Diabetes leads to micro-vascular and macro-vascular complications which includes diabetic nephropathy (DN) and cardiovascular dysfunctioning (CVD) respectively. By transition through some common pathways like reactive oxygen species (ROS) and oxidative stress, renin angiotensin aldosterone system (RAAS) activation; diabetes leads to DN and CVD which reveal that both complications cross-talk with each other. The available therapeutic options for the management of DM and its related complications have been faced with many challenges that necessitate the need for a more effective alternative approach. One such alternative therapeutic option is to use micronutrients in DN and CVD. Micronutrients includes vitamins and minerals which are needed only in minuscule amounts for proper growth and development of the body. Many studies have been conducted to investigate the independent role of several vitamins and minerals on diabetic complications which shows that, molecules involved in insulin signalling cascade are the main target for micronutrients to treat DN and CVD. Specific selected micronutrients like Vanadium, Selenium, Chromium, Magnesium, Vitamin D, Vitamin E and Vitamin B shows their effect by activating and deactivating the positive and negative regulators in insulin signalling cascade respectively. Therefore micronutrients has potential to act as a replacement therapy for insulin because of their insulin-mimetic effect or at least serve the purpose of add-on therapy in diabet es and related complications. The aim of this review is to provide an update on molecular and cellular mechanisms of DN, CVD, micronutrients and their overall cross-talk by which micronutrients may provide beneficial effects in alleviating DN and CVD.
INTRODUCTION:
Diabetes describes a group of metabolic disorders characterized and identified by the presence of hyperglycaemia in the absence of treatment. The heterogeneous etiopathology includes defects in insulin secretion, insulin action or both, and disturbances of carbohydrate, fat and protein metabolism.\(^1\) According to study, overall prevalence of diabetes in India to be 7·3% and the prevalence of prediabetes to be 10·3%.\(^2\) Diabetes is the leading cause of death and India’s fastest growing disease. Findings of some study shows that the mortality rate is three times higher in individuals with diabetes having age between 51-70 years as compared to those without diabetes and there is an increased risk for mortality as the degree of diabetes and its related complications worsens.\(^3\) Diabetes is not a single disorder. Diabetes leads to two types of complications namely; micro-vascular complications which includes diabetic retinopathy, diabetic nephropathy and diabetic neuropathy in which small blood vessels gets damaged. Second complication, being macro-vascular complications which include cardiovascular disease in which arteries gets damaged. Molecular and cellular mechanisms behind all these complications are complex and overlapping.\(^4\)

DIABETIC NEPHROPATHY
Diabetic nephropathy (DN) or diabetic kidney disease is one of the major micro-vascular complication of diabetes characterized by the presence of pathological quantities of urine albumin excretion, diabetic glomerular lesions and decrement in glomerular filtration rate (GFR) in diabetics. DN leads to both structural and functional changes; Mesangial expansion, thickening of the basement membrane and nodular glomerulosclerosis (Kimmelstiel–Wilson nodules) are observed in glomeruli. Tubular hypertrophy is present in early DN but eventually interstitial fibrosis with tubular atrophy develops, along with arteriolar hyalinosis. In advanced cases, there is an infiltrate of macrophages and T-lymphocytes; podocyte loss and reduced endothelial cell fenestration are observed ultrastructurally.\(^5\) Functional changes include early glomerular hyperfiltration, increased albumin excretion with advancing nephropathy, increasing proteinuria and declining GFR.\(^6\) Diabetic nephropathy develops in series of stages namely; stage 1 is characterized by early hyperfiltration and hypertrophy, stage 2 is glomerular lesions without clinical disease, stage 3 is incipient diabetic nephropathy, stage 4 is overt diabetic nephropathy and stage 5 is end-stage renal failure with uremia due to diabetic nephropathy.\(^7\) Blood urea nitrogen, serum creatinine, GFR estimation, proteinuria and albuminuria are measures currently used to assess the presence and progress of diabetic nephropathy.\(^8\) Some biomarkers of tubular dysfunction are Neutrophil Gelatinase-Associated Lipocalin, kidney injury molecule 1, N-acetyl-b-d-glucosaminidase, liver-type fatty acid binding protein, β2-microglobulin and stable microprotein α-1 microglobulin.\(^9\)

