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Review Article

# Therapy for Type 2 Diabetes Mellitus: Targeting the 'Unlucky Thirteen'

N. P. Somasundaram[1*], A. M. Wijesinghe[1]

[1]*National Hospital of Sri Lanka, Colombo, Sri Lanka*

**Corresponding author: Dr. N. P. Somasundaram, Consultant Endocrinologist, National Hospital of Sri Lanka, Colombo, Sri Lanka,*

*Tel: +94776029924; Email: noelsomasundaram@gmail.com*

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## Abstract

Glycemic control in diabetes mellitus is a key strategy to prolong survival and mitigate complications of diabetes. Understanding the pathophysiological mechanisms contributing to diabetes has resulted in newer targets and is clinically useful for personalized management plans. Pathophysiological mechanisms causing and playing a role in diabetes has continued to unravel at a rapid pace; starting with two factors (Insulin resistance and Insulin deficiency), then triumvirate (addition of hepatic gluconeogenesis), ominous octet (addition of deranged adipocyte metabolism, decreased incretin effect, increased glucagon secretion, increased renal glucose reabsorption and central appetite dysregulation) and recently dirty dozen (addition of Dopamine, Vitamin D, Testosterone and Renin angiotensin system). In this article we add a thirteenth mechanism; role of gut, contributing to the pathogenesis through facilitation and alteration of caloric absorption and the gut microbiome. New targets of drugs such as Sodium Glucose co-transporter 1 inhibitors of gut absorption are under development and future research on manipulation of gut microbiome will prompt development of novel therapies for the glycemic control.

We also propose that the choices of therapies will undergo a drastic change with the understanding of the role of glucagon, which had been given a minor role in the pathogenesis of diabetes, with insulin action taking the center stage. The newer Glucagon antagonists have demonstrated something that has not been thought possible; the ability to suppress metabolic manifestations of total insulin deficiency in Type 1 animal models with absence of catabolic consequences and ketoacidosis. This implies an extremely important role of glucagon in pathogenesis of diabetes and will transform our understanding of the 'central role' of insulin and the role that glucagon plays in the absence of insulin. The development of glucagon receptor antagonists will become a harbinger of a paradigm shift in the understanding of pathogenesis as well as management of diabetes mellitus.

**Keywords:** Insulin deficiency; Insulin resistance; Triumvirate; Ominous octet; Dirty dozen; Unlucky thirteen

### Abbreviations

ACEI : Angiotensin Converting Enzyme Inhibitor;

ARB : Angiotensin II receptor Blocker;

AMPK : 5' Adenosine Monophosphate –Activated Protein Kinase;

DKA : Diabetic Keto Acidosis;

DPP4 : Dipeptidyl Peptidase 4;

FA : Fatty acids;

GIP: Glucose dependent Insulinotrophic Polypeptide;

GLP1: Glucagon Like peptide 1;

GLP1A : Glucagon Like peptide 1 Agonists;

ID: Insulin Deficiency;

IFG: Impaired Fasting Glucose;

IGT: Impaired Glucose Tolerance;

IR: Insulin Resistance;

MF: Metformin;

PPAR: Peroxisome Proliferator Activated Receptors;

RAS: Renin Aldosterone System;

SGLT1: Sodium Glucose Cotransporter 1;

SGLT2 : Sodium Glucose Cotransporter 2;

SU: Sulfonylurea;

SUR: Sulfonylurea Receptor;

T1DM: Type 1 Diabetes Mellitus;

T2DM: Type 2 Diabetes Mellitus;

## Introduction

The global burden of diabetes is in a relentless upsurge; with more than one tenth of global health expenditure is spent on diabetes [1]. In order to face this pandemic of diabetes and its effects on individuals and on world economy, it is mandatory to have comprehensive understanding of its pathogenesis to develop novel therapeutic and preventive strategies. The understanding of pathogenesis of diabetes had also evolved from the historical dual pathology of insulin deficiency (ID) and insulin resistance (IR) to the "Triumvirate", "Ominous Octet" and then to "Dirty Dozen" which in turn was pursued by development of drugs with new targets of action. Here we present "Unlucky Thirteen in Diabetes" with one addition to the dirty dozen and how it can be utilized as a new platform in the understanding and treatment of diabetes.

### *Insulin deficiency (ID) and insulin resistance (IR)*

Pathogenesis of diabetes was initially attributed to two main mechanisms; pancreatic beta cell failure with defective insulin secretion and resistance to action of insulin leading to reduced glucose uptake by peripheral tissues especially skeletal muscles. In type 1 diabetes it is the insulin deficiency due to beta cell failure and in type 2 diabetes (T2DM) both ID and IR plays a role.

