List of Publications and Presentations

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1. **Palit SP**, Patel R, Jadeja SD, Rathwa N, Mahajan A, Ramachandran AV, Dhar MK, Sharma S, Begum R. (2020) A genetic analysis identifies a haplotype at adiponectin locus: Association with obesity and type 2 diabetes. Sci Rep., 10:2904. (IF:3.99)

2. **Pramanik S**, Rathwa N, Patel R, Ramachandran AV, Begum R. (2018) Treatment Avenues for Type 2 diabetes and Current perspectives on Adipokines. Curr Diabetes Rev., 14: 201-221.

3. Patel R, **Palit SP**, Rathwa N, Ramachandran AV, Begum R. (2019) Genetic variants of Tumor Necrosis Factor-α and its levels: A Correlation with Dyslipidemia and Type 2 Diabetes Susceptibility. Clin Nutr., 38:1414-1422. (IF:6.40)

4. Rathwa N, Patel R, **Palit SP**, Parmar N, Rana S, Ansari MI, Ramachandran AV, Begum R. (2020) β-cell replenishment: Possible curative approaches for diabetes mellitus. Nutr Metab Cardiovasc Dis., 30:1870-81. (IF:3.70)

5. Rathwa N, Parmar N, **Palit SP**, Patel R, Ramachandran AV, Begum R. (2020) Intron specific polymorphic site of vaspin gene along with vaspin circulatory levels can influence pathophysiology of type 2 diabetes. Life Sci., 243:117285. (IF:3.64)

6. Rathwa N, Patel R, **Palit SP**, Jadeja SD, Narwaria M, Ramachandran AV, Begum R. (2019) Circulatory Omentin-1 levels but not genetic variants influence the pathophysiology of Type 2 Diabetes. Cytokine, 119:144-151. (IF:2.95)

7. Rathwa N, Patel R, **Palit SP**, Ramachandran AV, Begum R. (2019) Genetic variants of resistin and its plasma levels: Association with obesity and dyslipidemia related to Type 2 Diabetes susceptibility. Genomics, 111:980-985. (IF:6.20)

8. Patel R, Rathwa N, **Palit SP**, Ramachandran AV, Begum R. (2018) Association of melatonin & MTNR1B variants with type 2 diabetes in Gujarat population. Biomed. Pharmacother., 103:429-434. (IF:3.74)

Manuscripts under communication

1. Repurposing statin and L-glutamine to replenish β-cells in hyperlipidemic mouse model.

Oral/ Poster Presentations

1. **Pramanik S**, Patel R, Rathwa N, Parmar N, Dalvi N, Ramachandran AV, Begum R. "L-glutamine and Pitavastatin: resuscitating the dying β-cells" at 9th International Conference on 'Nextgen genomics, biology, bioinformatics and technologies (NGBT) held at Mumbai, India on 30th September -2nd October, 2019. *(*Received YUVA scholarship award for participation in the conference*).
2. **Palit SP**, Patel R, Rathwa N, Dalvi N, Ramachandran AV, Begum R. L-glutamine and Pitavastatin: a therapeutic approach to revive the insulin gold mine. Poster presentation delivered at ICRED- 2019, 37th Annual Conference of the International Conference on Reproductive Biology and Comparative Endocrinology (19-21 January 2019) at School of Liberal Studies and Education, Navrachana University, Vadodara-391410, Gujarat, India (*Received Best Poster Award*).
3. **Pramanik S**, Patel R, Rathwa N, Ramachandran AV, Begum R. Haplotype at adiponectin locus and its remarkable association with type 2 diabetes. Oral presentation delivered at International Conference on 'Proteins, miRNA and Exosomes In Health and Diseases' held at The M. S. University of Baroda, Vadodara, Gujarat, India on 11th - 13th December, 2018. (*Received 1st prize for best poster*)
4. **Pramanik S**, Patel R, Rathwa N, Patel N, Rana S, Ramachandran AV, Begum R. "Adiponectin: a watchdog in inflammation induced metabolic disorder" at "Immunocon-2017. 44th Annual Conference of the Indian Immunology Society (IIS)" held at Institute of Science, Nirma University, Ahmedabad, Gujarat-382481, India, 14th – 16th Dec 2017 (*Received Best Poster Award*).
5. **Pramanik S**, Patel N, Rana S, Ramachandran AV, Begum R. Association of Adiponectin Genetic Variants with Type 2 Diabetes. Poster presentation delivered at International Conference on Reproductive Biology and Comparative Endocrinology & The 35th Annual Meeting of The Society for Reproductive Biology and Comparative Endocrinology, 9-11 February 2017 held at Department of Animal Biology, University of Hyderabad, Hyderabad, India.
6. **Palit SP**, Rathwa N, Patel R, Rana S, Patel N, Ramachandran AV, Begum R Association of Adiponectin and Resistin genetic variants with Type 2 Diabetes. Poster presentation delivered at Two-day National Symposium on Omics…to Structural Basis of Diseases, 30 Sept. and 1 Oct. 2016 held at The M. S. University of Baroda, Vadodara, Gujarat, India
7. Patel R, **Palit SP**, Rathwa N, Parmar N, Dhimmar H, Pancholi DA, Ramachandran AV, Begum R. "Melatonin and DPP-IV inhibitor: A novel combinatorial approach for β-cells regeneration" at 9th International Conference on 'Nextgen genomics, biology, bioinformatics and technologies (NGBT) held at Mumbai, India on 30th September -2nd October, 2019.
8. Patel R, **Pramanik S**, Rathwa NN, Parmar NR, Dhimmar H, Pancholi DA, Ramachandran AV, Begum R. Melatonin and DPP-IV inhibitor: A novel combinatorial approach for β-cell regeneration. Poster presentation delivered at American Diabetes Association 79th

Scientific Sessions (7-11 June 2019) at Moscone Center, San Francisco-94103, California, USA.

9. Rathwa N, **Palit SP**, Patel R, Dhimmar H, Ramachandran AV, Begum R. Genetic Variants of Omentin-1 and its levels: Association with Type 2 Diabetes Susceptibility in Gujarat population. Poster presentation delivered at International Conference on 'Proteins, miRNA and Exosomes in Health and Diseases' held at The M. S. University of Baroda, Vadodara, Gujarat, India on 11-13 December 2018.
10. Rathwa N, **Palit SP**, Patel R, Dhimmar H, Bhati H, Parmar N, Ramachandran AV, Begum R. Genetic Variants of Omentin-1 And Vaspin: Association with Obesity And Dyslipidemia Related To Type 2 Diabetes Susceptibility. Poster presentation delivered at International Conference on Reproductive Physiology and Comparative Endocrinology & The 36th meeting of SRBCE, 20 – 22 January 2018 held at BITS Pilani, KK Birla Goa Campus, Goa, India.
11. Parmar N, Patel R, **Pramanik S**, Rathwa N, Shetty S, Patel N, Ramachandran AV, Begum R. "Evaluation of genetic variants of LEPTIN and LEPTIN RECEPTOR as risk factors for T2D in Gujarat population" at 9th International Conference on 'Nextgen genomics, biology, bioinformatics and technologies (NGBT) held at Mumbai, India on 30th September -2nd October, 2019.
12. Rathwa N, Patel R, **Palit SP**, Parmar N, Ramachandran AV, Begum R. GABA in combination with CR as possible therapeutic approach for ameliorating insulin resistance and favoring β-cell regeneration in Type 2 Diabetes. Poster presentation delivered at NextGen Genomics, Biology, Biochemistry and Technologies (NGBT) Conference (Sep 30th to 2nd Oct 2019) at Taj Lands End, Mumbai India.
13. Rathwa NN, Patel R, **Pramanik S**, Parmar NR, Ramachandran AV, Begum R. Calorie restriction in combination with GABA ameliorates type 2 diabetes. Poster presentation delivered at American Diabetes Association 79th Scientific Sessions (7-11 June 2019) at Moscone Center, San Francisco-94103, California, USA.
14. Patel R, Rathwa N, **Palit SP**, Parmar N, Dhimmar H, Ansarullah, Vasu V, Ramachandran AV, Begum R. β-cell regenerative potential of melatonin and DPP- IV inhibitor in amelioration of T1D. Oral presentation delivered at International Conference on Reproduction, Endocrinology and Development (19-21 January 2019) at School of Liberal Studies and Education, Navrachana University, Vadodara-391410, Gujarat, India.
15. Rathwa N, Parmar N**, Palit SP**, Patel R, Dhimmar H, Ramachandran AV, Begum R. Genetic Variants of Omentin-1 and Vaspin: Association with Type 2 Diabetes Susceptibility. Poster presentation delivered at International Conference on Reproduction, Endocrinology and Development (19-21 January 2018) School of Liberal Studies and Education, Navrachana University, Vadodara-391410, Gujarat, India.
16. Rathwa N, Patel R, **Palit SP**, Parmar N, Ansarullah, Bhaskaran RS, Ramachandran AV, Begum R. Therapeutic potential of γ-aminobutyric acid and calorie restriction in type 2 diabetic mouse model. Poster presentation delivered at International Conference on Reproduction, Endocrinology and Development (19-21 January 2018) School of Liberal Studies and Education, Navrachana University, Vadodara-391410, Gujarat, India.
17. Patel R, Rathwa N, **Palit SP**, Parmar N, Ansarullah, Ramachandran AV, Begum R. Replenishing β-cells with Melatonin & DPP-IV inhibitor: An in-vivo study. Poster

presentation delivered at International Conference on ‘Proteins, miRNA and Exosomes In Health and Diseases’ held at The M. S. University of Baroda, Vadodara, Gujarat, India on 11 -13 December 2018.

18. Rathwa N, Parmar N, **Palit SP**, Patel R, Ramachandran AV, Begum R. Association of Vaspin levels and its Genetic Variants with Type 2 Diabetes Susceptibility. Poster presentation delivered at International Conference on ‘Proteins, miRNA and Exosomes in Health and Diseases’ held at The M. S. University of Baroda, Vadodara, Gujarat, India on 11-13 December 2018.

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REVIEW ARTICLE

Treatment Avenues for Type 2 Diabetes and Current Perspectives on Adipokines

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Abstract: ***Background*:** Diabetes has turned into a pandemic disorder that is affecting millions of people worldwide. Industries are aggressively racing and pursuing research towards the discovery of antidiabetic drug and the current global sale of such drugs are ever on the increase. However, in spite of such massive level of expenditure thereof, WHO projects that by 2030, diabetes will rank as the 7th leading cause of mortality.

***Objective*:** It is in this context that we have reviewed here the various approaches available and possible towards diabetes management. This review also includes the WHO guidelines for controlling the glycemic levels, which must be known and followed by clinicians for a better diabetes management.

***Conclusion*:** Despite having a wealth of FDA-approved therapeutic options for type 2 diabetes majorities of the patients are not able to achieve the appropriate glycemic control due to various factors. The development of new options with actions at multiple foci of diabetic manifestation and better efficacy may potentially help in improving the current scenario of T2D management.

Keywords: Type 2 diabetes, therapeutics, gut and brain derived molecules, adipokines, small molecule drugs, phytochemicals.

1. INTRODUCTION

While the past history of mankind has, and is still witnessing the loss of human life due to malnutrition, the modern world is trying to combat diseases caused by unhealthy and excessive eating patterns in developed and developing countries. Although progress in science and technology has enhanced the quality of life, on the flip side, affluence has decreased physical activity especially amongst the economically privileged section of the society. This has led to the increased incidence of lifestyle related disorders such as obesity, impaired lipid profile, hypertension, and diabetes proving to be of great concern to public health. Although sedentary life style and modern dietary patterns have been related with type 2 diabetes (T2D), the interaction of genetic factors has also been suggested to have a role in diabetes manifestation [1].

Prediction based on current trend indicates that by 2030 there would be about 552 million obese and diabetic individuals. Current trends suggest that obesity and T2D have assumed pandemic proportions [2]. India alone is home to more than 65.1 million diabetics [3].

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1.1. Clinical Journey of T2D

T2D is characterized by hyperglycemia resulting from insulin resistance, eventual pancreatic β-cell failure and decreased incretin function [4]. By the time T2D is diagnosed, islet function is often reduced by 50% as compared to healthy controls [5]. The involvement of impaired α-cell function has also been implicated in the pathophysiology of T2D. A hampered α-cell functioning leads to a constitutive rise in the glucagon levels which keeps the blood glucose high even post meal [6].

Research has shown that 74% predisposition towards T2D is due to lifestyle and only 26% due to genetic factors (https://cpmc.coriell.org/about-the-cpmc-study/health-conditions-and-drug-response/type-ii-diabetes/risk-factors-type2-diabetes).

Till the present, several factors have been associated with T2D like obesity, inflammation, mitochondrial dysfunction, hyperinsulinemia, lipotoxicity/hyperlipidemia, genetic background, endoplasmic reticulum (ER) stress, aging, oxidative stress and steatosis [7]. An extremely high correlation between the T2D and obesity has been established in which obesity (adiposity) has been shown to be the major cause of insulin resistance and consequent diabetic manifestations [8]. T2D is a classical metabolic disease, but it is also associated

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Table 1. Glycemic index (WHO).

Sr no.	Blood Sugar Classification		Fasting (mmol/L)	2-h Post-glucose Load (mmol/L)
1	Normal		5.5	4.4- 7.7
2	Pre-diabetic	Impaired glucose tolerance	<7.0	7.8-11.0
		Impaired fasting glucose	6.1-6.9	<7.8
3	Diabetic		≥7.0	≥11.1

with a 2-4 fold increased risk of cardiovascular disease [9-12].

A recent report in 2013 established that the rising levels of obesity in South Asians are largely due to nutrition, lifestyle and demographic transitions, ever more due to faulty diets and physical inactivity, in the background of genetic predisposition [13]. In the backdrop of rich nutritious diet, T2D results from dysfunctional carbohydrate metabolism.

1.2. The Multi-Tasking Hormone: Insulin

Insulin is a metabolic hormone produced by pancreatic β-cells present in the islets of Langerhans. It is released into the blood stream in response to a rise in blood glucose level post meal and exhibits diversified effect on various tissues. Primarily, insulin mediates glucose uptake by muscle, fat, and liver cells; and it also stops glucose production in the liver by inhibiting gluconeogenesis. Alongside, insulin also stimulates the liver and muscle tissue to store excess glucose in the form of glycogen. In a healthy person, these functions together maintain the blood glucose and insulin levels in a harmonic balance [14].

