Co-administration of pramlintide and insulin via an automated dual-hormone artificial pancreas system to regulate glucose levels in adults living with type 1 diabetes: a randomized, controlled, crossover trial

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Abstract

Type 1 diabetes is a chronic disease caused by the autoimmune destruction of pancreatic beta cells responsible for the release of various hormones that regulate the blood glucose level. One of the main challenges in treating type 1 diabetes lies in achieving normal blood glucose levels using insulin therapy, while reducing the risk of hypoglycemia. Hybrid closed-loop systems are a promising therapy choice for patients with type 1 diabetes as they have been shown to improve glycemic control by increasing the time in the target glucose range and reducing the risk of hypoglycemia. These systems are composed of a glucose sensor, an infusion pump, and a dosing algorithm that regulates insulin delivery according to the sensor readings. Nonetheless, one of the main challenges in maintaining tight glucose control in a hybrid closed-loop system occurs at mealtimes when postprandial glucose level surges require timely and precise insulin adjustments.

This thesis explores the advantages pertaining to glycemic control associated with the incorporation of pramlintide into a hybrid closed-loop system, alongside its potential to improve patient satisfaction and reduce the overall burden of diabetes management. Pramlintide is a synthetic analog of amylin, a hormone co-secreted with insulin by pancreatic β-cells, which plays a key role in postprandial glucose regulation. It modulates glycemic control by slowing gastric emptying, suppressing glucagon secretion, and enhancing satiety, thereby reducing postprandial glucose excursions. Integrating pramlintide into a hybrid closed-loop system offers a multifaceted approach to insulin therapy, promoting more stable blood glucose levels while potentially improving patient adherence and reducing the overall burden of diabetes management.

We enrolled 23 adults with type 1 diabetes (33.1 (12.7) years, HbA1c 7.2 (0.7) %) that underwent two interventions in a randomized order: 29 days of rapid insulin-plus-pramlintide hybrid closed loop delivery followed by 29 days of rapid insulin-plus-placebo hybrid closed loop delivery, or vice versa. Time in glucose target range (3.9-10 mmol/L) was not found to be significantly different between both groups (placebo 69.9 (10.3) % vs pramlintide 71.8 (10.0) %; non-inferiority p=0.21). In participants with suboptimal glycemic control (time in range <70% on placebo, n=11), pramlintide significantly improved time in range (65.7 (6.9) % vs. 61.5 (6.6) %, p = 0.028) and reduced mean glucose (9.5 (0.6) mmol/L vs 9.0 (0.5) mmol/L, p = 0.009). Participants reported higher occurrence of adverse gastrointestinal symptoms (1.7 (0.5)) with the insulin-and-

pramlintide system compared to the insulin-and-placebo system (1.2 (0.2), p = 0.00002).

Résumé

Le diabète de type 1 est une maladie chronique causée par la destruction auto-immune des cellules bêta pancréatiques responsables de la libération de diverses hormones qui régulent le taux de glucose dans le sang. L'un des principaux défis du traitement du diabète de type 1 consiste à l'atteinte des niveaux glycémiques normaux grâce à l'insulinothérapie, tout en réduisant le risque d'hypoglycémie. Les systèmes à boucle fermée constituent une option thérapeutique prometteuse pour les patients atteints de diabète de type 1, ayant démontré leur capacité à améliorer le contrôle glycémique en augmentant le temps passé dans la plage cible et en réduisant le risque d'hypoglycémie. Ces systèmes sont composés d'un capteur de glucose, d'une pompe à perfusion et d'un algorithme de dosage qui régule l'administration d'insuline en fonction des données du capteur. Néanmoins, l'un des principaux défis du maintien d'un contrôle glycémique strict dans un système à boucle fermée survient lors des repas, lorsque les pics de glucose postprandiaux nécessitent des ajustements d'insuline rapides et précis.

Cette thèse explore les avantages du contrôle glycémique relatifs à l'intégration du pramlintide dans un système à boucle fermée, ainsi que son potentiel à améliorer la satisfaction des patients et à réduire le fardeau global de la gestion du diabète. Le pramlintide est un analogue synthétique de l'amyline humaine, une hormone co-sécrétée avec l'insuline par les cellules bêta pancréatiques, et joue un rôle clé dans la régulation postprandiale du glucose. Ce dernier module le contrôle glycémique en ralentissant la vidange gastrique, supprimant la sécrétion de glucagon et augmentant la sensation de satiété, réduisant les excursions glycémiques postprandiales. L'intégration du pramlintide dans un système à boucle fermée constitue une approche complémentaire à l'insulinothérapie, favorisant une stabilisation du taux de glycémie tout en améliorant potentiellement l'adhérence des patients au system, réduisant ainsi le fardeau global de la gestion du diabète.

Nous avons recruté 23 adultes diabétiques de type 1 (33.1 (12.7) ans, HbA1c 7.2 (0.7) %) qui ont suivi deux interventions dans un ordre randomisé : 29 jours d'administration d'insuline rapide et pramlintide en boucle fermée, suivis de 29 jours d'administration d'insuline rapide et placebo en boucle fermée, ou vice versa. Le temps passé dans la plage cible de glucose (3,9-10 mmol/L) n'a pas été différent entre les deux groupes (placebo 69,9 % (10,3) vs pramlintide 71,8 % (10,0) ; p =

0,21). Chez les participants ayant un contrôle glycémique sous-optimal (temps dans la plage < 70 % avec placebo, n = 11), l'ajout du pramlintide a amélioré le temps passé dans la plage cible (65,7 (6,9) % vs 61,5 (6,6) %, p = 0,028) et réduit la glycémie moyenne (9,5 (0,6) mmol/L vs 9,0 (0,5) mmol/L, p = 0,009). Les participants ont signalé une occurrence plus élevée de symptômes gastro-intestinaux (1,7 (0,5)) avec le système d'insuline-pramlintide par rapport au système d'insuline-placebo (1,2 (0,2), p = 0,00002).

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Contributions of Authors

In this following thesis, we designed and conducted a 23-participant clinical trial investigating the safety and efficacy of a novel insulin and pramlintide hybrid closed-loop system in outpatient settings.

My contribution to this thesis was to conduct the study, including participant recruitment, training and follow up, as well as managing Research Ethics Board and Health Canada submissions and communications. I also closed the study and wrote the manuscript.

Dr Ahmad Haidar, developed the novel algorithm investigated, oversaw the study's design and conduct, and co-wrote the manuscript.

Dr Michael Tsoukas, principal investigator, contributed to the study's design, supervision, and conduct, admitting and following participants for the duration of the studies, as did all the other co-investigators.

Chapter 1. Introduction

1.1. Thesis Objective and Hypothesis

Type 1 diabetes is a chronic autoimmune disease characterized by the destruction of pancreatic islets' beta-cells responsible for the secretion of various hormones, predominantly insulin, a key factor in plasma glucose level regulation. Hybrid closed-loop systems (HCL) constitute a promising therapy choice for patients with type 1 diabetes, as highlighted by previous studies, through concomitantly increasing the time in the target glucose range and reducing the risk of hypoglycemia. The hybrid closed-loop system is composed of three components: a glucose sensor, an infusion pump, and a dosing algorithm that controls insulin delivery based on the sensor's readings. In these systems, automation of hormone delivery is combined with manual user input, as participants are still required to announce meals to the system by entering the estimated carbohydrate content to ensure appropriate prandial insulin dosing.

Nonetheless, one of the main challenges in attempting to maintain tight glucose control in a hybrid closed-loop system occurs at mealtimes. To attempt to improve HCL system performance, we infused two hormones using two separate pumps: insulin and pramlintide. The latter is an experimental medication that has been designed to work like the hormone amylin, which in healthy individuals is secreted by the pancreas at mealtimes to slow down the appearance of glucose in the blood. Previous work has shown that a hybrid closed loop system delivering both hormones has the potential to better normalise glucose levels, especially after meal consumption.

I conducted a randomized, controlled, open-label, crossover trial to compare the performance of a novel rapid insulin-and-pramlintide hybrid closed-loop system with that using rapid insulin-and-placebo hybrid closed-loop system in adults with type 1 diabetes in an outpatient, free-living, unsupervised setting.

We hypothesize that the rapid insulin (lispro or aspart)-and-pramlintide hybrid closed loop system will improve glucose control and quality of life compared to the insulin-and-placebo hybrid closed loop system.

1.2. Thesis Outline

This thesis initially outlines the fundamental aspects of type 1 diabetes, including its

pathophysiology, epidemiology, and associated complications. Followingly, it reviews current treatment options, focusing on insulin therapies and technologies such as continuous glucose monitors (CGMs) and closed loop systems, highlighting the innovative combination of insulin and pramlintide to achieve superior glucose control, along with a review of their corresponding mechanism of action and clinical outcomes derived from previous studies. The manuscript presented in this thesis details a randomized controlled crossover trial comparing the efficacy of a dual-hormone hybrid closed-loop system using insulin-and-pramlintide against an insulin-and-placebo hybrid closed-loop system in adults with type 1 diabetes. Subsequently, the discussion explores the clinical implications of pramlintide in closed-loop systems, its impact on glucose regulation, and the importance of carbohydrate counting. Additionally, quality of life and the challenges faced by users of these systems are assessed through data provided by surveys. The thesis concludes by delving into potential advancements in closed-loop technologies, including novel therapies such as Volagidemab, GLP1-RAs, SGLT2 inhibitors, and immunotherapies.

Chapter 2. Background and Literature review

2.1. Type 1 diabetes

2.1.1. Physiology

Glucose ($C_6H_{12}O_6$), the most abundant monosaccharide, constitutes, through both aerobic and anaerobic cellular respiration, the central component of energy metabolism pertaining to mammals particularly.[1] Furthermore, as human beings are renowned for their substantial cerebral capacity, a considerable portion of this glucose-based energy metabolism is dedicated to the growth and appropriate functioning of the human brain.[2] Naturally, a plethora of mechanisms ensure the appropriate regulation of both glucose level and distribution throughout the body, many of which can be originally attributed to the endocrine portion of the pancreas, the pancreatic islets ("Islets of Langerhans"). The main types of cells that constitute these islets, by decreasing order of frequency, are divided into the insulin and amylin-producing β-cells (centrally located); the α-cells responsible for glucagon secretion and the δ-cells that govern somatostatin release.[3]

In effort to maintain the blood glucose level within the physiological range of 4-6 mmol/L (70-100 mg/dL),[4] the dynamic balance between the aforementioned secretions, particularly insulin and glucagon, plays a crucial role in several major biochemical mechanisms to either increase or decrease glycemia.[5] Importantly, in response to a rise in the blood glucose level, reaching normoglycemia would mainly rely upon insulin's effects at the level of several organs including the liver, skeletal muscles and adipose tissue through tyrosine kinase receptors activation, stimulating the conversion of glucose into pyruvate (glycolysis) or glycogen (glycogenesis) or even in the form of triglycerides in combination with fatty acids. Concomitantly, amylin, which is co-released with insulin, inhibits glucagon release and decreases the absorption of glucose from the gastrointestinal tract into the bloodstream, all the while decreasing appetite. In addition, somatostatin also constitutes a negative regulator of glucagon secretion.[6] Moreover, insulin itself also serves to inhibit the release of glucagon.[7] Interestingly, following the oral intake of glucose, hormones known as incretins, mainly GIP and GLP1, are secreted at the level of the intestinal tract and have been shown to correlate with enhanced insulin release when compared with other routes of glucose supplementation, mainly intravenously.[8]

Conversely, increasing the blood glucose level following an episode of hypoglycemia would

require the direct action of glucagon upon the liver, promoting both glycogenolysis, the degradation of glycogen into glucose molecules, and gluconeogenesis, the formation of glucose from non-hexose molecules through the activation of the phosphoenolpyruvate carboxykinase enzyme. Concurrently, glucagon inhibits glycogenesis (glycogen synthase activity) and glycolysis (phosphofructokinase-1 activity), all the while promoting lipolysis, the degradation of triglycerides into glycerol and fatty acids into the blood.[9]

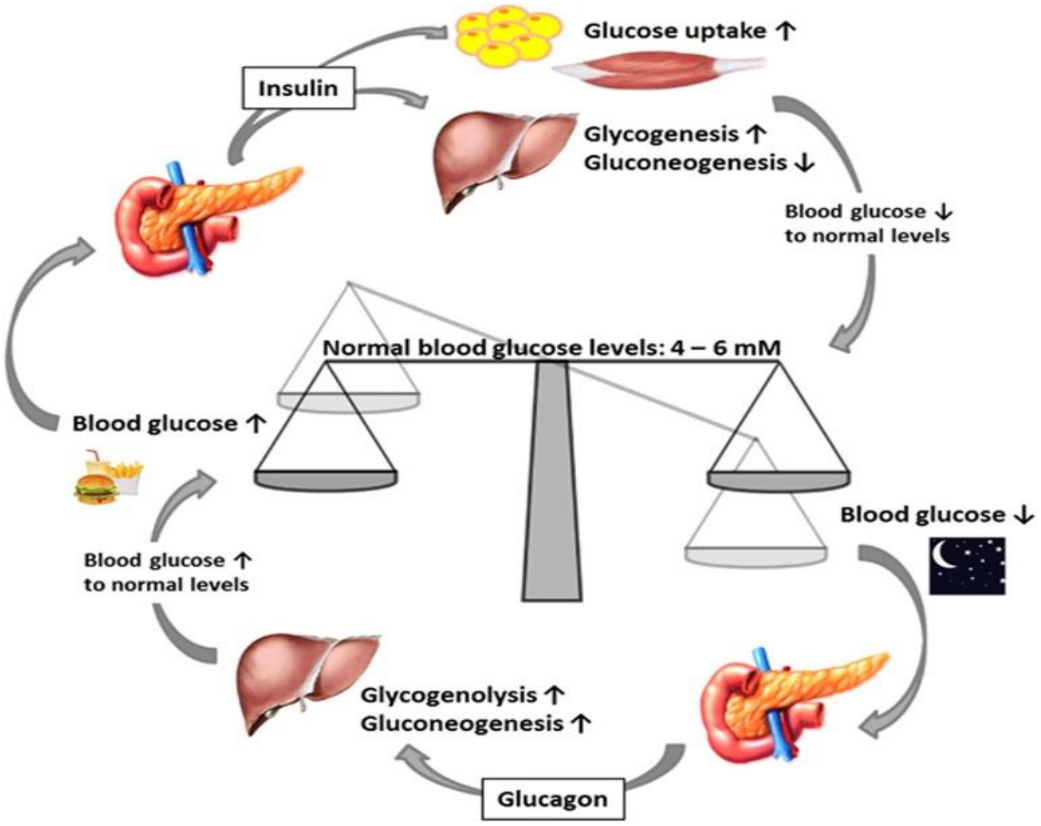

Figure 1. Maintenance of blood glucose levels by glucagon and insulin. When blood glucose levels are low, the pancreas secretes glucagon, which increases endogenous blood glucose levels through glycogenolysis. After a meal, when exogenous blood glucose levels are high, insulin is released to trigger glucose uptake into insulin-dependent muscle and adipose tissues as well as to promote glycogenesis.[3]

2.1.2. Pathophysiology

Diabetes mellitus encompasses metabolic illnesses characterized by a common key symptom: Chronic hyperglycemia. Naturally, either impaired insulin secretion, effect, or typically both constitute the root causes.[10]

Interestingly, the characteristics of both types of diabetes may coexist in one subject, making the distinction between type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM) difficult, thus making way for the so-called “double diabetes”, “hybrid diabetes”, or “type 1.5 diabetes”. Double diabetes is defined as the occurrence of insulin resistance, a hallmark of metabolic syndrome, in people with a pre-existing T1DM diagnosis. Moreover, DD carries a much-increased risk of micro- and macroangiopathic complications—complications that are unrelated to glycemic control. The prevalence of DD may likely rise in light of the global obesity pandemic along with the incremental increase in T1DM incidence.[11]

Although the precise cause of T1DM is still unknown, a substantial genetic susceptibility associated with particular HLA (DR and DQ) alleles has been emphasized throughout several studies, as this correlation has been found to be stronger in younger patients.[12] Pancreatic islet autoantibodies in the bloodstream indicate the possibility of T1DM development or its current state. These antibodies include zinc transporter isoform 8 (ZnT8), insulinoma antigen 2/islet tyrosine phosphatase 2 (IA-2), glutamic acid decarboxylase isoform 65 (GAD65), and islet cell cytoplasmic antibodies (ICA) and insulin (IAA). Notably, the majority of IAAs are seen in children.[13] The most prevalent autoantibody found in adulthood is GAD65. The likelihood of acquiring T1DM increases with the quantity and titer of detectable antibodies.[14]

T1DM and T2DM are the two primary forms of diabetes that have been identified, among others. This categorization scheme is predicated on a number of variables that distinguish patients with T1DM from T2DM, including age at the onset of the disease, obesity, presence of metabolic syndrome, insulin resistance, degree of pancreatic β-cell function loss, presence of particular autoantibodies linked to β-cell destruction, presence of a systemic subclinical inflammatory state, blood C-peptide concentration, and need for insulin therapy to survive.[15] A patient with T1DM is commonly portrayed as a young, thin individual who has lost 90–100% of their β-cell function and has required insulin therapy since the onset of the disease due to a direct lack of insulin secretion and synthesis.[16]