In type 2 diabetes, where the main pathology is insulin resistance, the treatment is targeted to minimize insulin resistance or to improve insulin sensitivity. This can be achieved with both non pharmacological and pharmacological methods. Physical exercise had shown to improve insulin sensitivity, especially in skeletal muscles and has shown to improve glycemic control in T2DM patients [2, 3].

Metformin is a biguanide, which activates 5' adenosine monophosphate –activated protein kinase (AMPK) in skeletal muscles and hepatocytes. Activation of AMPK leads to increased glucose uptake in skeletal muscles. In the liver, apart from inhibiting hepatic gluconeogenesis, AMPK modulates adipokine secretion and also suppress Acetyl Co A Carboxylase activity and in addition suppresses steroid regulatory element binding protein 1 (SREPB 1) leading to increased fatty acid (FA) oxidation and improving insulin sensitivity through inhibiting genetic expression of lipogenic enzymes. The resultant reduction in glucotoxicity and lipotoxicity attenuate insulin signaling defects and thereby improve glycemic control. In addition to its beneficial effects on glycemic control, AMPK activation strongly suppresses cellular proliferation in malignant as well as non-malignant cells. This is done through regulation of cell cycle through mammalian target of rapamycin complex 1 (mTORC1) and upregulation of p53 –p 21 axis. Further, reduction of hyperinsulinemia by AMPK action results in amelioration of the insulin induced stimulatory effects on cellular proliferation.

Insulin secretagogues, sulphonylurias (SU) and glinides act through binding to SU receptor. Principal action of SU is the inhibition of ATP-sensitive potassium ($K_{ATP}$) channel on beta cell membrane, which causes depolarization of the β-cell membrane and triggers the opening of voltage-gated $Ca^{2+}$ channels, eliciting $Ca^{2+}$ influx and a rise in intracellular $Ca^{2+}$, which stimulates the exocytosis of insulin-containing secretory granules. The $K_{ATP}$ channel comprise of two different types of protein subunits; an inwardly rectifying K+ channel (Kir6.x), and a sulfonylurea receptor (SUR). More than one isoform exists for both Kir6.x (Kir6.1, Kir6.2) and SUR (SUR1, SUR2A, and SUR2B). In most tissues, Kir6.2 serves as the pore-forming subunit, but it associates with different SUR subunits; it associates with SUR1 in pancreas and brain; SUR2A in heart and skeletal muscle; and SUR2B in brain and smooth muscle. In the vascular smooth muscle, the $K_{ATP}$ channel is comprised of Kir6.1 in association with SUR2B. Variation in the subunit composition of the $K_{ATP}$ channel accounts for the different metabolic and drug sensitivities of $K_{ATP}$ channels in different cells. Numerous choices of SUs are available depending on the duration and place of action, metabolism and route of excretion to individualize the treatment.

### *The Triumvirate - Hepatic gluconeogenesis*

The theory of two was made "The triumvirate" when Dr. Ralph DeFronzo proposed the third mechanism in 1987; increased hepatic glucose synthesis [4]. In early course of T2DM, pancreatic beta cells are healthy and are able to secrete sufficient amount of insulin to offset the insulin resistance in muscles and liver. However beta cell secretory capacity decreases over time and this results in hepatic glucose synthesis especially during sleeping hours leading

to fasting hyperglycemia [4]. Metformin inhibits hepatic gluconeogenesis through activation of AMPK in addition to its effects of increased cellular glucose uptake and improving insulin sensitivity.

**Ominous Octet**

Ominous Octet was proposed in 2008, with five additional core defects responsible for T2DM; deranged adipocyte metabolism, decreased incretin effect, increased glucagon secretion, increased renal glucose reabsorption and central appetite dysregulation.

***Deranged adipocyte metabolism***

There is considerable evidence that deranged adipocyte metabolism plays a role in pathogenesis of T2DM [5, 6, 7]. Insulin is an anti lipolytic hormone which leads to elevated serum free fatty acid (FFA) levels in insulin deficiency states or in insulin resistance in adipose tissue [5,6, 7]. Chronically increased plasma FFAs stimulate hepatic gluconeogenesis, induce muscle/hepatic IR [5, 8, 9], and impair insulin secretion [10, 11] through multiple mechanisms and contribute to hyperglycemia.

Thiazolidinedione (TZD) class of drugs acting through binding and modulation of the activity of a family of nuclear transcription factors - peroxisome proliferator activated receptors (PPAR), causing improvements in insulin sensitivity in liver, muscle and adipose tissue. Their action on insulin sensitivity may be secondary to the lowering of circulating lipids by PPAR-γ activation and by secretion insulin-sensitizing hormones such as adiponectin by adipocytes. In addition to the glycemic control and reduction in macro vascular complications, some studies have demonstrated that TZDs retard the progressive beta cell deterioration and loss [12].

