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Treatment of type 2 Diabetes through oral delivery of insulin: best outcomes are to come yet?

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Abstract

Type 2 diabetes is responsible for most cases of diabetes and its prevalence is increasing in many countries. Characterized by hyperglycemia, inherited or acquired insulin resistance, loss of insulin function or by quantitative and qualitative changes in insulin secretion. Ultimately, the treatment for a large number of patients with diabetes involves subcutaneous insulin administration, although there is still controversy over when to initiate and intensify insulin therapy. However, this route of administration is often painful, uncomfortable, and inconvenient, as many patients will have to self-administer multiple subcutaneous injections, with a higher risk of hypoglycemia, peripheral hyperinsulinemia, and weight gain. Furthermore, erythema, pruritus, lipodystrophy, are often seen at the injection site.

Although oral insulin administration is an ideal route for patients with diabetes, due to its high simplicity and convenience, several limitations must be overcome, such as the rapid degradation of insulin in the gastric fluid and the low oral bioavailability.

Numerous strategies have been undertaken to improve these limited parameters, such as chemical structure modification, mucoadhesive polymers, addition of absorption promoters and enzyme inhibitors. In addition, nanoparticles are promising systems for the delivery of insulin and bypass several physiological barriers.

Keywords Type 2 diabetes, Oral route delivery, Pathophysiology of Diabetes, Nanotechnology, oral insulin

Resumo

A diabetes tipo 2 é responsável pela maioria dos casos de diabetes e a sua prevalência está a aumentar em muitos países. Caracteriza-se por hiperglicemia, resistência à insulina herdada ou adquirida, defeito na secreção insulínica ou por alterações quantitativas e qualitativas na secreção de insulina. Em última análise, o tratamento para pessoas com diabetes envolve administração subcutânea de insulina, embora, em muitos casos, ainda haja controvérsia sobre quando iniciar e intensificar a terapêutica com insulina. No entanto, essa via de administração pode ser dolorosa, desconfortável e inconveniente, pois a maioria dos pessoas terá que auto administrar várias injeções subcutâneas, com maior risco de hipoglicemia, hiperinsulinemia periférica e aumento de peso. Além disso, no local de injeção é frequente haver eritema, prurido, e lipodistrofia.

A administração de insulina por via oral seria a via ideal para pessoas com diabetes, devido à sua grande simplicidade e conveniência, contudo, várias limitações devem ser superadas, como a rápida degradação da insulina a nível gástrico e a sua baixa biodisponibilidade oral.

Inúmeras estratégias têm sido tentadas para aumentar a absorção da insulina, como a modificação da estrutura química, polímeros mucoadesivos, adição de promotores de absorção e inibidores de enzimas. Além disso, as nanoparticulas constituem sistemas promissores para a administração de insulina, ultrapassando diversas barreiras fisiológicas.

Palavras-chave: Diabetes tipo 2, administração por via oral, fisiopatologia da diabetes, nanotecnologia, insulina oral

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Acronyms

ACE - American College of Endocrinology

ADA- American Diabetes Association

AMI – Acute Myocardial Infarction

COPD- Chronic Obstructive Pulmonary disease

DM – Diabetes Mellitus

DPP-4 - Dipeptidyl peptidase-4

FDA – Food and Drug Administration

FEV1 – Forced Expiratory Volume in the First Second

FVC – Forced Vital Capacity

GFR – Glomerular Filtration Rate

GLP-1 – Glucagon-like peptide-1

GLUT-4 – Glucose transporter type 4

HbA1c – Glycated Hemoglobin

NPH – Neutral Protamine Hagedorn

PLGA- Poly Lactic co-Glycolic Acid

RCT – Randomized Controlled Trial

SGLT2 – Sodium-glucose co-transporter-2

T1D – Type 1 Diabetes Mellitus

T2D – Type 2 Diabetes Mellitus

1. INTRODUCTION

1.1. Pathophysiology of Diabetes

Diabetes Mellitus (DM) is a chronic metabolic condition, with two main forms, Type 1 Diabetes Mellitus (T1D) and Type 2 Diabetes Mellitus (T2D) (Artasensi et al., 2020; Wong et al., 2016).

T1D is characterized by having a rapid onset and typically presents clinically with polyuria, polydipsia, weight loss and ketosis. It is caused by the autoimmune destruction of insulin-producing β-pancreatic cells in the islets of *Langerhans*, mediated by T cells, resulting in an insulin deficiency (Wong et al., 2016).

T2D is responsible for the majority of diabetes cases found in clinical practice (about 90-95%) (Artasensi et al., 2020). It is a complex, chronic, multifactorial and progressive disease, generally characterized by inherited or acquired insulin resistance, loss of insulin function and by quantitative and qualitative changes in insulin secretion (Khan et al., 2020; Wong et al., 2016).