The development of T1DM may be divided into three main stages, including “the asymptomatic

stage 1", where ≥ 2 pancreatic autoantibodies are present, however exhibiting both normal glucose tolerance and fasting glucose levels. Pancreatic autoantibodies, which are typically numerous, and dysglycemia, which is defined as either impaired glucose tolerance (7.8–11.1 mmol/L two hours after a 75 g glucose load) or impaired fasting glucose (5.6–6.9 mmol/L), constitute the principal diagnostic criteria for "stage 2" along with an HbA1c of 5.7% to 6.4%. Nonetheless, people continue to exhibit no symptoms. "Stage 3" diabetes is characterized by any of the following: fasting glucose levels ≥7 mmol/L, glucose levels ≥11.1 mmol/L two hours after eating 75 g of glucose during an oral glucose tolerance test, and/or a HbA1c of ≥6.5%. Hyperglycemia is defined as random glucose levels ≥11.1 mmol/L as well as clinical symptoms. When a person does not exhibit the typical signs of hyperglycemia or a hyperglycemic crisis, two tests should be run (either concurrently or at separate periods) to confirm the diagnosis.[17]

T1DM typically manifests as abrupt hyperglycemic symptoms, such as polyphagia, polydipsia, polyuria, impaired vision, nocturnal enuresis, inadvertent weight loss, weariness and weakness. These symptoms are particularly common in children. It may develop into a medical emergency if it is not assessed and treated rapidly. Electrolyte imbalances may also accompany hyperglycemia. DKA may develop in these people if therapy is not received; in such cases, hospitalization, treatment with intravenous fluids, insulin, potassium, and close observation are necessary. Approximately one-third of young patients have DKA. Compared to juvenile diabetes, adult-onset diabetes has a more varied symptom onset, and DKA is less common.[18]

2.1.3. Epidemiology

Although T1DM may occur at any age, this disease constitutes one of the most common chronic illnesses in children.[12] T1DM, which accounts for 5% to 10% of all cases of diabetes, has been steadily rising in both incidence and prevalence. Globally, 9.5% of persons have T1DM, with an incidence of 15 cases per 100,000. Furthermore, the incidence varies greatly according to the region at hand, as China and Venezuela have the lowest reported incidence, whereas Finland and other Northern European countries exhibit the highest reported incidences, with a greatly significant discrepancy (400 times higher than the previously mentioned low-incidence countries).[19]

The major genetic risk factors for T1DM lie within the Major Histocompatibility Complex (MHC) locus, which encodes cell surface receptors that deliver antigenic peptides to CD4+ and CD8+ T lymphocytes. T1DM is a highly polygenic disease with over 90 identified risk loci as of early 2023. 90% of T1DM cases in Europe had haplotypes of class II HLA-DR and -DQ genes DRB1*0301-DQA1*0501-DQB1*0201 (DR3) and DRB1*04:01/02/04/05/08-DQA1*03:01-DQB1*03:02/04 (DR4). It is believed that these high-risk DR3/DR4 haplotypes raise the risk of T1DM by changing the dynamics that govern peptides-MHC II binding and autoantigen delivery to T cells.[20]

2.1.4. Diagnosis

Naturally, glycemia is one of the most crucial factors: Time in range (TIR, typically 3.9-10 mmol/L; TIR targets are lower during pregnancy and higher in those who are frail and/or have complex comorbidities or limited life expectancy); time below range (TBR; <3.9 mmol/L; level 1 hypoglycemia is 3–3.8 mmol/L and level 2 hypoglycemia is <3 mmol/L); time above range (TAR; typically >10 mmol/L; level 1 hyperglycemia is 10.1 to 13.9 mmol/L and level 2 hyperglycemia is >13.9 mmol/L); and glycemic variability (% CV; coefficient of variation).[21]

Moreover, every three to six months, HbA1c should be checked. The previous two- or three-months' glycemic management is reflected in the HbA1c (approximating the lifespan of a red blood cell). A target HbA1c of less than 7.0% is the goal, with higher targets in the case of frailty, cardiovascular disease, severe hypoglycemia in the past, or even other comorbidities. When attainable safely (i.e., without increasing the risk of hypoglycemia), lower objectives are employed. An annual lipid profile, urine albumin to creatinine ratio, serum creatinine, and eGFR are among the additional laboratory testing. In addition to AST, ALT, TSH, vitamin B12, vitamin D, and serum potassium should be checked at least once and as clinically advised if one is using an ACE-Inhibitor, ARB, or diuretic medication. If the prior findings were unusual, these tests might be conducted more regularly. As individuals with T1DM are more likely to experience additional autoimmune conditions, screening for autoimmune illnesses such as include autoimmune thyroid disease, primary adrenal insufficiency, celiac disease and rheumatoid arthritis should be taken into consideration when clinically appropriate.[22]

As T1DM is considered a T-cell-mediated autoimmune illness,[23] the presence of inflammatory cells within and surrounding the pancreatic islets, known as insulitis, has long been recognized as the pathologic hallmark of T1DM.[24] The primary mechanisms disrupted during inflammation are protein breakdown and HLA loading machinery, indicating that insulitis not only causes β-cell malfunction but also increases β-cell exposure to immune surveillance,[25] thus precipitating further development of T1DM.[26] Generally, the infiltration of a minimum three Langerhans islets by at least 15 CD45+ lymphocytes/ islet constitutes the main definition of this entity.[27] Separately, certain tests for the afore-mentioned autoantibodies, including GAD65, IA-2, and IAA might be carried out in order to corroborate the diagnosis of T1DM.[28] Nonetheless, increasing evidence suggests that the role of insulitis in determining diabetes symptoms at the outset of the disease along with the corresponding amount of beta cell loss may have been overestimated.[29] Additionally, monitoring certain biomarkers associated with β-cell stress and mass may provide some information pertaining to both the health state of β-cells and the advancement of T1DM.[30]

Although insulin levels aren't usually used to diagnose T1DM, they can be useful in circumstances where the type of DM to be diagnosed may be ambiguous. In fact, as T1DM causes the autoimmune death of pancreatic beta cells, insulin and C-peptide levels are typically low or nonexistent. Particularly, less than 5 µU/mL (0.6 ng/mL) of C-peptide indicates T1DM, whereas larger levels point to T2DM in which some insulin synthesis is still present.[31]

2.1.5. Complications and Management

Acute

A plethora of complications can be encountered with T1DM. Importantly, diabetic ketoacidosis (DKA), which is typically observed in T1DM patients, may occur in approximately 4% of patients annually, or 8.0–51.3 instances per 1,000 T1DM patients.[32] Significantly lower insulin concentrations in DKA lead to hyperglycemia and ketosis, which is caused by an increase in counter-regulatory hormones such as catecholamines, cortisol, glucagon, and growth hormones, mainly through an increase in hepatic glycogenolysis and gluconeogenesis, accompanied by a reduction in the absorption of glucose by peripheral organs. Resistance to any leftover insulin is also fueled by free fatty acids and hormone imbalances. Osmotic diuresis brought on by increased blood glucose levels causes severe dehydration and electrolyte loss.[33] Subsequently, free fatty

acids produced by adipose cells in response to this shortage of insulin are oxidized by the liver to yield ketone bodies, resulting in a state of metabolic acidosis characteristic of DKA.[34]

The chief symptoms of DKA consist of nausea, emesis, Kussmaul breathing, sweet smelling (acetone "fruity") breath, abdominal pain, hypothermia, hypotension, tachycardia, poor skin turgor, weakness, and altered mental state.[32] Diagnosis of DKA relies upon the following criteria: blood glucose levels typically exceeding 250 mg/dL (13.8 mmol/L), arterial blood pH less than 7.30 indicating metabolic acidosis, ketones present in urine and blood, and a high anion gap.[32] Management comprises intravenous (IV) fluid administration used for fluid resuscitation to treat dehydration and return blood volume to normal, followed by insulin therapy administered IV to simultaneously stop the synthesis of ketone bodies and lower blood glucose levels. Notably, to avoid hypokalemia, insulin should be administered if serum potassium levels are verified to be ≥ 3.3 mEq/L. Finally, we have acidosis correction, which is usually treated with insulin and hydration. After an hour of treatment, bicarbonate therapy is rarely recommended and only taken into consideration if the pH is still less than 7.0.[35]

One uncommon yet potentially fatal side effect of diabetic ketoacidosis is cerebral edema. Typically, it can appear a few hours after starting ketoacidosis treatment. However, cerebral edema can also happen prior to beginning any kind of treatment.[36] If there is a suspicion of cerebral edema, mannitol or hypertonic saline ought to be employed. Additionally, blood glucose levels should be progressively corrected—ideally by no more than 50–75 mg/dL per hour—to reduce the danger of cerebral edema.[37]

Another significant complication, cardiac arrhythmias, are mostly brought on by the impact of dysglycemia as well as the underlying cardiovascular risks linked with diabetes. Diurnal variations in arrhythmia susceptibility were observed, as daytime dysregulations were found to be the most impactful as per Hageqlvist et al.[38] This discrepancy may be due to several mechanisms, including, at first, hyperglycemia and inflammation. In fact, extended periods of hyperglycemia may result in anatomical and electrical alterations in the heart, raising the possibility of arrhythmias, mainly through the production of advanced glycation end products that can harm cardiac cells. Second, autonomic neuropathy: as the autonomic nervous system is responsible for controlling heart

rhythm and rate, this could result in arrhythmias. Third, electrolyte imbalances such as hyperkalemia may also lead to arrhythmias, thus prompting the necessary regulation of serum potassium levels.[39] As such, the risk of arrhythmias, such as atrial fibrillation, must be mitigated by maintaining appropriate blood glucose levels.[40]

Lastly, the deleterious effect of DM on the patient's quality of life cannot be undermined. Poor immune response and the availability of free iron in the circulation constitute the main causes for the increased risk of infection, along with the high plasma glucose levels and an acidic pH. An example of a such infections would be Mucormycosis which, although uncommon, may amount to terrible outcomes, including reduced T lymphocyte counts, neutrophil dysfunction, leukocyte apoptosis, and compromised dendritic cell function. In order to lower the burden of this disease, there is a need to raise awareness about mucormycosis infection and its effects on community health.[41]

Chronic

The nuclear factor-κB (NF-κB) pathway is a characteristic pro-inflammatory signaling pathway in several nucleated cells. Several studies have established the role of NF-κB in platelet immunological inflammation, despite the fact that the signaling mechanism in platelets is not fully known. There is a partial cross-link between the platelet NF-κB pathway and the diabetic microangiopathy development signaling pathway, which could be explained by the platelets' ability to exacerbate vascular endothelial cell damage through the production of several chemokines.[42]

DM is renowned for having several detrimental effects throughout the body, including premature loss of muscle strength and mass, complications from neuropathy or vasculopathy, overweight/obesity, insulin resistance, inflammatory cytokines, and endocrine changes.[43]

Separately, T1DM patients experience gradual vision loss due to a particular diabetic microangiopathy called diabetic retinopathy and its frequency is strongly associated with the length of diabetes in the individual patients.[44] Additionally, diabetic retinopathy can be classified into two basic categories: proliferative diabetic retinopathy and non-proliferative diabetic

retinopathy, which are defined by abnormalities of the retina.[45]

Furthermore, prolonged diabetes mellitus, inadequate glucose regulation, and unchecked hypertension represent significant risk factors for the onset of diabetic nephropathy. It is identified by two or more episodes of chronic albuminuria on early morning urine samples that were at least three months apart. When albuminuria exceeds 300 mg in a 24-hour period or 200 micrograms per minute, it is considered persistent. Patients with diabetic nephropathy typically present with fatigue, edema in the pedal region caused by hypoalbuminemia and nephrotic syndrome, and foamy urine (more than 3.5 g of protein per day). However, diabetic nephropathy is less common in patients with T1DM when retinopathy is absent.[46]

Over time, the tiny blood arteries that nourish the body's nerves (Vasa nervosum) may sustain damage from hyperglycemia. Hence, the nerve fibers may be harmed or perhaps perish.[47] Nerves in the lower limbs are most frequently damaged by diabetic neuropathy. Symptoms might include discomfort and numbness in the hands, feet, and legs, depending on which nerves are affected. In addition, it may result in several problems pertaining to the cardiovascular system, digestive tract, and urinary tract.[48]

Importantly, non-enzymatic glycation and sorbitol buildup are the two main underlying mechanisms that are responsible for almost all these chronic complications. On one hand, the process by which glucose binds itself spontaneously to proteins, lipids, and nucleic acids without the aid of enzymes, particularly in case of persistent hyperglycemia, is referred to as non-enzymatic glycation, or the Maillard reaction, resulting in Advanced glycation end-products (AGEs), which leads to damage at the level of the blood vessels, kidneys and nerves.[49] On the other hand, these same complications may occur through the polyol route, in which the enzyme aldose reductase transforms surplus glucose into sorbitol, leading to its accumulation. This polyol route is especially active in tissues including the kidneys, nerves, and the lens of the eye that have low insulin dependency.[50]

Table 1
Complications of diabetes

Ophthalmologic	Neuropathic	Nephropathic	Cardiovascular
Retinopathy	**Sensory neuropathy**	**Microalbuminuria**	**Coronary heart disease**
Moderate nonproliferative retinopathy	Acute sensory neuropathy	**Macroalbuminuria**	**Cerebrovascular disease**
Mild nonproliferative retinopathy	Chronic sensorimotor diabetic peripheral neuropathy	**Chronic kidney disease**	**Peripheral vascular disease**
Severe nonproliferative retinopathy	**Focal and multifocal neuropathies**		
Proliferative retinopathy	**Autonomic neuropathy**		
Glaucoma	Cardiovascular autonomic neuropathy		
Cataracts	Gastrointestinal neuropathy		
	Genitourinary neuropathy		

Table 1. The microvascular (ophthalmologic, neuropathic and nephropathic) and macrovascular (cardiovascular, cerebrovascular and peripheral vascular) complications of diabetes.[44]

2.2. Conventional Treatment Therapies

2.2.1. Insulins

As previously mentioned, the primary trigger for insulin release in β-cells is increased blood glucose levels after a meal.[51] Particularly, the facilitative glucose transporter GLUT2 (SLC2A2), found on the surface of β-cells, absorbs glucose from the bloodstream, thus promoting glycolysis which yields adenosine triphosphate (ATP) and raises the ratio of ATP to ADP. The ensuing closure of ATP-sensitive K+-channels (KATP-channels) disturbs the movement of K+ ions along their concentration gradient and results in the destabilization of the resting potential. Sequentially, the membrane depolarizes and voltage-dependent Ca+-channels open, allowing for an increase in intracellular calcium concentration, the ensuing fusion of insulin-containing granules with the membrane and releasing of their contents.[52]

Several types of insulin are currently being used clinically worldwide. Notably, the fast-acting

insulin is used to manage hyperglycemia in certain situations, mainly post-prandially. This category comprises the rapid-acting insulin analogs and the standard human insulin. The analogs' onset duration of action varies from 5 to 15 minutes, yet due to its dose-dependent nature, the typical 4-hour duration of action of rapid-acting insulin analogs may be subject to variation. Examples include Lispro (Humalog) and Aspart (Novolog). On the other hand, regular human insulin (for instance, Humulin R and Novolin R) requires approximately 4 hours to reach its peak effect, with a total duration of action lasting around 8 hours. Although a higher dose of ordinary human insulin accelerates its onset, a clear delay in its peak effect has been reported.[53]

In contrast, the intermediate acting insulin reaches the bloodstream more slowly, but its effects persist for a longer duration. Hence, its associated glycemic control is best achieved overnight and in-between meals. Importantly, two main types may be highlighted. First, human insulin NPH, including N. Humulin and N. Novolin, in which the onset requires two hours and the peak may be reached in four to six hours, or even twelve hours in certain situations. Accordingly, a higher dose of NPH would result in both a longer duration of action and a relatively delayed peak in its effect. The second type consists of pre-mixed insulin, whose components and effects combine those of intermediate- and rapid-acting insulins.[53]

At last, long-acting insulin has a relatively low peak and gradual blood sugar release, but it stabilizes blood sugar through its "plateau" effect that lasts throughout most of the day. One example is Glargine (Lantus). It is essential for periods of fasting, overnight, or in-between meals. Currently, long-acting insulin analogs are mostly used, with an onset requiring approximately 2 hours and a duration of action spanning 12 to 40 hours.[53,54]

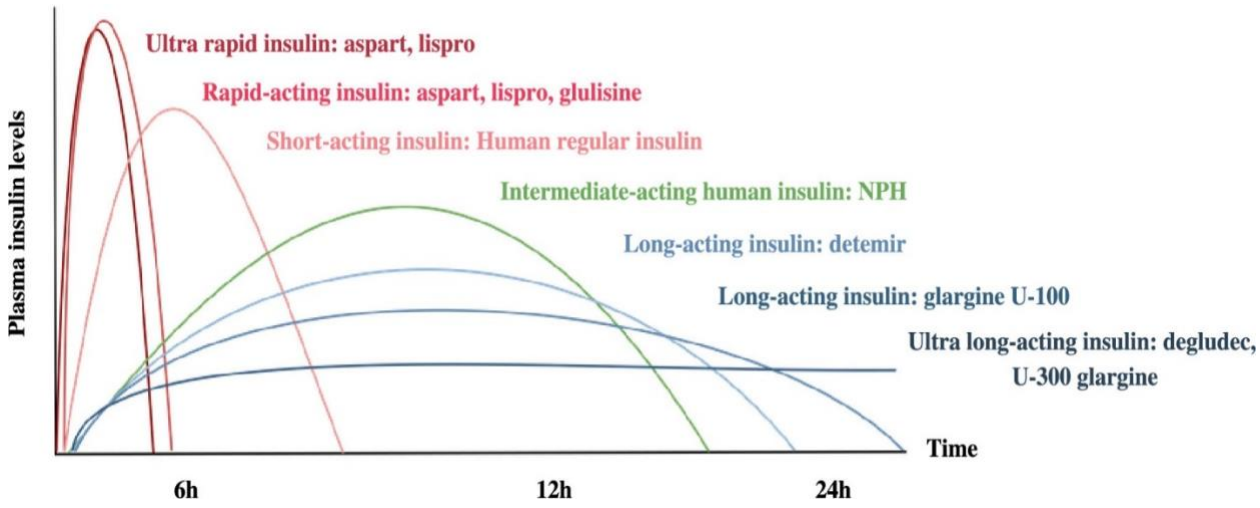

Figure 2. Insulin analogs' pharmacokinetic profiles. The spectrum of insulin analogs' pharmacokinetic properties from ultrarapid prandial to ultralong basal insulin.[53]

