oscillatory insulin secretion in glucose-stimulated β-cells[36]. The cascade of events leading to glucose-stimulated Ca^{2+} signals in β-cells starts when glucose is internalized by the transporter GLUT2 (or GLUT1 in humans)[32]. Undergoes glycolysis and oxidative metabolism, and increases the cell ATP/ADP ratio. ATP inhibits plasma-membrane KATP channel activity, resulting in cell depolarization and activation of voltage-dependant Ca^{2+} channels on the plasma membrane[37]. The local increase in sub plasma membrane Ca^{2+} regulates docking and fusion of secretory granules, resulting in insulin secretion[38, 39].

Importantly, this secretion is pulsatile in nature, and consequently creates oscillations in plasma insulin levels, which are important for insulin action on its target tissues.

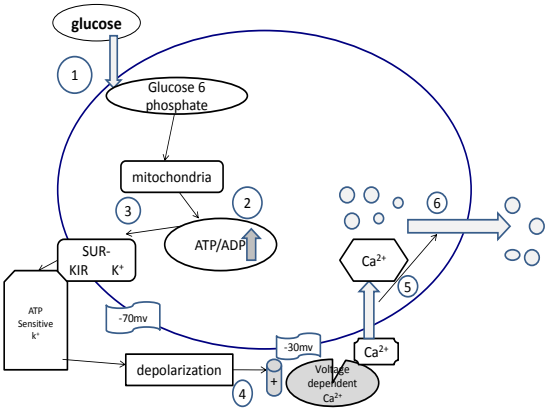

Fig: 3. Insulin secretion

1. Glucose is transported into the beta cell by type 2 glucose transporters (GLUT2). Once inside, the first step in glucose metabolism is the phosphorylation of glucose to produce glucose-6-phosphate. This step is catalyzed by hexokinase; it is the rate limiting step in glycolysis, and it effectively traps glucose inside the cell.
2. As glucose metabolism proceeds, ATP is produced in the mitochondria.
3. The increase in the ATP**:** ADP ratio closes ATP-gated potassium channels in the beta cell membrane. Positively charged potassium ions (K^+) are now prevented from leaving the beta cell.
4. The rise in positive charge inside the beta cell causes depolarization.
5. Voltage-gated calcium channels open allowing calcium ions (Ca^{2+}) to flood into the cell.
6. The increase in intracellular calcium concentration triggers the secretion of insulin via exocytosis.

This pulsatility is controlled by oscillations in cytosolic Ca^{2+}, among other factors, and this in turn is triggered by the oscillatory nature of glucose metabolism[40]. These oscillations in Ca^{2+} are dependent on both voltage-gated extracellular Ca^{2+} influx as well as the sequestration of cytosolic Ca^{2+} into the ER via SERCA pumps. There are different types of oscillations: slow, fast, spiking, and fast receptor-induced, and they are controlled by different stimuli (glucose, IP3, cAMP) and result in different frequencies and durations of intracellular Ca^{2+} increase. However, all of the intricate mechanisms in insulin secretion have not been fully elucidated. Other aspects of cellular metabolism, including pyruvate cycling, NADH, cAMP, and PLC mediated agonists, may also play a part in glucose-stimulated insulin secretion[41].

Role of Vitamin C: Vitamin C and glucose molecules help in the insulin-mediated tunneling mechanism into cells through the membrane. High glucose levels obstruct the vitamin C entry into the cells.The importance of vitamin C for blood sugar regulation has been demonstrated in both humans and animals. According to GAA pathway (Glucose-Ascorbate Antagonism) both the molecules glucose and ascorbate require help from the pancreatic hormone insulin before they can penetrate cell membranes using special (pumps). The name of this process in which both glucose and vitamin C (the reduced form) passes through cell membranes is insulin-mediated uptake.
Dr. Ely suggested that the insulin-mediated uptake of glucose and vitamin C occurs using white blood cells. In the White blood cells more insulin pumps are present and they contain 20 times amount of vitamin C as ordinary cells. This explains that both glucose and vitamin C molecules compete with each other, but all things are not equal. There is (fight & flight) response that favors glucose entry into cells at the expense of vitamin C. Because of this antagonism between sugar and Vitamin C, they recommend a low-carbohydrate, low-processed sugar diet[42].
Vitamin C is also known as ascorbic acid. It is water soluble vitamin with an antioxidant property. Antioxidants mean such type of factor which increases the level of blood cholesterol, level of blood sugar, smoking, and radiation. Free radicals will generate in our body by these factors. These free radicals give harmful chemical reaction in our body. That is, they cause heart disease, cancer, diabetes aggravation. The role of antioxidants (some substances which cut the chain of oxidation of these chemicals), as in case of diabetes, uncontrolled blood sugar level occurs which is result of bad oxidation reaction.
There is some general study done on vitamin C in reference to diabetes maintaining the integrity of blood vessels, vitamin C has been shown to inhibit three different biochemical processes that are associated with end-organ damage in diabetics. First,

