This is an Accepted Manuscript, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. This Accepted Manuscript will be replaced by the edited, formatted and paginated article as soon as this is available.

You can find more information about Accepted Manuscripts in the Information for Authors.

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal's standard Terms & Conditions and the Ethical guidelines still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this Accepted Manuscript or any consequences arising from the use of any information it contains.
PREDIABETES: GROUNDS OF PITFALL SIGNALLING ALTERATION FOR CARDIOVASCULAR DISEASE

PREDIABETES AND CARDIOVASCULAR DISEASES

Author names and affiliations

Sameer N. Goyal*¹, Shaikh Haiderali¹, Navya Reddy M¹, Dharamvir Singh Arya², Chandragouda R. Patil¹

¹Cardiovascular Pharmacology Division, Department of Pharmacology, R.C.Patel Institute of Pharmaceutical Education and Research, Shirpur- Dist-Dhule, Maharashtra, India

²Department of Pharmacology, All India Institute of Medical Sciences, New Delhi-110029, India

*Correspondence Address: Sameer Goyal Ph.D. (AIIMS, New Delhi)

Associate Professor, Department of Pharmacology
R.C.Patel Institute of Pharmaceutical Education and Research,
Shirpur, Dist-Dhule, Maharashtra, INDIA
Landline: 02563 255 189| Mobile: +91 955 291 6993
Fax: 02563-251808
E mail: goyal.aiims@gmail.com
Abstract

Impaired glucose metabolism either in prediabetes or diabetes mellitus is one of the detrimental root causes of premature mortality throughout the world. Uncontrolled Prediabetes coincides with the induction of diabetic mellitus and associated cardiovascular diseases (CVDs). Needless to mention, impaired glucose metabolism, including impaired fasting glucose (IFG) and impaired glucose tolerance (IGT) have been known identically or in combination as prediabetic stage but by itself is not diabetes mellitus. Impaired β-cell function, insulin resistance, increased level of FFAs, hyperinsulinemia and down-regulation of GLUT-4 are the critical impairments during prediabetes. Vascular endothelium sustains the free flow of blood in vessels by normalizing vascular tone by releasing numerous endothelial-derived factors. However, in recent studies a marked impairment in endothelial-derived factors has been observed in prediabetes. Thus, the impaired endothelial-derived factors could make prediabetic patients more vulnerable to cardiovascular disease pathology. Nobel laureates, Robert Furchgott, Louis Ignarro and Ferid Murad (1998) discovered a novel signalling molecule; Nitric oxide (NO), identified as endothelium-derived relaxing factor. This imperative mediator has potent vasodilatory, anti-platelet, anti-proliferative, and anti-inflammatory actions in vessels. Endothelium-derived NO generation is mediated through the activation of PI3-K-Akt-eNOS-NO signalling pathways. Therefore, conspicuous destruction in PI3-K/Akt-eNOS-NO signalling has been revealed in prediabetes and render individuals more susceptible to CVDs. several researche has defined prediabetes as a platform for diabetes mellitus and associated CVDs. But the molecular alteration during Prediabetes is unclear; although the signalling modulator may be imperative issue and may open a prerequisite new vista for novel research. In this review, we have critically discussed the possible signalling alteration in Prediabetes.

Keywords: Prediabetes, PI3K/AKT signalling, signalling alteration, cardiovascular diseases, IGT, IFG
Main text