Figure 1. Molecular mechanism of Diabetic nephropathy resulting from hyperglycemia.
Multiple hemodynamic and metabolic pathways are activated in response to hyperglycaemia. Increased glucose level affects overall kidney functions. In glomerulus, glomerular hyper-perfusion and hyperfiltration decreases afferent arteriolar resistance. Increased glucose in glomerular ultra-filtrate stimulates sodium glucose transporter 2 (SGLT2) gene with consequent increased proximal tubular absorption of filtered sodium and glucose. SGLT2 in the apical membrane of the proximal tubular epithelial cells is responsible for absorption of 90% of the glucose in the ultra-filtrate.[11] As a result, distal tubular sodium delivery decreases and hence distal tubular macula densa pays less energy in sodium absorption. Decreased energy expenditure decreases adenosine activity with consequent vasodilatation of afferent arterioles. [12] The increased glucose absorption raises intracellular glucose availability with consequent increased activity of polyol pathway that leads to increased fructose synthesis. Fructose metabolism leads to increased intracellular uric acid (UA) synthesis. [13] UA stimulates nicotinamide adenine dinucleotide phosphate (NADPH) oxidase enzyme in glomerular mesangial cells causing increased intracellular oxidative stress that ultimately leads to renin angiotensin system (RAS) activation, endothelial and mitochondrial injury, adenosine triphosphate (ATP) depletion and increased epithelial-mesenchyme transition (EMT). Excess fibroblasts infiltrate the interstitium with consequent progressive interstitial fibrosis. [15] ROS also activates protein kinase C (PKC), mitogen activated protein kinase MAPK and nuclear factor-κB (NF-κB) which eventually results in overproduction of extracellular matrix (ECM) proteins (Fig.1; part 1). [16] Fibroblast growth factor 23 (FGF23) is a phosphatonin responsible for renal phosphate elimination. A study has shown that, FGF23 mRNA is not detected in the kidneys of normal rats but starts to appear in the kidneys of diabetic rats at 2, 4, 6, 8 months of age and increases thereafter. [17] FGF23 inhibits 1-α hydroxylase gene with consequent decreased calcitriol synthesis. An inverse relationship between calcitriol and renin levels was displayed. [18] These findings disclose the cross talk between FGF23 and RAS. Intrarenal RAS genes expression is induced in diabetes.[19] Mechanical strain increases angiotensin II (Ang II) production and up-regulates angiotensin I receptor (ATIR) in podocytes.[20] Increased Ang II maintains and aggravates glomerular hypertension. In cultured podocytes, Ang II causes in-vitro loss of nephrin; the protein necessary for the proper functioning of the renal filtration barriers which includes the fenestrated endothelial cells, glomerular basement membrane and the podocytes of epithelial cells. [21] Notch1 couples Ang II with nephrin down regulation. Notch1 is a transmembrane receptor that plays a role in cell differentiation and renal development. Activation of Notch1 receptor leads to the release of the active Notch1 intracellular domain (ICN1). Another transcription factor within the cytoplasm is triggered by notch 1 receptor signaling. This factor is called the snail. Upon notch1 signaling, both ICN1 and snail translocate to the nucleus and share in repression of nephrin expression and podocyte apoptosis (Fig.1; part 2). [22] During further pathophysiologic progression, there is processing of the increased Ang II activity which eventually causes hypertrophy of mesangial cells and tubular epithelial cells. It also promotes production of the pro-sclerotic cytokine; transforming growth factor-beta (TGF-β) which has been identified as one of cause for glomerular sclerosis. [23] α-Klotho is a multifunctional trans-membrane protein highly expressed in the kidney that serves as the cofactor for FGF-23 to bind to its cognate receptor and regulate phosphorus and vitamin D metabolism. Increasing evidence demonstrates that the renoprotective action of Klotho is through inhibition of intrarenal RAS. It has further been shown that Klotho may inhibit intrarenal RAS by targeting the Wnt/β-catenin signaling system. Klotho directly binds multiple Wnts, including Wnt1, Wnt4, and Wnt7a. Others have shown that Klotho exerts a direct inhibitory effect on Wnts synthesis in adrenal glands. It is likely that Klotho may exert a multitude of actions to mitigate the activation of intrarenal RAS as well as systemic aldosterone production. Overall, strong evidence demonstrates the suppression of renal Klotho expression in diabetic renal disease, and more vigorous functional studies are needed.
to define the renoprotective action of this antiangiogenic protein as well as its relationship with intrarenal RAS.\textsuperscript{[24]} Many studies have demonstrated that hyperglycemia can trigger the activation of phosphatidylinositol-3 kinase (PI3K) and protein kinase B (PKB, also called AKT) pathways, which subsequently lead to the activation of mammalian target of Rapamycin (mTOR). Activated mTOR induces the synthesis of matrix proteins responsible for basement membrane thickening and mesangial matrix accumulation. In addition, mTOR is incriminated in renal fibrosis. In addition, mTOR stimulates infiltration of the kidney interstitium by macrophages through monocyte chemotactic protein-1 (MCP-1) upregulation.\textsuperscript{[25]} Some groups of researchers have found that tubular epithelial cells in diabetes induce renal MCP-1 production which is regulated by RAS. The so formed MCP-1 is responsible for macrophage recruitment resulting in renal fibrosis and indirect promotion of extracellular matrix formation. By observing the role of MCP-1 in the pathophysiology of diabetic nephropathy we can undoubtedly say that it is a promising therapeutic target for treating diabetic nephropathy (Fig.1; part 3).\textsuperscript{[26]} Although it is conceptually easier to describe above all these pathways individually, these pathways overlap and interact with one another in vivo, and enhance one another’s biophysiological effects.