***Incretins***

The fact that oral glucose ingestion elicits a much higher insulin response than during an intravenous glucose infusion is attributed to incretin effects [5, 13, 14]. Glucagon Like peptide 1(GLP-1) act via stimulation of insulin secretion from pancreatic beta cells in glucose dependent manner [5, 15] and suppression of glucagon secretion from alpha cells, which in turn inhibits liver from releasing excess glucose [16]. GLP-1 also delays gastric emptying resulting in reduction of the rate of carbohydrate absorption [5, 16, 17]. In addition, GLP-1 results in feeling of fullness and satiety [16] and is therefore associated with control of weight gain. The incretin effect is substantially reduced in patients with T2DM and in addition there is resistance of beta cells to the stimulatory effects of GLP-1 and GIP on insulin secretion [5, 18, 19]. GLP-1 has short half-life in plasma (1-2 minutes) due to amino terminal degradation by the enzyme dipeptidyl peptidase IV (DPP4). Multiple pharmacologic techniques have been developed recently to harness the potential of GLP 1 signaling to treat diabetes, which include GLP-1 agonists (GLP1A) and DPP4 inhibitors. GLP1A are synthetic peptides with 10 fold higher activity than native GLP-1 and is resistant to DPP4 degradation. They are shown in trials to cause an HbA1C reduction 1 – 1.5 % with modest weight loss [20, 21, 22]. Sitagliptin, Saxagliptin and Vildagliptin are DPP4 inhibitors, which increase postprandial GLP1 and GIP levels resulting in reduction of HbA1C by approximately 0.7% [23, 24, 25].

***Glucagon***

The other major factor implicated in pathogenesis of diabetes is glucagon. Henquin and colleagues showed that pancreatic beta cell mass is reduced in diabetes compared to non-diabetic patients, where as there is no reduction in alpha cell mass [26]. Glucagon hormone assays had demonstrated that hyperglucagonemia is present in untreated T1DM [27]. In addition, it has been shown that while insulin levels progressively decline over the course of the disease, the basal plasma glucagon levels remained elevated in T2DM patients [26]. Basal glucagon levels are elevated in patients with T2DM patients compared to non-diabetics [28]. And, fasting glucagon levels are elevated and the post prandial glucagon levels are not suppressed, but paradoxically elevated. This elevated blood glucagon levels increase hepatic gluconeogenesis resulting in elevation of fasting and post prandial glucose levels [28, 29]. Following somatostatin infusion, plasma glucagon levels was shown to decline by 44% in association with a 58% decrease in basal hepatic glucose production. When somatostatin was infused into alloxan-diabetic dogs [30] or in insulin-deprived humans with T1DM [31], hyperglucagonemia was suppressed and hyperglycemia was markedly decreased, even though insulin had been reduced or discontinued. Animal studies has shown that selective decrease in glucagon levels resulted in a rapid fall in glucose production [32], whereas a selective increase in glucagon caused a rapid rise in hepatic glucose output [33]. These results conclusively demonstrated the prime role of glucagon in the pathogenesis of hyperglycemia in type 2 diabetes [28].

Studies with glucagon receptor–null (*Gcgr*$^{-/-}$) mice indicate that glucagon mediates the catabolic effects of insulin deficiency [34]. In these *Gcgr*$^{-/-}$ mice, who exhibit no response to glucagon at any concentration, total β cell destruction did not result in any of the diabetic metabolic abnormalities thought to be caused by ID whereas destruction of β cells in wild-type controls resulted in the catabolic consequences of ID, with death due to ketoacidosis. The insulin-deficient *Gcgr*$^{-/-}$ mice did not become hyperglycemic or hyperketonemic, and their livers did not show increase either in phospho–cAMP response element–binding protein (p-CREB; a mediator of glucagon action) or in the gluconeogenic enzyme phosphoenolpyruvate carboxykinase, both of which are elevated in uncontrolled diabetes. This implies that the glucagon excess is the main pathology causing diabetes and DKA, not insulin deficiency itself. Another study showed Immunoneutralization of endogenous glucagon with monoclonal glucagon antibody normalizes hyperglycaemia in moderately streptozotocin induced-diabetic (insulinopenic) rats [35].

A number of glucagon antagonists including peptide glucagon receptor antagonists and non-peptide glucagon receptor antagonists are currently under development and are in initial stages of experiments. The administration of glucagon receptor antagonists leads to a reduction in blood glucose levels in normal and diabetic rodent models [36].

In 2001, a Phase 1 study of a glucagon receptor antagonist (GRA) - BAY 279955 (Bayer) conducted in 14 healthy men showed that Bay 27-9955 is an effective and safe glucagon antagonist in humans [37]. This observation was followed by few trials using orally administered small molecule glucagon receptor antagonist MK-0893, MK 3577 and LGD-6972 (Ligand) in healthy subjects and T2DM patients. It was noted that all compounds were found to be effective at lowering plasma glucose/ HbA1c in a dose dependent manner. And higher the dose, there were trends for increases in LDL-cholesterol, LFTs and blood pressure.