1. Introduction

Impaired glucose metabolism is an overt sign of insulin dependent and non-dependent diabetes mellitus over a period of many years which is the major culprit of mortality with towering prevalence in developed and developing countries [1]. Prediabetes has been firstly expressed by the ‘World Health Organization’ in 1980, as impaired glucose tolerance [1]. According to “American Diabetes Association” average blood glucose measurement level in prediabetes for A1C test, fasting plasma glucose (FPG) test and oral glucose tolerance test (OGTT) is to be 5.7% to 6.4%, 100 mg/dl to 125 mg/dl and 140 mg/dl to 199 mg/dl respectively [2]. However, a huge irony of fate is about its awareness. The latest statistics suggest, 1 out of 3 US adults and older have prediabetes, but only 11% are aware of their status [3]. Generally prediabetes defined as the transitional metabolic states between normal and diabetic glucose homeostasis [4]. Impaired glucose metabolism, including impaired fasting glucose (IFG) and impaired glucose tolerance (IGT) is referred to individually or in combination as prediabetic stage but by itself is not diabetes [5]. IFG predominantly concerns to hepatic insulin resistance sensitivity, whereas muscle insulin resistance is responsible for IGT [6]. Hepatic insulin resistance and unreliable insulin secretion in isolated IFG bring excessive production of fasting hepatic glucose creation which instigates fasting hyperglycemia [7]. Thus, diminishing of early insulin response to combination of hepatic insulin resistance consequently increase the plasma glucose level. But the defence of late insulin secretion combined with normal muscle insulin sensitivity permit glucose levels to restore to the primary value in isolated IFG [6]. In contrast, hepatic insulin resistance and, late insulin secretion become imperfect in isolated IGT, which result in prolonged hyperglycemia after glucose intake [4]. Insulin resistance and impaired β-cell function are the crucial imperfections examined in diabetes mellitus, and both these defects are noticed in IGT and IFG [8]. These important risk markers are major contributors of diabetes, retinopathy, neuropathy, vascular endothelial dysfunction and increased risk of cardiovascular pathology [9] figure 1.

Endothelium is an innermost lining of blood vessels which is principally involved in the regulation of vascular tone and maintenance of free flow of blood through vessels. C-type Natriuretic peptide (CNP) and endothelin-1 (ET-1) are potent vasodilator and vaso-contracting endothelium derived factors which legalize the vascular homeostasis. However, evidences suggest the decreased level of endothelial-derived factor CNP, increased level of ET-1 and thickness of tunica intima are the key signalling alteration in IGT associated atherosclerosis and cardiovascular complication [10]. In-addition, phosphatidylinositol 3-kinase (PI3K)
followed by protein kinase-B (PKB or Akt) signalling cascade is widely demonstrated for cardio protection via activation of endothelial nitric oxide synthase (eNOS). This triggered eNOS provoke the generation and bioavailability of novel biosensor nitric oxide (NO) which has potent vasodilatory, anti-platelet, anti-proliferative, and anti-inflammatory actions in vessels [1]. Though, some recent findings suggest the alleviation of this innovative PI3K-Akt-eNOS-NO survival pathway during prediabetes condition [11].

Hypertriglyceridemia is a predisposed risk factor allied with atherosclerosis, vascular endothelial dysfunction (VED), and cardiovascular diseases (CVDs) [12]. In spite of this, increased level of triacylglycerol, blood pressure and decreased activity of high density lipoprotein (HDL) -associated phospholipase A₂ has been studied in prediabetic subjects [13]. Moreover, reactive oxygen species (ROS) and tumor necrosis factor alpha (TNF-α) are key triggering molecule in pathogenesis of endothelial dysfunction and associated cardiovascular disorders [14]. However, ROS and TNF-α are markedly over diligent in prediabetes. In-addition to TNF-α; an increased plasma levels of granulocyte–monocyte colony-stimulating factor (GM-CSF) interleukin-5 (IL-5), interleukin-6 (IL-6) and interleukin-7 (IL-7) has been registered in prediabetes [15]. Thus, activated pro-inflammatory cytokine and dendritic cells (DCs) plays a crucial role in the development of cardiovascular diseases [16]. In spite of these signalling variation, sensitization of α-1 and α-2 adrenoreceptor signalling also has been perceived in prediabetes. Furthermore, this sensitization of α-1 and α-2 adrenoreceptor revealed the contribution of prediabetes to associated coronary vasoconstriction and cardiac ischemia [17]. Prediabetes associated alteration in gene expression also asserted in the pancreatic islets resulting further down-regulation of several genes participated in glucose metabolism and insulin signalling pathways in next generation [18]. Taken together, the evidences in terms of gradual decay in beta-cell function or increased insulin resistance and alteration in survival signalling pathways could impact significantly the action of drugs for treating cardiovascular complications associated with prediabetes. The present review delineates crucial pathophysiology and various signalling pathways identified to be involved in the pathogenesis of prediabetes.