**DIABETIC CARDIOVASCULAR DYSFUNCTIONING**

Diabetes affects the heart in three ways: (1) coronary artery disease (CAD) due to accelerated atherosclerosis; (2) cardiac autonomic neuropathy (CAN); and (3) diabetic cardiomyopathy. Diabetic cardiomyopathy is defined as myocardial dysfunction occurring in patients with diabetes in the absence of CAD, hypertension, or valvular heart disease.\textsuperscript{[27]} Diabetic cardiomyopathy is characterised by myocardial hypertrophy, myocardial fibrosis and the increased extracellular matrix in the interstitium of the ventricular wall is type VI collagen, reactive with periodic acid Schiff. At the cellular level, diabetic cardiomyopathy is associated with abnormalities in fatty-acid metabolism and calcium homeostasis and these changes can result in stiffer ventricular walls and impaired myocyte contractility.\textsuperscript{[28]} Diabetic cardiomyopathy develops in three stages namely early, middle and late. The early stage is asymptomatic, where the heart becomes hypertrophic and has diastolic dysfunction with normal ejection fraction (EF). The middle stage is recognized by increased left ventricle size, wall thickness and mass, which is accompanied by diastolic dysfunction and a slight decrease in systolic function (EF < 50%). The progression from middle stage to late stage disease is associated with microvascular changes, CAD and CAN, which impairs both systolic and diastolic functions. This stage is identified by increased left ventricle size, wall thickness and mass, dilatation, fibrosis and microangiopathy.\textsuperscript{[29]} The presence and progress of diabetic cardiovascular dysfunctioning is assessed by measuring cardiac biomarkers. Several myocardium-specific proteins have been reported as useful biomarkers for congestive heart failure, myocardial infarction and other heart diseases. These biomarkers include cardiac troponins I and T, cardiac natriuretic peptides, creatine kinase isoenzyme MB and lactate dehydrogenase.\textsuperscript{[30]}

**Figure 2. Molecular mechanism of CVD resulting from hyperglycemia.**
Different pathogenic mechanisms of CVD possibly induced by increased ROS and oxidative stress, AGEs, mitochondrial dysfunction and consequences of polyol, hexosamine pathway and RAAS activation induced by hyperglycemia which collectively leads to CVD.

Multiple molecular mechanisms have been suggested to contribute to development of CVD, including excessive ROS formation and oxidative stress, mitochondrial dysfunction, increased advanced glycation end-products (AGEs), inflammation, cell death, increased flux of glucose into the alternate metabolic pathway such as hexosamine pathway which results in impaired diastolic relaxation, polyol pathway which results in DNA damage and cardiomyocyte apoptosis and RAAS activation.