A Phase 2 study assessed, ISIS-$GCGR_{Rx}$ (injectable glucagon receptor antagonist) in T2DM patients who had uncontrolled blood sugar despite treatment with metformin therapy. Thirteen weeks of ISIS-$GCGR_{Rx}$ added to their doses of metformin had robust and sustained, dose-dependent, statistically significant mean reductions in HbA1c and significant reductions in serum fructosamine and fasting plasma glucose levels. ISIS-$GCGR_{Rx}$ was generally well tolerated in the study without abnormalities in liver enzymes, incidents of symptomatic hypoglycemia, elevation of LDL-C, blood pressure or body weight gain [38].

In 2015, Kazda and colleagues conducted a randomized phase 2 study of a Glucagon Receptor Antagonist, LY2409021 has showing that LY2409021 in type 2 diabetes patients has produced a significant dose dependent reductions in mean HbA1c levels and serum glucose levels compared to placebo over a period of 12-weeks. Change from baseline in HbA1c level was -0.83% at 10 mg, -0.65% at 30 mg, and -0.66 % at 60mg vs. placebo 0.11%. Increases in the levels of aminotransferases, fasting glucagon, and total fasting GLP1 were observed. However they returned to baseline after drug washout and the incidence of hypoglycemia had been minimal. None of the patients in LY2409021 treatment groups had developed clinically significant changes in plasma lipid measurements, blood pressure, heart rate or ECG changes compared with the placebo group [39]. These studies are harbingers of novel research in using glucagon as main target in diabetes.

***Renal glucose reabsorption***

Kidneys also play a significant role in pathogenesis of diabetes. About ninety percent of the filtered glucose is reabsorbed through the high capacity Sodium Glucose co Transporter 2 (SGLT2) in the convoluted segment of the proximal tubule of nephron, and the remaining 10% of the filtered glucose is reabsorbed by the Sodium Glucose co Transporter 1 (SGLT1) in the straight segment of the proximal tubule. The result is that no glucose appears in the urine. Non diabetics would start to excrete glucose in urine when plasma glucose level is more than 180 mg/dL, however in diabetes mellitus, the threshold for spilling glucose into urine is much elevated resulting in worsening of hyperglycemia [40]. In addition, cultured human proximal renal tubular cells from T2DM patients demonstrated markedly increased levels of SGLT2 mRNA and protein and a fourfold increase in the uptake of alpha-methyl-D-glucopyranoside (AMG), a non metabolizable glucose analog [41] implying renal conservation of glucose in diabetic patients.

SGLT 2 inhibitors have been shown to be effective as monotherapy for treatment of T2DM in several trials with improved glycemic control, reduced body weight and blood pressure [42, 43]. They can be safely combined with other oral hypoglycemic agents such as metformin [44, 45] sulphonylurias [44, 45], TZD [46] and DPP4 inhibitors [47] without dose adjustments and showed similar efficacy in blood sugar control and body weight reductions or neutralization of weight gain caused by TZDs [46] without additional risks of hypoglycemia. Add on therapy of empagliflozin and also dapagliflozin to obese, difficult-to-treat patients with T2DM inadequately controlled on high MDI insulin doses has similar glycemic improvements, weight reductions and also reduced the insulin requirements without significant increase in hypoglycemia [48].

In addition to its effect of renal glucose excretion, canagliflozin and dapagliflozin has shown to improve beta cell function [49] and to improve insulin sensitivity [51].

The beneficial effects of SGLT 2 inhibitors on cardiovascular mortality and morbidity was recently established for empagliflozin in EMPA-REG (Empagliflozin, Cardiovascular Outcomes, and Mortality in Type 2 Diabetes) trial and for dapagliflozin [52, 53]. EMPA-REG trial has shown that among patients with type 2 diabetes at high risk for cardiovascular events, those receiving empagliflozin had a lower rate of deaths from cardiovascular causes, nonfatal myocardial infarction, or nonfatal stroke than did patients receiving placebo.

There is recent concern of potential of using SGLT 2 inhibitors in T1DM patients. Henry and colleagues published a randomized, double-blind, placebo-controlled pilot study, a 2-week, dose-ranging, proof-of-concept study randomly which assigned 70 adults with type 1 diabetes who were receiving treatment with stable doses of insulin, to dapagliflozin or placebo [54]. Patients with type 1 diabetes demonstrated acceptable short-term tolerability and increases in urinary glucose excretion. Within the dapagliflozin groups, dose-related reductions in 24-h glucose, glycemic variability, and insulin dose were suggested, which provide hope that SGLT2 inhibition may prove in larger randomized controlled trials to be efficacious in reducing hyperglycemia in T1DM. However a recent study with canagliflozin in type 1 diabetes patients has shown to be associated with an increased incidence of serious adverse effects of diabetes ketoacidosis (DKA) in patients who are inadequately controlled with insulin [55]. These studies implies the potential for use of SGLT2 inhibitors for type 1 diabetes, however thorough monitoring

and caution should be implemented when prescribing for both clinical and research purposes.