2. Prevalence and pathophysiology

Prediabetes is the foremost trigger feature of diabetes mellitus and is a pivotal bench mark of VED and CVDs. Prevalence of prediabetes is rapidly growing in the population of developed and developing countries [19]. The fact sheet of National Health and Nutrition Examination Survey (NHANES) described, 35% of US aged ≥ 20 years and 50% of those ≥ 65 years suffered with prediabetes in 2005–08, defined on the measurement of HbA1c concentrations. Estimated yield of 2010, 79 million adults were calculated from the percentages of the
entire US population [20]. As per 2011 report of the International Diabetes Federation (IDF), the prevalence of this insidious disease has been estimated to reach 472 million by the year 2030 [4]. Unreliable generation, bioavailability and sensitivity of insulin with respect to plasma glucose level leads to hyperglycaemia. The increased production of fasting hepatic glucose is carried out by virtue of hepatic insulin resistance and variable insulin secretion in isolated IFG which instigates the fasting hyperglycaemia [7]. However, weaker early insulin response and hepatic insulin resistance consequently increase the plasma glucose level. In-fact this accumulated plasma level could be restored by late insulin secretion in isolated IFG [6]. In-contrast, the imperfection in hepatic insulin resistance and late insulin secretion in IGT result in prolonged hyperglycemia and prediabetes [4]. Free fatty acids (FFAs) and cytokines release, directly worsen insulin sensitivity. FFA provokes ROS and blunts the activation of insulin receptor substrate 1 (IRS-1). This alleviated IRS-1, consequently debilitates PI3K-Akt signalling and glucose transporter-4 (GLUT-4) in skeletal muscle and adipose tissue therefore, down-regulation of GLUT-4 leads to insulin resistance and diabetes mellitus [21]. Obesity-linked insulin resistance could increase the level of cholesterol and that may be a core basis of prediabetes, diabetes mellitus and associated CVDs [2]. Obesity also could promote an inflammatory pathway in pancreatic islets and lead to β-cell dysfunction. Thus, β-cell dysfunction may develop prediabetes and diabetes [22]. Hence β cell damage lead to increased insulin release leading to hyperinsulinemia. Hyperinsulinemia accelerates insulin resistance and induces rise in circulating cholesterol [23]. In this manner, the increased level of FFAs, insulin resistance, cholesterol, β-cell dysfunction; hyperinsulinemia and down-regulation of GLUT-4 are indeed pitfall causes of prediabetes onset.

IFG and IGT are the precursors of type 2 diabetes and associated CVD’s in individuals with prediabetes. Moderate to severe insulin resistance and impaired first and second phase insulin secretion occurs in muscle of subjects with isolated IGT whereas, mild insulin resistance in liver, impaired first phase insulin secretion and almost normal muscle insulin sensitivity is seen in individuals with IFG [24]. Apart from insulin resistance, excess fatty acids, adipokines etc released from adipose tissue in individuals with prediabetes predisposes diabetes and associated cardiovascular diseases [9]. These adipokines and glucose activate specific intracellular signaling pathways in endothelium, resulting in endothelial dysfunction in diabetes [25]. Epidemiological studies, have shown that pre-diabetes confers an increased risk of cardiovascular disease. More or less 70% of individuals with prediabetes progress into diabetic state with an additional risk for cardiovascular diseases as per an ADA expert panel [26]. Prediabetes associated Hyperglycemia mediated adverse effects through multiple pathways such as polyol, hexosamine, protein kinase C, and glycation pathways are the major
factors contributing to cardiovascular risk by generating reactive oxygen species. Postprandial hyperglycemia negatively contributes to endothelial dysfunction imparting high CVD risk than fasting hyperglycemia by several mechanisms like activation of NFκB and expression of monocytes - macrophages and smooth muscle cells [27]. Moreover, Kathryn reported the role of the β unit of Transforming growth factor (TGFβ) in ventricular remodelling by activating the Akt–mTOR–p70S6K1 pathway and also by reactive oxygen species generation assisting further transforming of prediabetes into diabetes and cardiac dysfunction [28]. Haemoglobin A1c levels between 5.7 -6.4% in prediabetic patients are diagnostic markers of future risk of diabetes and associated CVD’s [27].