Physiologically, insulin is secreted at basal levels between meals to keep a check on the hepatic glucose output. However, post meal it is secreted at higher levels to aid glucose uptake and this occurs in two phases. In the first phase, it reduces basal glucagon secretion, and in the second, *i.e.* 10 min after glucose exposure, the secretion is sustained until normoglycemia is achieved [15]. In T2D, the first phase of insulin response is almost eliminated or is severely blunted [16]. The loss of β-cell function appears to be accompanied by a reduction in β-cell mass [5] which regulates overall insulin secretion. Due to impaired insulin release, the blood glucose levels tend to remain high post meal that is eventually compensated by the second phase of insulin release achieving normoglycemia [16].

Thus, the decreased insulin function leads to chronic hyperglycemia (during both fasting and postprandial stages) and acute glycemic fluctuations. Table **1** represents the classification of diabetics and pre-diabetics based on the glycemic index as prescribed by the World Health Organization (WHO) [17].

1.3. Insulin Resistance

Insulin resistance is a pathophysiological condition wherein insulin-induced glucose uptake is impaired in the insulin-responsive tissues *i.e.* liver, muscle and adipose tissues evoking pre-diabetes/impaired glucose tolerance. Pre-diabetes is associated with high blood HbA1C levels and is the first pathophysiological condition to set in [18], eventually leading to hyperinsulinemia as the β-cells produce a large amount of insulin in an effort to control blood glucose levels [7]. Unchecked/ undetected pre-diabetic stage in the due course of time develops into T2D as the β-cells get exhausted in the process of compensating for the insulin resistance [19].

Most individuals with insulin resistance remain unaware that they are in their pre-diabetic stage for many years until they develop T2D, which is a serious lifelong disorder.

Obesity and the malfunctioning of β-cells have been firmly associated with each other but the molecular pathway is still undefined [20]. Adiposity or obesity has been directly coupled with adipose tissue inflammation and is marked by amplified pro-inflammatory cytokines such as TNF-α. However, it is a mystery whether the low-grade chronic inflammation is adequate to cause islet dysfunction [21-24]. Other factors such as glucolipotoxicity, amyloidosis, failure of β-cell expansion and dedifferentiation and β-cell apoptosis, have also been associated with obesity [18, 25-28]. Thus, though T2D is a multifactorial, polygenic disorder, obesity seems to play a major role in the onset of this disorder [29].

1.4. Obesity: A Cause of Insulin Resistance

Since the discovery of insulin in 1920 and its role in T2D, it has been used as a mono-hormonal therapy for treating diabetic patients [14]. However, the unraveling of additional hormones having glucoregulatory effect has expanded our horizon for search towards innovative therapies for T2D management.

Obesity, caused due to an over accumulation of adipose tissue, is not just a cosmetic concern but a medical condition as well. Excessive body (adipose) accumulates over a period of time to an extent that it starts having a negative effect on one's well-being [30]. In 1962, J. Neel theorized the "thrifty gene hypothesis" to partially explain the rise in obesity-related diseases in the world [31]. The hypothesis tries to explain that, various genes that promote the efficient utilization and storage of fuel might have been selected by nature to favor the survival of the human race during famines. Whereas today, in time of food abundance, the "same genes" make human predisposed to obesity and T2D [32]. Hormonal or other disturbances/imbalances in the early developmental periods may also lead to a thrifty gene phenotype predisposing individuals to diabetes in the adult stage on exposure to potential diabetogenic agents/conditions [33]. Adipose tissue, apart from serving as a store house of en-

ergy, also secretes bioactive peptides, termed 'adipokines/ adipocytokines', which act locally and distally by autocrine, paracrine and endocrine modes [34]. They interact with central and peripheral organs such as brain, liver, and skeletal muscles thus playing an important role in many physical processes [35]. Till date, over 100 adipokines have been identified and studied like leptin, resistin, adiponectin, visfatin, omentin-1, TNF-α, IL-6, etc. Increased production of most adipokines in obese individuals influences multiple functions such as appetite and energy balance, immunity, insulin sensitivity, angiogenesis, blood pressure, lipid metabolism and homeostasis [36].

Researchers have also found a gripping statistics for a substantial number of T2D patients being lean with BMI <25 [37-39]. Such cases of T2D have been found to be associated with malnutrition [40, 41], smoking [42], alcoholism [43], predisposition to genetic modulators [44], and also impaired adipose expandability [45]. Genome-wide association studies (GWAS) have identified approximately 50 genetic loci to be associated with T2D in lean and obese individuals [46-48]. The road towards the development of T2D remains many and since the cure is still obscure, the approach is restricted to T2D management by achieving glycemic targets.

2. GLYCEMIC TARGETS FOR T2D PATIENTS

The most important goal to prevent and delay diabetes-related complications is to maintain the glycemic target. It has been noticed that achieving glycosylated hemoglobin (HbA1c) level below 6.5% reduces microvascular complications in T2D [49]. Intensive control of blood-glucose levels using sulphonylureas or insulin drastically reduces the risk of complications in patients with T2D when compared with conventional treatment [49, 50].

The WHO [17] recommends four options for diagnosing diabetes as shown in Table **2**.

Table 2. Glycemic targets for T2D patients.

Glycemic Targets	
Fasting Plasma glucose	<7 mmol/L
2-hour plasma glucose	< 11.1 mmol/L
HbA1c	< 6.5%
Random plasma glucose	<11.1 mmol/L

Though the disease can be taken care of by using various anti-diabetic drugs or subcutaneous injections, they do not offer the extent of glycemic control provided by functional pancreatic β-cells.

3. LIFESTYLE INTERVENTIONS FOR THE TREATMENT OF DIABETES

Interventions involving dietary and physical activity changes are widely used and appear to be the most successful approaches for improving long-term weight maintenance and health status [51]. Exercise as a physical activity is suggested to serve as first line therapy for obesity and diabetes [52]. Exercise is known to contribute to glucose homeostasis and improve diabetic manifestations thus decreasing the incidence of diabetes significantly [53, 54]. It brings about significant changes in molecules of insulin signaling pathway and glycogenesis (GLUT4, protein kinase B (PKB), glycogen synthase (GS)) along with lipid profile markers *i.e.* reduction in plasma LDL, total cholesterol, triglyceride levels and TC/HDL ratio [55-57]. It also decreases the levels of pro-inflammatory cytokines like IL-6, C-reactive protein (CRP), TNF-α and IL-1β [58-60] and modulates adipokines such as leptin, resistin, apelin and ghrelin [61, 60]. Interestingly, exercise appears to be a new *modus operandi* for adipose tissue remodeling and modulation of uncoupling protein 1 (UCP1) in brown adipose tissue for improving diabetic manifestation [62]. A profound change in white adipose tissue (WAT) in response to exercise training is the mechanism by which the whole-body metabolic health is improved. Exercise also increases the number of beige cells in WAT that express UCP1, Tbx1, Tmem26, and Cd137 as well as markers of vascularization (*e.g.*, Vegfa, Pdgf, Angptl2) [63]. Moreover, it also promotes mitochondrial biogenesis in skeletal muscle helping ameliorate diabetic manifestations [64, 65]. Hence, exercise induced protein molecules apart from exerting a favorable influence on overall heath can also improve glucose and lipid metabolism and so could serve as a novel therapeutic target. Besides physical activity, people nowadays are also inclined towards calorie restricted diet.

Calorie restriction (CR) is fast developing as a new dietary intervention even though it has its own limitation such as its result reproducibility. CR is described as a reduction in caloric intake, typically by 20-40% of ad libitum consumption while maintaining sufficient intakes of protein and micronutrients to avoid malnutrition [66, 67]. Ideally, dietary treatment should aim to ensure adequate growth and development by reducing excessive fat accumulation and avoiding the loss of lean body mass taking care of overall well-being and preventing cyclical weight regain [68]. There are various dietary approaches for weight loss *i.e.*, low-fat, high-protein, low glycemic index and calorie restricted diets. In this context, *Dietary Guidelines* have recommended certain foods to be consumed less - "foods to reduce" (*i.e.*, saturated and trans fats, cholesterol, sodium, added sugar, refined grains, alcohol) and foods to be consumed more - "foods to increase" (*i.e.*, fruits, vegetables, whole grains, low-fat dairy and protein foods, oils). There is no standard definition of a "high-protein diet;" however, intake of protein greater than 25% of the total energy or 1.6 g/kg per day of body weight can be considered high [69]. As carbohydrates vary in the degree to which they raise blood glucose and insulin levels, a term "glycemic index" (GI) has been coined indicative of the property of carbohydrate-containing food [70]. A low GI diet is a precise blend of low-fat and low-carbohydrate dietary regime. Recommendations for this dietary approach are based not only on GI but also takes into account the nutritional content of the diet as a whole [71].

CR attenuates the degree of oxidative stress [72] and increases expression of genes involved in mitochondrial function and biogenesis such as *PPARGC1A, TFAM,* and *SIRT1* [73]. Elevated rate of whole body fat oxidation in response to calorie restriction was observed along with decreased lev-

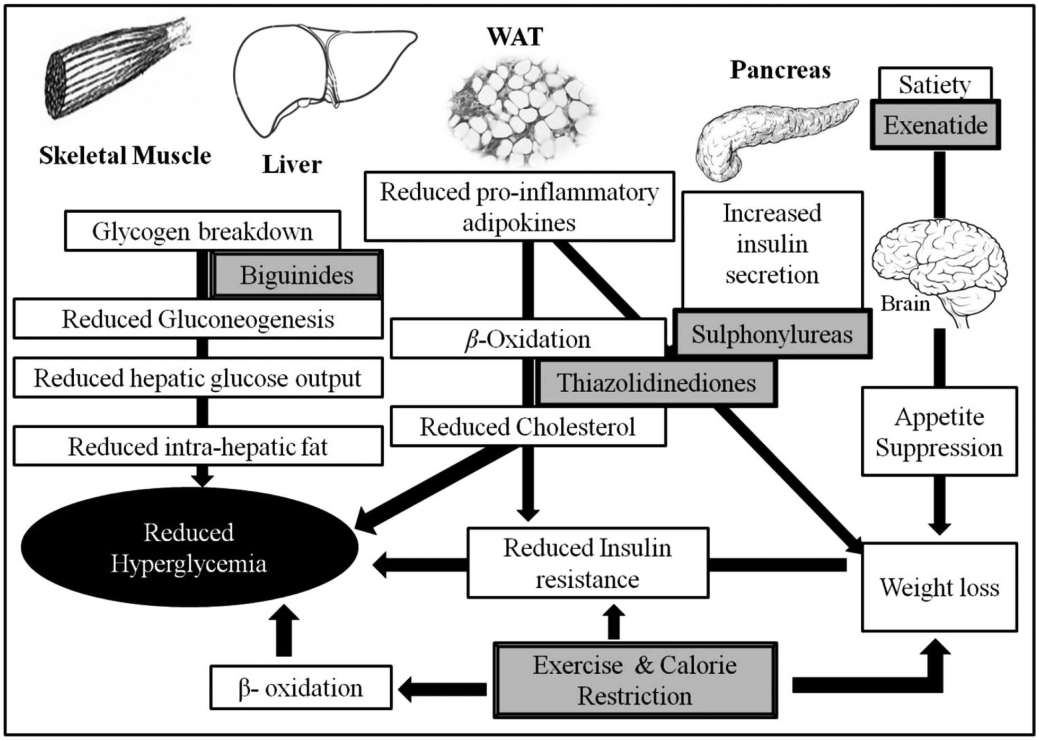

Fig. (1). An illustration showing plethora of effects of exercise, calorie restriction and drugs contributing to reduced hyperglycemia.

els of fatty acid synthesis in liver [74], improved fasting glucose levels besides offering protection from other cardio metabolic risk factors [75], and also reduced pro-inflammatory adipokines [76]. The tight direct relationship between the phosphorylation (and therefore activation) of ERK and $p70^{S6K}$ along with the phosphorylation of $IRS1^{S612}$ and $IRS1^{S632/635}$ implicates ERK and MTOR/ $p70^{S6K}$ as the kinases responsible for the phosphorylation of these sites in the liver as observed in obesity-induced insulin resistance. However, CR diminished activities of these kinases ameliorating insulin resistance [77] as shown in Fig. (**1**).

Thus CR was and still is the traditional first-hand method to control T2D. However, the CR regime would vary from person to person and thus needs to be designed in a person-specific manner. Conversely, strict caloric restriction may work negatively by increasing the risk of hypoglycemia [78], leading to a decreased bone density, ketosis, *etc* [79].

4. THERAPEUTIC MODALITIES AND TARGETS

4.1. Current Strategies (Synthetic Small Molecule Drugs/ Oral Hypoglycemic Agents)

The characteristics of most widely used monotherapeutic modalities are tabulated in Table **3**. The choice of initiating a glucose lowering strategy is based on the level of glycemic control required. When the level of glycemia is high (*e.g.*, A1C >8.5%), therapeutants with a rapid glucose-lowering capacity, or potentially earlier initiation of combination therapy, are recommended. Similarly, when glycemic levels are closer to target goals (*e.g.*, A1C <7.5%), CR or medications with lower hypoglycemic potential may be considered [80]. Below is an overview of traditional and newer/emerging agents used in T2D.

4.1.1. Biguanides

Metformin is the most widely prescribed blood-sugar-lowering drug in the world and is the first line of medication for T2D. It belongs to a class of drugs called biguanides. American Diabetes Association (ADA) and European Association for the Study of Diabetes (EASD) have jointly recommended metformin as the initial drug to be prescribed if nutritional therapy and exercise prove to be inadequate [81]. Metformin limits glucose production from liver by inhibiting gluconeogenesis and glycogenolysis while increasing insulin sensitivity so that glucose is taken up by muscle, fat, liver, and other types of cells. Metformin monotherapy on an average lowers A1C by approximately 1.5% and it is generally well-tolerated, with the most frequent undesirable effects being gastrointestinal in nature [80]. The major advantage of metformin is that it does not cause hypoglycemia while having positive effect on serum lipids and lipoproteins as compared to other classes of small molecule [82-84]. Metformin non-competitively inhibits the redox shuttle enzyme mitochondrial glycerophosphate dehydrogenase and mitochondrial complex I, resulting in an altered hepatocellular redox state, reduced conversion of lactate and glycerol to glucose, and decreased hepatic gluconeogenesis. Alternatively, it has been shown that in mouse hepatocytes, metformin leads to the accumulation of AMP, which inhibits adenylate cyclase, reducing the levels of cAMP and protein kinase A (PKA) activity, and further inhibiting phosphorylation of crucial protein targets of PKA, while blocking glucagon-dependent glucose release from hepatocytes [85-88]. Further, AMPK which is activated by metformin might play a key role in long-term effects of metformin by improving lipid metabolism and mitochondrial function in the liver [89].