Increased glucose metabolism due to hyperglycemia leads to an increase in oxidative stress by generation of mitochondrial ROS. Overproduction of superoxide by the mitochondrial respiratory chain and the consequent oxidative stress results in reduction of myocardial contractility and eventually myocyte fibrosis. High glucose-induced ROS can be generated by both enzymatic and non-enzymatic pathways. The non-enzymatic pathways include mitochondrial electron transport chain, glucose autoxidation, increased flux through the hexosamine biosynthetic pathway, accumulation of AGEs, enhanced receptor for advanced glycation end-products (RAGE) and angiotensin II receptor type 1 signaling and elevated levels of free fatty acid. Conversely, the enzymatic pathways include NADPH oxidase, xanthine oxidase, uncoupled nitric oxide synthase, cytochrome P450, cyclooxygenase, lipoxygenase and myeloperoxidase. ROS and oxidative stress cause cellular DNA damage and acceleration of cardiomyocyte apoptosis. An important biomarker of oxidative stress is lipid peroxidation which is defined as a process under which oxidants such as free radical species attack lipids, resulting in lipid peroxyl radicals and lipid hydroperoxides that damage cell membranes. In diabetic heart, lipid peroxidation produces highly reactive aldehydes, including malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE). Experimental studies showed increased levels of MDA and 4-HNE in the myocardium of diabetic rats, suggesting that peroxidative damage may be involved in the pathogenesis of CVD. The increase in 4-HNE due to oxidative stress may contribute to myocardial damage and dysfunction by interfering with protein and subcellular organelles like mitochondria by forming adducts (Fig. 2). DNA damage induced by oxidative stress also activates poly ADP ribose polymerase (PARP), a DNA reparative enzyme. PARP diverts glucose metabolism from its usual glycolytic pathway (through inhibition of glyceraldehyde phosphate dehydrogenase) into alternative biochemical pathways that result in generation of various mediators which causes hyperglycemia induced cellular injury, which include AGEs, increased flux of hexosamine and polyol and activation of the enzyme PKC. The activation of PKC and peroxisome proliferator activated receptor- gamma and alpha (PPAR-γ and PPAR-α) results in elevated levels of plasma FFA. Increased plasma FFA levels in patients with T2DM and obesity, result in increased cardiac fatty acid (FA) uptake and triglyceride (TG) accumulation leads to cardiac steatosis (increased myocardial lipid content), has also been proposed as an important cause for CVD (Fig. 2). Extensive evidence suggests that mitochondrial dysfunction plays a critical role in the development of CVD. Mechanisms underlying the mitochondrial dysfunction and impairment in mitochondrial morphology include altered energy metabolism-induced mitochondrial uncoupling, oxidative stress, impaired mitochondrial Ca\(^{2+}\) handling, cell death and altered mitochondrial dynamics and biogenesis (Fig. 2). Chronic hyperglycemia can lead to increased flux of glucose into the alternate metabolic pathway known as hexosamine pathway that is implicated in many adverse consequences of diabetes. Increased glucose metabolism in the hexosamine pathway is associated with disruption of normal cardiomyocyte calcium flux linked to reduced sequestration of calcium in the sarcoplasmic reticulum. This results in reduction in myocardial performance and impaired diastolic relaxation, a possible mechanism for CVD (Fig. 2). Polyol pathway is also activated by chronic hyperglycemia and glucose is converted to sorbitol by the action of the enzyme aldose reductase (AR) in the presence of NADPH that is oxidized to NADP\(^+\). NADPH is a co-factor essential for regeneration of reduced glutathione, an important scavenger of ROS in the body, and increased utilization of NADPH in the polyol pathway disturbs the redox balance of cells. The
consequent increase in oxidative stress can lead on to DNA damage and cardiomyocyte apoptosis. Sorbitol can also glycate proteins that results in formation of AGEs, the mediators of tissue injury in diabetes (Fig. 2). AGEs are products of the non-enzymatic reaction of glucose with protein amino groups or lipids. Accumulation of AGEs in the diabetic heart causes irreversible glycosylation of structural proteins, including Ca²⁺ channels and can lead to increased myocardial stiffness. Besides, AGEs can increase myocardial collagen cross-linkages, leading to increased myocardial fibrosis and subsequently impaired cardiac relaxation and diastolic and systolic dysfunction. AGEs also contribute to inflammation via activation of transcription factors, such as NF-κB. NF-κB dependent genes in turn trigger several pathways that induce production of pro-inflammatory cytokines such as tumour necrosis factor-α and cause myocardial damage. In a study, NF-κB blockers were found to attenuate mitochondrial oxidative stress and protect against cardiac dysfunction in diabetic mice (Fig. 2).

Miller and colleagues demonstrated that during the early stages of diabetes there was an increase in plasma renin activity, mean arterial pressure and renal vascular resistance. Renin is the importance enzyme in the activation of the RAAS cascade. There are several mechanisms whereby diabetes can promote tissue Ang II/AT1R actions. Firstly, hyperglycemia directly stimulates local Ang II production in cardiomyocytes, cardi fibroblasts and endothelial cells as well as in murine and human diabetic heart tissues. Studies on cardiac myocytes suggest that the mechanism whereby hyperglycemia increases local Ang II in the heart is the generation of intracellular Ang II by intracellular chymase and/or internalized prorenin. Then, intracellular Ang II could directly produce oxidative stress and cellular apoptosis and/or enhance RAAS components expression through a positive feedback mechanism. The second mechanism underlying AngII/AT1R activation in diabetes is that high glucose concentrations can enhance the tissue response to Ang II and vice versa. Interestingly, several works have demonstrated that hyperglycemia can also stimulate aldosterone secretion by increasing local Ang II. Xue and Siragy observed an upregulation of renal aldosterone synthase in diabetic rats, which was significantly reduced by AT1R blockade. The third way whereby diabetes promotes Ang II tissue actions is through the several metabolic abnormalities associated with hyperglycemia. These include AGEs, which form after prolonged hyperglycemia and oxidative stress, dyslipidemia, and low-grade inflammation. All of them can in fact stimulate the AngII/AT1R pathway by upregulating AT1R expression. Another intriguing mechanism whereby diabetes enhances AngII/AT1R actions is angiotensin converting enzyme 2 (ACE2) downregulation, which does not only promote Angiotensin II actions but also reduce local Ang 1–7 leading to an imbalance of the RAAS (Fig. 2). This perception is supported by the works of Tikellis and colleagues, who showed that the induction of diabetes was associated with a significant reduction of ACE2 expression and ACE2 activity in the heart and the vasculature together with a significant increase in circulating Ang II and a significant reduction of Ang 1–7 levels. Similar changes were reported in the kidney of diabetic mice.