3. Endothelium associated signalling alteration during prediabetes

Endothelium is an interior layer of blood vessel which regulates numerous biological functions [29][24]. Being an innermost layer, it regulates the vascular tone and homeostasis by maintaining endothelium-derived relaxing (e.g., Acetylcholine, ATP and ADP, substance P, bradykinin, histamine, thrombin, serotonin) and contracting factors (e.g., Endothelin-1, arachidonic acid, Ca+ ionophore A23187, thromboxane A2, prostaglandin H2, and superoxide anions) [309]. Endothelium-derived hyperpolarizing factor, CNP originate from endothelium wall of vessel and act as endothelial relaxing factor [31]. Suppression of endothelial dependent vasorelaxation and expression of endothelial dependent vasocontraction leads to impairment in regulation of vascular homeostasis [32]. CNP, an endothelium-derived hyperpolarizing factor; promotes cellular cGMP level by binding to G-protein receptor and Natriuretic peptide receptor-B [33]. CNP potently increase the level of IL-1β, TNF in response to lipopolysaccharide [34]. Thus, lower level of CNP is robustly correlated with VED, hypertension, atherosclerosis, thrombogenesis, re-stenosis and left ventricular diastolic dysfunction [35]. In-addition, increased level of endothelin-1, a potent vasocontracting factor also exaggerate VED and associated cardiovascular diseases [35]. Regrettably, unsteadiness has been observed in both of these endothelial derived factors during prediabetes and insulin resistance which is the pitfall hallmark of VED and associated cardiovascular disorder. Clinical evidences revealed the substantial reduction of CNP and considerably higher level of endothelin-1 during prediabetes and thickness of endothelial layer which address endothelial dysfunction [6] figure 2. This premise is supported by an imbalance of these endothelial derived factors in diabetes mellitus [36]. Thus, the disparity in endothelial function during prediabetes because of impaired release of endothelium-derived relaxing and contracting factors may show the way of cardiovascular complication.

4. PI3K/PKB signalling alteration during prediabetes
PI3Ks, a family of kinases, are stimulated by tyrosine kinase receptors and G-protein coupled receptors. Akt/PKB, is a family of serine/threonine kinase [37]. PI3-K-Akt-eNOS-NO signalling has a pivotal role in the regulation of cardiovascular physiology [38]. PI3K/Akt signalling pathway is involved to invoke the stimulation and activation of eNOS, and associated NO formation in endothelial cells [39]. PI3K pathway also plays a vital role in the regulation of vascular endothelial function via activation of Akt/PKB, which consequently increases the phosphorylation of eNOS, and NO generation [40]. Several evidences suggested the regulating role of PI3K-Akt signalling cascade in cardiomyocyte survival, angiogenesis, and inflammation [41]. Several investigators have confirmed the fundamental role of PI3K/AKT signalling in cardiovascular protection.

*Prosopis glandulosa*, (A honey mesquite tree) belong to Fabaceae (or legume) family, exerts cardio-protective and anti-hypertensive effects against prediabetes rat model via activation of PI3-K-Akt signalling pathways [42]. Statins are potent inhibitors of 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase, which is a rate-limiting enzyme for the synthesis of cholesterol, and are extensively used in the treatment of cardiovascular abnormalities including dyslipidemia, atherosclerosis and hypertension. This cardiovascular defensive action of statins highlights up-regulation of PI3-K-Akt-eNOS pro-survival pathways [43]. Kobayashi et al., 2009 also imply the involvement of PI3-K-Akt associated eNOS production in the improved cardiac functioning and remodelling in cardioprotective effects of statins [44]. Carbamylated erythropoietin, a carbamoyl derivative of erythropoietin, shows cardiovascular protective action by reducing myocardial apoptosis. Intriguingly, this attenuated cardiomyopathy by Carbamylated erythropoietin involves stimulation of PI3K-Akt signaling pathways in rat myocardial cells [45].