Table 3. Characteristics of most widely used monotherapeutic modalities.

Group	Class	Generic Name	Side Effects
Biguanides	Sensitizer	Metformin	Weight loss, GI upset
Thiazolidinediones		Rosiglitazone Pioglitazone	Weight gain Peripheral edema
Alpha glucosidase inhibitors	----	Acarbose Miglitol	GI upset
Sulfonylureas	Secretagogue	Chlorpropamide Glibenclamide Glimepiride Glipizide Tolazamide Tolbutamide	Hypoglycemia Weight gain
Glinides		Nateglinide Repaglinide	Weight gain
Exenatide	GLP-1 analog	Byetta	Weight loss
Dipeptidyl peptidase-4 inhibitors	DPP-4 inhibitors	Sitagliptin Saxagliptin Linagliptin	----

4.1.2. Sulfonylureas

Sulfonylurea is an insulin secretagogue *i.e.* it lowers glucose levels by triggering insulin secretion from *β*-cells. It closes the potassium channels by binding to adenosine triphosphate (ATP)-sensitive potassium channels and thereby leads to subsequent opening of calcium channels resulting in the exocytosis of insulin. Though the first generation sulfonylureas were efficacy wise similar to metformin, they were however known to cause severe episodes of hypoglycemia. The second-generation sulfonylurea agents (*e.g.*, glipizide, glimepiride) have comparatively lesser side effects [90]. Sulfonylureas bind to and close ATP-sensitive K^+ channels (KATP) on pancreatic beta cell membrane which depolarizes the cell by preventing potassium ions from exiting. This depolarization opens voltage-gated Ca^{2+} channels leading to Ca^{2+} influx. This rise in intracellular calcium leads to increased fusion of insulin granulae with the cell membrane, and therefore increased secretion of (pro) insulin [91].

4.1.3. Glinides

Glinides (*i.e.*, repaglinide, nateglinide) are a similar class of insulin secretagogues like sulfonylurea but bind differently and have a shorter circulating half-life. It depolarizes *β*-cell membrane leading to insulin granule exocytosis and also acts as peroxisome proliferator-activated receptor-gamma (PPARγ) agonist leading to glucose uptake [92, 93]. This necessitates frequent administration. Like metformin and sulfonylurea, glinides too have a similar efficacy of reducing A1C by 0.5 – 0.8% and they also pose a risk of weight gain [80]. Moreover, they have not been associated with episodes of hypoglycemia [94, 95].

4.1.4. α-Glucosidase Inhibitors

The mode of action of α-Glucosidase inhibitors (*e.g.*, Acarbose & Miglitol) is very different from the above classes discussed. They work by reducing the rate of digestion of polysaccharides in the proximal small intestine and thus indirectly lower the postprandial glucose levels. However, compared with metformin and sulfonylureas, they are less effective in lowering glucose reducing A1C by only 0.5% to 0.8%. These drugs function as high affinity reversible inhibitors of alpha-glucosidase, particularly pancreatic alpha-amylase and membrane-bound intestinal alpha-glucosidase. Pancreatic alpha-amylase hydrolyzes complex carbohydrates to oligosaccharides in the lumen of the small intestine while, intestinal glucosidase hydrolyses oligosaccharides, trisaccharides and disaccharides to glucose and other absorbable monosaccharides in the brush border of intestinal villi. The inhibition of these enzymes thus reduces the rate of formation of "absorbable sugars" and thus delays the rise in blood glucose concentration following meals (postprandial). This action therefore results in attenuation of postprandial plasma glucose (30-35% reduction), as well as insulin, gastric inhibitory polypeptide and triglyceride peaks [96]. *α*-Glucosidase inhibitors are commonly associated with increased gastric complications [80].

4.1.5. Thiazolidinediones (TZDs or Glitazones)

TZDs mediate their effect *via* the activation of peroxisome proliferator–activated receptor (PPARγ) largely present in adipose tissue which, modulates the expression of several genes involved in glucose and lipid metabolism, inclusive of those that code for adipocyte fatty acid binding protein, lipoprotein lipase, fatty acid transporter protein, fatty acyl-CoA synthase, malic enzyme, glucokinase and the GLUT4 [97]. Activation of PPARγ is reported to induce adi-

pogenesis and adipocyte differentiation after the activation of C/EBP-α and synergizing with it [98]. TZDs (*i.e.* pioglitazone, rosiglitazone) also increase insulin sensitivity of glucose disposing tissues. Pioglitazone treatment was reported to increase (PPAR)-γ coactivator-1α and mitochondrial transcription factor A leading to mitochondrial biogenesis. Further, it also increases the expression of genes in the fatty acid oxidation pathway such as carnitine palmitoyltransferase-1, malonyl-CoA decarboxylase, and medium-chain acyl-CoA dehydrogenase [99]. TZDs are mostly used as part of a combination therapy. The most common adverse effects associated with TZDs include weight gain, fluid retention, increased subcutaneous adiposity, macular edema, heart failure, and bone fractures [100].

4.2. Current Strategies (Synthetic Large Molecule Drugs)

4.2.1. Insulin

Amongst the various strategies, insulin is the most effective in lowering glycemia and reduces elevated A1C to, or close to, the therapeutic goal. However, because T2D patients are insulin resistant, generally a large dose is required. Insulin therapy has beneficial effects on the triglyceride and HDL-cholesterol levels but is also known to cause weight gain [101] and hypoglycemic episodes. Compared with NPH (Neutral Procaine Haledon) and regular insulin, insulin analogues with longer pharmacokinetic profiles (*e.g*. insulin glargine), as well as, analogues with very short durations of action (*e.g*. insulin lispro), decreases the risk of hypoglycemic episodes [80].

4.2.2. Incretins

Incretins are a class of enteric hormones which regulate blood glucose by stimulating insulin secretion indirectly from the *β*-cells and, the decline of *β*-cells in T2D has been linked to their impaired action [5]. The major ones in this class are glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) secreted from endocrine K and L cells respectively in the small intestine in response to a rise in glucose levels. They then activate G protein–coupled receptors on pancreatic *β*-cells thereby stimulating insulin secretion [102]. GLP-1 also inhibits glycogenesis by decreasing the secretion of glucagon. Furthermore, GLP-1 is known to have an effect on the central nervous system like delayed gastric emptying and a feeling of satiety. In contrast, GIP has an effect only on glucagon secretion [103]. What makes GLP-1 a favorable agent is its property to induce insulin secretion in response to the raised blood glucose level post meal. This reduces the chances of adverse side effects such as sudden hypoglycemia [104]. Studies on T2D individuals have shown increased insulin secretion and concomitant decreased glucagon secretion on treatment with GLP-1 receptor agonists [5].

4.2.3. DPP 4 Inhibitors

Dipeptidyl-peptidase IV (DPP-4) is a ubiquitous serine protease acting on a variety of substrates ranging from hormones to chemokines to neuropeptides [105]. In the enteric system it cleaves GLP-1 and GIP secreted from gastric mucosa, trimming down their half-life to few minutes in plasma. DPP-4 inhibitors are being used to sustain the rise in GLP-1 level post meal in fasting conditions as well, thus keeping a check on hyperglycemia [106]. DPP-4 has also been identified as a novel adipokine with a significantly high expression in visceral fat of obese subjects impairing insulin signaling at Akt level in the glucose disposal organs [107]. The same group has also shown the augmented release of DPP-4 (by 50%) in response to obesity-related TNF-*α* elevation and an inhibition of the anti-lipolytic action of Neuropeptide Y (NPY) [108].

Strategies such as the development of DPP-4 resistant GLP-1 analogues (*e.g.*, exenatide, liraglutide) as well as molecules that inhibit the enzymatic activity of DPP-4 (*e.g.*, sitagliptin, vildagliptin, saxagliptin) have already been extensively attempted [109, 110].

4.3. New and Emerging Therapies

Over the counter, oral drugs discussed till now mostly stimulate insulin release, suppress hepatic glucose output and assist glucose disposal but they only work towards diabetes management by controlling hyperglycemia [110]. With reference to both type 1 and type 2 diabetes, wherein there is *β*-cell loss sooner or later, the therapeutic focus has now shifted from merely controlling glycemic targets to regeneration or preservation of *β*-cell mass. A lot of work has been carried out in this context in the past few decades and as a result, a large number of agonists (*e.g*. Betatrophin) have been identified through high throughput screening that induces *β*-cell replication in animal models. In this context, work from our own group has shown flavonoid mixture from *Oreocnide integrifolia* (Urticaceae), a folklore plant, to have significant insulin secretagogue, insulinomimetic and cytoprotective effects [111]. But unfortunately, very few such molecules have been found to induce *β*-cell replication at a substantial rate from a therapeutic point of view in humans [112].

Of late, hormones and other protein molecules have also gained a great deal of focus as therapeutic agents by virtue of their biological significance encompassing an array of various functions as illustrated in Fig. (**2**) [113].

4.3.1. Brain: The Seat of Hunger and Satiety

The long posited theory, that brain was critical in the negative feedback regulation of appetite and body weight was found to be true as it was found that brain requires an incessant supply of glucose for meeting its energy demands [114] making it the highest consumer of glucose [115]. Due to this strict dependence on glucose, brain exerts regulation on blood glucose levels through an array of intercoordinating hormones (leptin, ghrelin, NPY, glucagon-like peptide-1, insulin, *etc*.) to achieve a precise physiological balance [116-118]. While NPY and ghrelin are secreted in response to activation of the nutritional prompt "feeding center" by a fall in the blood glucose level, the rest are secreted in response to activation of "satiety center" by a rise in the glucose level, making the brain a "dual-core" system [115]. Also, reduced plasma levels of brain-derived neurotrophic factor (BDNF) have been associated with impaired glucose metabolism and type II diabetes in human subjects [119]. Simultaneously, caloric restriction and exercise have been shown to elevate BDNF levels by various mechanisms [120, 121]. Increased insulin-stimulated tyrosine phosphorylation

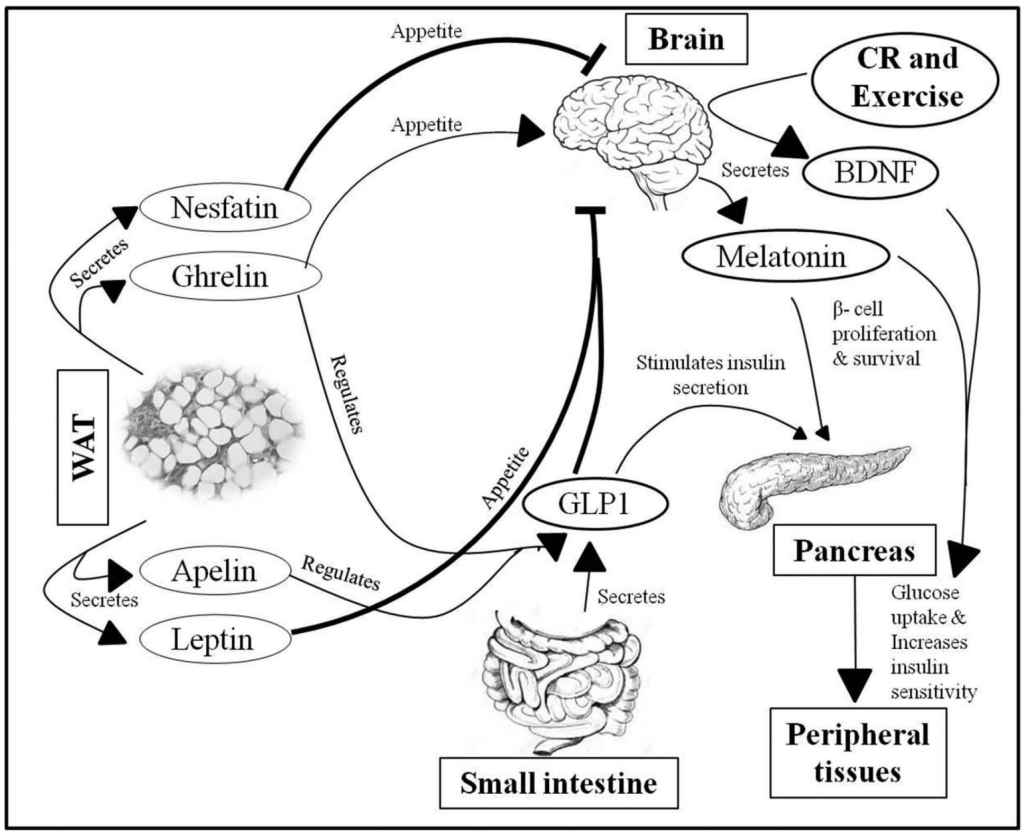

Fig. (2). The Gut – Brain axis in diabetic regulation.

of insulin receptors in liver and PI3-kinase activity in liver, skeletal muscle and brown adipose were demonstrated in *db/db* mice when administered with BDNF for 14 days [122]. Any disturbance in the energy homeostatic balance leads to conditions like hyperglycemia or T2D.

4.3.1.1. Melatonin

Melatonin, referred as the hormone of darkness, is mainly secreted by the pineal gland with a night time high and day time low [123]. Its primary role has been identified in maintaining body homeostasis and biological clock *i.e*. the sleep-wake cycle [124]. It is thus, referred as "Sleep hormone" too. Apart from regulating the "Clock genes" it has also been strongly linked to T2D [125]. Many past studies from our group have shown hypoglycemic and promotion of peripheral glucose uptake effects of melatonin in the vertebrate series based on melatonin administration and pinealectomy [126-128]. Recently, it was further shown that melatonin supplementation exhibited greater potency than estrogen replacement therapy in overcoming diabetogenic metabolic dysregulation in ovariectomized/estrogen deficient rats [129]. Studies describing the effect of melatonin on various glucose responsive tissues expressing MT2 (Melatonin receptor Type 2) are available. Accordingly, in human adipocytes, it downregulates GLUT4 expression [130], in murine skeletal muscle it enhances insulin receptor substrate-1(IRS-1) phosphorylation [131] and in mouse liver it induces glucose release [132]. Interestingly, MT1 (Melatonin receptor Type 1) and MT2 receptors are also expressed on the pancreatic islet cells [133, 134]. In the *β*-cells, it decreases insulin secretion by inhibiting cAMP and cGMP pathways while, it enhances the secretion of insulin by increasing cytoplasmic Ca^{2+} concentration *via* phospholipase C/IP3 pathway activation [135, 136]. The modulatory effect of melatonin also extends to glucagon secretion from *α*-cells apart from insulin secretion from *β*-cells [137]. Thus, a reduction in melatonin could potentially contribute to the genesis of diabetes as, a functional inter-relationship between melatonin and insulin is revealed in diabetic patients [138, 139]. Additional evidences from experimental studies are available for melatonin promoted insulin receptor tyrosine phosphorylation and production of insulin growth factor [140]. Shi *et al*. [141] further demonstrated insulin resistance and glucose intolerance in individuals with a disturbed internal circadian system which could however, be re-established by melatonin supplementation [142, 143]. Our group had earlier demonstrated increased GLUT4 expression in the muscle tissue of diabetic rats upon melatonin supplementation. In other studies, the anti-diabetogenic effects of melatonin as a de-programmer of early neonatal corticosterone induced thrifty phenotype for adult diabetogenic manifestations had also been recorded [33, 144].