2.2.2. Insulin therapy

In order to support basic metabolism, low levels of insulin are continuously secreted. In response to high blood sugar, additional insulin is secreted in two phases, including an initial brief spike in secreted insulin, followed by a prolonged period of continued insulin secretion. Insulin therapy attempts to mimic these two responses, through the combination of various insulin formulations at different rates and durations, which culminated in improved glycemic control and reduced episodes of hypoglycemia.[55]

In the case of T1DM patients, insulin supplementation is required once the diagnosis is confirmed, to avoid the aforementioned associated life-threatening complications.[56] Insulin is generally administered as injections at specific times of the day (basal, long-acting) to stabilize blood glucose levels during fasting intervals, and before or after meals (bolus or prandial, fast-acting) to prevent post-prandial spikes in glycemia, in order to fulfill the usual treatment target (glycated hemoglobin levels < 7%).[57,58] A replacement dose of 0.5–1.0 units of insulin per kilogram of body weight per day is often needed in patients with T1DM. Nonetheless, during the early stages of T1DM ("the honeymoon period"), as some proportion of beta cells remain functional and continue to produce some insulin, patients require less basal exogenous insulin administration, ranging from 0.2 to 0.6 units per kg per day.[59]

Interestingly, nanotechnology is currently showing great promise for alternative approaches pertaining to the management of diabetes and may, soon enough, become part of the conventional insulin therapies. Graphene nanocomposites, quantum dots, and multiwalled carbon nanotubes enhance glucose and insulin monitoring. Cell therapies backed by nanofiber-based scaffolds or immunoisolation membranes, glucose-responsive nanogels and nanovesicles offer new possibilities for controlled insulin release.[60] In addition, the development of nanocarriers is easing the delivery of insulin orally, a technique that has traditionally been difficult because of the breakdown of insulin in the gastrointestinal tract. By shielding insulin from stomach acid and improving its absorption in the small intestine, these nanocarriers enable a less intrusive and more palatable mode of administration than traditional injections, hereby increasing patient compliance while reducing injection-related side effects.[61] Furthermore, nanotechnology-based implantable continuous glucose monitoring systems that offer real-time data on blood glucose levels are being developed. These technologies dismiss the need for frequent stressful manual monitoring and enable more precise insulin administration.[60]

As with any drug therapy administered, insulin-related side effects ought to be considered. The greatest risk associated with insulin therapy is hypoglycemia, which also poses a significant obstacle to reaching glycemic objectives in people with either T1DM or advanced T2DM. Notably, weight gain has also been reported. The recurrent hypoglycemia episodes, in which patients overeat in reaction to hunger and consume extra calories to address the low glucose level, may contribute to the weight increase. Reducing glycosuria can also stop calories from being lost in the urine.[59] These two side effects are of major concern in children.[62] As for pregnant women, insulin-induced hypoglycemia puts the health of both the mother and the fetus at risk.[63]

Rarely, hypersensitivity reactions to insulin or one of its components (protamine, for instance) may occur, causing either localized cutaneous manifestations such as erythema, pruritus, or wheals or severe life-threatening systemic symptoms, such as anaphylaxis.[59]

2.2.3. Daily Injections and Conventional Pump Therapy

At this level, a comparison between insulin injections and pumps could be made. In contrast to

traditional insulin injections, which typically require multiple daily injections for both basal (background) and bolus (meal-time) insulin, insulin pumps are small, computerized devices that deliver a continuous flow of rapid-acting insulin through a thin tube (cannula) inserted under the skin; allowing a steady supply of insulin that mimics the body's natural release of insulin.[64] Insulin pumps give users more dosing flexibility because they can be programmed to deliver bolus doses for meals based on carbohydrate intake and blood glucose levels at that specific moment. In contrast, traditional injections require a strict schedule and frequently result in less precise insulin delivery as users have to estimate their needs and manually administer doses. Additionally, the pump's infusion set only needs to be changed every two to three days, thus mitigating the need for needle sticks, whereas conventional injections require several shots per day. Moreover, a lot of contemporary insulin pumps are compatible with continuous glucose monitoring (CGM) devices, which enables automated modifications pertaining to insulin dosage in response to current glucose readings. Nonetheless, despite the mentioned disadvantages of traditional injections, this modality seems generally easier to administer and might constitute a better option for people who would rather not wear a device.[65]

2.2.4. Continuous Glucose Monitor

Importantly, conventional glycemic control metrics, including [A1C], cannot clearly indicate whether daily treatment adjustments are necessary. Intermittent self-monitored blood glucose (SMBG) offers more data to assist treatment decisions, but there are substantial obstacles to its utilization, including inconvenience and a lack of timely and consistent feedback. Furthermore, it's possible to overlook crucial information about glucose changes. The use of CGM provides significant effectiveness as shown by fewer episodes of hypoglycemia, longer durations of time in the target glucose range, and improved [A1C].

As CGM provides a constant, real-time picture of glucose patterns and levels, preventative care can be provided more readily. In addition, CGM reduces the need of uncomfortable and unpleasant fingerstick testing. Users can also receive alerts regarding incoming episodes of hyperglycemia or hypoglycemia. Mainly, CGM enhances glucose regulation and restricts the duration of hypo- or hyperglycemic episodes.[66]

Separately, the implementation of highly developed CGM constitutes an essential step in furthering the advancement of diabetes treatment technologies. A vast amount of work is still required in this domain. For example, patients' mobile devices, where accessible, and the clinic's current infrastructure ought to be used to facilitate data exchange between the patient and the physician. Standardized data organization has also become crucial. Systems that offer patients instant feedback and decision support tools for both patients and clinicians have shown better results than standard SMBG on its own.[66]

Globally, the use of CGM has increased, and a few big manufacturers, like Abbott and Dexcom, dominate the market. Nonetheless, the cost, insurance coverage, and governmental permissions represent some of the elements that affect adoption of this technology in different nations.[67]

Unfortunately, particularly when considering the hypoglycemic range, CGM accuracy is inferior to that of laboratory glucose testing. In addition, regular sensor replacement results in increased expenses over time. Blood glucose levels and interstitial fluid glucose levels naturally lag by five to twenty minutes. The insertion site may cause skin irritation, and the possibility of sensor dislodgment should be accounted for. Therefore, relying solely on the current CGM technology without any additional fingerstick testing may provide insufficient data, thus rendering the subsequent management inadequate.[67]

2.2.5. Predictive Low-Glucose Suspend

Predictive low-glucose suspend (PLGS) is a feature found in some insulin delivery systems, particularly in insulin pumps and CGMs. When blood glucose levels are expected to drop below a certain threshold within the next half hour, PLGS are programmed to immediately stop delivering insulin. This system is beneficent as hypoglycemia is a common and hazardous side effect of insulin therapy, and this proactive strategy attempts to lower its incidence and severity. Additionally, it enhances the effectiveness and safety of insulin pumps with sensors, as sensor-augmented insulin pump therapy (SAPT) with Low-glucose suspend (LGS) feature automatically interrupts insulin delivery when a hypoglycemic threshold is reached. This suspension can continue for up to two hours, making it a viable alternative for improving metabolic control while reducing nocturnal hypoglycemia.[68] The Tandem t:slim X2 and Medtronic MiniMed 670G

constitute two of the first commercially accessible devices with this predictive capability.[69]

The main benefit of adopting PLGS devices is a notable decrease in hypoglycemic episodes, especially in high-risk groups such as the elderly who lack awareness regarding hypoglycemia. Additionally, implementing the LGS property has been associated with decreased hypoglycemia-related anxiety levels in patients, thus enhancing their general well-being and increasing their compliance with diabetic treatment regimens.[70]

However, many patients may not be able to afford insulin pumps utilizing PLGS technology, especially those who live in low-income areas. Moreover, PLGS is not infallible and cannot stop every hypoglycemic incident, particularly during rapid decreases of blood glucose level.[68]

2.2.6. Automated Insulin Delivery System

The adoption of automated insulin delivery systems is growing worldwide. For example, the FDA has approved devices such as the Control-IQ and the bionic pancreas in the United States. A significant discrepancy in accessibility, however, can be explained by differences in legislative frameworks, insurance coverage, and healthcare systems.

Usually, automated insulin delivery systems comprise three essential parts: CGM, insulin pump, and algorithm for control. Glycemia-related data is transferred from the CGM to the control algorithm, which modifies insulin delivery through the pump, constituting a closed-loop device. In addition, certain systems might incorporate the aforementioned PLGS, in which insulin flow is inhibited in the case of imminent hypoglycemia.[71] Notably, the introduction of hybrid closed-loop systems reduces the need for frequent manual input, as this device which consists of the same aforementioned three main elements, automatically modifies basal insulin delivery according to the prandial carbohydrate count specified by the user.[72]

When compared to the conventional insulin administration modalities, studies have shown that hybrid closed loop systems can greatly lower both [A1c] and the amount of time spent in a hypoglycemic state. As the required number of human interventions is quite limited, a decreased level of daily stress, along with an improvement in quality of life, naturally ensue.[73]

Nonetheless, as no device of this complexity can be truly flawless, users still need to interact with the device and monitor their blood sugar levels because malfunctions may occur. Furthermore, the cost of acquiring and maintaining hybrid closed loop systems may present an insurmountable barrier to the majority of patients, particularly in areas with low funding for healthcare.[74]

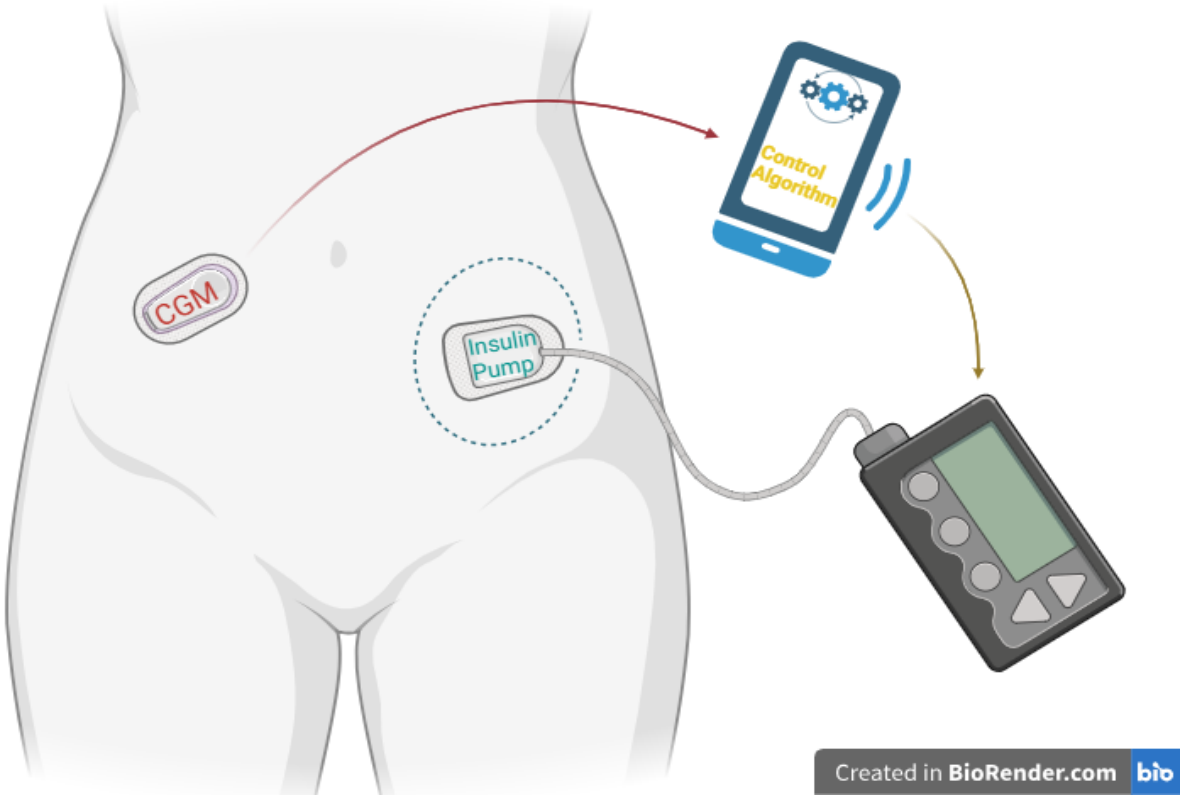

Figure 3. Diagram representing the basic functioning of a closed loop system comprised of a continuous glucose monitor, a control algorithm and an insulin pump. *(Created using BioRender website)*

2.3. Dual hormone Closed-loop systems

2.3.1. Pramlintide Mechanism of Action

Pramlintide, the first anti-diabetic peptide-based medication (Symlin®) since the discovery of insulin in 1921,[75] constitutes the sole amylin analog medication approved so far. However, the amyloidogenic qualities of pramlintide are significantly diminished as it differs from its human counterpart by three amino acids. For individuals with T1DM and T2DM who take insulin,

pramlintide is authorized since it has biological activity and potency comparable to human amylin.[76]

Administered subcutaneously, its bioavailability reaches approximately 35%, with approximately 40% of this drug being left unbounded to albumin or blood cells in the plasma. With a short half-life of 48 minutes, pramlintide is eliminated through primary metabolism in the kidneys.[77]

Pramlintide therapy has a wide variety of advantages, including decreased body weight and food intake, a decrease in glycated hemoglobin levels, and a reduction in the amount of insulin required to achieve glycemic control in both T1DM and T2DM.[78] In fact, patients receiving both insulin and amylin therapy ought to cut their usual insulin dosage by half, especially at mealtimes, mainly to prevent any insulin-induced hypoglycemic exacerbation. Nonetheless, pramlintide does not usually impair the counterregulatory responses to hypoglycemia.[79] Pramlintide also regulates glucose homeostasis by slowing stomach emptying, inhibiting postprandial plasma glucagon increases, and reducing caloric intake, similarly to amylin.[80]

Additionally, pramlintide use has been linked to a considerable decrease in postprandial markers of oxidative stress, such as nitrotyrosine, hyperglycemia, and oxidized LDL excursions.[81] Pramlintide also prevented a decrease in the overall radical-trapping antioxidant parameter (using Ghiselli's TRAP technique) as compared to a placebo, thus avoiding the associated decrease in postprandial glycemic regulation.[82]

Importantly, pramlintide does not seem to significantly affect blood pressure as neither hypertension nor noticeable hypotension are typical in clinical practice,[83] thus reducing the need to take additional precautions pertaining to this crucial cardiovascular factor while administering this medication.