A widely preferred clinical approach of inhibiting ischemia-induced cardiac apoptosis using *Panax notoginseng* saponins involves activation of PI3-K-Akt signaling pathways [46]. Likewise, Fibroblast growth factor 21, a hepatic metabolic regulator exerts a cardiovascular protective action in lean and obese rat hearts in response to ischemia-induced injury through autocrine-paracrine pathways. Interestingly, Fibroblast growth factor 21 associated cardioprotection against ischemia-induced injury is mediated through PI3K/Akt, ERK1/2 and AMPK pathways [47]. Moreover, naturally occurring antioxidant like grape seed proanthocyanidin, defends cardiomyocytes against I/R injury by activation of Akt-eNOS signalling [48]. SO2 which is believed to be a pollutant and toxic gas exerts cardiovascular protection against myocardial ischemia/reperfusion (I/R) injury in rats. SO2 preconditioning significantly reduces myocardial infarct size and myocardial caspase-3 and 9 activities. This protective effect of SO2 preconditioning is suggested to involvePI3K/Akt pathway [49]. Ref 45 reported that N-myc downstream-regulated gene 2 exerts novel action of PI3K/Akt signaling in insulin
mediating cardioprotection [50]. Cuadrado et al. (2011) demonstrated that labdane diterpenes which are well known for their cytoprotective and anti-inflammatory actions also provide cardio-protection against anoxia-reperfusion-induced injury by anti-apoptosis action in isolated heart by activating PI3K/AKT signalling [51]. Similarly, Sun et al. (2011) have reported, the cardioprotective actions of tanshinone IIA is also mediated through Akt-dependent pathway in diabetic cardiomyopathy [52]. Luteolin, a naturally occurring polyphenol flavonoid, exerts myocardial protection through the PI3K/Akt pathway in experimental model of ischemia/reperfusion [53]. β-cadenin targeted remote ischemic preconditioning exerts potent myocardial protection by activating the PI3K/Akt signalling pathway [54]. Activated protein C (APC) mediated cardioprotection against I/R injury also involves improvement of endothelial function via AKT1 activation [54]. Kallistatin balances the vascular homeostasis by attenuating endothelial apoptosis through activation of Akt-eNOS signaling pathway [55]. The PI3-K-Akt associated signalling cascade plays an imperative role in cardiac function, including cardiac contractility [56]. The PI3-K-Akt associated cardioprotective action against I/R injury offer unique opportunities for improving cardiac physiology [57]. Taken together, PI3-K-Akt-eNOS signalling activation plays an integral role in maintaining cardiovascular function, and preventing cardiovascular disease pathology.

However, studies revealed that PI3-K-Akt-eNOS signalling is substantially down-regulated in prediabetes condition [1]. Prediabetes associated metabolic changes including perturbed endothelial function, sub-clinical inflammation [58], changes in adipokines, expansion of atherogenic dyslipidemia, raised levels of free fatty acids (FFAs), and changes in thrombosis and fibrinolysis leads to cardiovascular abnormalities. IRS diminution has been observed in insulin resistance and β cell dysfunction, which leads to PI3K-Akt/eNOS pathway suppression and consequently generates the platform for vascular endothelial dysfunction and associated cardiovascular diseases [21]. A marked suppression in PI3-K-Akt-eNOS signalling in endothelial cells is noted in hyperglycemia [59]. In addition, hyperglycemia-associated impairment in PI3-K-Akt signaling might promote endothelial cell proliferative dysfunction in diabetes. In the vascular system, the endothelium-dependent relaxation is impaired in diabetes mellitus [59]. Ref 37 reported a significant down-regulation in PI3-K activity with consequent decline in phosphorylation of downstream targets, including protein kinase B (PKB)/Akt, mTOR and FoxO in prediabetes associated obese groups [42]. In addition, Hanson et al., (2010) demonstrated that, insulin dependent phosphorylation of human erythrocyte cAMP is controlled by PI3K and PDE 3 which may lead to endothelial shrinkage [60]. Moreover, Al-Suhaimi and Shehzad (2013), support the obstruction of PI3K-dependent pathway in insulin resistance, associated with leptin deficiency [61]. Since PI3-
K-Akt pathway regulates the function of vascular endothelium, its impaired activation in prediabetes might negatively influence the function of vascular endothelium, and might lead to cardiovascular disease pathology. Therefore, suppression of PI3-K-Akt-eNOS signalling might cause exaggerated cardiovascular disease pathology in patients with insulin resistance and prediabetes.