Some of the studies had reported episodes of euglycemic ketoacidosis with SGLT 2 inhibitors. A possible mechanism in which SGLT 2 inhibitors causing ketosis would be related to reduced insulin secretion from pancreatic beta cells in response to lowered blood sugar levels secondary to renal glucose excretion. Declining insulin levels stimulate lipolysis in the adipose tissue with release of FFA which are converted to ketone bodies by beta oxidation in the liver. In addition, reduced insulin levels diminish the activity of acetyl CoA carboxylase, which increase carnitine palmitoyl transferase I levels which again stimulate beta oxidation of FFA to ketones in the liver.

Common non-serious adverse effects observed with SGLT 2 inhibitors are increased risk of genital mycotic infections and lower urinary tract infections [52, 56]. Watts and colleagues recently published the increased prevalence of fractures of upper and lower limbs in patients treated with canagliflozin (2.7%) vs non canagliflozin (1.9%) treated patients in the CANVAS study, however this finding was not consistent in the same study analyzing pooled non CANVAS studies where there was no significant increase in fractures [57]. However, the cause of increased fracture risk with canagliflozin is unknown.

Use of SGLT 2 inhibitors in renal impairment is still under evaluation. It had been shown that canagliflozin and empagliflozin improved glycemic control and was generally well tolerated in T2DM patients with chronic kidney disease stage 2 to 3 [58].

A study on pharmacokinetics, safety and tolerability of empagliflozin in patients with hepatic impairment has concluded that Empagliflozin was well tolerated in diabetic patients with hepatic impairment. Increases in empagliflozin exposure were less than twofold in patients with hepatic impairment, thus no dose adjustment of empagliflozin is required in patients with hepatic impairment [59]. However more studies are required to establish long term safety and dose adjustments of SGLT 2 inhibitors in CKD and liver impairment.

### *Central appetite dysregulation*

Hypothalamic center for appetite control is dysfunctional in diabetes leading to increased appetite. GLP 1 receptors in brain are involved in appetite suppression. In diabetes, resistance to GLP 1 and CNS resistance to insulin contribute to weight gain; which can aggravate insulin resistance. Amylin is another neuroendocrine hormone, which is deficient in type 1 and type 2 diabetes. Its effect on appetite dysregulation appears to be mainly mediated via central pathways that include high-affinity binding sites in the area postrema in the hindbrain [60] and reduce appetite. Further it is also known that amylin has direct gut effects through a decrease in rate of gastric emptying [61]. Pramlintide is an amylin analogue subcutaneously administered and had shown to reduce HbA1c by 0.5 – 0.7% when added to insulin or other oral hypoglycemic agent [62].

### Dirty Dozen

In 2013 Kalra et al took a further step, describing "the Dirty Dozen" in diabetes with four additions to the octet; Dopamine, Vitamin D, Testosterone and Renin angiotensin system [63].

### *Dopamine*

Dopamine is a neurotransmitter in the brain which has lower plasma levels during insulin-resistant state and increase to normal following restoration of the insulin-sensitive state [64, 65]. Further, selective destruction of dopaminergic neurons in suprachiasmatic nuclei of hypothalamus causes severe IR in animal models [64, 65]. Conversely, systemic and intracerebral dopamine agonist (DA) -bromocriptine administration in insulin-resistant animals lead to a decrease in elevated ventromedial hypothalamus noradrenergic and serotonergic levels with a decline in hepatic gluconeogenesis, reduced adipose tissue lipolysis, and improved insulin sensitivity [65, 66]. Systemic bromocriptine administration improves glycemic control and dyslipidemia without change in body weight in type 2 diabetic and obese nondiabetic humans [67, 68]. Therefore it is postulated that hypothalamic dopamine is decreased in the early morning in diabetic patients causing increased hepatic gluconeogenesis and lipolysis resulting in glucose intolerance, insulin-resistance and dyslipidemia. Further a quick release formulation of bromocriptine administered within 2 hours of rising in the morning has shown to reduce HbA1c up to 1.2%, implying the role of DA in treatment of diabetes patients.