Separately, nausea represents the most frequently reported adverse effect associated with the use of pramlintide. On the other hand, pramlintide's associated decreased caloric intake, and subsequently, decreased body weight renders it a promising medication to treat obesity in addition to its main role in plasma glucose regulation.[84]

The entirety of these physiological effects is mediated by pramlintide interaction with a variety of receptor complexes, such as the calcitonin receptor (CTR) and, most importantly, three different types of highly selective receptor activity-modifying proteins (RAMPs).[77] First, RAMP1 increases amylin's and pramlintide's potency at the amylin receptor complex, especially when it comes to the synthesis of the second messenger, cAMP, as amylin and pramlintide's binding affinity significantly increases, which enhances signaling efficacy in specific cellular situations.[85] Additionally, empirical research suggests that, albeit to a lesser degree than RAMP3, RAMP1 might be involved in regulating energy balance and food intake among other metabolic responses, mainly by affecting the receptor's overall signaling profile.[86]

Second, in certain cellular contexts, RAMP2 is essential for the appropriate development of functional amylin receptors. Its significance for the physiological operation of amylin signaling is highlighted by the fact that its absence may result in a failure of receptor functioning. In contrast to RAMP1 and RAMP3, RAMP2 is required for the proper development of the embryo since its deletion causes fatal widespread edema, thus proving to be essential in general physiological integrity.[86]

Third, RAMP3 dramatically boosts the potency of amylin and pramlintide at the receptor complex, especially when it comes to triggering downstream signaling pathways and releasing intracellular calcium, to a greater degree than RAMP1, suggesting that RAMP3 is essential for amylin signaling in a variety of tissues for proper functioning. In particular, RAMP3 plays a crucial role in regulating pramlintide’s effects on hunger and glucose metabolism. Hence, RAMP3 clearly constitutes the cornerstone of amylin’s metabolic effects, as evidenced by the fact that its absence can reduce pramlintide's overall effectiveness.[86]

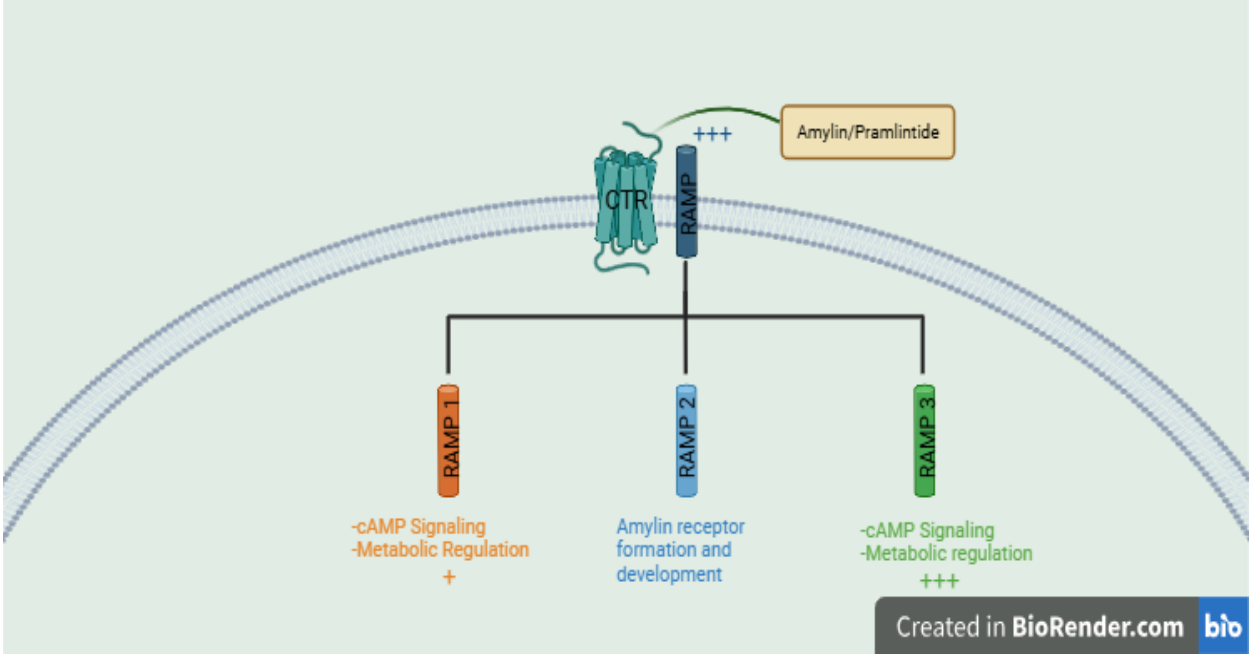

Figure 4. Diagram summarizing the different types of RAMPs with their corresponding predominant functions pertaining to amylin/pramlintide. (*Created using BioRender website)*

2.3.2. Insulin-and-Pramlintide Closed-Loop Systems

Pramlintide is prescribed as an adjuvant treatment in conjunction with insulin rather than as a stand-alone medication. It is currently being progressively integrated into closed-loop insulin delivery systems for the management of T1DM. As a matter of fact, glycemic control in T1DM is enhanced by the fixed molar ratio administration of pramlintide plus insulin.[87] This combination was not commonly employed in the past due to the necessity of the separate injection of both of its constituents. Interestingly, a novel strategy including a coformulation of pramlintide and insulin, the latter being stabilized via supramolecular approaches, is currently being developed. This coformulation remained stable for several days, and administering it was demonstrated to improve lunchtime glucagon suppression in diabetic pigs. Nonetheless, clinical trials about this coformulation have yet to be published, thus indicating the necessity for further development of this concept.[88]

Closed-loop systems fall into two primary categories. First, the Fully Closed-Loop Systems that dispense insulin at mealtimes automatically and without user involvement. However, due to the possible discrepancies in meals' timing and carbohydrate composition, these loops constitute a challenge for controlling post-prandial glycemic variations. Second, the Hybrid Closed-Loop

Systems that automate insulin delivery at night and in between meals, but they still require user-initiated prandial bolus configuration. It has been demonstrated that, when compared to conventional insulin therapy, these hybrid systems enhance both glycemic control and quality of life.[89]

An interesting study points out the specific role of pramlintide within this dual pramlintide-insulin administration, as patients with T1DM were treated with pramlintide before a meal in addition to their regular dose of insulin, aiming toward slower stomach emptying. Pramlintide decreased meal-induced glucose excursions when the meal was taken orally; however, pramlintide had no effect on the rising glycemia following an intravenous glucose load. These findings indicate that pramlintide did not alter post-absorptive glucose metabolism, although it did slow down the uptake of glucose from meal-derived carbs.[90]

In a 24-hour inpatient study, T1DM patients received a unique dual-hormone closed-loop device that delivered a fixed ratio of pramlintide:insulin. When compared to a rapid insulin-only system, the dual-hormone system (rapid insulin-and-pramlintide) increased duration in target range (84% vs. 74%), a benefit that can be attributed to better glucose control during the day. However, compared to insulin alone, there were greater reports of gastrointestinal complaints when pramlintide was used in closed-loop devices.[91]

Recent pilot studies have demonstrated encouraging outcomes, as significant decreases in postprandial hyperglycemia were observed with dual-hormone delivery system (using insulin and pramlintide) shown by an increased duration in the target glucose range without the need for carbohydrate counting.[92]

Unfortunately, a few barriers related to the practical implementation of these devices cannot be undermined. Mainly, since insulin and pramlintide require separate infusion reservoirs, pramlintide's active use in closed-loop systems is restricted. This complicates device design and patient care.[93] In an effort to improve patient compliance, avoid the need for separate injections, and enable the use of traditional single-chamber insulin pumps, the need for coformulations of pramlintide and insulin is currently being heavily emphasized.[94]

Separately, the notable focus on the combined properties of insulin and amylin has led to a recent innovative approach. Knowing that amylin is broken down and metabolized by insulin-degrading enzymes (IDE), a significant reduction in glycemia was reported upon IDE inhibition in preclinical mice models of metabolic illness by modifying the activities of both insulin and amylin. Nonetheless, since appropriate clinical trials have yet to be conducted, it is unknown whether this strategy will prove to be therapeutically effective.[95]

2.3.3. Previous Pramlintide and Amylin-analog-related Studies and Clinical Outcomes

A randomized crossover study was performed by Haidar et al to compare the use of a hybrid closed loop system with i) dual-hormone, rapid insulin and pramlintide (DAP); ii) regular insulin (Humulin R) and amylin (R-DAP); and iii) rapid insulin-alone in 12 patients with T1DM who were admitted three times for 3 meals ingestion and bedtime snack. By the end of this study, Haidar et al arrived to the following conclusion: A dual-hormone HCL system, as opposed to a first-generation insulin-alone HCL system, enhances glucose control and decreases glucose variability, especially during the day, by providing fast insulin and amylin in a constant ratio.[96] Similar results were reported in a more recent study, also conducted by Haidar et al, thus confirming the added benefits of combining pramlintide and rapid insulin when compared to the sole use of insulin in the HCL system.[91]

Besides, pramlintide exhibits several advantages for people with diabetes and obesity; however, due to its short half-life and potency, as well as the fact that it must be taken three times a day with each large meal, its effectiveness as a treatment has become limited. This was explored in a randomized, multicentered double-blinded study conducted on 204 obese people.[97] In an attempt to offer additional therapeutic effect other than the diabetic control and weight loss, stronger and longer-lasting amylin analogs have been created and tested.

Separately, a completely closed-loop device that combines pramlintide and faster-acting insulin aspart (Fiasp) that doesn't require meal input was developed by Tsoukas et al. As a conclusion of this trial, for the total percentage of time in the glucose target range, the Fiasp-alone hybrid closed-loop system did not largely outperform the Fiasp + pramlintide fully closed-loop system.

Moreover, even with the fully closed loop system, a significant portion of the participants' time was still spent within the target range. Interestingly, no side effects related to the hybrid device's use were reported.[98] A more recent study by Cohen et al determined that automated distribution of pramlintide and Fiasp may reduce carbohydrates counting without compromising glucose control.[99]

Recently, Cohen et al developed a unique closed-loop insulin and pramlintide device in which mealtime bolus was based off a predetermined meal size instead of the usual carbohydrate counting, which is the standard of care for determining prandial insulin needs in people with T1DM, however its negative effects upon quality of life have already been described. Followingly, this device was evaluated in comparison with insulin- placebo closed loop with either simple meal announcement or carbohydrate counting. As a result, simple meal announcements coupled with the insulin and pramlintide system reduced the need to count carbohydrates, albeit without compromising glucose management. Unfortunately, the Emotional Burden score did not indicate an improvement in quality of life.[100] Confirming results found in other studies,[98] a substantially higher incidence of adverse gastrointestinal symptoms, whether mild or moderate, was reported with the use of pramlintide.

Moving on to explore different amylin analogues other than pramlintide , let's start with an analogue of amylin with greater potency, effectiveness, and half-life (26 minutes) which is davalintide. Similarly to amylin and pramlintide, davalintide exhibits a strong affinity for amylin receptors while also demonstrating affinity for CTRs. Besides its analogous characteristics, davalintide demonstrates a significant homology with amylin (49% of amino acids). In mice, preclinical research has shown that davalintide resulted in a longer duration of receptor activation, as well as a higher decrease in food intake and body weight, when compared to either amylin or pramlintide.[101] In addition, mice given davalintide experienced glucoregulatory effects, such as a drop in fasting glucose levels. This high efficacy may be the result of the slow dissociation of davalintide from receptors such as CTRs.[102]

Separately, structural modifications are another way to improve the activity of amylin analogs. By connecting a polyethylene glycol (PEG) or glycosylation, for example, a longer half-life can be

achieved. Though additional studies are required to determine these modified peptides' therapeutic value, their extended activity and longer half-life seem to maintain the effects of pramlintide for a longer duration in-vitro.[103]

Importantly, current research has verified that dual agonists (amylin and calcitonin receptor agonists) have a greater potency in reducing food intake and body weight than amylin and its associated analogs. These dual agonists (DACRAs) are capable of long-lasting activation of both amylin and CTRs (calcitonin receptors). When DACRAs were compared, in a randomized controlled trial, to amylin and other amylin receptor analogs: rat amylin, rat calcitonin, obese high-fat diet and diabetic zucker fatty rats. In addition to the known metabolic beneficial role of amylin receptor activation, Larsen et al demonstrate that calcitonin receptor activation is important for blood glucose regulation in diabetes. These findings contribute to the understanding of the powerful metabolic benefits of DACRAs and highlight the potential of DACRAs as a treatment for obesity and diabetes. Plus, there was a significant improvement in terms of typical amylin-induced effects, such as reduction of food intake, gastric emptying rate, body weight, and glucagon secretion.[104] Furthermore, DACRA therapy improved the glucose homeostasis in obese animals as seen by decreased glycated hemoglobin, fasting blood glucose, and glucose levels following an oral glucose tolerance test.[105] As a result, DACRAs have been the subject of recent research since they are an effective medication for diabetes and obesity.

For instance, Cagrilintide (AM833), is a relatively nonspecific CTR and AMY agonist that is one of the most effective DACRAs currently available. When used alone, cagrilintide caused a noticeable and prolonged reduction of weight; when combined with the GLP-1 agonist semaglutide, AM833 caused even greater responses that outperformed the effects of the majority of other pharmacotherapies (Cagrilintide 0·16−4·5 mg had a half-life of 159–195 h, with a median tmax of 24–72 h, while semaglutide 2·4 mg had a half-life of 145–165 h, with a median tmax of 12–24 h. AUC [AM833]0–168 h ranged from 926 nmol × h/L to 24271 nmol × h/L, and Cmax ranged from 6·14 nmol/L to 170 nmol/L).[106] At least some of the actions of this and other long-acting amylin analogs appear to be mostly mediated by binding with CTR, although the precise mechanism of action of these compounds is still not entirely elucidated.[107]

Chapter 3. Thesis Manuscript

Co-administration of pramlintide and insulin via an automated dual-hormone artificial pancreas system to regulate glucose levels in adults living with type 1 diabetes: a randomized, controlled, outpatient, crossover trial

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Word count: 247 (Abstract), 3240 (Main text)
Tables: 6 (Main Text)
Figures: 5 (Main Text)
References: 15

Abstract

Background:

Hybrid closed loop (HCL) systems improve glycemic control in type 1 diabetes mellitus (T1DM), yet mitigating postprandial hyperglycemia remains a challenge. Pramlintide, an amylin analog, has shown promise in attenuating postprandial glucose excursions. Here, we evaluated the efficacy of insulin and pramlintide HCL system compared to insulin and placebo HCL system in an outpatient, unsupervised setting.

Methods:

We performed a randomized, controlled, crossover trial in adults with T1DM. Participants underwent two 29 days interventions in randomized order: (i) insulin-and-pramlintide HCL and (ii) insulin-and-placebo HCL. Basal-bolus insulin-and-pramlintide were delivered at a fixed ratio (1U:6μg).

Results:

Twenty-three participants completed the study and included in the final analysis (age 33.1 (12.7) years, HbA1c 7.2% (0.7)). Time spent between 3.9-10 mmol/L was similar between the insulin-and-pramlintide and insulin-and-placebo interventions (71.8% (10.0) vs. 69.9% (10.3), respectively, with $p = 0.21$). However, total insulin requirements were significantly lower using pramlintide (49.0 (28.1) U/day vs. 54.4 (32.3) U/day, $p = 0.006$), driven by reduction in bolus insulin (19.1 (13.5) U/day vs. 24.7 (16.8) U/day, $p = 0.0002$). Notably, in participants with suboptimal glycemic control (time in range <70% on placebo), pramlintide significantly improved time in range (65.7 (6.9) % vs. 61.5 (6.6) %, $p = 0.028$) and reduced hyperglycemia (32.3 (6.1) % vs. 36.7 (6.2) %, $p = 0.024$). Gastrointestinal symptoms were more frequent with pramlintide (1.7 (0.5) vs. 1.2 (0.2), $p = 0.00002$).

Conclusions:

The insulin-and-pramlintide HCL improved glucose control in patients who do not achieve glucose targets despite using insulin-and-placebo HCL.

Introduction

T1DM, also known as insulin-dependent diabetes mellitus, is a chronic autoimmune disease that results in complete insulin insufficiency due to the destruction of insulin-producing beta cells in the pancreas. Lifelong care is thus required in order to preserve glucose homeostasis and avoid long-term complications including retinopathy, neuropathy, and cardiovascular diseases. Conventional treatment methods entail multiple daily insulin injections or continuous subcutaneous insulin infusion through pumps.

Hybrid closed-loop systems play a pivotal role in the management of T1DM. They combine insulin pump treatment with continuous glucose monitoring (CGM) via a dosing algorithm. These systems reduce both hyperglycemia and hypoglycemia as well as improve quality of life compared to conventional treatments.[89] Despite these benefits, glycemic variability and hyperglycemia are still common, especially postprandially.[108,109]

Amylin supplements have been shown to enhance glycemic control and lessen postprandial blood sugar rises in individuals with T1DM.[110,111] Pramlintide, a synthetic analog of amylin, is an FDA-approved drug that can be used as an adjuvant insulin therapy. When taken as prescribed, pramlintide has the potential to lower HbA1c levels.[112]

We have developed and previously tested a closed-loop system that delivers both insulin and pramlintide based on CGM readings. Three configurations of the system were developed; a hybrid system that requires carbohydrate counting,[113] a system that only requires simple meal announcements,[100,114] and a fully closed-loop system.[92] The hybrid insulin-and-pramlintide system was assessed in an inpatient 24-hour study and was shown to improve glucose control over the insulin-alone system. In this study, we used a hybrid closed-loop system with carbohydrate counting, in which participants were asked to estimate and enter the amount of carbohydrates at mealtimes. Simple meal announcement (SMA) configuration with the addition of pramlintide in a hybrid closed loop system was addressed in a previous trial.[100] This is the first study to evaluate the incorporation of pramlintide in our hybrid closed-loop system equipped with the Euglide algorithm. A fully closed-loop configuration is currently being assessed in a separate trial. The purpose of this study is to assess the insulin-and-pramlintide system in outpatient free-living

settings.

Study Methods

Study Design

We conducted an open-label, randomized, controlled, crossover trial in 23 adult participants to compare insulin-and-pramlintide hybrid closed-loop system with insulin-and-placebo hybrid closed-loop system over 29 days each. Participants' usual rapid acting or ultra rapid acting insulin were used in both interventions. Interventions were separated by a 14-30-day washout period. Participants reverted to their usual insulin therapy during the wash-out period (Figure 1).