MICRONUTRIENTS

Micronutrients are one of the major groups of nutrients our body needed only in minuscule amounts for specific functions. They are the “magic wands of health” that enable the body to produce enzymes, hormones and other substances essential for proper growth and development. As tiny as the amounts are, however, the consequences of their absence are severe. Micronutrients include vitamins and minerals and they are further classified as water-soluble vitamins, fat-soluble vitamins, macrominerals and trace minerals. Vitamins are necessary for energy production, immune function, blood clotting and other functions. Minerals play an important role in growth, fluid balance, bone health and several other processes. Many studies have been conducted to investigate the independent role of several vitamins and minerals on diabetic complications where they act as a hypoglycaemic, anti-oxidant, anti-inflammatory and insulin-mimetic agents. Some vitamins and minerals target the molecules that are involved in the ROS and oxidative stress activation, inflammation, RAAS activation which are some common leading pathways involved in pathogenesis of DN and CVD. Finding of these studies reveal that micronutrients may collectively
be used for alleviation of both DN and CVD as they are interlinked.

1. Vanadium

The trace element vanadium’s chemical structure is similar to that of phosphorus, which appears to influence its biochemical actions. Vanadium compounds increases the activity of PI3K; enzyme involved in insulin signalling cascade; phosphorylates the substrate phosphatidylinositol 4,5-biphosphate (PIP2) to form phosphatidylinositol 3,4,5-triphosphate (PIP3) and increases its level by activation of the insulin-like growth factor I receptor (IGF-IR). Through inhibitory impact on phosphatase and tensin homologue (PTEN), vanadium compounds also increases the activity of PKB/Akt which is recruited by PIP3 (Fig.4). PTEN are the main negative regulator of the PI3K, dephosphorylates PIP3 to generate PIP2. The activated PKB phosphorylates a variety of proteins, particularly, forkhead box protein 01 (FOXO1) transcription factor, which diminishes the expression of enzymes involved in gluconeogenesis. (Fig.4) PKB activation by vanadium also increases the level of glucose transporter 4 (GLUT4) which transfers glucose in the cytoplasm of adipocytes or muscle cells and inactivates glycogen synthase kinase-3 (GSK-3), leading to stimulation of the synthesis of glycogen from glucose by glycogen synthase enzyme (Fig. 4). This result is also supported by findings by K. Vijay et al which shows that liver glycogen level is increased by using vanadium pentoxide nanoparticles in diabetic rats.

Protein tyrosine phosphatase-1B (PTP1B) is a negative regulator of the insulin signalling pathway which regulates the phosphorylation process between the insulin receptor (IR) and its substrate (IRS). Vanadium inhibits the activity of PTP1B and allows the IR to remain activated, that is, to retain the tyrosine phosphorylation of the IR-β subunit. So, by preventing dephosphorylation of the IR-β subunit, vanadium may ameliorate the activity of IR protein tyrosine kinase (PTK). The effects of vanadium occur through IRS-1 phosphorylation due to the potent protein tyrosine phosphatase (PTPases) inhibitory properties of vanadium salts. Vanadium also act as a modulator of mitogen-activated protein kinases (MAPK) pathways. Pandey and coworkers have demonstrated that vanadyl sulfate treatment resulted in an increased level of tyrosine phosphorylation of extracellular signal regulated kinase 1/2 (ERK 1/2), stimulation of MAPK kinase (MEK) and C-raf-1 activities, and activation of p21ras and ribosomal protein 6 kinase (S6K). PI3K inhibitors, wortmannin and LY294002, also have shown to block the vanadyl sulfate-mediated increase in MAPK activity and phosphorylation of ERK 1/2 and S6K. These results suggested that the vanadyl sulfate mechanisms are mediated by the PI3K-dependent stimulation of the Ras-MAPK and S6K pathways (Fig.4).

2. Selenium

Selenium, an essential biological trace element, is an integral component of several enzymes and acts as an anti-inflammatory and antioxidant micronutrient. Like vanadium, selenium and their nanoparticles has shown anti-hyperglycemic activity. Selenium shows insulin-mimetic effects by activation of Akt and other kinases of the insulin signaling cascade such as p70S6 kinase (Fig.4). Sodium selenate translocate glucose transporters to the plasma membrane and activate serine/threonine kinases, including the p70 S6 kinase resulting in glucose uptake in isolated rat adipocytes. Selenium also accelerate renal glucose excretion in rats.