vitamin C functions as an antioxidant. Second, this vitamin inhibits the intracellular accumulation of sorbitol. In one study, supplementation with 2,000 mg/day of vitamin C reduced erythrocyte sorbitol accumulation by 56.1% and 44.5% in healthy individuals and diabetics, respectively[43]. Third, vitamin C significantly reduced the glycosylation of proteins, when given to healthy volunteers at a dose of 1 g/day[44].in general A diabetic patient who has deficiency of vitamin c faces gum problem, muscles weakness, and difficulties in healing of different wounds (especially skin). Intake of vitamin C reduces all these problems as well as prevented scurvy. The vitamin C concentrations in plasma, platelets and white blood cells[45] were significantly lower in diabetics than in healthy controls[46]. Vitamin C deficiency in diabetics may be more pronounced within the cells than in plasma or other body fluids. That is because vitamin C is structurally similar to glucose, and may therefore compete with glucose for transport into cells. In the presence of elevated blood sugar, the uptake of vitamin C into cells appears to be impaired[47].Vascular changes resulting from scurvy resemble those seen in diabetics. A study of 20 diabetic patients found that 500 mg of ascorbic acid given twice daily led to significantly increased levels of ascorbic acid in the blood and decreased the albumin excretion rate, a key measure of disease progression in diabetics[48].A different study of vitamin C supplementation in rats found that vitamin C inhibits the action of interferon alpha, a substance that inhibits the release of insulin148. A randomized, double-blind study of 30 patients with type 2 diabetes found that supplementation with 1250 mg of vitamin C per day slowed the progression of kidney disease that developed as a complication of diabetes[49]. A new study has found that vitamin C decreases oxidative stress and improves blood vessel function in diabetic patients[50]. A randomized, double-blind study of 30 patients with type 2 diabetes mellitus found that improved function of arteries was an important finding in the prevention of complications in this condition. An additional study found evidence that vitamin C reduces oxidative stress and improves blood vessel function in diabetics. These studies suggest that long-term supplementation with vitamin C may help prevent many of the complications of diabetes.

Some other study done in role and action of vitamin C in type 2 diabetic animals and humans;Guinea pigs fed with vitamin C-deficient diet developed diabetic glucose tolerance curves, glycosuria, and decreased pancreatic insulin content[51]. A study of diabetic rats found that vitamin C supplementation leads to protection against oxidative processes108. A newer study found that vitamin C supplementation decreases insulin resistance and improves glucose regulation in diabetic mice138. Diabetic blood sugar curves were also seen in humans with vitamin C deficiency; these values returned to normal after supplementation with vitamin C (Prevention and Treatment of Diabetes with Natural Therapeutics) A study of 56 outpatients with non-insulin-dependent diabetics found that 2 grams per day of vitamin C led to improved glycemic control and fasting blood glucose levels in addition to having a favorable effect on cholesterol and triglycerides[51].

Take vitamin C in rich fresh fruits and vegetables like: broccoli, currant, and sprout tomatoes, cabbage, citrus fruits (lemon, orange), strawberries, cantaloupe, red peppers, parsley, and potatoes or other source of vitamin C is diabetes in these supplements. Do not exceed the dose of 250 mg per day.

Conclusion: The oscillations in the intracellular Ca^{2+} levels regulate the docking and fusion of the secretory granules and hence the insulin secretion within the β-cell.
Vitamin C is structurally similar to glucose, and may therefore compete with glucose for the transport into cells. And it has been observed that the cells that can't absorb glucose are not absorbing vitamin C either. As blood glucose levels rise, vitamin C uptake is greatly diminished throughout the body, even in cells with undamaged insulin pumps. This may lead to serious health consequences like blindness, wounds that won't heal, limb amputation, etc.
In summary, we want to develop one hypothesis to examine the extracellular effects of Ca^{2+} signals and vitamin C in MIN6 cells of mouse strain, using a combination of conventional and new live-cell imaging approaches. Importantly, Ca^{2+} release from the β-cell ER in response to vitamin C will directly measure in mouse cell line and evaluate what is the response of insulin secretion in type 2 diabetes as well as other disease where altered Ca^{2+} signaling is adversely affecting cell survival.

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