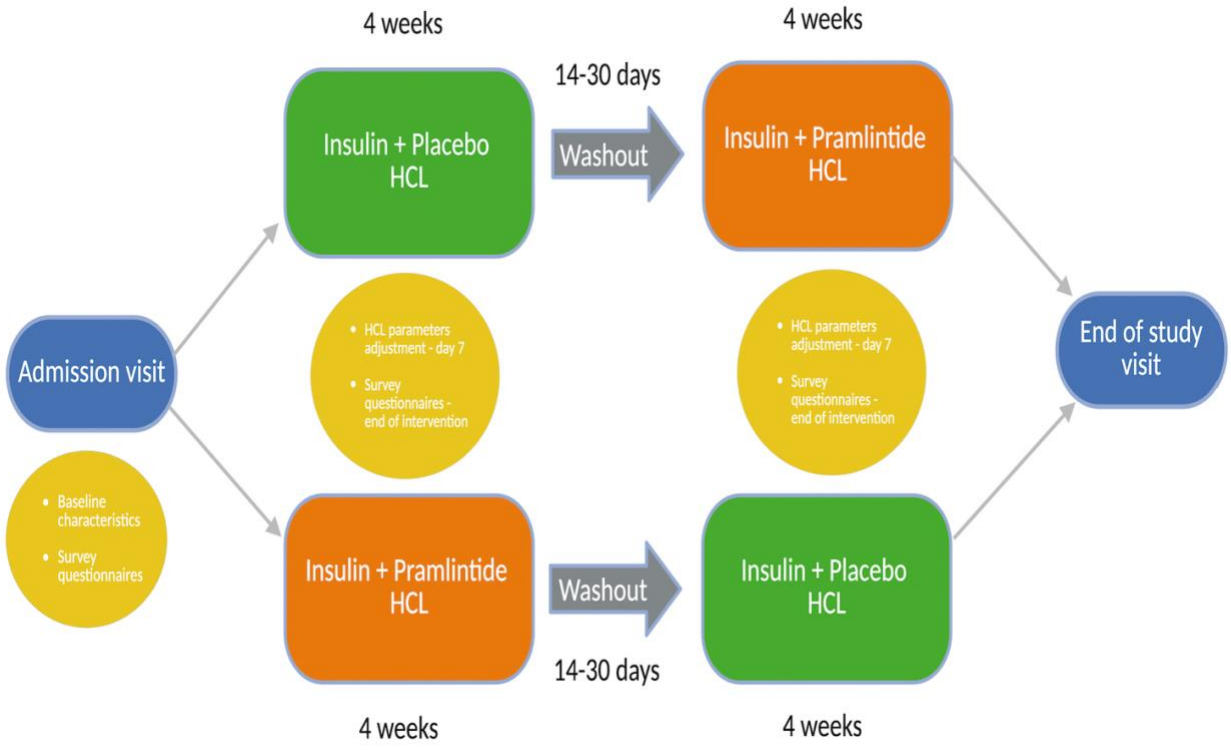

Figure 1. Study design

Participants

Participants were recruited at McGill University Health Centre. Eligible participants were adults aged 18 years or older with a clinical diagnosis of T1DM for at least 12 months and have been using an insulin pump for at least six months and actively performing carbohydrate counting.

Main exclusion criteria were pregnancy, breastfeeding, gastroparesis (counterindication to the use of pramlintide), use of anti-hyperglycemic medication, hydroxyurea medication, or medication that alters gastrointestinal-motility, the occurrence of a severe hypoglycemic episode or diabetic ketoacidosis (DKA) episode within 3 months of admission, and any other serious medical illness likely to interfere with study participation.

All eligible participants signed a written informed consent form before the start of study-related procedures. Prior to study initiation, approval was received from the McGill University Health Centre's research ethics board and Health Canada. The study was conducted in accordance with the standards of the Declaration of Helsinki.

Study Procedures

Upon enrolment, participants attended a 1–2-hour initial visit. During the visit, participants reviewed the study protocol, were provided with study devices, and undertaken a comprehensive medical history review, including current medications, dietary supplements, and past medical treatments or procedures. Furthermore, participants' weight, height, and insulin therapy parameters (e.g., total daily dose, basal rates, and carbohydrate ratios) were recorded and a blood sample was collected to measure baseline HbA1c if recent results (< 2 weeks) were not available. At the end of the initial visit, participants were randomly assigned to receive either 29 days of hybrid closed-loop delivery with insulin and pramlintide followed by 29 days of hybrid closed-loop delivery with insulin and placebo, or vice versa. A block balanced randomization was used to determine the order of the interventions (block size six).

Prior to the start of the first intervention, participants were trained on the hybrid closed-loop system to ensure competency with study devices. Participants were instructed to change the pump's catheters and reservoirs every 48 hours, change the glucose sensor every 10 days, and treat hypoglycemia and hyperglycemia as per their usual practice.

The hybrid closed-loop system was composed of three components: a glucose sensor (Dexcom G6, Dexcom, CA, USA), two infusion pumps (YpsoPump, Ypsomed, Switzerland), and a

cellphone (Google Pixel 2) containing a dosing algorithm app (euGlide, McGill University) to control pumps' deliveries based on glucose sensor readings. One pump was used for insulin delivery and the other was used for pramlintide/placebo delivery, which were delivered at a fixed ratio of 0.6 units (6 μg) per 1 unit of insulin.

The euGlide app is an Android-based application that receives real-time glucose data from the Dexcom G6 CGM and wirelessly controls the delivery of the two pumps using Bluetooth connectivity. The app employed an adaptive model predictive control algorithm[115] to calculate basal insulin and pramlintide infusion rate every 10 minutes and employed a standard bolus calculator to determine prandial boluses at mealtimes (Figure 2). The app was initialized with programmed basal rates, total daily insulin dose, and carbohydrate ratios of participant's usual therapy. The app did not deliver automated correction boluses outside mealtimes; instead, it controlled glucose levels by adjusting the basal rates as aggressively as needed.

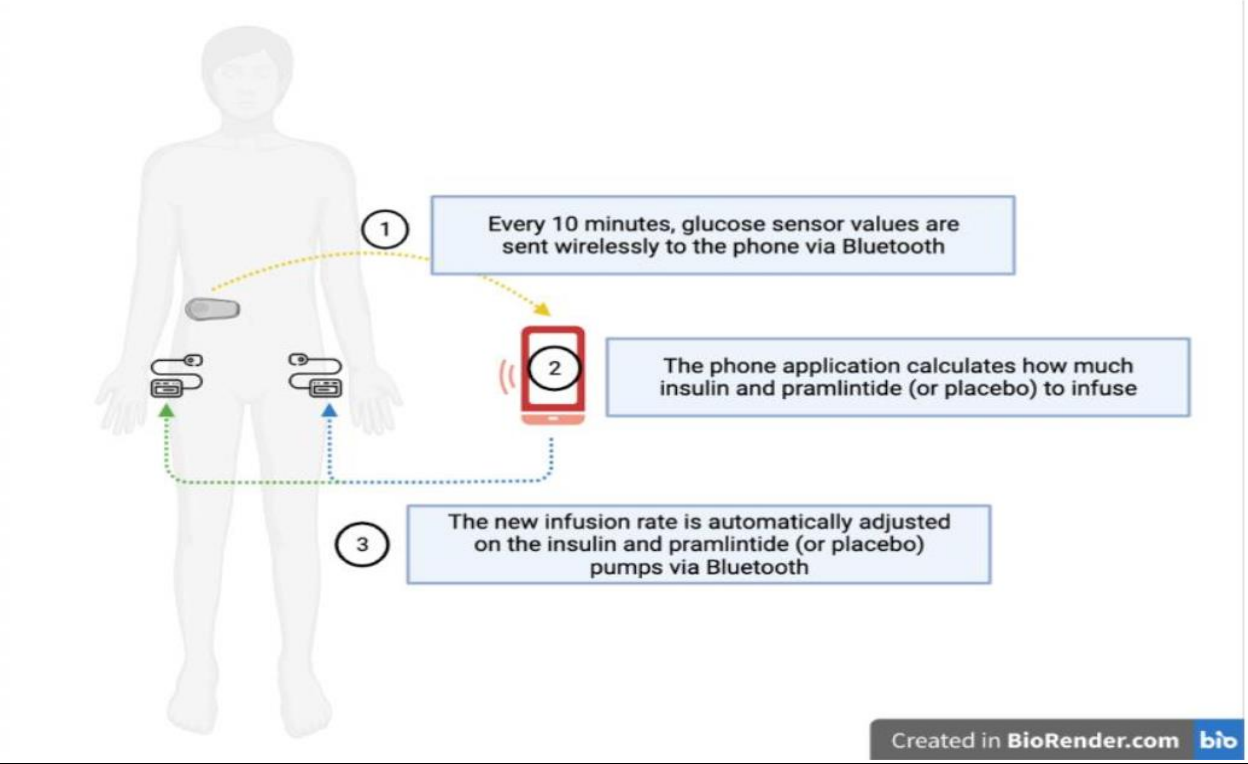

Figure 2. Hybrid closed loop (HCL) system

The closed-loop system was operated in hybrid mode; that is, participants were instructed to enter

the amount of carbohydrate of the meals and snacks they ingest, enabling the app to calculate and administer prandial boluses. Glucose targets were set to 6.0 mmol/L unless the exercise function was enabled by the participant, raising the targets to 9.0 mmol/L. Participants could administer correction boluses at any time.

During the interventions, the study team made remote follow-ups on days 1, 7, and 14 (+/- 1 days) to resolve potential technical issues with the system and record adverse events. The study team performed data reviews on day 7 (+/- 1 days) to adjust therapy parameters for safety reasons only, if needed. The study coordinator was on call throughout the interventions to provide technical support. No restrictions were imposed on food intake or physical activity.

Quality of Life Assessment

Participants were asked to complete the following quality of life surveys to assess their satisfaction with the system at baseline and following each intervention: Type 1 Diabetes Distress Scale (DDS), the Hypoglycemia Fear Survey – II (Worry Subscale), INSPIRE questionnaire for adults, the Diabetes Bowel Symptoms Questionnaire (DBSQ), and the Treatment Satisfaction Questionnaire (TSQ).

Study endpoints

The pre-specified primary endpoint of the study was the proportion of time for which glucose levels were in the target range between 3.9 and 10.0 mmol/L. The pre-specified secondary endpoints included time spent at glucose levels between 3.9 and 7.8 mmol/L, below 3.9 and 3.0 mmol/L, higher than 7.8, 10, 13.9 and 16.7 mmol/L, mean glucose level, total insulin delivery (basal and bolus amounts), and standard deviation and coefficient of variance of glucose levels as measures of glucose variability. Safety endpoints were measured as the number of hypoglycemic events defined by a blood glucose level below 3.0 mmol/L for at least 15 minutes, gastrointestinal symptoms, and adverse events. Adverse events were classified as mild, moderate or severe depending on severity, while mild symptoms were defined as discomfort noticed but no disruption of normal daily activity and moderate symptoms as sufficient discomfort to reduce or affect normal daily activity. Endpoints were evaluated for the entire 29-days periods.

Sample Size and Statistical Analysis

The study aimed to recruit 26 participants to ensure 21 complete the two interventions, providing 80% power to detect a difference of 11% in the primary endpoint at a 5% significance level assuming a standard deviation in the paired differences of 17%. Participants who did not complete both interventions were not included in the final analysis, were not counted toward the recruitment goal, and were replaced in the enrollment process.

The effects of interventions on continuous outcomes were analyzed using linear mixed-effect models that accounted for the interventions' sequence and participant-level intra-correlation. Residual normality was evaluated, with nonparametric methods applied as needed for skewed data. A 5% significance threshold was applied to the secondary analyses with no formal corrections for multiplicity.

Results

Participants characteristics

27 participants were recruited for the study, of which 4 were not included in the final analysis. One participant dropped out after the first intervention while the other three participants were excluded for technical mistakes that impacted the integrity of the data. As a result, the final analysis included a total of 23 participants. The latter who completed that study were aged 33.1 (12.7) years, 11 males (48%) and 12 females (52%), had a weight 77.7 (17.7) kg, BMI 26.5 (5.7) kg/m^2, duration of diabetes 17.5 (8.7) years, HbA1c 7.2 (0.7)%, and daily insulin dose 0.69 (0.2) U/kg/day. (Table 1).

Characteristics	Participants
Age, years	33.1 (12.7)
Male, n (%)	11 (48%)
Female, n (%)	12 (52%)
Weight, kg	77.7 (17.7)
BMI, kg/m²	26.5 (5.7)
HbA1c, %	7.2 (0.7)
Duration of diabetes, years	17.5 (8.7)
TDD, U/(kg/day)	0.69 (0.2)

Total daily insulin, U/day	55.6 (32.2)
Total daily basal insulin, U/day	26.3 (14.0)
Total daily bolus insulin, U/day	29.3 (20.6)

All values are shown as **mean (SD)**, except for gender

Table 1. Baseline participant characteristics (n=23)

Glucose and Insulin Outcomes

The time in target range with the insulin-and-pramlintide system was 71.8 (10.0)% compared to 69.9 (10.3)% with the insulin-and-placebo system (p = 0.21). Mean glucose level with the insulin-and-pramlintide system was 8.5 (0.9) mmol/L compared to 8.7 (1.0) mmol/L with the insulin-and-placebo system (p = 0.02). The standard deviation and coefficient of variance of glucose levels were not different between the two arms (p = 0.09 and 0.30, respectively), but time spent below 3.9 mmol/L was slightly higher by 0.4% (~ 6 min/day) with the insulin-and-pramlintide system (p = 0.01). Time below 3.0 mmol/L was low and not different between interventions (0.4 (0.1, 0.7)% versus 0.2 (0.1, 0.4)%, for pramlintide and placebo, respectively, p = 0.22 , Table 2). Glucose profiles and basal hormonal deliveries are reported in Figure 3 and 4 respectively.

Outcome	Insulin + placebo (n = 23)	Insulin + pramlintide (n = 23)	p-value
Time spent at glucose levels (%) (mmol/L)			
3.9–10.0	69.9 (10.3)	71.8 (10.0)	0.21
3.9–7.8	46.7 (11.2)	47.6 (10.7)	0.58
<3.0	0.2 (0.1, 0.4)	0.4 (0.1, 0.7)	0.22
<3.9	1.8 (1.1, 2.4)	2.2 (1.3, 3.8)	0.01
>7.8	52.8 (11.5)	51.4 (11.4)	0.36
>10.0	28.3 (10.4)	25.8 (10.4)	0.11
>13.9	6.2 (4.7, 11.3)	7.3 (2.1, 9.1)	0.02
>16.7	2.0 (0.8, 4.1)	1.6 (0.5, 3.3)	0.10
Mean glucose (mmol/L)	8.7 (1.0)	8.5 (0.9)	0.02
SD of glucose (mmol/L)	3.2 (0.7)	3.0 (0.6)	0.09

CV of glucose (mmol/L)	36.5 (5.2)	35.6 (4.7)	0.30
Total Insulin (U/day)	54.4 (32.3)	49.0 (28.1)	0.006
Total basal insulin (units)	29.8 (17.2)	29.8 (16.1)	0.94
Total bolus insulin (units)	24.7 (16.8)	19.1 (13.5)	0.0002
Total Carbohydrates (g/day)	170.5 (72.2)	147.6 (72.7)	0.02

Data presented are **mean (SD)** or **median (Q1, Q3)**

Table 2. Overall comparisons of insulin-and-placebo hybrid closed-loop system with insulin-and-pramlintide hybrid closed-loop system (n=23)

Total insulin use was lowered by around 10% with the insulin-and-pramlintide system (49.0 (28.1) U/day) compared to the insulin-and-placebo system (54.4 (32.3) U/day, p = 0.006). This reduction in total insulin was driven by the reduction in bolus insulin (19.1 (13.5) U versus 24.7 (16.8) U, p = 0.0002), while basal insulin was not different between the two arms (mean 29.8–29.8 U, p=0.94, Table 2). Total carbohydrate intake was lower in the pramlintide group compared to the placebo group (147.6 (72.7) g/day vs. 170.5 (72.2) g/day, p = 0.02), further highlighting pramlintide's ability to reduce appetite and consequently lower carbohydrate intake.

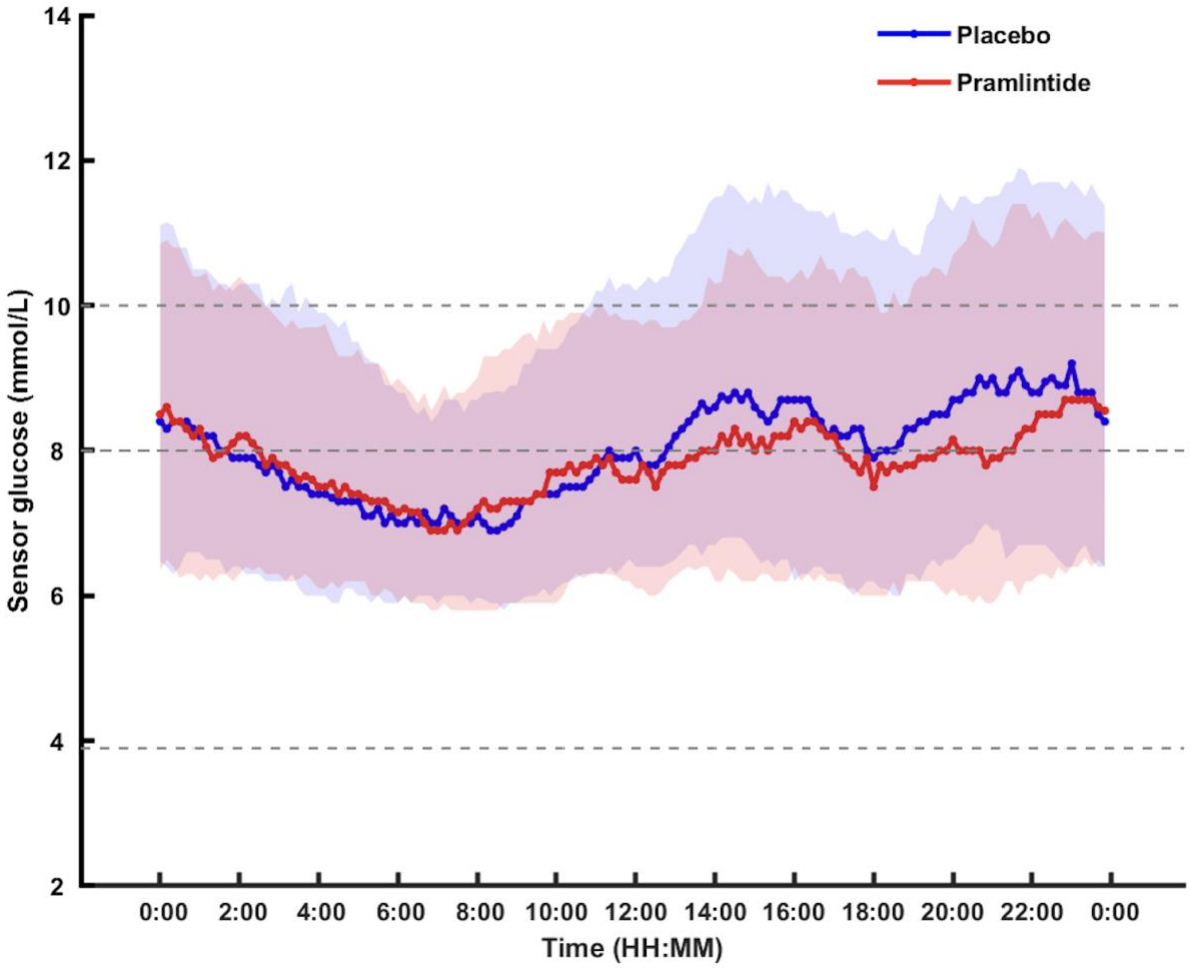

Figure 3. Median [IQR] profiles of mean glucose levels during both interventions. Insulin-and-pramlintide (red), insulin-and-placebo (blue)