5. Hypertriglyceridemia associated signalling alteration during prediabetes

Triglyceride (TG), a central core of lipoprotein, transported in plasma and regulates the endothelial function. In-spite of optimal LDL-C reduction on statin monotherapy, risk of continuous atherogenic dyslipidemia remained high. This signifies the significant contribution of additional factor in atherosclerosis [62]. Triglyceride-rich lipoproteins (TRLs); including VLDL, VLDL remnants and CM remnants independently promote atherogenesis. Partial hydrolysis of TRLs produces remnant species in presence of lipoprotein lipase [62]. These remnant species uptake cholesterol esters through the action of cholesterol ester transfer protein and leads to accumulation of lipid and foam cells generation. This continuous accumulation of foam cells in artery wall causes fatty streaks, and atherosclerosis [62]. Both clinical and preclinical studies suggest the activation of numerous proatherogenic responses such as augmented recruitment, attachment of monocytes -and macrophage-derived inflammatory proteins, including TNF-α, IL-1β, monocyte chemotactic protein-1, intercellular adhesion molecule-1 and matrix metalloproteinase-3 (MMP-3), increased expression of adhesion molecules and direct vascular cytotoxicity via lipolytic byproducts have been produced by TRLs and their remnants [63]. In-addition, TRLs also associate with apo C-III content which leads to activation of PKC followed by down-regulation of IRS/PI3K/PKB/NO signalling pathway [64]. Therefore, these evidences proved that high level of TG is an important biomarker involved in the promotion of several atherogenic lipoprotein [65]. However, this central core of lipoprotein, TG is elevated in prediabetes condition which may produce the mentioned signalling alteration, atherosclerosis and associated cardiovascular diseases [66]. Hypertriglyceridemia associated atherosclerosis is widely documented predictor of cardiovascular disease by decreasing the lipoprotein concentration [67].

6. TNF-α and ROS associated signalling alteration during prediabetes

Cytokine, TNF-α, kick off the regulation of numerous cellular signals comprising NF-kappa B, MAP kinase, necrosis and apoptosis. The pleiotropic action of TNF-α is to conduct the production of ROS by initiation of NADPH oxidase [68]. The activation of TNF-α stimulates NADPH oxidase and augments the level of ROS. ROS are involved in a complex array of cellular cascades. Insulin resistant, β-cell dysfunction and
obesity are the mediators of activation of TNF-α and ROS associated signalling alteration in prediabetic condition [15]. ROS may decrease insulin releasing substrate-1 (IRS-1) followed by down-regulation of PI3K-Akt/eNOS pathway, insulin resistance, hyperinsulinemia and GLUT-4 downregulation leading to vascular endothelial dysfunction and associated cardiovascular diseases. Moreover, [21], TNF-α itself activates several pro-inflammatory cytokines by increasing GM-CSF, IL-5, IL-6, IL-7 and dendritic cells [15]. Thus, an increase in the level of IL-6 and GM-CSF in prediabetes condition may be a sign of impending cardiovascular pathology. In-addition increased C-reactive protein (CRP), which is a bio-marker of inflammatory processes, may indicate endothelial dysfunction and cardiovascular events in diabetic condition. More recently Angelidi et al. (2014) reported CRP, as a potential novel marker of vascular integrity, atherosclerosis and cardiovascular risk. Eventual scientific data suggest CRP to be an individual future risk predictor for cardiovascular diseases, in healthy individuals, and patients with coronary syndrome. These pivotal evidences clear the fact that CRP activation and its presence in plasma may indicate the existence of cardiovascular diseases. Increase level of CRP has been observed in prediabetes condition which supports the presence of endothelial and cardiovascular dysfunction. This cardiovascular abnormality associated with inflammatory pathway may be mediated by prediabetes signalling alteration by pro-inflammatory cytokines. Hence, these pro-inflammatory cytokines and ROS activated signalling alteration cascades may be critical root causes for cardiovascular disorder in prediabetic condition.