Therefore, the existence of melatonin receptors on islet cells may be targeted to design pharmacotherapy for T2D. Melatonin is currently under intensive scrutiny in experimental animal models of diabetes, obesity, and metabolic syndrome [145].

4.3.2. GUT: The Second Brain

4.3.2.1. Ghrelin

Ghrelin (a gut – brain peptide) is synthesized mainly by the P/D cells of human gastric mucosa [146]. However, ghrelin is also found in many other tissues like the hypo-

thalamus, brain cortex, pituitary, adrenal, hippocampus and pancreas [147, 148]. In obese individuals ghrelin is found to be attenuated [149].

Ghrelin possesses growth hormone-releasing activity, adipogenic activity, and orexigenic activity by acting as a ligand to the growth hormone secretagogue receptor 1a (GHSR) in CNS [148, 150-152] and, its level is controlled by a number of factors like food intake, insulin and glucagon levels. Apart from playing a role in regulating energy homeostasis, the presence of ghrelin and its receptors on the pancreatic α and β-cells indicates its additional glucoregulatory role [153]. In a study by Tong and colleagues, it was reported that both supraphysiological and physiological doses of ghrelin in healthy individuals suppressed insulin secretion leading to an impaired glucose tolerance but interestingly, the insulin sensitivity was unaltered in the latter dose [154, 155]. In another independent study by Vestergaard *et al*. [156], intramuscular administration of ghrelin enhanced glucose uptake and lowered blood glucose level. They had earlier shown this effect of ghrelin to be without any effect on the insulin signaling pathway [157]. Thus, the above studies are suggestive of the pharmacological potential of ghrelin by its effect on Glucose-stimulated Insulin Secretion (GSIS) and insulin sensitivity. Ghrelin has also been demonstrated to suppress glucose-induced insulin release *via* GTP-binding proteins and delay K+ efflux thereby regulating insulin release and glycemia [158]. There are also reports of ghrelin priming intestinal L cells for the production of GLP1 incretin hormone which improves glucose tolerance by stimulating insulin production by β-cells and decreasing glucagon production by α-cells [159]. Maintaining a critical level of serum ghrelin by its neutralization using antagonists or anti-ghrelin antibodies might be worthwhile to study. Since insulin is known to inhibit ghrelin, usage of insulin mimetics to regulate ghrelin level could also be a novel approach towards T2D management.

4.3.3. Adipokines

4.3.3.1. Adiponectin

ADIPOQ gene located on chromosome 3q27 codes for the 30 kDa adiponectin protein [160]. The protein is exclusively expressed in white adipose tissue. Adiponectin is found in various polymorphic forms in plasma. It is found in 3 major oligomeric forms; a low–molecular weight (LMW) trimer, a mid–molecular weight (MMW) hexamer, and a High–Molecular Weight (HMW) 12- to 18-mer [161, 162].

Normal level of adiponectin in the circulation is 2-20 μg/mL and forms 0.05% of total serum protein. Apart from its insulin sensitizing action, adiponectin is also responsible for free fatty-acid combustion *via* PPARα activation and increasing AMP: ATP ratio by AMPK activation and thus plays a pivotal role in energy metabolism [163]

The primary mechanism by which adiponectin enhances insulin sensitivity appears to be through increased fatty acid oxidation and suppression of gluconeogenesis thus decreasing the triglyceride content in liver and skeletal muscle, and enhancing insulin sensitivity [163, 164]. The mechanism of action of adiponectin on muscle appears to be through activation of AMP kinase (AMPK) and PPARα [165, 166]. In the case of liver, adiponectin promotes glucose uptake and stalls gluconeogenesis and, activates fatty acid oxidation and decreases inflammation *via* PPARα [167]. Interestingly, it has been found that adiponectin levels are compromised in obese, insulin-resistant rodent models [168] and also in obese rhesus monkeys that develop T2D [169]. More interestingly, reduced insulin sensitivity in conjunction with decreased plasma adiponectin level was also noted in these animal models [169]. Similar observations were reported in obese humans as well, particularly those with visceral obesity [170-172]. In humans, caloric restriction and physical exercise have been shown to increase circulating adiponectin levels significantly and also to attenuate the TNF-α to adiponectin ratio [173, 174].

Restoring the adipokine level or increasing AMPK and PPARα levels may in this context prove beneficial. Many such molecules have already been studied, each having its own limitations. Recently, a small-molecule adiponectin receptor agonist- Adiporon was reported to improve insulin sensitivity without altering insulin secretion [175]. However, activating adiponectin alone or increasing AMPK level might not be an ultimate answer to β-cell loss. Among the several adipokines, adiponectin has of late attracted a good deal of attention by virtue of its antidiabetic and antiatherogenic effects [176].

4.3.3.2. Resistin

Resistin gene was originally identified present on chromosome no. 19 of mouse in 2001. Resistin (12.5 kDa) is an unusual hormone in the sense that it has 11 cysteine residues out of a total of 114 amino acids [177]. In serum, resistin circulates predominantly as trimers and hexamers, with the trimer being the most bioactive form [178]. Resistin is expressed at very low levels in human adipose cells whereas; high levels are expressed in spleen, bone marrow, mononuclear leukocytes and macrophages [178-180]. Some studies have suggested that mature human adipocytes lack resistin expression, while preadipocytes do [181, 182]. Infusion of resistin in Sprague-Dawley rats resulted in weakened hepatic insulin sensitivity and glucose metabolism [183] and, chronic elevated circulating resistin levels led to increased fasting glucose, weakened glucose tolerance and decreased hepatic insulin sensitivity [184]. Resistin was also reported to induce SOCS3, resulting in the suppression of insulin-mediated signaling in adipocytes [185].

However, the function of resistin in humans has been inconclusive [186-188]. Many studies have shown a positive correlation between elevated serum resistin level and insulin resistance and obesity in humans. Sheng *et al.* [189] observed resistin to be expressed in human hepatocytes while Tsiotra *et al.* [190] and Gharibeh *et al.* [191] observed that resistin caused insulin resistance in female subjects with T2D and obese T2D patients. However, contradictory results from human studies indicate resistin gene expression and its circulating levels to be both increased and unchanged in obesity and insulin resistance [192-195]. Resistin was identified as a pro-inflammatory adipokine mediating its action *via* TNF-α by activation of NF-κB pathway [196] and recruitment of immune cells [197]. And recent study unraveled that resistin binds to adenyl cyclase associated protein 1receptor which increases cAMP and PKA activity [198]. In a study by Steppan *et al*. [199], blocking of resistin action with neutral-

izing antibody was found to improve whole-body insulin sensitivity in diet-induced obese (DIO) mice while, antisense oligodeoxynucleotide against resistin mRNA completely reversed hepatic insulin resistance in animals [200]. It is likely that resistin is a biomarker for and/or contributes to insulin resistance in specific populations.

4.3.3.3. Omentin-1

Omentin-1 is a novel 40 kDa fat depot-specific adipokine (gene bank accession number- AY549722) which has been identified from a cDNA library of visceral omental adipose tissue [201], located on 1q21.3 chromosome locus [202]. It is also known variously as intelectin-1, intestinal lactoferrin factor, endothelial lectin HL-1 or galactofuranose binding lectin. In humans, omentin is expressed as two homologous proteins, omentin-1 and -2, encoded by two separate genes located adjacent to one another on 1q22-q23. Omentin-1 is the major circulating isoform in human plasma. Omentin-2 shows 83% amino acid identity with omentin-1 [203]. Omentin mediated glucose uptake occurs *via* the phosphorylation of Akt at physiological concentrations [204, 205].

Omentin gene expression in visceral adipose tissue and circulating omentin level were reported to be decreased in obese subjects [203] associated with impaired glucose tolerance (IGT) and in T2D subjects [206-208]. However, circulating omentin levels were found to be elevated in patients with nonalcoholic fatty liver disease [209]. Omentin receptor, target tissues, and signaling mechanism remain obscure as yet, but the above studies are indicative of its potential as a therapeutant.

4.3.3.4. Vaspin

Vaspin, a visceral adipose tissue (VAT) derived serine protease inhibitor has an insulin-sensitizing effect and belongs to the serpin superfamily (Serpina12). It was found in the VAT of Otsuka Long-Evans Tokushima Fatty rat (OLETF) typified with central obesity and T2D [210]. Vaspin acts as a circulating serpin, which serves as a ligand for a cell-surface receptor complex, GRP78/MTJ-1, and exerts anti-inflammatory action in ER induced stress [211]. In another study, Nakatsuka *et al*. [212] showed vaspin to serve as a ligand for a cell-surface voltage-gated anion channel complex in endothelial cells thereby exerting anti-apoptotic, proliferative, and protective effects on the endothelium of rat models with T2D. Furthermore, vaspin also protects endothelial cells by its inhibitory action on NF-κB [213].

Increased serum vaspin was found to be associated with obesity in young Korean men [214] and also with BMI, triglycerides, fasting insulin and insulin resistance in pubertal obese children [215]. However, administration of recombinant vaspin in obese mice showed to improve glucose tolerance and insulin sensitivity suggesting the rise in vaspin levels to be a compensatory rise in response to obesity and insulin resistance. Interestingly, it was also higher in healthy females as compared to healthy males demonstrating sexual dimorphism [216]. On the other hand, several studies have failed to show even a simple correlation between serum vaspin levels and BMI [217] and insulin sensitivity [218] or with T2D [219].

Interestingly, vaspin influences insulin-induced glucose uptake *in vivo*, but not *in vitro*. Vaspin probably modulates insulin action only in the presence of its target proteases, which most probably trigger altered insulin sensitivity. Therefore, identification of vaspin's target protease is the major challenge for future studies related to vaspin. Unraveling the proteases might lead to the development of novel anti-diabetic therapy, which may improve insulin sensitivity in patients with T2D.

4.3.3.5. Leptin

Leptin is synthesized and secreted by the adipose tissue in proportion to the amount of fat deposition [220-222]. However, it mediates its action *via* brain as an anorexigenic hormone [223]. It was found that injection of recombinant leptin on daily basis into normal mice reduced their appetite while increasing their energy expenditure. This resulted in the elimination of fat deposits in a short span of time without causing hypoglycemia [224, 225]. These results made leptin a potent anti-obesity drug. However, it was soon, also, observed that leptin levels were already elevated in obese individuals [149]. Detailed leptin-based clinical trials by NIH show significant hyperleptinemia under obese conditions (www.clinicaltrials.gov and http:// www.ncbi.nlm.nih.Gov/ pubmed/). This suggests of a possible leptin resistance [226-228] which can be due to three possible reasons: i) inefficient/weakened transport of leptin across the blood-brain-barrier (BBB), ii) diminished neuronal leptin signaling in the target neurons, and iii) impaired downstream signaling cascade of target cells [229].

Leptin regulates body weight and neuro-endocrine functions apart from appetite through its receptors in CNS [220, 221]. Leptin's effect on body weight has been shown to be *via* GABAergic neurons in mice [230]. Though the mechanism through which leptin functions remains still obscure, a substantial amount of data strengthens its glucoregulatory effect [231, 232]. Thus, leptin may serve as a therapeutic solution for lean as well as obese type 2 diabetics by means of a "Brute force" effect (exogenous leptin given despite leptin resistance).

4.3.3.6. Nesfatin

Nesfatin-1was discovered for the first time in 2006 as a satiety hormone [233] and further studies [234] provided evidence for it to be another hormone involved in the regulation of energy metabolism. Specifically, it is secreted by the peripheral adipose tissue, gastric mucosa, pancreatic endocrine β-cells, and testis [235]. Intraperitoneal injection of nesfatin-1 in rats reportedly suppressed food intake in a dose-dependent manner [236]. It is also shown to work independent of the leptin pathway [233], thus making nesfatin-1 a possible mode of treatment in obese individuals with leptin gene mutation or leptin resistance [237]. There are a number of theories that explain its action; the first being by activation of the melatonin pathway and, the second being by inducing NPY secretion [235, 237-239].

Additionally, it was found that there was a significant decrease in food intake and body weight on a continuous infusion of nesfatin-1 into the third ventricle of brain in rats [233]. Downregulation of gluconeogenesis and promotion of peripheral glucose uptake were the attendant effects noticed

by such infusions [240]. However, in multiple clinical studies, it was noted that plasma nesfatin-1 levels were higher in T2D patients and was associated with the homeostasis model assessment of insulin resistance (HOMA), BMI and plasma insulin. This paradoxical elevation of plasma nesfatin-1 in T2D patients is hypothesized to be a compensatory upregulation to recompense for the metabolic stress imposed by obesity or a possible nesfatin-1 resistance [241]. Improper understanding of nesfatin-1 action precludes detailed elucidation of its role in T2D and glucoregulation. However, nesfatin-1induced activation of β-cell Ca^{+2} channels and the resultant increased insulin secretion [242] opens up avenues to explore the feasibility of nesfatin-1 in the amelioration of T2D.

4.3.3.7. Visfatin

Visfatin has recently been identified as a novel adipokine and also as a pre β-cell colony-enhancing factor [243]. Much greater expression in visceral fat tissue has formed the basis for its name [244]. However, some other studies showed visfatin to be expressed by the macrophages infiltrating adipose tissue in response to the inflammatory signals emanating from various other tissues [245, 246]. Visfatin was found to be insulin mimetic in action as it increased glucose uptake in adipocytes and myocytes. It also exerted other actions like suppressing hepatic glucose release and stimulating hepatic triglyceride accumulation and, increasing its own synthesis in pre-adipocytes in mice models [247]. Visfatin was found to exert its effect by activating insulin receptors *via* a different binding site, causing receptor phosphorylation and the activation of the downstream signaling molecules [248]. In a meta-analysis study, volunteers categorized as overweight/obese or type 2 diabetic, Chang and colleagues observed the plasma visfatin concentrations to be increased [249]. This provides hope for its exploitation as a possible diagnostic marker for diabetes.