DM affects the quality of life of individuals, leading to significant morbidity and premature mortality. It is diagnosed based on analytical parameters of hyperglycemia and/or elevated hemoglobin glycated (HbA1c), with multiple etiological processes that are at its base. These processes influence the phenotype of diabetes in the presentation and progression of the disease (namely response to pharmacological treatment and microvascular complications, such as nephropathy, retinopathy, peripheral neuropathy and lower limb amputations and macrovascular complications such as coronary heart disease with or without Acute Myocardial Infarction (AMI), stroke and heart failure) (Pearson, 2019; Wong et al., 2016).

T2D is known to be a polygenic and highly heterogeneous disease. Currently, there is a lack of biomarkers to clarify subtypes in the category of T2D (Pearson, 2019). Some studies have stratified patients with diabetes into clusters based on variables (glutamate decarboxylase antibodies, age at diagnosis, BMI, HbA1c, and homoeostatic model assessment estimates of β-cell function and insulin resistance) with different disease progression and risk of

diabetic complications. This stratification can help to adapt and target early treatment to the patients who benefit most from it (Ahlqvist et al., 2018).

On the other hand, it is important to realize that some individuals considered to have T2D, may have in fact, another etiology, namely latent autoimmune type 1 diabetes/latent autoimmune diabetes in adults (LADA) (destruction of β-pancreatic cells by pancreatic auto antibodies) whose incidence demonstrated to be equal regardless of the individual's age (Thomas et al., 2018). In addition, the diagnosis of monogenic diabetes is important through genetic testing as some patients may transition to non-insulin treatment. It is known that only 3% of individuals over 30 years of age have monogenic diabetes, and individuals with a high probability of monogenic diabetes can be selected based on the measurement of pancreatic auto antibodies and C peptide (Wong et al., 2016).

The large-scale genetic sequencing allowed us to realize that although there are etiological variants that can be found, they explain only a small part of the phenotypic variation of T2D, the majority of the genetic variation being explained by multiple common variants (Pearson, 2019; Wong et al., 2016). In the study by Mahajan *et al.* performed in 1 million people, 80 rare low-frequency variants were identified, which in total explained 1.1% of the phenotypic variance. On the other hand, 323 common variants explained 16.3% of the phenotypic variance (Mahajan et al., 2018).

According to McCarthy *et al.,* T2D is the result of defects in multiple etiological pathways: the function of β-pancreatic cells, the action of insulin in the liver, muscle and fat, the secretion / action of glucagon, the secretion / action of incretins and the distribution of fat (Figure 1). For some individuals, the defect in each of these pathways may be subtle but the several affected pathways may be sufficient to result in diabetes. For other individuals, diabetes can result from a major defect in only one or two pathways (McCarthy, 2017).

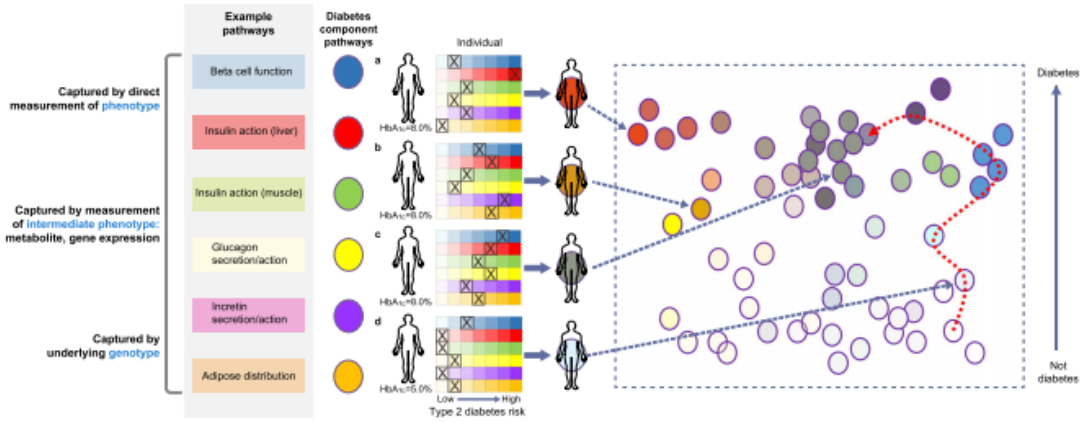


Figure 1. Multiple etiologic pathways of DM2. Adapted from MCCARTHY, 2017

1.2. Type 2 diabetes: prevalence and costs

Diabetes is considered one of the most frequent endocrinological diseases and in many countries, its incidence has increased due to habits inherent to socioeconomic development, namely sedentary lifestyle and increased availability and consumption of processed foods (Khan et al., 2020).