7. **Gene expression alteration during prediabetes**

As per finding of several research, hyperglycemia and increased activity of TGFβ1 is observed in prediabetes. D’Souza et al. (2011) evidenced, both hyperglycemia and increased activity of TGFβ1 regulate gene expression of extra cellular matrix (ECM). Moreover, author also reveals the increased expression of Collagen type 1α and fibronectin by measuring increased fibrotic deposition in GK rats (prediabetic Goto–Kakizaki rat) [69]. Thus, the increased level of extra cellular matrix regulator gene leads to the expression of connective tissue growth factor by fibroblast differentiation. Matrix metalloproteinases (MMP), an endopeptidases is involved in the development of plaque, atherosclerosis and cardiovascular pathology [70]. In-addition, MMP-2 and MMP-9 is significantly upregulated in genetically modified prediabetic rats [69]. Furthermore, it has been disclosed that, prediabetes could be inherited trans generationally by human germ cells. Wei and his coworkers have demonstrated that, the offspring of prediabetic fathers shows altered gene expression in the pancreatic islets. Prediabetic paternal cytosine methylation has been observed as a significant alteration in their offspring.
epigenomic profile. These alterations produce reproducible changes in methylathion with consequent insulin signalling gene. The methylome sequence of spermetazoa is distorted in prediabetic paternal patient and that cause malformed insulin signalling gene in pancreatic islets of offspring [71]. Thus, down-regulation of glucose metabolism and insulin signalling regulator gene has been observed in offspring of prediabetic parents. Hence, these observation uniquely demonstrate that prediabetes associated gene alteration leads to serious changes such as insulin signalling, glucose metabolism, increased fibrotic deposition and the development of plaque and atherosclerosis.

8. Miscellaneous signalling alteration during prediabetes

Apart from the above discussed signalling pathways, several other pathways are also involved. Many laboratories demonstrated the role of sympathetic α-adrenergic receptor in regulating coronary blood circulation [72]. Baumgart et al. (1999) suggested that, the reduction in coronary blood flow occurs through α-1 and α-2 adrenoreceptor activation induced vasoconstriction [73]. Furthermore, the author suggests that augmented microvascular constriction occurs through epicardial α-1 and α-2 adrenergic receptor activation, which is associated with atherosclerosis and can cause myocardial ischemia [74]. Recent extensive evidence is in congruence with the improvement in coronary blood flow, myocardial function and metabolism by blockade of α-1 and α-2 adrenoceptors. A large contribution of α-2 adrenoceptor than α-1 in the constriction of the resistive microcirculation is also experimentally substantiated [75]. These existing evidences reveal the impact of α-1 and α-2 adrenergic receptor in microvascular constriction and associated cardiovascular diseases. Unluckily, prediabetes is associated with sensitization of α-1 and α-2 adrenergic receptor signalling that could reduce control of coronary blood flow [76]. Augmented level of Ang II, a peptide hormone critically leads to congestive heart failure related morbidity and mortality [77]. Ang II considerably activates angiotensin-II receptor, type I (AT-1R) and leads to cardiac hypertrophy by activating various transcription pathways [78]. Zhang et al. (2005) observed, the increased level of Ang II during prediabetic metabolic syndrome, which can significantly increase coronary vasoconstriction both in vitro (isolated coronary arterioles, 60-110 µm) and in vivo (anesthetized open-chest dog) [79]. Increased concentration of leptin in obese individuals associated with prediabetes, is an independent risk factor for attenuating coronary dilation, which results in coronary vascular diseases and VED [80].

9. Therapeutic implications of Prediabetes:
Adapting a balanced diet and non-sedentary lifestyle such as exercise in daily routine contributes to reduction in haemoglobin A1c levels rapidly. Medications such as anti-cholesterol, anti-lipase, GLP-1 analogues are used to control obesity which is referred as a major risk factor in prediabetes [27]. Metformin and acarbose treatment which have been implicated in 1st line prediabetic therapy since 4 decades offer promising protection [81]. Apart from the above drugs, DPP-4 inhibitors, insulin and insulin secretagogues such as sulfonylureas, incretins and its analogues offer β cell protection and improve insulin sensitivity through cellular growth and regeneration [82]. NF kB mediated inflammation leads to insulin sensitivity, atherogenecity and increased C-reactive protein concentrations hence, pharmacological targets of NF kB such as Aspirin seem to benefit prediabetic subjects. PPARγ receptor activators such as Thiazolidinediones (TZD) have shown beneficial effects by increasing the expression of glucoregulatory molecules and enhanced insulin sensitivity [83]. Chromium elicits therapeutic benefits in patients with insulin resistance by de-phosphorylation of protein tyrosine phosphatise which is a negative regulator of insulin receptor [84]. Eventhough, such medications exist, there are drawbacks such as high cost of medications, patient compliance and most of all the glycemic rebound which occurs immediately after cessation of the medications. Hence, there is a need for novel pharmacological targets which can bias these drawbacks and can be used as a first line therapy in prediabetes as well as diabetes. Recent studies have revealed that, overexpression of miRNA29a/b/c increases diabetic manifestations and apoptosis in islets of NOD mice [85]. This data may lead to a new direction of research in this field. Likewise, Decreased NO bioavailability occurs due to eNOS dysfunction by the enzyme arginase. Hence, inhibitors of arginase and activators of eNOS may be of use in preventing endothelial dysfunction.