4.3.3.8. Retinol Binding Protein 4 (RBP4)

RBP4 is a carrier protein of retinol (vitamin A alcohol) in circulation. It is bound to transthyretin in circulation and its physiological function is to prevent the kidney excretion of retinol [250].

The first key link between RBP4 and diabetes was the observation of an eminently higher plasma level of RBP4 in obese and T2D mice and humans [201] and, alleviation of insulin resistance in diet-induced obesity by an induced experimental decrease in RBP4 [251]. Yang's group also showed the expression of gluconeogenic enzyme (phosphoenolpyruvate carboxykinase) in liver and attenuation of insulin signaling in muscle by preventing insulin receptor substrate-1phosphorylation and activation of phosphatidylinositol-3-kinase in mice by RBP4.

RBP4 has also been reported to cause adipose tissue inflammation by the activation of JNK inflammatory pathway leading to the priming of antigen presenting cell (APC) activation and consequent overshooting of the balance towards differentiation of adipose tissue resident APCs into M1 macrophages exhibiting increased pro-inflammatory gene expression [252-255]. Thus, reducing the RBP4 levels can be a potential therapeutic strategy by means such as Transthyretin Antisense Oligonucleotides [256] or Anti-TNF-α therapy [257].

4.3.3.9. Apelin

Apelin was detected in adipose tissue [258] and later shown to be produced and secreted by adipocytes [259]. Apelin has been identified as an adipokine which increased during adipogenesis [259, 260]. One of the main regulators of apelin is insulin and, a close relation between insulin and apelin has been shown both *in vivo* and *in vitro* [259]. The expression of apelin in adipocytes has been shown to be increased in various mouse models of obesity associated with hyperinsulinemia. The pattern of apelin expression in adipocytes paralleled the plasma levels of insulin in mice under conditions of fasting and re-feeding. Even in *in vitro* studies involving cultured adipocytes (3T3F442A) and isolated human adipocytes, expression and release of apelin is shown to be increased on insulin treatment. Apelin receptors (APJ) find wide expression in various tissues such as stomach, heart, lung, skeletal muscle, etc. along with being expressed in hypothalamus [259, 261, 262]. One of the first actions of apelin noted was its role in energy metabolism and, the same group also demonstrated its action on intestinal glucose absorption in a murine model [263]. It was also reported to promote glucose uptake by the enterocytes by overexpression of GLUT2 channels and upregulation of GLP1 secretion [264, 265]. In peripheral tissues, apelin exhibits a glucoregulatory action by stimulating Akt and AMPK phosphorylation [264, 266]. Consequently, it was also shown that apelin treatment improved mitochondrial biogenesis [267] and insulin sensitivity in insulin-resistant obese mice [263]. Adding to the apelin quest, Xu *et al.* [268] demonstrated apelin facilitated GLUT4 translocation in C57BL/6J mice.

As apelin receptors also exist on β-cells, it is assumed to have a paracrine or autocrine regulatory action on insulin secretion thereby preventing hyperinsulinemia and contributing to improved insulin sensitivity [269]. Apelin was also shown to inhibit lipolysis in 3T3 L1 cells [270] and *in vivo* studies indicated it to be through activation of AMPK [271]. Consistent with these findings, many studies have shown increased plasma apelin concentrations in obese and/or diabetic subjects with higher insulin levels [261].

All the reported data put together suggests that apelin could play a major role in glucose homeostasis by increasing insulin sensitivity and insulin secretion along with a concomitant suppression of lipolysis.

4.3.3.10. Adipsin

Adipsin was the first adipokine to be studied [272] and, it functions in the complement pathway as factor D [273, 274]. Adipsin cleaves factor B only when in complex with C3b, catalyzing the formation of the C3 convertase (C3bBb), which can act on C3 to liberate C3a. C3a stimulates insulin secretion by localizing Ca^{2+} in β-cells only in the presence of elevated glucose levels. In addition, the half-life of C3a is very short as it is rapidly inactivated by serum carboxypeptidases to its inactive form- C3a-desArg [275]. Its levels are known to drastically decline in obese and diabetic animal models [276]. Interestingly, restoration of adipsin expression increased post prandial insulin levels in T2D patients. It was also noted that the circulating levels of adipsin were

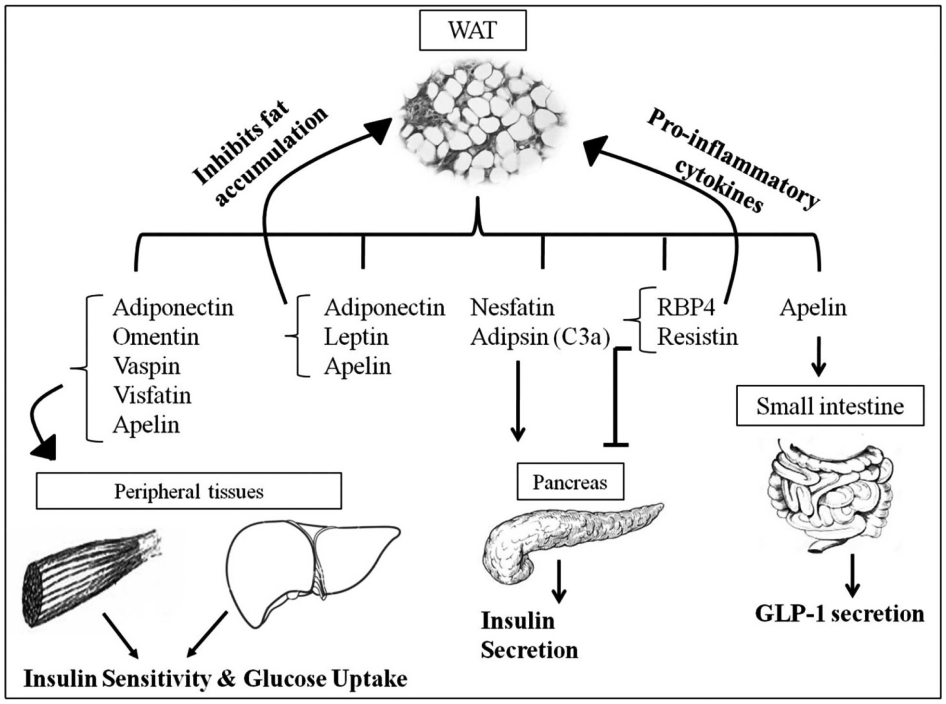

Fig. (3). An illustration of the interplay of various adipokines in maintaining glucose homeostasis.

significantly decreased in T2D patients with β-cell failure when compared with T2D patients without the evidence of β-cell failure. This observation suggested that adipsin dysregulation in diabetic patients might be one of the factors leading to β-cell insufficiency. Thus, adipsin seems to fill in the gap between adipose tissue metabolism and pancreatic β-cell function. Adipsin levels may prove to be a predicting biomarker to help a physician identify individuals at highest risk of impending β-cell failure [275].

The characteristic glucose-dependent insulin secretagogue property makes C3a an ideal drug having an in-built negative feedback coordination reducing the chances of hypoglycemia. However, strategies to overcome its short half-life need to be evaluated and its usage at the cost of generating mitochondrial oxidative stress and its long-term effects needs to be assessed in detail [276].

By and large, every adipokine discussed in this section plays a role in the maintenance of glucose homeostasis as representated in Fig. (**3**).

4.3.4. Phytochemicals

Antidiabetic plants are known to be ubiquitously present worldwide. The extracts from these antidiabetic plants exert wide ranging effects such as stimulate B cell regeneration/proliferation, exert hypolipidemic and antioxidative effects, modulate glucose metabolism, alleviate diabetic complications and also act as insulin mimetics [277]. The active ingredient in the plant extracts are, for example, bassic acid (*B. sartorum)* and, natural flavonoids like quercetin and kaempferol (*E. alatus*) and many others. They possess the capacity to reduce hyperglycemia by promoting glucose uptake and glycogen synthesis [278-280]. Dieckol, a compound isolated from *E. cava*, too exhibits antidiabetic properties by inhibiting α-glucosidase and α-amylase as with acarbose, a pharmaceutical antidiabetic [281]. Though the exact mechanism is still obscure, a study carried out by Kang *et al*. [282], suggested Dieckol to mediate its action *via* AMPK and Akt signaling pathways. In addition, the root extract of *B. aristata* and Comatin, an active ingredient from *C. comatus* were found to reduce insulin resistance and enhance glucose homeostasis [283, 284]. Studies in this direction have also demonstrated the favourable influence of not only a poly-herbal extract in rats but even single plant extract and its isolated flavonoid rich fraction on various facets of carbohydrate metabolism and β-cell neogenesis in a T2D mouse model [111, 285-287]. The active compounds obtained from medicinal herbs and their properties are shown in Table **4**.

The medicinal plants with antidiabetic properties also have bioactive compounds like (−) epicatechin (a flavonoid), marsupin (benzofuranone), and pterosupin (a dihydrochalcone) which have been shown to decrease blood glucose level in diabetics as effectively as metformin [253, 353, 354]. A sulfated flavonoid from *P. discolor* extract was reported to inhibit aldose reductase in experimental animals and, clinical trials of the same showed anti-hyperglycemic effect [355]. There are also other active compounds like amorfrutins isolated from licorice (*G. foetida)* which mediate their action *via* activation of PPAR γ, a central player in glucose and lipid metabolism [288]. Vanillin and 4-hydroxybenzaldehyde (Table **4**) are shown to reduce insulin resistance by decreasing adipogenesis and increasing fatty acid oxidation and leptin signaling in obese rats [289]. Further, cytopiloyne has been reported to bring about insulin release from β-cells by increasing the levels of DAG and PKCα and promoting Ca^{2+} influx [290]. Additionally, capsaicin, an active constituent of chili pepper has been shown to activate AMPK in 3T3-L1 preadipocytes [322]. EGCG acts in multiple ways as mentioned in Table **4**. It affords protection against β-cell death mediated *via* islet amyloid polypeptide (IAP) *in vitro* [356] and also activates AMPK in adipocytes [322]. Resveratrol,

Table 4. **Active antidiabetic compounds from plants and their properties.**

Plant Name	Active Compound	Property	Refs.
G. uralensis	Amorfrutin 1-4	Regulates Insulin Resistance	[288]
G. elata	Vanillin, 4 hydroxy-benzaldehyde	Reduces Insulin Resistance	[289]
C. verum, C. Zeylanium C. aromaticum	Cinnamaldehyde	Reduces Insulin Resistance	[290]
T. foenum-graceum	Diosgenin Galactomannan Trigoneoside Xa, Xb, X1b, XIIa, XIIb, XIIIa, Ia, Ib, Va G hydroxylisoleucine	Reduces Insulin Resistance	[291, 292]
T. divaricate E. microphylla	Conophylline	Regulates β-cell function	[293-296]
roxburghii	Kinsenoside	Regulates β-cell function	[297]
N. stellata	Nymphayol	Regulates β-cell function	[298]
S. marianum	Silybin Silydianin, Silychristin	Regulates β-cell function	[299-307]
B. pilosa	3-β-D-glucopyranosyl-1-hydroxy-6(E)-tetradecene-8,10,12-triyne 2-β-D-glucopyranosyloxy-1-hydroxy-5(E)-tridecene-7,9,11-triyne 2-β-D-glucopyranosyloxy-1-hydroxytrideca-5,7,9,11-tetrayne (cytopiloyne)	Regulates β-cell function	[308, 309, 290]
Dietary fibers from roots of A. tequilana	Inulin/Raftilose	Regulates GLP-1 function	[310-312]
japonica	Butyl-isobutyl pthalate	Glucose absorption in gut	[313]
B. vulgaris	Berberine	Decrease hyperglycemia, Increase insulin resistance, Increase pancreatic β-cell regulation, decrease lipid peroxidation	[314-319]
M. charantia	Momordicin	Decrease blood glucose	[320]
P. Clausseni-anum	2',6'-dihydroxy-4'-methoxychalcone	Decrease blood glucose	[321]
Capsicum plants	Capsaicin	Regulates insulin resistance and β-cells	[322, 323]
P. ginseng	Ginsenoside Rb1, Rb2, Rc, Rd, Re, Rf, Rg1	Regulates insulin resistance and β-cells	[324, 325]
longa	Curcumin Turmerin	Regulates insulin resistance and β-cells	[326, 327]
I. paraguariensis	3,5,-o matesaponin2	Increase GLP1 production	[328]
Z. officinale	Gingerol Shogaol	Regulates insulin receptor signaling Increase islet cell proliferation and insulin sensitivity	[329, 330]
C. sinensis	Epigallocatechin 3 gallate (EGCG)	Islet protection, Increase insulin secretion and insulin tolerance, Decrease gluconeogenesis Insulin mimetic action	[331-333]

(Table 4) Contd…

Plant Name	Active Compound	Property	Refs.
I. okamurae	Diphlorethohydroxy carmalol	α-glycosidase and α-amylase inhibitor	[334]
G. max	Genistein	Increase islet mass and insulin sensitivity, Activates PKA, ERK1/2, AMPK	[322, 335-338]
	Glyceollin I, II, III	GLP-1 and insulin secretion, β-cell function	[339]
A. linearis	Aspalathin Rutin	Regulates insulin tolerance and β-cell function, α-glucosidase inhibitor	[340-344]
A. vera	Aloresin A	Decrease α-glucosidase and insulin resistance	[345]
E. jambolana	FIIc	Antidiabetic and antioxidant	[346]
Rupestris C. aerea	Phenol, 2,4-bis (1,1-dimethylethyl) and z, z-6,28-heptatriactontadien-2-one	α-amylase inhibitor and antioxidant	[347]
S. sonchifolius (ECU44)	4,5-di-O-caffeoylquinic acid (CQA) and 3,5-di-O-CQA	α-amylase and α-glucosidase inhibitor	[348]
P. integerrima	Pistagremic acid	α-glucosidase enzyme inhibition	[349]
H. thebaica	Luteolin 7-O-[6"-O-α-Lrhamnopyranosyl]-β-D-galactopyranoside and chrysoeriol 7-O-β-D-galactopyranosyl(1→2)-α-L-arabinofuranoside	Ameliorate glucose and insulin tolerance, Reduces AST and ALT levels of liver	[350]
E. jambolana	LH II	Antidiabetic	[351]
E. addisoniae	2'"-dimethyldihydropyrano [5'",6'"] and isoflavanone and 2,3-dihydroauriculatin	Tyrosine phosphatase 1B (PTP1B) inhibitor	[352]

commonly found in plants has potential to activate AMPK and other downstream molecules which are shown to decrease insulin resistance in diabetic mice [357, 358].