### *Vitamin D*

Vitamin D has been shown to stimulate insulin secretion by regulating intracellular calcium, modulating pancreatic beta-cell insulin release and prevention of apoptosis [69, 70]. This was especially seen in T1DM patients where significantly low vitamin D levels (<10 ng/ml) were associated with higher insulin requirements suggesting the possibility of an insulin secretory action of vitamin D [71]. This is also in good agreement with the observation by Hypponen et al, that children receiving 2000 IU of vitamin D from age 1 year onwards had 80% reduced risk of getting T1DM [72]. In addition, immunomodulatory effects of vitamin D may also be a possible mechanism offering protection against autoimmune disease like type 1 diabetes. A serum vitamin D level of less of <20 ng/ml has shown to be associated with new-onset obesity in both adults and children/adolescents; in this study the highest incidence of obesity was found with a serum vitamin D level of <17 ng/ml [73, 74]. As low levels of vitamin D is associated with a higher risk of metabolic syndrome and its components, supplementation with this hormone has been shown to have multiple beneficial effects [75]. Few pilot studies have demonstrated that vitamin D

supplementation may help improve insulin sensitivity and markers of metabolic syndrome [76].

However, there is conflicting evidence by Wagner and colleagues showing that vitamin D treatment has no effect on beta cell function, insulin sensitivity or glucose homeostasis in subjects with abnormal glucose homeostasis. There, 44 subjects were randomized to 30,000 IU vitamin D3 once weekly or placebo for 8 weeks and there was no significant difference between vitamin D treated group and placebo with regards to insulin response, insulin sensitivity, glucose tolerance and HbA1C. Therefore future more large scale randomized controlled trials are needed to prove the role of vitamin D in pathogenesis of diabetes mellitus [77].

### *Testosterone*

There is emerging evidence that low serum testosterone levels is associated with T2DM and metabolic syndrome. In 2007 Pitteloud et al showed that serum testosterone levels positively correlated with insulin sensitivity and the subjects with hypogonadal testosterone levels had a threefold higher prevalence of the metabolic syndrome than their eugonadal counter partners [78]. Also, androgen replacement in hypo gonadal men has shown to improve insulin sensitivity and glycemic levels, reduce insulin requirements and improvement in the other metabolic parameters [79, 80].

### *Renin aldosterone system*

Renin aldosterone system (RAS) blockage with ACE inhibitors (ACEI) and Angiotensin receptor blockers (ARB) plays a major role in management of diabetic nephropathy. Multiple studies have looked into the effects of ACEI and ARB on insulin sensitivity in hypertensive patients, with or without diabetes. Some studies with ACEI in hypertensive non-diabetic individuals showed a slight but significant increase in insulin sensitivity [81], while some failed to reveal any significant change [82, 83]. Postulated mechanisms for RAS blockage resulting in improved insulin sensitivity include; improvement of blood flow & microcirculation in skeletal muscles and facilitating insulin signaling at cellular level and improvement of insulin secretion by pancreatic beta cells. Two large, prospective, placebo-controlled randomized clinical trials whose primary outcome is the prevention of type 2 diabetes had been conducted: the DREAM (Diabetes Reduction Approaches with ramipril and rosiglitazone Medications) trial with the ACE inhibitor Ramipril, showed that among persons with impaired fasting glucose (IFG) or impaired glucose tolerance (IGT), the use of Ramipril for 3 years did not significantly reduce the incidence of diabetes or death but did significantly increase regression to normoglycaemia [84]. NAVIGATOR (Nateglinide And Valsartan in Impaired Glucose Tolerance Outcomes Research) trial with the ARB Valsartan showed that among patients with IGT