Conclusion

It is well known fact that prediabetes plays a key role in pathogenesis of diabetes mellitus and CVD by altering several signalling pathways. Hence, there is an escalated interest in finding the most promising therapeutically targetable signalling cascade involved in CVD in prediabetes patients. In fact, recent bench and clinical studies have suggested that physical exercise and some therapeutic agents may have protective role in prediabetes. The involvements of molecular mechanisms and downstream effectors that provoke the development of cardiovascular risk have remained largely unclear. Moreover, a multipronged intervention is always most effectual in exploiting new molecular entities. Thus, present review throws the light on the signalling alterations associated with prediabetes generated CVDs. Several studies have shown that the prediabetic heart associated with alteration or downregulation of CNP, ET-1, PI3-K-Akt-eNOS, TG, TNF-α,
ROS, α-1 and α-2 adrenoreceptor, leptin and Ang II pathways. However, there is a need to meticulously trace out the targetable signalling perturbations in the metabolic disorders associated with the attenuation of cardioprotection during prediabetes. A paradigm shift in the generation of novel therapeutic approach to prevent the pathogenesis of CVDs which can be translated to clinical scenario in near future is warranted.

Acknowledgment

The authors gratefully acknowledge the financial support received under Young Scientist Research Scheme (File No. SB/YS/LS-114/2013) of Science and Engineering Research Board (SERB), Department of Science and Technology, New Delhi, India.

Conflict of interest

The authors declare that there are no conflicts of interest

Search Methodology

Database searches using Google Scholar, Pubmed, and Science Direct were conducted until 21st July 2014 to include up-to-date documented information in the present review article. The search was limited to English language papers. For data mining, the following MESH words were used in the databases mentioned above: Prediabetes, IGT, IFG, Signalling mechanism, PI3K/AKT signalling, eNOS, Diabetes, Myocardial infarction, Apoptosis, Reactive oxidative stress, Caspase, Antiapoptotic, OGTT, iIGT. In almost all cases, the original articles were obtained and the relevant data was extracted.

Reference

Diabetes Care, 2007, 30, 7539.


[19] Filippatos TD1, Rizos EC, Tsimihodimos V, Gazi IF, Tselepis AD, Elisaf MS, 
*Lipids*, 2013, **48** (6), 547-55.

estimates and general information on diabetes and prediabetes in the United States, 
2011. Atlanta, GA: US Department of Health and Human Services, Centers for 
Disease Control and Prevention., 2011.


[22] Chentouf M, Dubois G, Jahannaut C, Castex F, Lajoix AD, Gross R, 

2012, **32** (9), 2068-76.


[37] Masure S, Haefner B, Wesselink JJ, Hoefnagel E, Mortier E, Verhasselt P, 


[40] Gao F, Gao E, Yue TL, Ohlstein EH, Lopez BL, Christopher TA, Ma XL,


[57] Dhanasekaran A, Gruenloh SK, Buonacorsii JN, Zhang R, Gross GJ, Falck JR,


[76] Dincer UD1, Araiza AG, Knudson JD, Molina PE, Tune JD, *Microcirculation j.*, 2006, 13(7), 587-95.


Figure 1: Prediabetic complication pathways leading to myocardial dysfunction
254x190mm (96 x 96 DPI)
Figure 2: Prediabetic pathways involved in progression of heart failure
254x190mm (96 x 96 DPI)