Since diabetic manifestations involve free radical associated damage in beta cells and their apoptosis accompanied with insulin resistance and hyperglycemia a combination of these well-studied phytochemicals can effectively target the pathophysiological conditions and prove to be a better treatment paradigm than either/alone.

CONCLUSION

Type 2 diabetes is a metabolic disorder that can be prevented/ controlled through lifestyle modification, diet control, and weight management. Despite the presence of several treatment options to aid the control and management of this disorder, majority of patients with T2D do not achieve appropriate glycemic control and also suffer from major or minor side effects. Since a one stop solution seems more lucrative, most pharma and biopharma companies seem to be in a competitive race for developing novel drugs with minimal side effects. Though a total cure is still elusive, newer insight into the pathophysiology of the disease is coming to light. While synthetic small molecule drugs pose long-term side effects, modulating adipokine levels seem to be the promising approach to evade side effects. As adipokines have intricate involvement and functions in the regulation of appetite, satiety, energy expenditure and physical activity, they are the most promising contenders which, can serve as tools for weight loss interventions in the future. T2D being characterized by hyperglycemia, hyperlipidemia, and hyperinsulinemia as mentioned earlier, interventions at any one or more of these triad manifestations along with β-cell regeneration may not only help manage T2D but also alleviate the disorder to a greater extent. Though researches as of now project adipokines as potent therapeutic agents, a lack of in-depth knowledge about the mechanism(s) at the molecular level poses a major limitation. Filling up this lacuna followed by clinical studies seems the urgent need for generating highly specific therapeutic modalities.

Till then, as cure is still not in the visible realm, management of the disease tailored to improve the quality of life of individuals with T2D seems the current need.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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A genetic analysis identifies a haplotype at adiponectin locus: Association with obesity and type 2 diabetes

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Adiponectin is a prime determinant of the status of insulin resistance. Association studies between adiponectin (*ADIPOQ*) gene single nucleotide polymorphisms (SNPs) and metabolic diseases have been reported earlier. However, results are ambiguous due to apparent contradictions. Hence, we investigated (1) the association between *ADIPOQ* SNPs: −11377C/G, +10211T/G, +45T/G and +276G/T for the risk towards type 2 diabetes (T2D) and, (2) genotype-phenotype association of these SNPs with various biochemical parameters in two cohorts. Genomic DNA of diabetic patients and controls from Gujarat and, Jammu and Kashmir (J&K) were genotyped using PCR-RFLP, TaqMan assay and MassArray. Transcript levels of *ADIPOQ* were assessed in visceral adipose tissue samples, and plasma adiponectin levels were estimated by qPCR and ELISA respectively. Results suggest: (i) reduced HMW adiponectin/total adiponectin ratio in Gujarat patients and its association with +10211T/G and +276G/T, and reduced *ADIPOQ* transcript levels in T2D, (ii) association of the above SNPs with increased FBG, BMI, TG, TC in Gujarat patients and (iii) increased GGTG haplotype in obese patients of Gujarat population and, (iv) association of −11377C/G with T2D in J&K population. Reduced HMW adiponectin, in the backdrop of obesity and *ADIPOQ* genetic variants might alter metabolic profile posing risk towards T2D.

Metabolic Syndrome (MS) is the new wave of diseases that has hit the human population in the last few decades-the Metabolic Syndrome Era. It has become pandemic and with obesity and type 2 diabetes (T2D) clubbed under the MS umbrella, millions of people around the globe have come under its grip. Though obesity and T2D are ubiquitous, there exists a pattern of prevalence based on ethnicity. A recent report has identified demographic transitions, nutrition and lifestyle in the backdrop of genetic predisposition as the chief factors responsible for the rising trend of obesity associated amongst South Asians[1]. Over accumulation of visceral adipose tissue (AT) has been identified as one of the major driving factors towards T2D. Adipose tissue is an important regulator of metabolic homeostasis by virtue of the adipokines (pro-inflammatory and anti-inflammatory) that it secretes. In obese conditions, the fine-tuned balance between the pro- and anti-inflammatory adipokines gets altered leading to various metabolic disorders[2]. These bioactive peptides act locally and distally to calibrate and fine tune various metabolic pathways. Adiponectin is one such calibrator which is abundantly expressed in white adipose tissue[3]. It circulates in three polymorphic forms, low molecular weight (LMW), moderate molecular weight (MMW) and high molecular weight (HMW). Interestingly, the ratio of plasma HMW adiponectin to total adiponectin is more strongly correlated with plasma glucose levels than any of the forms alone[4]. Adiponectin gene (*ADIPOQ/APM1/GBP28*) locus, 3q27, has been strongly associated with a variety of metabolic disorders like- impaired glucose tolerance, obesity, dyslipidemia and T2D[5–7]. Studies undertaken on different ethnic groups have shown positive association of certain SNPs of the adiponectin gene with T2D[3,8–11]. However, T2D being a multi-factorial and polygenic metabolic disorder[12], significant variations have been reported concerning the genetic architecture

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underlying T2D amongst different ethnic populations[13,14]. The SNPs to be studied were selected based on the following criteria: (1) validated SNPs for frequency in Genome Wide Association Studies (GWAS), (2) SNPs with scientific evidence for their role in augmented protein synthesis. *ADIPOQ* comprises of 2 introns and 3 exons encoding for the 30 kDa adiponectin protein[15]. Four SNPs were studied, −11377C/G (*rs266729*) in promoter, +10211T/G (*rs17846866*) in intron 1, +45T/G (*rs2241766*) in exon 2 and +276G/T (*rs1501299*) in intron 2, to examine their association with T2D. Since Indian population is relatively non-homogenous, we conducted our study in native Gujarat, and Jammu and Kashmir (J&K) population independently. We also aimed to study the genotype-phenotype association of the above-mentioned SNPs with Fasting Blood Glucose (FBG), Body Mass Index (BMI), plasma lipid profile and T2D.

Materials and Methods

Study subjects. Two ethnically different populations of India, one from the western Indian state of Gujarat and another from the northern Indian state of J&K were included in the present study. This study was carried out in agreement with the Declaration of Helsinki as approved by the Institutional Ethical Committee for Human Research (IECHR), Faculty of Science, The Maharaja Sayajirao University of Baroda, Vadodara, Gujarat, India (FS/IECHR/2016-9) and Institutional Ethics Review Board (IERB), Shri Mata Vaishno Devi University, Katra, J&K, India (Smvdu/IERB/13/23). It was ensured that at least five previous generations of the study subjects were of the respective ethnicities. Blood collection camps were conducted to guarantee the involvement of all the socio-economic strata in the study. The importance of the study was explained to all the participants and written consent was obtained from all patients, and age and sex-matched control subjects. The study group of Gujarat population included 475 diabetes patients (211 males and 264 females) and 493 control subjects (250 males and 243 females) while, the study group of J&K included 507 diabetes patients (282 males and 225 females) and 300 controls (140 males and 160 females) between the age group of 30 to 67 years. The T2D patients recruited for the study displayed FBG > 125 mg/dL[16]. Patients suffering from autoimmune diseases or cancer were excluded from the study. Samples of visceral (omental) adipose tissue were taken from individuals of Gujarat population undergoing bariatric surgery and fasting clinical parameters of all the study subjects are as described previously[17]. A detailed family history of the patients was recorded based on a questionnaire to collect information on first- and second-degree relatives and their history of T2D. The controls selected showed FBG < 110 mg/dL with no prior history of T2D. They were healthy and disease or infection free. The study subjects included both obese and lean individuals and their BMI (weight in kg/height in m^2) was calculated by recording height and weight.

Blood collection and DNA extraction. FBG levels were measured by prick method using glucometer (TRUEresult® - Nipro). Blood was obtained from diabetic and ethnically matched controls as per our previous study[17]. Plasma was used for lipid profiling and assaying plasma HMW adiponectin and total adiponectin levels. PBMCs were separated for DNA extraction by phenol-chloroform method. DNA was stored at −20 °C for further analysis.

Screening of *ADIPOQ* SNPs. Samples from Gujarat population were genotyped by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) for −11377C/G, +10211T/G and +276G/T. The PCR reaction mixture had a total volume of 20 µL as per our previous study[17] with primer dependent annealing temperatures (Table S1). The amplified products were checked by electrophoresis on a 2.0% agarose gel stained with ethidium bromide. Details of the restriction enzymes (Fermentas, Thermo Fisher Scientific Inc., USA) and digested products are mentioned in Table S1. 15 µl of the amplified products were digested with 1U of the corresponding restriction enzyme in a total reaction volume of 20 µl as per the manufacturer's instruction. The digestion products with 50 base pair DNA ladder (HiMedia, India) were resolved on 3.5% agarose gels stained with ethidium bromide and visualized under UV transilluminator i.e. E-Gel Imager Life Technologies (Fig. S1A–C) and uncropped images of the gels are as in Fig. S3. More than 10% of the samples were randomly selected for confirmation and the results were 100% concordant (analysis of the chosen samples was repeated by two researchers independently) and further confirmed by sequencing. *ADIPOQ* +45T/ G (*rs2241766*) SNP was genotyped by TaqMan real time PCR using the pre-designed assay ID c__26426077_10 for allelic discrimination, containing specific probes for each allele marked with VIC and FAM fluorescent dyes (ThermoFisher Scientific, USA). Real-time PCR was performed in 10 µl volume using LightCycler®480 Probes Master (Roche Diagnostics GmbH, Mannheim, Germany) following the manufacturer's instructions. A no-template control (NTC) was used with the SNP genotyping assay. Samples with each genotype were analyzed together as an internal control. J&K samples were genotyped for −11377C/G (*rs266729)*, +45T/G *(rs2241766)* and +276G/T*(rs1501299)* in a panel using High-throughput genotyping MassArray platform (SEQUENOM)[18]. The success rate of SNP genotyping was > 95%. As a quality control measure of SNP genotyping, three duplicate samples and a negative control was included in each 96 well plate. The concordance rate for genotyping was 99.5%. Further values for SNP +10211T/G *(rs17846866)* were imputed using CEU data from 1000 genome (Phase 3) as reference dataset and analyzed using PLINK ver 1.07 as the samples were exhausted.

Plasma parameters. In Gujarat population plasma total cholesterol (TC), triglycerides (TG), and high-density lipoprotein cholesterol (HDL-c) levels were measured using commercial kits (Reckon Diagnostics P. Ltd, Vadodara, India). Low-density lipoprotein cholesterol (LDL-c) was calculated using Friedewald's (1972) formula[19]. Human total adiponectin and HMW adiponectin ELISA Kits (Elabioscience Biotechnology Inc., USA) with a sensitivity of 0.47 ng/mL and 3.75 ng/mL respectively were used to estimate the levels of total adiponectin and HMW adiponectin in patients and controls. The plasma samples used were freeze-thawed only once. All the

plasma estimations were carried out in duplicates with % coefficient of variation within 10%. The plasma samples from J&K population were assayed for various biochemical parameters at a commercial clinical laboratory.

Determination of adiponectin transcript levels. RNA isolation and cDNA synthesis: Total RNA was isolated from visceral adipose tissue (VAT) using Trizol method. RNA integrity and purity were verified by 1.5% agarose gel electrophoresis/ethidium bromide staining and O.D. 260/280 absorbance ratio of 1.9 respectively. To avoid DNA contamination, RNA was treated with DNase I (Puregene, Genetix Biotech) before cDNA synthesis. Transcriptor High Fidelity cDNA Synthesis Kit (Roche Diagnostic GmbH, Mannheim, Germany) was used to prepare cDNA using one microgram of total RNA isolated, according to the manufacturer's instructions in the Eppendorf Mastercycler gradient (USA Scientific, Inc., Florida, USA). The expression of *ADIPOQ* and *GAPDH, IPO8* and *ACTB* (reference) transcripts were measured by Light-Cycler® 480 Real-time PCR (Roche Diagnostics GmbH, Manneheim, Germany) using gene- specific primers (Eurofins, Bangalore, India) as shown in Table S1. Real-time PCR was performed using Light-CyclerH 480 SYBR Green I Master (Roche Diagnostics GmbH, Mannheim, Germany) and carried out in the Light-CyclerH 480 Real-Time PCR (Roche Diagnostics GmbH, Mannheim, Germany) as per our previous study[17].

Statistical analyses. The normally distributed data for baseline parameters were analyzed by unpaired t-test while Mann-Whitney test was used for data not following normal distribution. Evaluation of the Hardy-Weinberg equilibrium (HWE) was performed for all the SNPs in patients and controls by comparing the observed and expected frequencies of the genotypes using chi-square analysis. The distribution of the genotypes and allele frequencies of *ADIPOQ* SNPs for patients and control subjects were compared using the chi-square test with 2×2 contingency tables respectively using GraphPad Prism 5 software. The genotypes have been analyzed in an additive, dominant and recessive model as there was low genotype frequency of the homozygous minor alleles (<10% frequency). *P* values less than 0.0125 for genotype and allele distribution were considered as statistically significant as per Bonferroni's correction for multiple testing. The strength of association of the *ADIPOQ* SNPs with the risk for T2D was assessed by odds ratio (OR) with 95% confidence intervals (CI). Haplotypes and linkage disequilibrium (LD) coefficients ($D' = D/D_{max)}$ and r^2 values for the pair of the most common alleles at each site were obtained using http://analysis.bio-x.cn/myAnalysis.php[20]. Association studies of SNPs with other parameters were performed using analysis of variance (ANOVA) and Kruskal Wallis test. Adjustments for the possible confounding effects of age, sex, and BMI were also done for the samples. Relative gene expression of *ADIPOQ*, and *GAPDH, IPO8* and *ACTB* levels and fold change ($2^{-\Delta\Delta Cp}$ values) in T2D patients and control groups were plotted and analyzed by unpaired t-test. All the analyses were carried out in GraphPad Prism 5 software. *P* values less than 0.05 were considered significant for all the association studies. To predict the functional impact of non-coding polymorphisms, ENCODE prediction tool (https://www.encodeproject.org/) was employed[21].

Results

Clinical parameters differed significantly between controls and patients in both the populations of Gujarat and J&K (Tables S2 and S3). Patients had significantly higher FBG ($p < 0.0001$). Moreover, obesity related factors like BMI, TC, TG and LDL-c were significantly elevated ($p < 0.0001$, $p = 0.0360$ and $p = 0.001$, respectively) while HDL-c was significantly decreased ($p < 0.0001$) in patients as compared to controls in Gujarat population while in the J&K population BMI ($p = 0.015$), FBG ($p < 0.0001$) and TG ($p = 0.001$) levels were significantly higher in T2D patients.