Selenium shows hypoglycaemic effect through glutathione peroxidase-1 (GPx-1) expression. GPx-1 was the first identified and the most abundant selenoproteins and its overexpression in wild-type animals afforded protection from streptozotocin (STZ) treatment. For example, mice that overexpressed GPx-1 were protected from STZ-induced β-cell damage. GPx-1 overexpression in db/db mice, a model of increased genetic diabetes susceptibility, led to initial amelioration and then reversal of hyperglycemia without the use of hypoglycemic agents. Another selenoprotein, selenoprotein P (Sepp1) and also GPx-1 inhibits the excessive production of ROS in β-pancreatic cells and selenium improves insulin sensitivity due to its antioxidant action in peripheral organs (Fig.4). In the liver cells, selenium regulate glucose-6-phosphatase (G6Pase) and glycogen phosphorylase enzymatic activity, thereby influences hepatic glucose production and also promotes the transcription and synthesis of proinsulin, insulin promoter factor 1, and increases glucagon-like peptide-1 receptor (GLP-1R) expression. By acting through different mechanisms on these different cells and organs, selenium improves insulin resistance.
primary rat hepatocytes or 3T3 L1 adipocytes. Selenium induced the expression of \(\beta\)-subunit of the insulin receptor and IRS-1; with induction of two more proteins associated with insulin signalling, p42 and p44 MAP kinases. These data which showed that selenium not only increased the phosphorylation of proteins identified in the insulin signaling cascade but also affected the overall phosphorylation state of the cell (Fig.4). [73]

3. Chromium

Chromium is one of the vital micronutrient obtained from diet which serves to potentiate insulin action and maintain normal glucose tolerance. Chromium shows insulin-mimetic action by increasing the number of insulin receptors, binding of insulin to cells and affecting protein phosphorylation-dephosphorylation reactions. Chromium increases insulin sensitivity and phosphorylation of the insulin receptor by activating insulin tyrosine kinase, thereby increases the activity of downstream effectors of insulin signalling cascade, PI3K and Akt and enhance GLUT4 translocation to the cell surface and glucose uptake due to cholesterol efflux mediated by chromium. Chromium also inhibits PTP-1B, which causes inactivation of insulin receptor and alleviates ER-stress within the cells, rescuing IRS from c-Jun N-terminal kinase (JNK)-mediated serine phosphorylation and subsequent ubiquitination (Fig.4). Chromium Picolinate upregulates 5′-adenosine monophosphate (AMP) – activated protein kinase (AMPK) to increases glucose uptake and decreases lipogenesis and fatty acid oxidation (Fig.4). [74,75] Some study provide evidence that chromium picolinate (CrPic) and chromium histidinate (CrHis) may have a protective role against diabetic nephropathy through the nuclear factor erythroid-2-related factor 2 (Nrf2) pathway and also through an anti-inflammation effect by NF-κB inhibition (Fig.4). Moreover, a greater reduction in NF-κB level and greater increases in the levels of nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha (IκBα) and Nrf2 in diabetic rats supplemented with CrHis than rats supplemented with CrPic suggest that CrHis has more favorable effects than CrPic. [76] Doddigarla et al. stated that CrPic and melatonin each alone and in combination decreased blood glucose levels in high carbohydrate diet-fed male rats. [77] CrPic exerts its antihyperglycemic and insulin-sensitizing actions through increased GLUT4 expression [78] and through the regulation of lipid and carbohydrate metabolism. [79,80] CrPic is also effective in reducing hyperglycemia in type 2 diabetes and in suppressing lipid peroxidation by enhancing antioxidant mechanisms. [81]