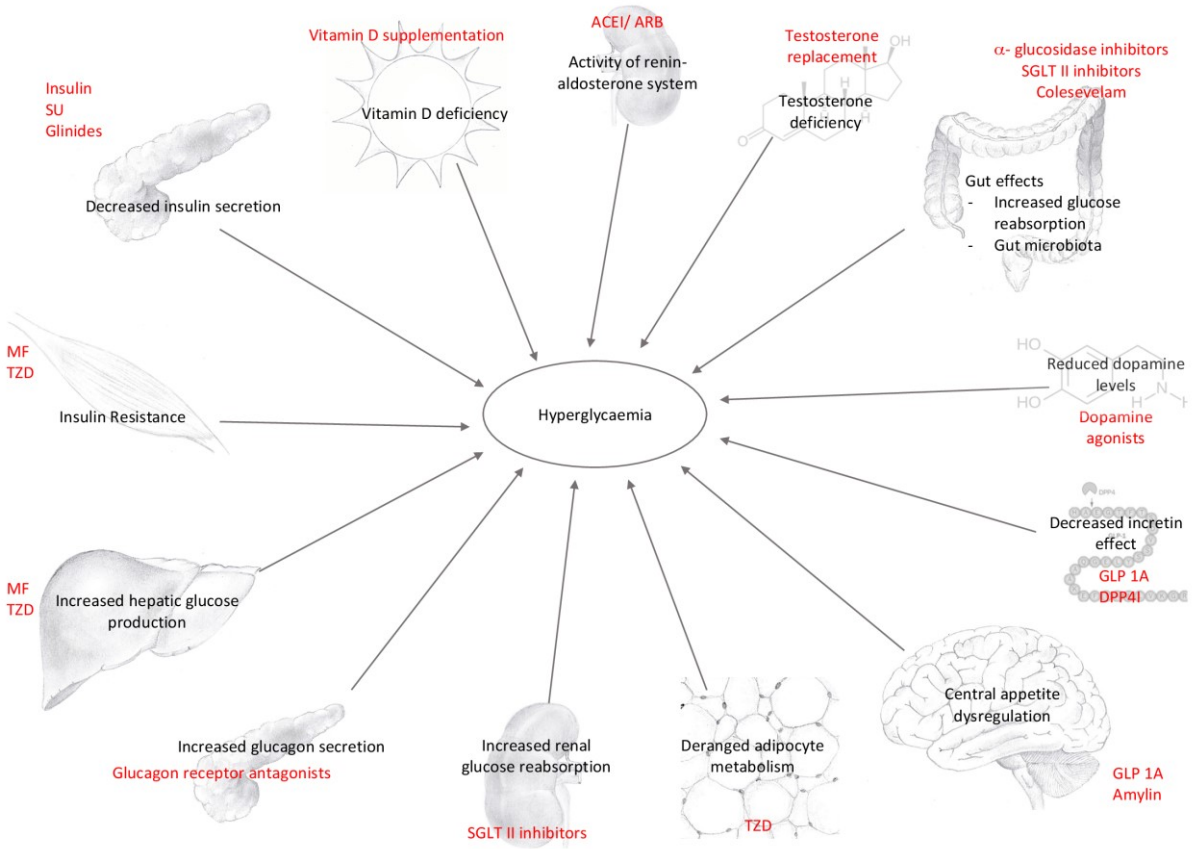


**Figure 1.** Unlucky Thirteen in Diabetes SU, sulfonylurea; MF, metformin; TZD, thiazolidinediones; SGLT, sodium glucose co transporter; GLP1A, glucagon like peptide 1 agonists; DPP4i, dipeptidyl peptidase 4 inhibitors; ACEi, angiotensin converting enzyme inhibitor; ARB, angiotensin II receptor blockers

and cardiovascular disease or risk factors, the use of valsartan for 5 years, along with lifestyle modification, led to a relative reduction of 14% in the incidence of diabetes but did not reduce the rate of cardiovascular events implying the possible role of RAS in pathogenesis of diabetes [85].

**Unlucky Thirteen (Figure 01)**

As the Dirty dozen doesn't complete the full spectrum of pathogenesis of diabetes, we propose a thirteenth mechanism in the pathogenesis of diabetes –the role of gut in diabetes mellitus.

***Gut in Diabetes Mellitus***

The primary driver in the diabetic pandemic across the world is an increase in mean caloric intake. Hence it is appropriate that this is also the thirteenth unlucky pathophysiologic mechanism.

Even though contribution of GI absorption of carbohydrates had been known for several years, its contribution was not effectively utilized as a target for oral hypoglycemic agents. The only class of drug which utilize this mechanism for treatment of diabetes is alpha glucosidase inhibitor (AGI) – Acarbose, Voglibose, Miglitol. AGIs delay carbohydrate absorption from proximal small intestine and thus have a lowering effect on postprandial blood glucose and insulin levels. A Cochrane systematic review and meta-analysis has shown that AGIs have clear beneficial effects on glycemic control and post load insulin levels [86].

Within the GI tract, Sodium Glucose Co Transporter 1 (SGLT-1) is the transporter responsible for glucose absorption and SGLT-1 is also involved in 10% of renal glucose reabsorption. Studies in mice treated with SGLT-1 inhibitor LX 2761 (Lexicon) demonstrated reductions in fasting and post prandial glucose and a reduction in HbA1C of 0.7% with no GI side effects, no increase in glycosuria, and an increase in circulating levels of GLP-1 and peptide YY, hormones that suppress the appetite [87]. In humans, the combined SGLT-1/ SGLT-2 inhibitor LX4211 (sotagliflozin) has shown to increase urinary glucose excretion, delay intestinal glucose absorption, and increase circulating GLP-1 levels [88, 89, 90]. Sands and colleagues described that 33 patients who were treated with sotagliflozin or placebo in a randomized, double-blind trial showed that sotagliflozin improved glycemic control and the continuous glucose monitoring profile (by 0.55%) with bolus insulin dose reduction (of 32.1%), weight loss (1.7Kg), and no increased hypoglycemia in type 1 diabetes [91]. This implies the contribution of GI carbohydrate absorption in pathogenesis of diabetes.

Coleselvelum is a novel second generation bile acid sequestrant which was observed to mediate modest reductions in glucose in T2DM when used as an adjunct to other agents. It provides HbA1c reduction of about 0.5%. The exact mechanism of action is not yet identified and potential mechanisms include effect on bile acid receptors in the intestine as well as in the liver to reduce endogenous glucose production [92].