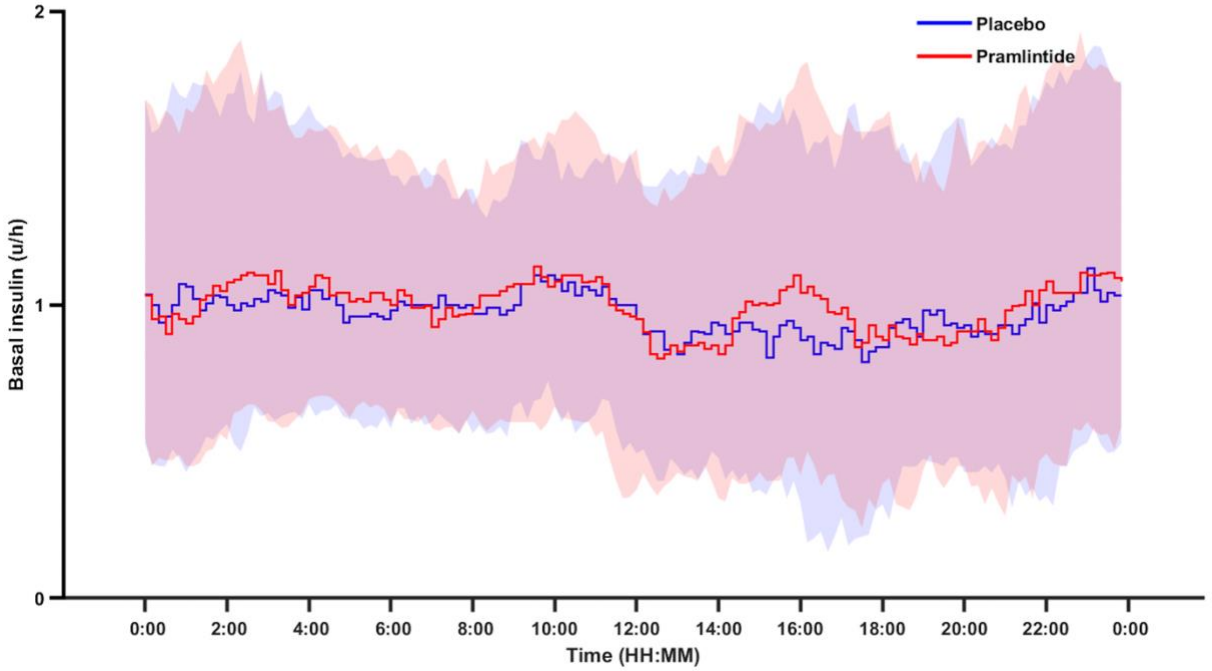

Figure 4. Basal hormonal deliveries during both interventions. Insulin-and-pramlintide (red), insulin-and-placebo (blue)

Gastrointestinal symptoms were the most commonly reported adverse events during the pramlintide phase, primarily including nausea, loss of appetite, abdominal discomfort, and bloating. These events were classified according to predefined severity criteria. Mild symptoms were reported during the pramlintide intervention on 11 instances while moderate symptoms were reported twice. No severe gastrointestinal events were observed.

When the analysis was restricted to those who did not achieve the target of > 70% in the time in range outcome while on the insulin-and-placebo system (n=11 out of 23), glucose benefits were more pronounced with the insulin-and-pramlintide system. For those participants, the time in target range increased from 61.5 (6.6)% in the insulin-and-placebo arm to 65.7 (6.9)% in the insulin-and-pramlintide arm ($p = 0.028$). Mean glucose level was decreased from 9.5 (0.6) mmol/L in the insulin-and-placebo arm to 9.0 (0.5) mmol/L in the in the insulin-and-pramlintide arm. Times spent in hypoglycemia below 3.9 mmol/L and 3.0 mmol/L were not different between the two arms ($p = 0.27$ and 0.88 respectively, Table 3).

Outcome	Insulin + placebo (n=11)	Insulin+pramlintide (n=11)	p-value
Time spent at glucose levels (%) (mmol/L)			
3.9–10.0	61.5 (6.6)	65.7 (6.9)	0.028
<3.0	0.2 (0.2,0.4)	0.2 (0.1, 0.7)	0.88
<3.9	1.5 (0.95, 2.1)	1.7 (1.3, 2.7)	0.27
>10.0	36.7 (6.2)	32.3 (6.1)	0.024
Mean glucose (mmol/L)	9.5 (0.6)	9.0 (0.5)	0.009
SD of glucose (mmol/L)	3.8 (0.6)	3.5 (0.6)	0.055
CV of glucose (mmol/L)	39.4 (4.9)	38.0 (4.6)	0.24
Total Insulin (U/day)	63.8 (42.1)	57.2 (38.7)	0.007
Total basal insulin (units)	33.7 (20.4)	34.6 (21.1)	0.17
Total bolus insulin (units)	30.1 (22.5)	22.6 (18.7)	0.005

Data presented are **mean (SD)** or **median (Q1, Q3)**

Table 3. Overall comparisons of insulin-and-placebo hybrid closed-loop system with insulin-and-pramlintide hybrid closed-loop system for patients with less 70% TIR in the placebo arm (n=11)

Given that insulin-to-carbohydrate ratios and basal rates were systematically adjusted on Day 7 based on glycemic data from the first week of intervention, we conducted a secondary analysis using only data from the last three weeks of each intervention. In the overall cohort (n=23), results were consistent with the full-period analysis: mean glucose (8.7 (1.1) mmol/L vs. 8.6 (0.9) mmol/L, p = 0.32) and TIR (69.6 (12.0) % vs. 70.8 (10.3) %, p = 0.56) did not differ significantly between the placebo and pramlintide phases. However, total insulin (53.9 (32.3) U vs. 49.7 (28.4) U, p = 0.04) and bolus insulin (24.2 (17.0) U vs. 19.5 (13.9) U, p = 0.002) were significantly lower with pramlintide (Table 4).

Outcome	Insulin + placebo (n=23)	Insulin+pramlintide (n=23)	p-value
Time spent at glucose levels (%) (mmol/L)			
3.9–10.0	69.6 (12.0)	70.8 (10.3)	0.56
<3.0	0.2 (0.1,0.4)	0.3 (0.1, 0.8)	0.45

<3.9	1.7 (1.1, 2.4)	2.1 (0.9, 3.3)	0.05
>10.0	28.6 (12.1)	27.0 (10.8)	0.43
Mean glucose (mmol/L)	8.7 (1.1)	8.6 (0.9)	0.32
SD of glucose (mmol/L)	3.2 (0.7)	3.1 (0.7)	0.34
CV of glucose (mmol/L)	36.1 (5.1)	35.6 (5.1)	0.54
Total Insulin (U/day)	53.9 (32.3)	49.7 (28.4)	0.04
Total basal insulin (units)	29.7 (17.1)	30.2 (16.0)	0.65
Total bolus insulin (units)	24.2 (17.0)	19.5 (13.9)	0.002

Data presented are **mean (SD)** or **median (Q1, Q3)**

Table 4. Overall comparisons of insulin-and-placebo hybrid closed-loop system with insulin-and-pramlintide hybrid closed-loop system during the last three weeks of intervention (n=23)

Among participants who had <70% TIR during the placebo phase (n=11), no statistically significant differences were observed in TIR (60.9 (10.1) % vs. 65.2 (7.3) %, p = 0.17) or mean glucose (9.5 (0.8) mmol/L vs. 9.1 (0.5) mmol/L, p = 0.09) between the placebo and pramlintide phases during the last three weeks. However, bolus insulin requirements were significantly lower with pramlintide (22.7 (19.1) U vs. 29.2 (23.2) U, p = 0.02) (Table 5).

Outcome	Insulin + placebo (n=11)	Insulin+pramlintide (n=11)	p-value
Time spent at glucose levels (%) (mmol/L)			
3.9–10.0	60.9 (10.1)	65.2 (7.3)	0.17
<3.0	0.2 (0.1,0.5)	0.2 (0.1, 0.8)	0.96
<3.9	1.4 (1.0, 2.2)	1.8 (1.1, 2.6)	0.42
>10.0	37.4 (9.9)	32.9 (6.7)	0.16
Mean glucose (mmol/L)	9.5 (0.8)	9.1 (0.5)	0.09
SD of glucose (mmol/L)	3.7 (0.6)	3.5 (0.7)	0.16
CV of glucose (mmol/L)	38.8 (4.5)	38.0 (5.4)	0.47
Total Insulin (U/day)	63.0 (42.5)	57.6 (38.9)	0.07
Total basal insulin (units)	33.8 (20.4)	34.9 (20.9)	0.15

Total bolus insulin (units)	29.2 (23.2)	22.7 (19.1)	0.02

Data presented are **mean (SD)** or **median (Q1, Q3)**

Table 5. Overall comparisons of insulin-and-placebo hybrid closed-loop system with insulin-and-pramlintide hybrid closed-loop system during the last three weeks of intervention for patients with less 70% TIR in the placebo arm (n=11)

Survey outcomes

According to the Diabetes Bowel Symptom Questionnaire, participants reported higher occurrence of adverse gastrointestinal symptoms (1.7 (0.5)) with the insulin-and-pramlintide system compared to the insulin-and-placebo system (1.2 (0.2), p = 0.00002). Of note, participants reported a lower rate of adverse gastrointestinal symptoms while using the insulin-and-placebo system compared to baseline (1.2 (0.2) vs 1.5 (0.4), respectively, p = 0.004). Regarding the Diabetes Distress Scale survey (DDS), moderate distress was reported during both interventions, with higher emotional burden scores during the insulin and pramlintide intervention (2.3 (0.9) vs 2.0 (0.6), p = 0.03).

There were no differences between the two interventions in the hypoglycemia fear survey-II, the INSPIRE survey, or the Diabetes Treatment Satisfaction Questionnaire (Table 6).

	Baseline	Insulin +placebo	Insulin + pramlintide	Insulin-and-pramlintide minus insulin-and-placebo, p value
Diabetes Bowel Symptom Questionnaire (overall) ‡	1.5 (0.4)	1.2 (0.2)	1.7 (0.5)	0.00002
Diabetes Distress Scale (overall) §	2.1 (0.7)	2.0 (0.6)	2.3 (0.9)	0.03
Hypoglycemia Fear Survey-II (overall) ‡	2.1 (0.5)	1.9 (0.6)	2.0 (0.7)	0.76
INSPIRE ‡	3.9 (0.7)	3.7 (0.6)	3.6 (0.7)	0.5
Diabetes Treatment Satisfaction Questionnaire	4.3 (0.6)	N/A	3.7 (0.7)	N/A

Data are presented as **mean (SD)**
‡ Scores range from 1-5 on Likert scale

§ Scores range from 1-6 on Likert scale; scores between 2.0-2.9 indicate moderate distress, scores ≥ 3 indicate high distress
DTSQs scores range from 6 = very satisfied to 0 = very dissatisfied

Table 6. Survey scores at baseline and following each intervention (n=23)

Discussion

Our randomized, controlled, crossover study aimed to compare our novel rapid insulin-and-pramlintide hybrid closed loop system with rapid insulin-and-placebo HCL system in 23 adults with type 1 diabetes in an outpatient, free-living, unsupervised setting. Promising results were obtained regarding the combined use of insulin and pramlintide pertaining to glycemic control levels with a concomitant reduction in bolus insulin needed for participants with lower control using the insulin and placebo hybrid closed loop system. To explore whether changes in carbohydrate intake might account for the observed improvements in glycemic control, we plotted individual changes in daily carbohydrate intake versus changes in time in range (TIR) between the pramlintide and placebo interventions (Figure 4).

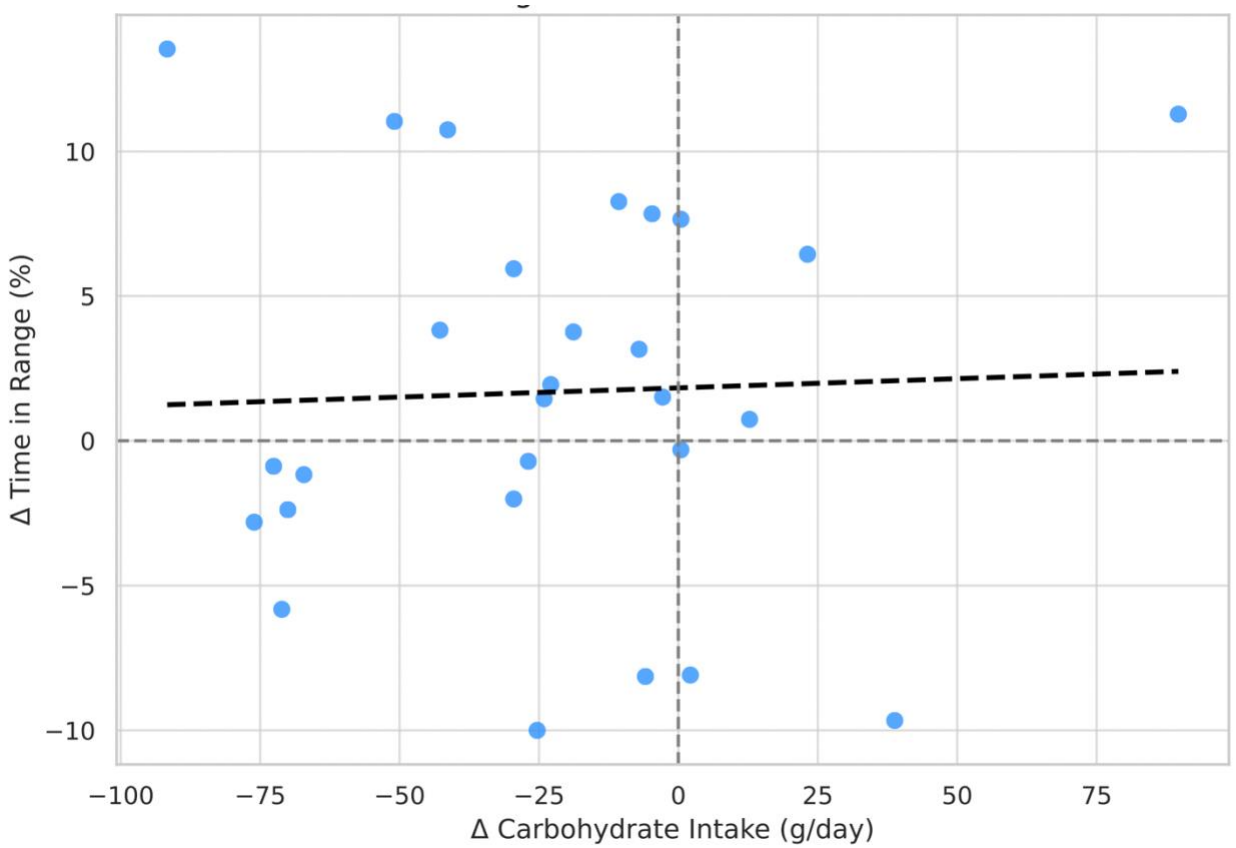

Figure 5. Associations between changes in carbohydrate intake and glycemic control following pramlintide administration (pramlintide – placebo)

While some participants consumed fewer carbohydrates during the pramlintide intervention, this reduction was not specifically associated with improved TIR. Notably, several participants demonstrated enhanced glycemic control despite increased carbohydrate intake, while others showed minimal or no change in TIR despite dietary reductions. These observations suggest that the glycemic benefits observed with pramlintide are unlikely to be solely due to appetite suppression or reduced carbohydrate intake, and may instead reflect physiological mechanisms such as delayed gastric emptying or attenuation of postprandial glucose excursions.

In this study, 11 of the 24 participants achieved a time in target range (3.9-10 mmol/L) lower than 70% during the placebo intervention. Nonetheless, within this particular subgroup, time spent in target range was significantly higher with the pramlintide intervention (65.7 (6.9)% vs 61.5 (6.6)%, p = 0.028), emphasizing pramlintide's benefits in improving glycemic control in cohorts characterized by suboptimal time in target range.[116]Additionally, within this specific cohort, a lower time spent in hyperglycemia (~1 hour less per day) was noted in the pramlintide group compared to the placebo group, mostly due to the rapid glucose lowering effect of pramlintide postprandially by slowing glucose absorption from the gastrointestinal tract and reducing glucagon secretion. By delaying gastric emptying, pramlintide enables postprandial glucose levels to better match insulin pharmacokinetics, reducing the risk of immediate post-prandial hyperglycemia followed by delayed hypoglycemia.