Association of *ADIPOQ* SNPs with T2D. The genotype and allele frequencies of the *ADIPOQ* SNPs are summarized in Table 1. The distribution of genotype frequencies for all the polymorphisms investigated was consistent with Hardy-Weinberg Expectations (HWE) ($p > 0.05$) in both the populations. Analysis of the genotype frequencies of +10211T/G (*rs17846866*) and +276G/T (*rs1501299*) SNPs using an additive model revealed them to be significantly associated ($p < 0.0001$) while the promoter 11377C/G (*rs266729*) and exonic +45T/G (*rs2241766*) SNPs were not associated with T2D (Table 1). Further, in Gujarat population a significant association was detected for the intron 1 +10211T/G (*rs17846866*) when analyzed in the recessive model (OR = 1.797, 95% CI = 1.369–2.359, $p < 0.0001$) with T2D. Likewise, the intron 2 + 276G/T (*rs1501299*) SNP was also found to be significantly associated in the recessive model (OR = 2.05, 95% CI, 1.57–2.65, $p < 0.0001$) as shown in Table 1. However, in J&K population, only promoter −11377C/G (*rs266729*) polymorphism was found to be associated ($p = 0.0101$; OR = 1.47, 95% CI = 1.09–1.96) with T2D in the recessive model (Table 1). The frequency of mutant alleles for +10211T/G (*rs17846866*) and +276G/T (*rs1501299*) was noted to be significantly higher in diabetic patients as compared to that of control subjects (OR = 2.33 and OR = 1.726, respectively) in Gujarat population.

Haplotype and linkage disequilibrium analysis of *ADIPOQ* SNPs. A haplotype evaluation of four polymorphic sites of *ADIPOQ* was performed in Gujarat population. The estimated frequencies of the haplotypes differed significantly between patients and controls (global $p = 7.76 \times 10^{-12}$) as shown in Table S4. The disease susceptible haplotypes were CGTG ($p = 0.0003$), CGTT ($p = 6.32 \times 10^{-5}$), GGTT ($p = 0.0207$) and GGTG ($p = 0.0030$) (Table S4). Furthermore, the GGTG ($p = 3.87 \times 10^{-5}$) haplotype in particular was found to be significantly higher in obese patients as shown in Table 2. The LD analysis revealed that the four SNPs investigated were in low to moderate LD association (Fig. S2). Haplotype and LD analyses were not performed in the J&K population as only −11377C/G (*rs266729*) was found to be associated with T2D and the genotypes of +10211T/G (*rs17846866*) were imputed.

***ADIPOQ* expression and plasma HMW adiponectin/total adiponectin ratio in patients and controls.** A significant reduction in *ADIPOQ* transcript levels was observed in Gujarat T2D patients as compared to controls after normalization with *GAPDH* expression ($p = 0.0187$) as suggested by mean ΔCp values (Fig. 1A).

SNP	N	Genotype		Allele		Odds Ratio [95% CI] (*p*-value)			
						Allelic	Additive	Dominant	Recessive
Gujarat Population									
rs266729		CC	CG + GG	C	G	1.23 [0.95–1.59] (0.118)	0.2644	1.46 [0.72–2.95] (0.1443)	1.28 [0.92–1.77] (0.1432)
Controls	286	155	131	427	145				
T2D Patients	285	137	148	402	168				
rs17846866		TT	TG + GG	T	G	2.33 [1.85–2.93] (<0.0001)	<0.0001	1.46 [0.15–2.02] (<0.0001)	1.79 [1.36–2.35] (<0.0001)
Controls	493	363	130	847	139				
T2D Patients	475	289	186	687	236				
rs2241766		TT	TG + GG	T	G	0.86 [0.64–1.18] (0.3722)	0.6704	0.74 [0.22- 2.55] (0.6325)	0.86 [0.61- 1.21] (0.3954)
Controls	467	362	105	822	112				
T2D Patients	359	287	72	642	76				
rs1501299		GG	GT + TT	G	T	1.72 [1.42–2.09] (<0.0001)	<0.0001	1.99 [1.28–3.08] (0.0018)	2.05 [1.57–2.65] (<0.0001)
Controls	489	255	216	692	250				
T2D Patients	464	172	298	579	361				
Jammu and Kashmir Population									
rs266729		CC	CG + GG	C	G	1.34 [1.05–1.69] (0.0168)	0.0365	1.26 [0.67–2.36] (0.2294)	1.47 [1.09–1.96] (0.0101)
Controls	290	151	139	423	157				
T2D Patients	503	309	194	787	219				
rs17846866[#]		TT	TG + GG	T	G	0.95 [0.70–1.29] (0.3827)	—	—	0.95 [0.71–1.27] (0.3663)
Controls	300	141	159	206	94				
T2D Patients	507	232	275	343	164				
rs2241766		TT	TG + GG	T	G	0.72 [0.52–1.02] (0.0613)	0.2041	0.646 [0.23–1.83] (0.2039)	0.71 [0.49–1.04] (0.0788)
Controls	299	251	48	545	53				
T2D Patients	507	400	107	894	120				
rs1501299		GG	GT + TT	G	T	1.09 [0.86–1.40] (0.2248)	0.7452	1.12 [0.59–2.13] (0.3670)	1.12 [0.83–1.51] (0.2247)
Controls	289	170	119	443	135				
T2D Patients	502	309	193	786	218				

Table 1. Genotype and allele frequencies distribution of *ADIPOQ* SNPs in T2D patients in Gujarat and J&K population. [#]Values were Imputed using CEU data from 1000 genome (Phase 3) as reference dataset and analyses was carried out in PLINK ver 1.07.

Haplotype *rs266729, rs17846866, rs2241766, rs1501299*	Obese Patients (Frequency %) (n = 330)	Lean Patients (Frequency %) (n = 150)	*p* for Association	*p* (global)	Odd Ratio [95%CI]
C G T G*	24.49 (0.129)	61.62 (0.081)	0.0397	2.26×10^{-8}	1.68 [1.020~2.780]
C G T T*	15.12 (0.080)	25.66 (0.034)	0.0053		2.48 [1.285~4.799]
C T G G	12.57 (0.066)	35.80 (0.047)	0.2851		1.43 [0.738~2.791]
C T T G*	53.25 (0.280)	273.96 (0.361)	0.0317		0.67 [0.474~0.968]
C T T T*	17.77 (0.094)	133.56 (0.176)	0.0051		0.47 [0.283~0.809]
G G T G*	15.34 (0.081)	16.02 (0.021)	3.87×10^{-5}		4.10 [1.993~8.434]
G T T G*	14.89 (0.078)	106.21 (0.140)	0.0219		0.51 [0.293~0.917]
G T T T*	19.89 (0.105)	39.53 (0.052)	0.0072		2.14 [1.215~3.774]

Table 2. Haplotype frequencies in lean and obese patients in Gujarat population. *Indicates haplotypes significantly associated with obesity induced T2D. Frequency <0.03 were ignored in the analysis. The haplotypes in J&K population could not be assessed as the data for +10211T/G (*rs17846866*) was imputed.

The $2^{-\Delta\Delta Cp}$ analysis showed approximately 0.84 fold decrease in the expression of *ADIPOQ* transcript levels in patients as compared to controls (Fig. 1B). Similar results were obtained for *ADIPOQ* transcript levels when normalized with *IPO8* ($p = 0.0184$) and *ACTB* ($p = 0.0344$) (Fig. S4A,C). The $2^{-\Delta\Delta Cp}$ analysis of the same showed approximately 0.87 and 0.82 fold reduction in the expression of *ADIPOQ* transcript levels in patients as shown in (Fig. S4B,D). Further, there was no significant difference observed between *ADIPOQ* transcript levels and its SNPs ($p > 0.05$) as shown in Fig. 1C. Plasma HMW adiponectin and total adiponectin levels, and their ratio monitored in 37 controls and 45 patients showed significant decrease ($p < 0.001$) in Gujarat patients as compared to controls (Fig. 1D). Healthy females showed higher HMW adiponectin/total adiponectin ratio than healthy males ($p < 0.001$) (Fig. 1E). A significant drop in the ratio was observed in diabetic males and females when compared with their healthy counterparts ($p < 0.05$ & $p < 0.01$ respectively) (Fig. 1E). There was no significant reduction in the HMW adiponectin/total adiponectin ratio between healthy lean and obese individuals. However, the

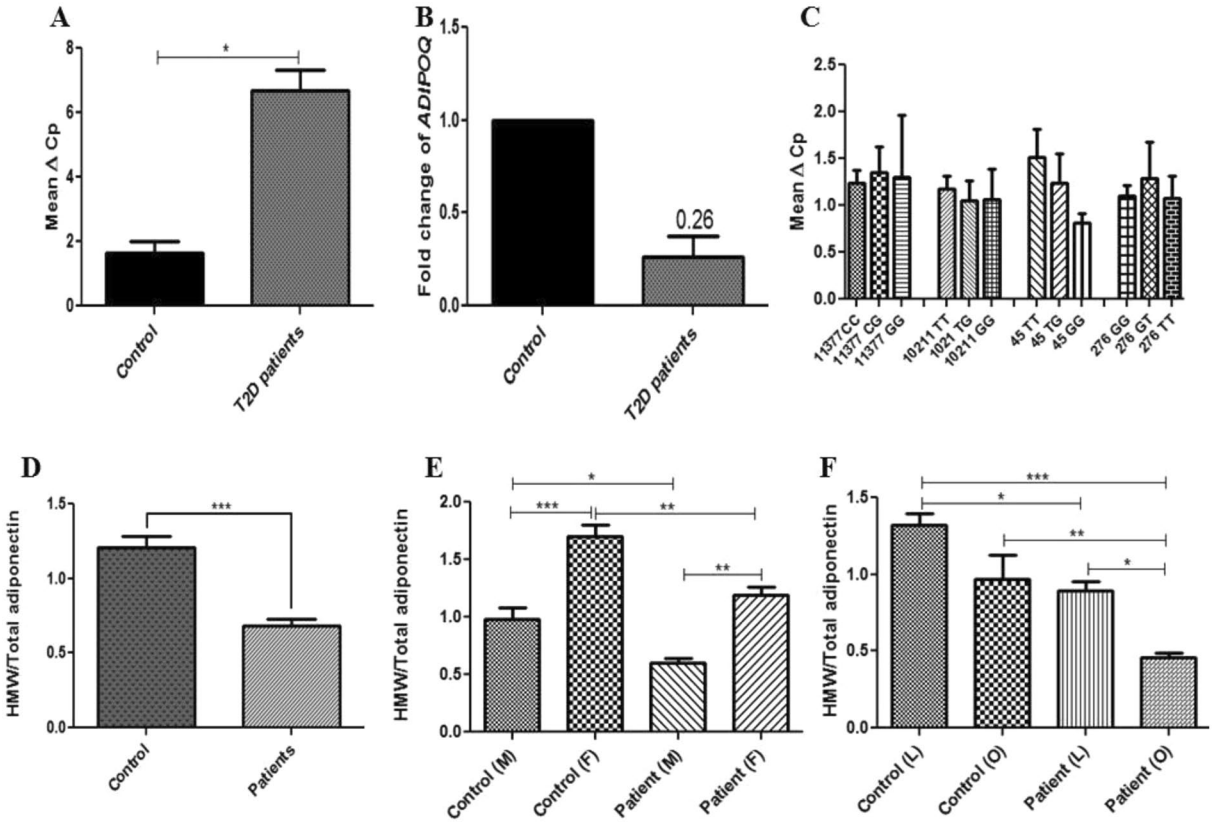

Figure 1. *ADIPOQ* transcript levels and plasma adiponectin levels in Gujarat population. (**A**) Relative gene expression of VAT *ADIPOQ* in controls and patients: Significant decrease in *ADIPOQ* transcript levels was observed in patients (Mean $\Delta Cp \pm SEM$: 1.639 ± 0.3829 v/s 6.681 ± 0.6558; $p = 0.0187$), (**B**) Relative fold change of *ADIPOQ* expression in controls and patients. Expression of *ADIPOQ* transcripts in T2D patients as compared to controls was decreased by 0.84 fold as determined by the $2^{-\Delta\Delta Cp}$ method. (Controls n = 14; T2D patients n = 10). (**C**) Association of *ADIPOQ* polymorphisms with *ADIPOQ* transcript levels. No association between *ADIPOQ* polymorphisms and *ADIPOQ* transcript levels ($p > 0.05$). HMW adiponectin/total adiponectin ratio in (**D**) controls versus patients. Plasma HMW adiponectin/total adiponectin ratio in patients were significantly lower than in controls, (**E**) control and diabetic males and females. HMW adiponectin/total adiponectin ratio in control and patient females were significantly higher than in control and patient males and (**F**) lean (L) and obese (O) control and diabetic subjects. Obese patients showed significantly reduced HMW adiponectin/total adiponectin ratio (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$). (Controls n = 37; T2D patients n = 45).

obese patients showed a significant drop compared to lean patients ($p < 0.05$) (Fig. 1F). Lean and obese diabetic individuals showed reduced HMW adiponectin/total adiponectin ratio as compared to their respective controls ($p < 0.05$, $p < 0.01$). The drop in the plasma adiponectin ratio was further accentuated in obese diabetic patients ($p < 0.001$) (Fig. 1F).

Association of *ADIPOQ* SNPs and their genotypes with metabolic parameters and HMW adiponectin/total adiponectin ratio. As shown in Table 3, in Gujarat population, the GG genotype of −11377C/G was associated with increased levels of TG, LDL-c and HDL-c (females). The GG genotype of +10211T/G was significantly associated with FBG, BMI, TG, TC, HDL-c and HMW adiponectin/total adiponectin ratio while the TT genotype of +276G/T was significantly associated with increased FBG, BMI, TG, TC and LDL-c and, decreased HDL-c ($p > 0.05$). Further, +45T/G was not associated with any of the parameters in Gujarat population. However, no significant association of the metabolic parameters was observed with the polymorphisms in J&K population (Table S5).