***Gut microbiota***

In addition to gut glucose absorption, role of colonic microbiome on pathogenesis of diabetes is under focus. Quin and colleagues had demonstrated that patients with type 2 diabetes exhibited a moderate intestinal dysbiosis, which included decrease in butyrate producing *Roseburia intestinals* and *F prausnitzii*, while healthy control samples were enriched with various butyrate producing bacteria (*Clostridiales sp, Eubacterium rectale, F prausnitzii, R intestinalis*) [93]. It is postulated that the gut dysbiosis in T2DM exert enrichment in membrane transport of sugars, branched chain amino acid and sulfate reduction, decreased butyrate biosynthesis and modifications in the secretion of the incretins. And also possibility of an increase in oxidative stress response which may represent a link to the pro inflammatory observed in T2DM patients. A similar study by Karlsson and colleagues in post-menopausal females exhibited increase in the abundance of four *Lactobacillus* species including *Lactobacillus gasseri*, *Streptococcus mutans* and some *Clostridiales* such as *Clostridium clostridoforme* and again decreases in at least five other *Clostridium* species. The common observation to both of these cohorts are that *C clostridoforme* and *Lactobacillus* species were increased whereas *Roseburia*, a major butyrate producer, was decreased in type 2 diabetes [94].

Further, a study by Vrieze and colleagues demonstrated an improvement in insulin sensitivity in individuals with the metabolic syndrome six weeks after infusion of intestinal microbiota from lean individuals [95]. All of these studies point to the possible role of colonic micobiome in pathogenesis of diabetes and potential for probiotics and prebiotics as new strategies to modulate the gut microbiota for therapeutic purposes in diabetes.

Interestingly, a recent studies on metformin by John B. Buse and colleagues demonstrated that the primary glucose lowering effect of metformin resides in the gut, not in the circulation by comparing a new formulation of delayed release preparation of metformin (Met DR – target ileum where absorption is low) versus conventional metformin preparations (Metformin immediate release and extended release where absorption occurs in duodenum and jejunum.) Even though Met DR had lower plasma metformin concentrations than the conventional preparations, it produced a more significant and sustained reduction in plasma glucose suggesting a role of unabsorbed metformin in lower gut [96]. The action of metformin in the gut is postulated to be mediated through increase in GLP1, peptide YY, and action on Farnisoid X receptor (FXR) [97, 98].

As described above, the gut exerts several mechanisms in pathogenesis of diabetes which would lead to development of novel therapeutic agents, and we propose the gut as the thirteenth mechanism for pathogenesis of diabetes making it the Unlucky Thirteen.

**Targeting Unlucky Thirteen in treatment of Diabetes**

Broadening of pathophysiology of diabetes mellitus has opened up options for many novel drug targets. Metformin which has the longest established safety profile has retained its place as first line agent despite new targets [99] particularly through new data on its non-glycemic benefits: anticancer effects [100], weight reduction [101], favorable lipid profile [102], and neuro protective effects [103]. Metformin has also been shown to be beneficial as a treatment modality in pre diabetes [104], polycystic ovarian syndrome [105] and nonalcoholic fatty liver disease [106].

Even though still at experimental level, we believe that glucagon will emerge as a key therapeutic modality because of its central role in pathogenesis of diabetes mellitus. Both injectable and oral formulations of glucagon receptor antagonists have shown glycemic benefits in preclinical and phase 2 studies.

In this article we have proposed gut as the thirteenth mechanism in pathogenesis of diabetes. Even though gut has shown to have a key role in glycemic control by restrictions on caloric intake, glucose absorption and gut microbiota, this pathogenic role is not currently adequately utilized as a treatment modality. Alpha glucosidase inhibitors are currently prescribed as add on therapy to diabetes, however the gastrointestinal side effects had made it unpopular among diabetes patient. SGLT 1 inhibitors and SGLT 1 & 2 combinations has shown promising results in reducing both fasting and post prandial hyperglycemia and these can be utilized as promising hypoglycemic agents in future with novel clinical experience. Bile acid sequestrants such as coleselvelum has also demonstrated beneficial effects on glycemic control and would be considered as add on to therapy. Recent studies on colonic microbiota has linked gut dysbiosis and development of type 2 diabetes, making transplantation of intestinal microbiota as a future therapeutic option in diabetes.

## Conclusion

With the current rapid upsurge in incidence of diabetes, it is mandatory to develop novel therapeutic agents to offset this health burden. This can be addressed by developing new target (pathogenesis) based therapy or targeting existing pathogenic factors which are currently underutilized as therapy but act as major pathogenic factors in development of diabetes. Therefore further development of glucagon receptor antagonists and gut related therapeutic agents for treatment of diabetes should be encouraged.

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**Author Contribution**

Both authors contributed in preparation of the manuscript

**Conflicts of interest**

None

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