These results concur with various studies. Notably, Haidar et al investigated whether the addition of pramlintide could potentially improve glycemic control when the sole use of insulin is frequently suboptimal in maintaining an adequate glucose level.[91] This study confirmed that the combination of pramlintide and rapid insulin in a hybrid closed loop system can significantly improve glycemic control in type 1 diabetes patients, without increasing the risk of hypoglycemia. Nonetheless, similarly to our findings, a higher rate of gastrointestinal adverse effects (moderate nausea) was also noted pertaining to the combined use of the pramlintide and insulin compared to rapid insulin-alone HCL system.

Haidar et al previously investigated the added benefits of combining pramlintide and rapid insulin when compared to the sole use of insulin especially during day time, showing a 10% difference in time in range with a rapid insulin-and-pramlintide system compared to a rapid insulin-alone system (84% (13%) vs 74% (SD 18%), p = 0.0014.[91] On the other hand, in a study that evaluated the potential benefits pertaining to glycemic control associated with the addition of glucagon to the insulin regimen, the use of a single-hormone system comprising insulin was found to achieve adequate glycemic control without a major risk of hypoglycemia, especially at night.[117] Interestingly, Tsoukas et al reported an increased rate of hypoglycemic episodes using the hybrid Fiasp-alone system compared to the fiasp plus pramlintide fully closed-loop system.[98] However, a higher number of gastrointestinal side effects was reported with the fully closed loop system comprising pramlintide, in agreement with our results. Nonetheless, the fiasp plus pramlintide fully closed loop system failed to demonstrate superiority in time within glucose target range when compared to the fiasp-alone hybrid closed loop system, which further illustrates the need to explore pramlintide's added benefits in a fully closed loop setting.

To explore whether glycemic control improved over time, we conducted a secondary analysis limited to the final three weeks of each intervention. Results were consistent with the full 4-week analysis, suggesting that glycemic control with pramlintide was sustained after initial parameters adjustment. Longer duration studies are needed to determine whether glycemic benefits associated with the incorporation of pramlintide into HCL systems evolve with extended use.

Our study has several limitations. First, the relatively small sample size may have limited the generalizability of our findings when compared to the broader type 1 diabetes population. Second, half of our study population achieved a time in range >70% with placebo, possibly hindering the potential pramlintide-associated improvement due to ceiling effect. Participants in this study had relatively good baseline glycemic control, which could limit the generalizability of the findings to individuals with suboptimal glucose control. Third, the pramlintide/ placebo infusion via a second pump added complexity to the participants' diabetes management compared to usual care. In addition, socioeconomic status, educational background, and other social determinants of health were not assessed, limiting our ability to evaluate the diversity and representativeness of the study

population. On that note, HbA1c and body weight were not collected at the end of the study, limiting our ability to assess long-term glycemic improvement potentially associated with pramlintide use. Although GI symptoms were reduced after the 1st week of pramlintide intervention, future research should systematically assess symptom burden over longer duration to determine whether gastrointestinal effects persist, diminish with continued use, or vary by patient characteristics, as these symptoms may significantly impact treatment adherence in real-world settings. Future research should explore co-formulation options for improved convenience and ease of use.

Our study had several strengths too. Our study was conducted in a real-world, free-living, unsupervised outpatient setting, making the findings clinically relevant and generalizable to daily diabetes management when compared to other studies conducted in restricted and controlled environments. Additionally, the randomized crossover design of the study allows for each participant to serve as their own control, reducing inter-individual variability and strengthening the statistical power of the findings.

Our study shows that pramlintide did not degrade glucose control compared to an insulin-and-placebo hybrid closed loop system and, in fact, improved glycemic control in individuals with suboptimal glucose regulation at baseline. Further optimization of the system, along with larger studies, is needed to expand on these findings and assess its long-term benefits in real-world settings.

Acknowledgements

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Author Contributions

MAT, LL, JFY, NG, MRP and AH supervised the study. EC and AH designed the study. MAT, JEM, LL, JFY, NG, MRP, EC and AH conducted the studies. JR and AJ carried out the statistical analyses. AH designed the dosing algorithm. AH had full access to the data and takes responsibility for the integrity of the data analysis.

Author Disclosure Statement

M.A.T. received research support from AgaMatrix, consulting fees from Sanofi, and speaker honoraria from Eli Lilly, Novo Nordisk, Boehringer Ingelheim, Janssen, and AstraZeneca. L.L. has pending patents in the field of artificial pancreas, received consulting fees from Dexcom and Insulet, and has received support for clinical trials from Merck, AstraZeneca, and Sanofi. J.-F.Y. received research support from Sanofi, Bayer, and Novo Nordisk and consulting fees and speaker honoraria from Sanofi, Eli Lilly, Novo Nordisk, Boehringer Ingelheim, Janssen, Takeda, Abbott, Merck, and AstraZeneca. M.-R.P. has received speaker honoraria from Medtronic Diabetes Canada and Abbott Diabetes Care. A.H. has acted as a consultant for Eli Lilly and has received drugs, supplies, equipment and other in-kind support from Tandem, Adocia, Dexcom, Eli Lilly and Ypsomed. A.H has pending patents in the artificial pancreas area. No other potential conflicts of interest relevant to this article were reported.

Data availability

The raw data can be shared by the corresponding authors upon reasonable request for academic purposes, subject to Material Transfer Agreement and approval from the Research Ethics Board of the McGill University Health Centre. All data shared will be de-identified.

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Chapter 4. Discussion

4.1. Carbohydrate counting and Pramlintide in AID Systems

4.1.1. Pramlintide in Automated Insulin Delivery systems

Hybrid closed-loop systems are a promising therapy choice for T1DM. These have been shown to improve glucose control in T1DM patients increasing the time in the target glucose range and reducing the risk of nocturnal hypoglycemia. However, one of the main challenges in maintaining adequate glucose control in a hybrid closed-loop system occurs at mealtimes. This is due to the rapid rate of carbohydrate absorption at mealtimes, leading to postprandial hyperglycemia.[127] To attempt to improve HCL system performance, we infused two hormones to regulate glucose levels: insulin and pramlintide. Pramlintide's main function, as a synthetic amylin analog, is to inhibit the release of glucagon postprandially, decelerate gastric emptying and increase satiety. Accordingly, the ensuing metabolic consequences coincide with the usual physiological β-cell secretion, thus rendering insulin's pharmacokinetics more suited to reduce potential post-prandial hyperglycemia along with delayed hypoglycemia.[75,128]

In a previous randomized, controlled crossover trial of 24 adults with type 1 diabetes, we evaluated a dual-hormone (insulin and pramlintide) closed-loop system over three 24-hours inpatient visits.[91] The study demonstrated a significant improvement in time spent within the glucose target range (3.9–10.0 mmol/L) with rapid insulin and pramlintide compared to rapid insulin alone: 84 (13) % compared to 74 (18) % with the rapid insulin-alone system ($p = 0.0014$). This improvement was mainly due to enhanced daytime glucose control (78% vs. 63%, $p = 0.0004$). Although mild gastrointestinal side effects were observed in some participants, most symptoms resolved during the study, suggesting pramlintide's promising role in hybrid closed-loop systems to more closely replicate physiological glucose regulation.

In a more recent randomized crossover trial, we compared three automated insulin delivery systems in 30 participants with type 1 diabetes over 14-day periods each: (i) an insulin-and-placebo closed-loop system with carbohydrate counting (CC), (ii) an insulin-and-placebo closed-loop system with simple meal announcements (SMA), and (iii) an insulin-and-pramlintide closed-loop system with SMA. The insulin-and-pramlintide system with SMA achieved the best glycemic outcomes, with 63% time in range (3.9–10.0 mmol/L) compared to 58% with insulin-and-placebo

with CC and 55% with insulin-and-placebo with SMA ($p = 0.031$). The study's qualitative analysis revealed that participants appreciated the system's simplicity and flexibility but raised concerns about mild gastrointestinal symptoms, which occurred in 47% of cases. These results underscore the benefits of pramlintide in improving glycemic control, particularly in mitigating postprandial hyperglycemia, even when carbohydrate counting is replaced by simplified meal announcements.[100]

We are currently performing a randomized controlled crossover trial in 30 adults with type 1 diabetes to evaluate the benefits of pramlintide in a fully closed-loop system.

4.1.2. Implications for Clinical Practice

As insulin pumps and CGM are incrementally becoming pillars in the clinical management of T1DM, carbohydrate counting's importance ought to be heavily emphasized. As previously highlighted, the pedagogical support provided by the caregivers and healthcare team to ensure that the autonomous patient can adequately calculate his carbohydrate intake is indispensable. Precisely monitored carbohydrate counting, when combined with real-time CGM, was associated with improved postprandial glycemic control. Carbohydrate counting also dismisses the ambiguity regarding required insulin dose adjustments for a wide array of food options, thereby mitigating the psychological burden associated with the concept of dietary restrictions and improving the quality of life of the T1DM patient.[122]

Notably, the advantages provided by individualized treatment have been repeatedly documented throughout healthcare literature. Accordingly, this approach also applies to structured and tailored educational programs regarding carbohydrate counting that improve diabetes management.[123] This comes as no surprise, as the understanding and execution of carbohydrate counting is subject to many variables such as the patient's mental health, emotions, motivation, and importantly, the patient's level of education.[124] Furthermore, the use of simplified tools such as food tracking apps can serve to alleviate the burden of manual carbohydrate counting in certain situations, thus facilitating its implementation in the patient's daily routine.[125] Thus, establishing an accurate algorithm to accurately evaluate carbohydrate count, especially in the setting of suboptimal demographics, has been linked to better overall outcomes in the associated precise calculation of

mealtime insulin boluses depending on the carbohydrate content entered in the algorithm.[126]

4.1.3. Importance of Carbohydrate Counting

Diabetes management, particularly for individuals with type 1 diabetes, requires a multifaceted approach to maintain good glycemic control. One of the key challenges faced by T1DM patients is the need to regulate postprandial blood glucose levels, which are heavily altered by carbohydrate intake. Hence, the appropriate estimation of carbohydrate intake constitutes a crucial factor in determining the adequate postprandial insulin bolus dosage, knowing that carbohydrates have the most significant and immediate impact on the blood glucose level.

Carbohydrate counting consists of calculating the specific amount of carbohydrates found within specific meals then to calculate the insulin-to-carbohydrate ratios adjusted post-meals in order to establish the adequate insulin bolus dose needed to mitigate postprandial hyperglycemia. Hence, carbohydrate counting constitutes an essential part of T1DM management and, if performed correctly by the patient, leads to improved glycemic control.[118,119]

Naturally, appropriate educational programs pertaining to accurate carbohydrate counting and its associated insulin dosage are of utmost importance.[120] Furthermore, a recent study highlighted the superiority, in terms of ensuing time-in-range, of accurate carbohydrate counting (80.3 (7.4) %) when compared to meal announcements with a preset of three carbohydrate amounts (73.5 (6.7) %) while using advanced insulin delivery systems.[121] The implementation of precise carbohydrate counting into incrementally suitable technological tools, such as a cellphone application, resulted in an overall better postprandial glycemic control while decreasing the potential risk of hypoglycemia when compared to traditional care.[119]

Finally, the importance of effective and efficient carbohydrate counting in the management of T1DM is readily emphasized throughout the literature. Nonetheless, variability in certain demographics and experience levels might impact the perceived complexity of carbohydrate counting, thus emphasizing the need for continuous support from caregivers and the healthcare community to promote awareness and adherence to carbohydrate counting.

4.2. Quality of Life

4.2.1. Importance of PROs

The implementation of Patient-Reported Outcomes (PROs) into the evaluation of hybrid closed-loop systems, especially those that incorporate both insulin and pramlintide, allows to scrutinize the acceptability of these advanced diabetes management technologies. PROs focus on the patient's experience, level of satisfaction with treatment, and general quality of life.

Dual-hormone hybrid closed loop systems that combine pramlintide and insulin have been found to improve glycemic control. For patients, this improvement is essential because it is directly linked to a lower risk of complications from diabetes.[91] Additionally, as an amylin analog, pramlintide helps decrease postprandial rise in glycemia and slow stomach emptying. This helps patients maintain better overall glycemic control without the need for excessive amounts of insulin, especially when it comes to controlling blood sugar levels after meals.[129]

As previously mentioned, delving further into the subjective assessment of efficacy and comfort pertaining to HCL systems technology is mandatory. For instance, that dual-hormone systems result in lower diabetes-related distress while improving overall satisfaction with the treatment when compared to traditional systems previously used, despite requiring more frequent insulin boluses.[93] This aligns with findings showing that better postprandial glucose control leads to reduced anxiety related to glycemic fluctuations, a key factor in long-term treatment adherence.

PROs are essential for detecting adverse effects that impact patient compliance but might not be noticeable in clinical trials. For example, more people experienced gastrointestinal issues when using a HCL system based on pramlintide-insulin-glucagon compared to a solely insulin-based HCL system.[130] Developers can then proactively remediate these problems with ongoing patient feedback.[130]

Findings from PROs can also help pinpoint the variables affecting treatment compliance. Patients are more likely to follow their treatment plan if they believe that a dual-hormone system greatly improves their quality of life by facilitating the management of diabetes, allowing for long-term health advantages associated with appropriate compliance.[89]

Studies suggest that the continuous improvement of hybrid closed loop systems that incorporate pramlintide and insulin may lead to better glycemic management without raising the risk of hypoglycemia. However, since real-world circumstances differ significantly from controlled clinical settings, additional research is required to validate the reproducibility of these findings in outpatient settings.[91]

PROs provide a holistic assessment of diabetes care, capturing both clinical efficacy and patient experience. As research progresses, real-world data from diverse patient populations will be essential in refining dual-hormone systems to enhance both glycemic control and long-term quality of life. Future studies should focus on optimizing system usability, addressing patient-reported challenges, and expanding access to ensure the successful integration of these technologies into everyday diabetes management, hopefully throughout all populations.

4.2.2. Survey outcomes

The insulin-and-pramlintide closed-loop systems have shown equivocal outcomes pertaining to the treatment of T1DM. For instance, as reported by Nwoko et al, the use of closed-loop systems was associated with improved quality of life metrics and a notable decrease in hypoglycemia-related anxiety and overall diabetes-related distress.[131] When compared to traditional insulin delivery methods, users of closed-loop devices report reduced distress ratings on the Diabetes Distress Scale (DDS), which is frequently used to evaluate this aspect.[131] Moreover, Fisher et al. evaluated how diabetes distress affected adults with T1DM glucose control and adherence to medication. Significant relationships between higher levels of distress and worse glycemic control were found when the Type 1 Diabetes Distress Scale was used to measure distress.[132] Notably, our study showed that patients subject to the treatment comprising insulin and pramlintide displayed a slightly higher level of distress than the ones on placebo, although not clinically significant ($p=0.33$)

Generally, T1DM patients frequently experience distress related to diabetes, which is defined by emotions of annoyance and exhaustion associated with managing their diabetes. This phenomenon has been measured using the Type 1 Diabetes Distress Scale, showing that some distress can be

reduced by the automation offered by closed-loop systems. After a year of use, participants in research assessing the Medtronic 670G hybrid closed-loop device reported a significant reduction in their diabetic distress scores.[133] This drop is explained by a reduced cognitive load, which frees patients in a manner that allows them to concentrate more on their everyday activities rather than continuous blood sugar checks.[133]

On the other hand, the Hypoglycemia Fear Survey-II (HFS-II) has shown lower scores associated with the implementation of closed-loop systems, which translates into less hypoglycemia-related anxiety in diabetic patients using this technology.[134,135] Importantly, the reliability of the HFS-II utilized among T1DM patients ought to be mentioned, through combined data from many cohort studies (n=777), as a significant correlation with psychological distress measures were confirmed.[136] For example, one study indicated that the HFS-II fear subscale (HFS-W), which measures fear regarding hypoglycemia, significantly decreased over time for individuals utilizing hybrid closed-loop systems. Naturally, an improvement in the emotional aspect related to the management of diabetes ensues.[135] Our study revealed that patients exhibited a slightly decreased level of hypoglycemia-related fear when using the insulin-and-pramlintide-based hybrid closed loop system compared to the baseline level, although these results were not found to be clinically significant ($p= 0.26$).

Separately, Bresson et al. investigated the connections between T1DM patients' cognitive hurdles to avoiding hypoglycemia and their concerns about it. Along with other assessments, participants (n=178) filled out the HFS-II, which showed that greater anxiety was linked to impaired hypoglycemia awareness.[134]

The degree of satisfaction of patients' display is frequently assessed using the Treatment Satisfaction Questionnaire for Medication. Very high levels of satisfaction with the usability and functionality of closed-loop systems have been reported in a study on patient satisfaction with home healthcare provider services, as shown in both early follow-up periods and after three months of use.[137] Similarly, our patients showed a good level of satisfaction on this scale with the combined use of pramlintide and insulin, yet somehow lower than the baseline level of satisfaction (3.7 vs 4.3, respectively, with $p = 0.003$).

As confirmed by our current study, the use of pramlintide in addition to insulin may cause gastrointestinal symptoms, which are evaluated using the Diabetes Bowel Symptoms Questionnaire. Although moderate gastrointestinal complaints have been documented in some studies, when properly controlled within the framework of closed-loop systems, these symptoms did not significantly affect the overall quality of life or treatment satisfaction.[100] Participants in our study had an increase in GI symptoms during the insulin and pramlintide intervention (1.7 vs 1.2, $p = 0.001$) compared to placebo, occurring most frequently during the first week of interventions and generally resolving within the following weeks of treatment. Similarly, mild-to-moderate gastrointestinal symptoms that gradually diminish over time have been previously noted with the use of pramlintide in T1DM patients.[138]

Furthermore, patient-reported outcomes for automated insulin delivery systems can be reliably evaluated using the INSPIRE questionnaire which was found to be valid across kids, adults, parents, and partners in a study that involved 750 participants from the Type 1 Diabetes Exchange Registry.[139] Plus, positive expectations about closed loop systems were successfully captured by the INSPIRE measures, offering insightful information about patient experiences.[140] Nonetheless, our patients showed a slight decrease in positive expectations following treatment using an insulin-and-pramlintide HCL system when compared to the baseline level ($p = 0.05$).