Bioinformatics analyses. ENCODE data base showed that −11377C/G (*rs266729*), +10211T/G (*rs17846866*), +45T/G (*rs2241766*) and +276G/T (*rs1501299*) do not overlap with any cis-Response Elements (cREs) or display any cREs within 2 kb. Further, eQTL database GTex shows TG and GG genotypes of *rs17846866* to have significantly reduced levels of plasma adiponectin similar to our findings. However, the eQTL data for the rest of the SNPs are not available. Analysis of *rs2241766*, a synonymous exonic SNP, revealed that the glycine residue at the 15th position remains unchanged (SIFT). Further, the change in codon usage was calculated by applying a relative synonymous codon usage (RSCU) approach to understand the relevance of ribosomal pause in reduced amount of protein being expressed. The delta Relative Synonymous Codon Usage (RSCU) value for

Genotype/ Allele	FBG (mg/dL)	BMI (Kg/m^2)	TG (mg/dL)	TC (mg/dL)	HDL-c (mg/dL)		LDL-c (mg/dL)	HMW adiponectin: total adiponectin (µg/mL)
					Male	Female		
***ADIPOQ* −11377 C/G (*rs266729*)**								
CC	124.50 (50.02)	25.37 (5.28)	123.00 (79.00)	161.70 (39.47)	36.81 (10.73)	45.17 (14.02)	93.83 (37.5)	0.97 (0.48)
CG	124.70 (51.02)	25.57 (5.95)	150.00 (102.00)	162.70 (39.52)	37.59 (9.30)	34.63 (9.96)	101.90 (39.36)	1.00 (0.54)
GG	124.10 (30.64)	26.36 (5.51)	166.00 (84.00)	156.40 (37.13)	39.75 (13.25)	26.56 (1.51)	101.40 (32.03)	0.64 (0.24)
***P* value**	0.6241	0.4906	**<0.0001**	0.8671	0.7369	**<0.0001**	**0.0087**	0.2055
***ADIPOQ* +10211T/G (*rs17846866*)**								
TT	130.00 (56.13)	25.60 (5.90)	135.80 (92.00)	151.60 (27.89)	42.79 (14.38)	43.18 (14.57)	96.86 (37.5)	1.50 (0.61)
TG	132.20 (55.11)	25.33 (5.20)	138.90 (78.00)	162.20 (38.97)	41.62 (21.49)	44.16 (13.51)	96.64 (46.54)	0.86 (0.39)
GG	148.10 (56.86)	27.82 (5.60)	166.40 (85.60)	175.60 (39.02)	37.76 (12.92)	34.22 (8.07)	99.20 (37.57)	0.82 (0.36)
***P* value**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**0.0141**	**<0.0001**	0.6024	**0.0001**
***ADIPOQ* +45T/G (*rs2241766*)**								
TT	155.40 (4.26)	26.82 (5.20)	164.00 (14.8)	163.80 (37.00)	36.62 (11.85)	40.53 (12.36)	95.79 (39.5)	0.98 (1.20)
TG	171.50 (12.96)	27.16 (5.29)	172.80 (20.3)	164.50 (44.91)	36.51 (11.00)	40.42 (14.46)	96.75 (39.26)	0.83 (0.38)
GG	122.50 (8.50)	30.05 (3.748)	103.90 (15.28)	185.70 (27.61)	34.57 (6.734)	41.27 (11.80)	94.87 (37.83)	0.82 (0.30)
***P* value**	0.3293	0.2619	0.6088	0.4735	0.9708	0.9936	0.9396	0.9284
***ADIPOQ* +276G/T (*rs1501299*)**								
GG	151.00 (53.88)	24.98 (4.53)	143.30 (78.00)	153.20 (29.34)	37.87 (12.34)	40.64 (12.52)	70.36 (27.13)	1.36 (0.63)
GT	166.90 (69.67)	27.69 (5.53)	165.20 (89.00)	154.70 (32.12)	35.78 (10.48)	39.25 (12.56)	92.99 (36.33)	0.93 (0.44)
TT	303.80 (94.54)	29.75 (4.23)	266.60 (90.00)	189.00 (25.96)	33.28 (11.93)	37.34 (6.34)	90.62 (34.1)	0.75 (0.33)
***P* value**	**<0.0001**	**0.0001**	**<0.0001**	**0.0001**	**<0.0001**	0.0831	**0.005**	**0.0006**

Table 3. Genotype-phenotype association analyses of *ADIPOQ* SNPs with metabolic parameters in Gujarat population. Data represented as Mean (SD).

the GGT to GGG codon change was calculated to be −0.31. However, no significant association of the +45T/G polymorphism was found with adiponectin levels.

Discussion

Our findings, for the first time, collectively suggest that *ADIPOQ* CGTG, CGTT, GGTT and GGTG haplotypes were associated with T2D, further GGTG was significantly associated with obesity induced T2D. Also, +10211T/G (*rs17846866*) and +276G/T (*rs1501299*) were strongly associated with obesity induced T2D susceptibility in Gujarat population; whereas in J&K population only −11377C/G (*rs266729*) was found to be associated with T2D. The difference in the association of variants can be attributed to the ethnic differences between the two populations. The findings in Gujarat population are further linked with reduced levels of HMW adiponectin and disease-associated risk factors like FBG, BMI and lipid parameters thereby suggesting their crucial role in metabolic disease susceptibility.

Obese phenotype has been associated with a reduction in the anti-inflammatory and a boost in the pro-inflammatory adipokines. Our previous reports suggest interleukin 1β (IL1β)[22], resistin[23] and TNFα[24] to play an important role in the development of obesity, islet dysfunction and decreased insulin secretion. On the contrary, adiponectin[2], omentin-1[25], melatonin[26] and vaspin[27] are known to enhance insulin sensitivity. The normal range of total adiponectin in healthy individuals is reported to be 2–20 µg/mL[28]. The characteristic short stature of South Asians combined with visceral adiposity leads to an increased weight per area distribution defined by body mass index predisposing those to metabolic diseases[1,29–31]. Genome-wide association studies have shown a close association between adiponectin, *ADIPOQ* SNPs, fasting hyperglycemia and various metabolic diseases though varying from population to population[32–34]. Earlier studies have shown promoter −11377C/G (*rs266729*) polymorphism to have a positive association with hypoadiponectinemia and risk of developing T2D[35] and is supported by the findings in J&K population. As opposed to this, we found this SNP not to be associated with T2D or BMI in Gujarat population supporting the work by Schaffler *et al.* who also reported the absence of transcription factor binding sites at or around this SNP site[36]. However, the GG genotype of −11377 C/G (*rs266729*) did show an association with increased serum triglycerides and LDL-c, and reduced HDL-c in females. In spite of not being associated with T2D, possibly an indirect effect of other SNPs could be the reason for the observed altered association of the −11377 C/G (*rs266729*) with the serum lipid levels.

Adiponectin gene expression in an adipose tissue is regulated by a 34 bp enhancer located in the first intron[37]. Therefore, the finding of +10211T/G (*rs17846866*) located close to the enhancer in the region of the first intron affecting lipid metabolism and adiponectin levels in the present study is of significance. Though the ENCODE data base doesn't show an overlap of this polymorphism with any cREs or display any cREs within 2 kb; eQTL database GTex shows TG and GG genotypes of +10211T/G (*rs17846866*) to have significantly reduced levels of plasma adiponectin similar to our findings. Additionally, this SNP is also seen to be associated with increased BMI, FBG, TG, TC and reduced HDL-c. To date, three independent studies, including ours, have established the association of +10211T/G (*rs17846866*) with three different Indian populations belonging to different

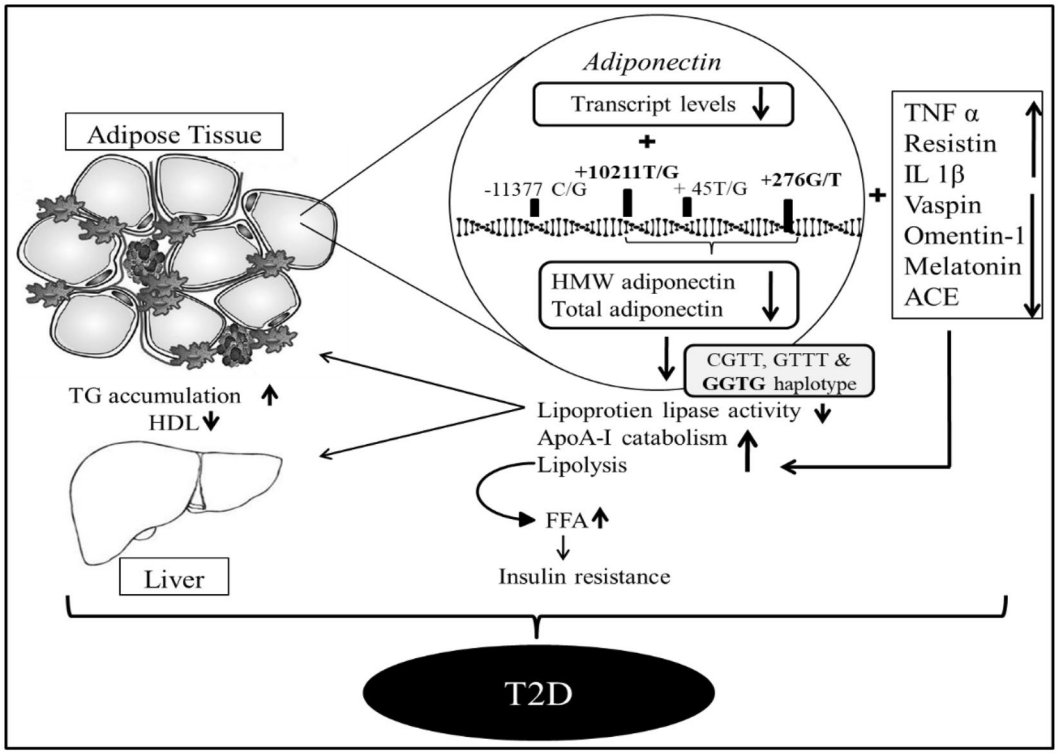

Figure 2. Role of *ADIPOQ* SNPs in T2D: The *ADIPOQ* CGTT, GTTT and GGTG haplotypes in presence of *ADIPOQ* +10211T/G (*rs17846866*) and +276G/T (*rs1501299*) along with decreased transcript, plasma HMW adiponectin and total adiponectin, and increased TNFα, FFA, resistin leads to altered metabolic profile thereby contributing to insulin resistance and T2D in Gujarat population.

demographical and geographical regions, thus further validating the significance of this SNP[10,11]. However, the results from J&K population did not reveal any such association. +45T/G (*rs2241766*) is a synonymous SNP with a codon change from GGT to GGG. Though studies on Chinese Han population found an association between +45T/G (*rs2241766*) and insulin resistance[38]; our results show no association between +45T/G (*rs2241766*) and T2D as supported by studies on Italian, French and Swedish populations[3,8,9]. We report a significant association of +276G/T (*rs1501299*) with T2D, and serum lipid profile in Gujarat population while no association was found in J&K population. Supporting our data from Gujarat population, similar results were obtained in earlier studies in German[39], Swedish[40], Italian Caucasian[41], French Caucasian[3] and South Indian populations[35]. However, the results of the study by Hara *et al.*[42] in Japanese subjects were in accordance with the results obtained in J&K population. In Gujarat population, the TT genotype conferred approximately double risk for developing T2D against the GG genotype in +276G/T (*rs1501299*). Furthermore, +276G/T (*rs1501299*) is also found to be linked with increased BMI, FBG, TG, and TC, and reduced HDL-c in males. These findings also suggest the association of +276G/T (*rs1501299*) with Non-Alcoholic Fatty Liver Disease (NAFLD), co-morbidity associated with T2D as supported by Wang *et al.*[43]. Additionally, we have also found increased levels of TNFα, Free Fatty Acids (FFA) and resistin in obese patients[17,44]. Since TNFα is shown to be an important regulator of adiponectin multimerization[45], our observations of increased TNFα, reduced adiponectin transcript and HMW adiponectin levels in obese patients are self-explanatory. We had also reported a rise in IL1β levels in obese diabetic patients[46], asserting the rise in pro-inflammatory adipokine and drop in anti-inflammatory adipokine in obesity-associated low-grade inflammatory condition. Further, adiponectin levels show sexual dimorphism[47] and our results further confirm this as females in general demonstrated a higher tendency of HMW adiponectin/total adiponectin ratio than males. Also, a significant drop in adiponectin ratio of lean diabetic individuals was observed which was further pronounced in obese diabetic patients. Moreover, the overall plasma HMW adiponectin/total adiponectin ratio tends to be lower in subjects with the homozygous mutant allele for +10211T/G (*rs17846866*) and +276G/T (*rs1501299*). In concordance with our findings, adiponectin levels were strongly and inversely associated with diabetes risk[48,49]. Alongside, we had also reported the prevalence of a significantly high number of angiotensin convertase enzyme (ACE) I/D polymorphism in the same population[50]. The ACE D allele has in particular been shown to be associated with increased angiotensin II[51] which may be further adding to the down regulation of adiponectin. We suggest that the reduced HMW adiponectin in particular is responsible for insulin resistance as, among the adiponectin isoforms, the HMW isoform binds to its receptor with maximum affinity leading to a potent activation of 5′ AMP-activated protein kinase (AMPK). Thus, the lowered HMW adiponectin may be partly responsible for developing T2D[52]. The increased level of TG may be due to a decrease in the lipoprotein lipase activity and Very Low-Density Lipoprotein receptor (VLDLr) expression levels, which have been proposed to be modulated by adiponectin[53]. While HDL-c levels and their particle size are inversely correlated with the

catabolic rate of apolipoprotein (ApoA-I), a direct role of reduced adiponectin with increased catabolism of the major ApoA-I present in HDL-c has been proposed[54], explaining how hypoadiponectinemia leads to decreased HDL-c levels. The correlation between hypoadiponectinemia and reduced HDL-c levels, as observed by us further strengthens the hypothesis. To summarize, +10211T/G (*rs17846866*) and +276G/T (*rs1501299*) are significantly associated with increased FBG, BMI, TG, TC and reduced HMW adiponectin/total adiponectin ratio. More importantly, the haplotype analysis reveals that individuals with GGTG haplotype in particular show an increased tendency towards obesity induced T2D[55] (Fig. 2). Thus, we may conclude that adiponectin gene is associated with T2D, nonetheless variation in the susceptibility loci within the gene depends on ethnic variation among different populations. However, further investigations to understand the mechanistic aspects of genetic variants regulating adiponectin levels are warranted in other cohorts.

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Author contributions

R.B. developed the concept. S.P.P. designed and performed the experiments. S.P.P., R.P. and N.R. contributed to data acquisition and data analysis was performed by S.P.P. S.D.J. contributed towards bioinformatics analyses and interpretation. A.M., M.K.D. and S.S. contributed to the data generation and analysis in J&K population. R.B. and A.V.R. contributed to the critical revision and approval of the article.

Competing interests

The authors declare no competing interests.

Additional information

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