4. Magnesium

The mineral magnesium functions as an essential cofactor for more than 300 enzymes. It is essential for all energy-dependent transport systems, glycolysis, oxidative energy metabolism, biosynthetic reactions, normal bone metabolism, neuromuscular activity, electrolyte balance and cell membrane stabilization. The activity of phosphoenolpyruvate carboxy kinase (PEPCK) and G6Pase, gluconeogenesis enzymes were found to be increased in magnesium deficiency. [82] Magnesium contributes to the activation of GLUT4 gene expression and translocation in muscle via the inhibition of FOXO1 gene and protein expression. Moreover, magnesium suppresses PEPCK enzyme via inhibition of FOXO1 gene expression in liver to prevent of hyperglycemia (Fig. 4). [83] In adipose tissue, Magnesium acts as an anti-inflammatory factor reducing interleukin-1 and TNF-α secretion. [84] One of the important marker in oxidative stress was malondialdehyde which was decreased after magnesium administration to improve renal dysfunction via lowering of blood urea nitrogen and creatinine in diabetic rats. [85] Magnesium also increased the IR, GLUT-4 and glutathione expression in magnesium treated group as compared to diabetic group. Morakinyo et al. findings provide a mechanistic basis for the use of magnesium supplements in the maintenance of glucose homeostasis in type 2 diabetes condition. [86] For the phosphorylation of IR and the activity of other signal kinases, intracellular magnesium concentrations are critical which operates together with ATP as a kinase substrate. Magnesium may exert regulatory influence on tyrosine kinase of IR and other enzymes, mediating the metabolic effects of insulin. [87] It plays a potential role in improving insulin sensitivity by increasing the affinity of the insulin receptor tyrosine kinase for ATP and regulates insulin secretion in pancreatic β-cells by acting as a cofactor for adenine nucleotides thereby influencing the rate of glucokinase; enzyme that convert glucose into glucose-6-phosphate. ATP produced in glycolysis process from glucose-6-phosphate binds to the Kir6.2 subunits and closes the ATP-sensitive potassium (KATP) channel; the main regulator of membrane potential in pancreatic β-
cells. Conversely, magnesium-ATP complex (Mg-ATP) binds to the sulfonylurea receptor 1 subunits of the channel and opens it. Inhibition of KATP channels by increased ATP levels results in depolarization of the membrane, activating \( \text{Ca}^{2+} \) influx via the voltage-dependent L-type \( \text{Ca}^{2+} \) channel that ultimately leads to the insulin vesicle release (Fig.3). 

![Figure 3. Regulatory role of magnesium in insulin release from pancreatic beta cells.](image)

Magnesium exerts beneficial effects on the cardiovascular system by acting on transmembrane ion transport pumps, improving glucose and insulin metabolism, enhancing endothelium-dependent vasodilation, improving lipid profile, and acting as an antihypertensive and anti-inflammatory agent. Additionally, magnesium is a natural calcium antagonist, is an essential cofactor in mitochondrial oxidative reactions, and has anticoagulant and antiplatelet properties. [89]

5. Vitamin B (Nicotinamide)

Water soluble Niacin (vitamin B3) occurs in two forms: nicotinic acid and nicotinamide. The active coenzyme forms (nicotinamide adenine dinucleotide [NAD] and NAD phosphate are essential for the function of hundreds of enzymes and normal carbohydrate, lipid, and protein metabolism. Nicotinamide has shown to prevent the development of diabetes in a cyclophosphamide induced NOD mouse model by inhibiting \( \beta \)-cell apoptosis and has proven to have free radical scavenging activity and consequently could well reduce DNA damage. [90] As nicotinamide is involved in NAD biosynthesis, it can restore the islet cell content of NAD towards normality and can also inhibit PARP, the DNA repair enzyme, thereby preventing cellular NAD depletion and inhibiting apoptosis. [91,92,93] Niacin has also proved its effect by decreasing oxidative stress parameters (Superoxide dismutase, catalase, glutathione) with reductions in fasting blood glucose levels and invigorating the glucose metabolism enzyme (Hexokinase, Glucose-6-phosphate, Fructose bisphosphatase) in diabetic treated group. This result was also supported by the liver and kidney tissues damage recovery by performing histological studies and cellular DNA damage was observed by performing comet assay in diabetic group in a study by Abdullah KM and colleagues. [94]

6. Vitamin D

1,25-Dihydroxyvitamin D3, the hormonal form of vitamin D, is an endocrine hormone with multiple physiological functions. Vitamin D deficiency have shown to induce insulin resistance, the main hallmark in the development of type 2 diabetes, but resupplementation of vitamin D reduced gluconeogenesis via AKT/FOXO1 mediated PEPCK and G6Pase (gluconeogenic enzymes) downregulation and enhanced glycogen synthesis via AKT/ GSK3\( \beta \) mediated glycogen synthase (GS) activation in mice; alongwith inhibition of hepatic lipid accumulation due to decreased expression of lipogenic genes Srebplc,
Acc and Fasn in findings by Mutt SJ and colleagues (Fig. 4). The RAS is the major pathway involved in pathogenesis of both DN and CVD which is negatively regulated by 1,25-Dihydroxyvitamin D3. Hyperglycemia-induced renal injury was more in the vitamin D receptor (VDR) lacking mice which also developed more severe albuminuria and glomerulosclerosis due to increased glomerular basement membrane thickening and podocyte effacement. Vitamin D3 suppressed high glucose-induced fibronectin production in cultured mesangial cells, increased renin, AT1R, angiotensinogen, ACE, TGF-β, and connective tissue growth factor (CTGF) which caused more severe renal injury; nephrin expression in cultured podocytes was also found to be increased by vitamin D in VDR knockout mice. These finding proves the renoprotective role of vitamin D3.