Although closed-loop systems constitute a promising beneficial technology in diabetes management and mitigate hypoglycemia-related anxiety, the significance of improvements in satisfaction and quality of life is still equivocal.[141] Such ambiguity could be due to disparities between study design characteristics and baseline population characteristics. Hence, additional research should aim toward standardizing PRO measurements across studies to allow for a more accurate assessment of the psychosocial impact of closed-loop systems.

4.2.3. Barriers and Challenges of Implementing a Dual-hormone hybrid closed-loop system

Any technology under development, including the promising insulin-and-pramlintide HCL systems, inevitably faces various impediments that limit its efficacy and widespread adoption, particularly when considering this technology's relative complexity. As a matter of fact, when

pramlintide is incorporated into HCL systems, the dosage algorithms become more complicated. Due to its effects on stomach emptying and glucagon suppression, pramlintide must be delivered with careful timing, in contrast to insulin, which is primarily used for glucose management. Thus, elaborating efficient algorithms that can modify dosages depending on real-time glucose measurements and meal patterns may be cumbersome.[91] In addition, patients must get comprehensive instructions regarding the adequate use of these dual-hormone systems for optimal results. Poor treatment regimen adherence brought on by inadequate education may reduce the system's potential benefits.[130]

Furthermore, pramlintide's ability to induce gastrointestinal symptoms including nausea and vomiting constitutes one of its major drawbacks. Patients taking pramlintide in addition to insulin experienced mild to moderate gastrointestinal effects in clinical trials, which may discourage them from pursuing the treatment, thus decreasing its overall efficacy.[91]

Importantly, the journey toward the official approval of an innovative medical device that incorporates a combination of drug classes represents quite a complicated procedure, as proof of safety and effectiveness are heavily emphasized and may significantly delay market availability and further experimental and clinical trials, thus halting the improvement of the HCL system.[129]

Knowing that current systems only allow separate administration of pramlintide and insulin, the compliance and adherence to treatment may be negatively impacted. The development of coformulations that enable the simultaneous administration of both hormones via a single device is currently under investigation. Nonetheless, one of the biggest associated scientific challenges is creating a stable formulation that preserves the effectiveness of both hormones.[91]

Finally, the assessment of dual-hormone hybrid closed loop systems has only occurred in well-controlled inpatient parameters in the majority of research. Naturally, outcomes do not necessarily translate to the real-world patient knowing that glycemic control is subject to a wide array of factors such as physical activity, meal schedule and overall lifestyle discrepancies. Additional research that incrementally mimics real-world parameters is needed to ensure sufficient consistency regarding the results obtained, both in the physical and psychosocial domains.[130]

Our study was performed in outpatient, free-living, unsupervised setting, demonstrating real-world data, which further reinforces the applicability of dual-hormone systems in everyday life, capturing real-world variations in glycemic responses, patient adherence, and usability challenges.

4.3. Future Directions for Closed-loop Systems

4.3.1 Barriers Towards a Fully Insulin-and-Pramlintide Closed-loop System

As research continues, multiple aspects of completely automated closed-loop devices that combine insulin and pramlintide are being considered to improve their efficiency, practicality and accessibility.

The requirements regarding user input for both meal announcements and carbohydrate counting in closed-loop systems constitutes one of their main current issues. Hence, future developments aim toward establishing completely automated systems that can precisely estimate carbohydrate consumption and modify the supply of insulin and pramlintide as necessary without the need for human input. Advanced algorithms that may recognize unexpected meals using patterns in glucose variations and data from CGMs, and preferably both, may be of assistance.[89,142] Additionally, algorithms that can estimate both meal timing and composition based on real-time glucose readings could facilitate the device's use and improve glycemic management, especially during postprandial periods.[89]

Furthermore, future closed-loop systems might include glucagon in addition to insulin and pramlintide to reduce the risk of hypoglycemia. This multi-hormonal strategy protects against episodes of low blood sugar while enabling more aggressive insulin doses. Research shows that glucagon supplementation can help stabilize blood glucose levels during times of substantial fluctuations, as right after meals or while exercising.[131] Thus, it is still crucial to conduct research on stable glucagon formulations for subcutaneous administration since this will make it easier to include into closed-loop systems.

Knowing that personalized treatment is incrementally becoming the goal of healthcare, the simplicity regarding the adoption of a specific regimen or device in a manner that is convenient

for the user is of utmost importance. In light of this aspect, improving both adherence and satisfaction with the use of closed-loop systems inevitably necessitates improving its connectivity with cellphones while incorporating streamlined user interface, adequate instructions to ensure complete awareness regarding all aspects of treatment and device use.[142]

As previously mentioned, closed-loop systems and their commercialization are significantly impacted by the ever-changing regulatory environment around diabetes-related drugs and management. Hence, facilitating FDA approval procedures, all-the-while ensuring adequate efficacy and safety, is mandatory and precedes the equally important goal of mitigating financial burdens through appropriate insurance coverage.[89] Concomitantly, long-term research evaluating cost-effectiveness, impact on quality of life and patient therapeutic outcomes related to the use of insulin-pramlintide systems in diabetes management is necessary.[131]

4.3.2. Volagidemab

A novel strategy to improve glycemic control, especially for T1DM patients, is the use of volagidemab, a glucagon receptor antagonist. Several potential paths for the successful incorporation of volagidemab inside the closed loop systems for diabetes management have become apparent as research advances.

Knowing that Volagidemab is currently undergoing phase II trials in hopes of elucidating its long-term effectiveness and safety in T1DM treatment, this monoclonal antibody constitutes a promising factor in improving glycemic regulation and lowering the need for insulin.[143] As for Volagidemab's mechanism of action, it targets glucagon receptors specifically and inhibits the ensuing cascade of glucagon-mediated metabolic changes. Mainly, as hyperglucagonemia (high glucagon levels) contributes to hyperglycemia in T1DM patients, Volagidemab works to lower blood glucose levels and decrease the overall requirement for exogenous insulin through the blockage of this glucagon receptor.[144] This approach is buttressed by the previous results associated with the use of glucagon receptor antagonists which serve to improve overall glucose management, especially by reducing postprandial hyperglycemia.[142]

The intricacies brought up by closed-loop systems comprising several hormones may require

sophisticated algorithms to handle, through efficiently balancing the effects of pramlintide, volagidemab, and insulin depending on specific patient responses, meal announcements, and real-time glucose readings. Accordingly, model predictive control and other advanced control techniques constitute viable alternatives to achieve this equilibrium.[145] Nonetheless, additional clinical trials encompassing a wide array of demographics and scenarios are essential for assessing the actual benefits and safety of closed-loop systems implementing Volagidemab. For example, research may assess the efficiency of these systems in controlling glycemic fluctuation during various meal kinds and daily activities.[146]

Additionally, developments in insulin delivery systems and CGM should be included into future closed-loop systems that use volagidemab. It is crucial to assess the long-term cost-effectiveness of closed-loop systems that incorporate volagidemab, just like with any new technology.[142] Finally, for multi-hormone closed-loop systems, obtaining regulatory approval can be an onerous process, especially with the incorporation of a monoclonal antibody, which imposes additional scrutiny pertaining to potential side effects and autoimmune reactions.

Pettus et al reported some common side effects pertaining to volagidemab, such as upper respiratory tract infection, dizziness, headache, nausea, hypertension and some laboratory abnormalities including elevated serum transaminases and LDL.[144] Nonetheless, hypoglycemia rates in both volagidemab and placebo groups were comparable. A Grade 3 hypoglycemic event necessitating emergency room admission was potentially related to the study treatment. The Phase 2 trial, although encouraging, did not reach the agreed-upon significance level of daily insulin usage reduction.

4.3.3. GLP1-Receptor Agonists

The incorporation of GLP-1 receptor agonists (GLP-1 RAs) into closed-loop systems would benefit its user through GLP-1's appetite-suppressing and glucagon-lowering effects, along with insulin's glucose-lowering activities which may improve glycemic control while reducing some of the drawbacks of insulin therapy alone, such as iatrogenic hyperinsulinemia, weight gain, recurrent episodes of hypoglycemia and increased cardiovascular risks.[147,148] Elucidating the precise processes through which GLP-1 RAs affect T1DM should be the main goal of future research. In

fact, it is currently agreed upon that GLP-1 analogs decrease stomach emptying, inhibit incorrect glucagon release, and increase glucose-dependent insulin secretion, culminating in better overall glycemic management in T1DM. Hence, creating more potent and efficient GLP-1 RA formulations, especially for T1DM patients, ought to be considered.[149]

The long-term effects of closed-loop systems that use GLP-1 RAs, such as their impact on PROs, time in range, HbA1c levels and adverse events ought to be established, partly through post-marketing surveillance depicting this system's function outside of regulated clinical settings, delineating areas of future improvements and modifications.[146,150] For instance, a recent study indicated that most adverse events consisted of gastrointestinal symptoms, yet some serious events of overt euglycemic ketosis were also noted, although none resulted in ketoacidosis.[147] Separately, an overall increased risk of diabetic ketoacidosis has also been noted during the use of closed-loop systems in general, especially in T1DM patients with an HbA1c of 8.5% or above.[151]

4.3.4. Sodium-Glucose Cotransporter-2 Inhibitors

According to recent research, the use of SGLT2i as an adjuvant treatment in T1DM patients can significantly lower HbA1c levels and daily insulin dosages. For instance, research has demonstrated that when SGLT2i are used in conjunction with insulin therapy, reductions of HbA1c by 0.39% and a 10% decrease in total daily insulin use are noted.[152]

SGLT2i, through promoting glycosuria, might contribute in mitigating postprandial hyperglycemia which constitutes one of the main issues in diabetes care. Increased time in range (TIR) during mixed meal testing is one of the encouraging outcomes of preliminary research using SGLT2i as adjuncts to closed-loop systems.[153] Accordingly, the appropriate integration of these drugs into closed-loop systems and their corresponding algorithms would plausibly improve postprandial glucose control without substantially disturbing glycemic stability as a whole.[154] Although quite advantageous through their nephroprotective and cardioprotective properties, SGLT2i still present with a risk of life-threatening DKA, especially in those with T1DM. Moreover, the risk of urinary tract infections and dehydration ought to be mentioned. Thus, mitigating these risks when incorporating SGLT2i into closed-loop systems is essential along with the establishment of patient education initiatives on DKA symptoms and prevention.[152,155]

In spite of SGLT2i's associated lower insulin needs and enhanced glycemic control in T1DM, long-term safety and effectiveness, primarily in both microvascular and macrovascular dimensions, still need to be studied, thereby ensuring that the advantages of SGLT2i outweigh the associated aforementioned risks and to set appropriate guidelines for their safe use in various populations.[146] Followingly, large-scale studies encompassing a wide array of demographics could also allow for the establishment of potential indications in the pediatric T1DM population, particularly individuals characterized by additional obesity or even insulin resistance.[156]

At last, the combination of SGLT2i and insulin in closed-loop systems, along with accurate CGM technology, could provide T1DM patients with multisystem protective effects, thus improving both diabetes and overall patient care, all-the-while decreasing the patient's stress and anxiety.[146]

4.3.5. Immunotherapies

As T1DM's underlying autoimmune processes progressively worsen the disease, the integration of immunotherapies ought to be considered. Furthermore, an immunotherapy that could also serve to optimize glucose control would concomitantly target part of the cause and the consequences of T1DM. Future research focusing on the combination of immunotherapies including immune-modulating drugs or monoclonal antibodies and closed-loop insulin delivery devices might demonstrate several synergistic benefits, particularly in newly diagnosed T1DM patients at an early disease stage, in order to mitigate the autoimmune-mediated insulitis and β-cell death, thus allowing for the maintenance of a certain amount of residual insulin. Consequently, the overall effectiveness and practicality of closed-loop systems may be improved, enabling better glycemic control and a diminished need for insulin. Optimal timing and dosage should be explored for maximal effectiveness.[157,158]

Nonetheless, the implementation of an immune component necessitates a highly individualized approach, as each patient's genetic and environmental characteristics should be taken into account. One of the main factors that could guide the selection of suitable immunotherapies and modifications to insulin delivery algorithms in closed-loop systems are biomarkers suggestive of the degree of autoimmune activity or remaining beta-cell function.[159] Although several

immunotherapies have shown promise in early-phase clinical trials for T1DM, several concerns regarding potential side effects and long-term efficacy when combined with closed-loop systems, particularly the overall effects on metabolism throughout the body and the potentially increased risk of hypoglycemia.[131]

The possible advantages of combination therapies that incorporate immunotherapy and diabetes medications such as SGLT2i or GLP-1RAs within closed-loop systems should also be investigated in future studies. These multidimensional amalgams could provide, either independently or synergistically, a plethora of benefits throughout several systems of the human body, yet the risk of potential adverse effects would be equally increased.[160] Finally, the effectiveness of every novel medicine depends on the patient's acceptance and adherence, thus emphasizing the need for raising T1DM patients' awareness regarding the advantages and disadvantages of immunotherapy in conjunction with closed-loop systems.[161]

Chapter 5. Conclusion

I performed a randomized, controlled, open-label, crossover trial to compare our novel rapid insulin-and-pramlintide hybrid closed-loop system with rapid insulin-and-placebo hybrid closed loop system in adults with type 1 diabetes in an outpatient, free-living, unsupervised setting. Subsequently, the findings suggest that this innovative system may serve as a complementary approach for glycemic management, particularly in individuals with suboptimal baseline control, while highlighting the need for further optimization to enhance patient adherence and satisfaction in real-world settings.

I recruited 23 adults (≥ 18 years of age) with type 1 diabetes to undergo a trial of 58 days in total which is subdivided into two main alternating periods: 29 days of using our hybrid closed loop (HCL) system with insulin-plus-pramlintide, preceded or followed by 29 days of HCL with insulin-plus-placebo. The sequential order of the interventions was random as we used a block-balanced randomization to determine the order for each participant. During both interventions, preprogrammed open-loop basal rates and carbohydrate ratios were updated on day 7 based on the 1st week's glucose control.

Time in target range was similar between the insulin-and-pramlintide group and the insulin-and-placebo group (71.8 (10.0), 69.9 (10.3) respectively, p = 0.21). On the other hand, median time in hypoglycemia (<3.9 mmol/L) was slightly higher for the insulin-and-pramlintide group (2.2% [1.3-3.8] compared to 1.8% [1.1-2.4], p= 0.01). However, this 0.4% difference translates to a 6-minute period over a 24-hour duration, making it clinically non-significant. Hence, although pramlintide's ability to improve insulin's postprandial glucose-lowering effect cannot be undermined, the concomitant slight increase in the risk of hypoglycemia must be considered. Nonetheless, no significant difference regarding the total number of hypoglycemic events was noted between the pramlintide and placebo arm (4.0 [1.0, 7.0]; 2.0 [1.0, 5.0] respectively, p = 0.60).

Total insulin and bolus insulin were greater in insulin + placebo group (54.4 (32.3) U/day; 24.7 (16.8) U/day respectively) than in insulin + pramlintide group (49.0 (28.1) U/day; 19.1 (13.5) U/day respectively) with a p = 0.006 and p = 0.0002 respectively. Thus, pramlintide's ability to

decrease mealtime insulin requirements without compromising glycemic control constitutes an additional benefit in optimizing the practicality of the treatment. Overall, only a few significant findings were noted according to the survey outcomes. In fact, participants using the insulin + placebo hybrid closed loop system had a lower rate of adverse gastrointestinal manifestations compared to insulin + Pramlintide and baseline groups (1.2 (0.2) vs 1.7 (0.5) and 1.5 (0.4), $p = 0.00002$ and $p = 0.004$ respectively). Although having shown promising results in the DTSQ, the insulin and pramlintide group had a lower rate of satisfaction with the use of our dual hormone hybrid closed loop system in comparison to baseline (3.7 (0.7) vs 4.3 (0.6), $p = 0.003$). Nonetheless, the adherence to the system was generally high, with participants completing ~98% of the scheduled interventions.

This study adopted a controlled, crossover design in which each participant served as their own control, and the system was tested in outpatient, free-living, unsupervised settings, providing real-world data on its effectiveness. Major limitations in the study included the relatively small sample size and a study cohort with good glycemic control at baseline (mean HbA1c 7.2%). In addition, over half of participants in the placebo group achieved a TIR >70%, good glycemic control with the use of our system, which may have limited the possibility of detecting a significant difference pertaining to the addition of pramlintide in this particular study population. Future research should focus on larger, more diverse populations and longer trial periods to confirm and expand upon these findings.

The ultimate goal is to have a fully automated closed-loop system that adjusts insulin delivery without requiring user input, especially at mealtimes. Accordingly, I am currently testing our Euglid algorithm in an outpatient randomized, controlled, crossover trial to assess the potential and effectiveness of a fully automated, dual-hormone (insulin-and-pramlintide) delivery system without carbohydrate counting while aiming to regulate glucose levels in adults with type 1 diabetes. I expect that our fully dual hormone closed loop system with no user input will reduce patient burden, maintain accurate glucose control, and ultimately improve both glycemic outcomes and patient satisfaction.

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