Another important target for vitamin D is decreasing the proinflammatory (p38MAPK, MCP-1) and profibrotic markers (TGFβ-1 and CTGF) involved in the DN pathogenesis; this may occur through expression of its VDR as well as the Klotho gene expression and through blocking of renin & Ang II expression (Fig. 4). Vitamin D and metformin combination in type 2 diabetic rats resulted in synergistic action by improving the cardiac functions, glycemic control, lipid metabolism and increased the expression of cardiac sarcoplasmic reticulum Ca²⁺-ATPase, beclin 1 and VDR in a study by El Sayed DM and colleagues.

7. Vitamin E:
This essential fat-soluble vitamin functions primarily as an antioxidant. Vitamin E attenuate tubular epithelial cells injury, apoptosis and prevent progression of EMT and tubulointerstitial fibrosis by depressing autophagic stress characterised by the accumulation of autophagic vacuoles and autophagy substrate which are induced by AGEs through blocking lysosomal-dependent degradation of autophagosomes in proximal tubules in DN. Interestingly, vitamin E treatment has shown to significantly reduce the cellular apoptosis and improved cell viability in-vivo and in-vitro. It has demonstrated renoprotective effect by decreasing the NF-κβ p65 subunit expression induced by ROS and Caspase-3 levels in kidneys of diabetic rats in dose dependent manner which provide the evidence that cellular apoptosis in DN is prevented by vitamin E (Fig. 4).

In findings by Zaulkffali AS et al., the combination of vitamins D and E has shown significant increase in GLUT4, p-AKT, reduced ROS level and vitamin D alone increases IR, P13K, GLUT3, GLUT4 expression levels as well as AKT phosphorylation and glucose uptake and Vitamin E alone increases p-Akt, reduces the ROS in insulin-resistant model induced in SK-N-SH neuronal cells which showed the insulin resistance mechanism of both vitamins D and E.

Figure 4. Insulin-mimetic mechanism and potential sites of micronutrients activity.

PTP-1B, PTEN, PHLPP, FOXO1, PEPCK, G6Pase, NF-κB, GSK3, ROS; negative signals of signalling cascade gets inhibited or deactivated by micronutrients. Legend: P-phosphorylation, Vd- vanadium, Se- selenium, Cr- chromium, Mg- magnesium, Vit D- vitamin D, Vit E- vitamin E, MN- micronutrients.
CONCLUSION
Multiple micronutrients deficiency disorders occurs because of poorly controlled diabetic conditions and thereby changes the health status and life style of people. Micronutrients deficiency conditions are widespread among 2 billion people in developing and in developed countries. So for the prevention of these conditions and thereby improving the health of patients; it is better to understand the cellular and molecular mechanism of micronutrients in DN and CVD. This review highlights the molecules involved in insulin signalling cascade as a therapeutic target and their overall cross-talk with diabetic complications and micronutrients. Therefore by considering beneficial effects of micronutrients in diabetes and its related complications specially DN and CVD, people with such complications should include vitamins and minerals in their natural daily diet. The selected micronutrients shows hypoglycaemic, anti-oxidant, anti-inflammatory activity which are beneficial properties of micronutrients to treat diabetes. Many studies have been conducted to investigate the independent roles of several vitamins and minerals for examples vitamin D, selenium etc. on diabetic complications. There is, thus, a future scope to use combination of vitamins and minerals for treatment of diabetic complications. There is also an opportunity to elucidate and establish the effective metabolic pathway which are involved in treating DN and CVD by using micronutrient formulations in animal models of diabetes and establish micronutrients as potential add-on therapy for diabetes and its complications thereby improving quality of life of diabetic patients.

REFERENCES
1) https://apps.who.int/iris/rest/bitstreams/1233344/ret rieve, Classification of DM 2019, WHO.


71) Harmon JS, Bogdani M, Parazzoli SD, Mak SSM, Oseid EA, Berghmans M, LeBoeuf RC, Robertson RP. Beta-Cell-specific overexpression of glutathione peroxidase preserves intranuclear MafA
92) Suarez-Pinzon WL, Mabley JG, Power R, Szabo’ C, Rabinovitch A. Poly (ADP-ribose) polymerase inhibition prevents spontaneous and recurrent autoimmune diabetes in NOD mice by inducing


How To Cite This Article:

Source of Support: Nil
Conflict of Interest: None declared

Your next submission with British BioMedicine Institute will reach you the below assets
- Quality Editorial service
- Swift Peer Review
- E-prints Service
- Manuscript Podcast for convenient understanding
- Global attainment for your research
- Manuscript accessibility in different formats (Pdf, E-pub, Full Text)
- Unceasing customer service

Track the below URL for one-step submission
http://www.britishbiomedicine.com/manuscript-submission.aspx