The global prevalence of diabetes in 2019 reached 9.3% (469 million people), in 2021 reached 10,5% (536.6 million people) and is expected to increase in 2030 to 10.2% (578 million people) and in 2045 to 10.9% (700 million people) (Saeedi et al., 2019; Sun et al., 2022; Zhou et al., 2020).

The prevalence is higher in men (10.8%) than in women (10.2%). The prevalence of diabetes is higher in more advanced age groups, with the highest prevalence (24%) being found in the age group from 75 to 79 years (Sun et al., 2022). One in two people with diabetes is unaware of having the disease. The prevalence is higher in urban areas compared to rural areas (12.1% and 8.3%, respectively, in 2021) (Saeedi et al., 2019). The number of people with diabetes living in urban areas is expected to increase to 13.9% as a result of globalization and an aging population.

The prevalence is higher in the middle-income countries and lower in low-income countries. The largest increase in people with diabetes in 2045 is estimated to be in middle-income countries, followed by high and low income countries (21.1%, 12.2% and 11.9%,

respectively). The country with the highest prevalence of diabetes is Pakistan (31%). Due to its high population numbers, the countries with the highest number of patients with diabetes are India, China and the United States (Khan et al., 2020; Sun et al., 2022; Wong et al., 2016).

The cost of treating diabetes is at least 3.2 times higher than the average per capita health expenditure, increasing to 9.4 times when diabetes complications are present. Total diabetes-related costs increased from $ 246 billion in 2007 to $ 673 billion in 2015 and are estimated to reach $ 2.2 trillion in 2030 (Bahman et al., 2019). Diabetes ranks 7th among the main causes of disability and lost years of life (DALYs) (Khan et al., 2020).

Furthermore, the prevalence of impaired glucose tolerance estimated in 2019 was 7.5% (374 million people), and it is expected to reach 8.0% in 2030 (454 million people) and 8.6% in 2045 (548 million people). The number of countries with high-quality studies on the prevalence of impaired fasting glucose is still limited. Nevertheless, this data demonstrate that diabetes control is one of the biggest global health challenges (Saeedi et al., 2019).

2. METHODS AND OBJECTIVES

This Master's Final Work aimed to review the existing literature and bibliography on T2D therapy, with special focus on insulin and its routes of administration. Currently available and under study oral insulin delivery strategies were explored.

The methodology was based on bibliographical research of scientific and review articles in English on the PubMed platform, using the keywords: type 2 diabetes, treatment, types of insulin, insulin administration routes, oral insulin and nanotechnology. Articles identified through the bibliographical references of the initially selected publications were also included.

Articles between 2000 and 2022 were selected, giving special relevance to the most recent articles.

3. TYPE 2 DIABETES THERAPY

Therapeutic objectives depend on the patient's age and his life expectancy, as well as his preferences, comorbidities, duration of diabetes, quality of life and cultural and socioeconomic factors (Landgraf et al., 2019).

Regarding non-pharmacological therapy, all individuals must initiate lifestyle changes, namely, individualized diet and physical activity plan (Forouhi et al., 2018; Taylor et al., 2019).

T2D pharmacological treatment should also be individualized according to the patients' profile, namely comorbidities. If the patient is unable to obtain adequate glycemic control for the therapeutic purpose, there must be therapeutic optimization with other classes of non-insulin antidiabetics or insulin. Again, these options have advantages and disadvantages, so they should be discussed individually, based on the cardiovascular risk profile and/or renal or cardiovascular complications and the patient's tendency towards (ElSayed, 2023; Landgraf et al., 2019).

Metformin improves liver insulin sensitivity, has good efficacy in reducing HbA1c, a known safety profile, low cost, low risk of hypoglycemia and may slightly reduce weight. The daily dose should be adjusted to renal function, being contraindicated when Glomerular Filtration Rate (GFR) <30mL/min, which should be checked every 3 to 6 months. (ElSayed, 2023; Landgraf et al., 2019; Lazarus et al., 2018).

Sulfonylureas (eg: glibenclamide, glipizide, gliclazide, glimepiride and gliquidone) are secretagogues (that stimulate the release of insulin by β-pancreatic cells), being low-cost drugs, having the greatest hypoglycemic potential of all non-insulin antidiabetics, presenting a more severe and lasting risk of hypoglycemia than the hypoglycemia caused by insulin, especially in elderly polymedicated patients. Except for gliclazide and gliquidone, all are contraindicated when GFR <30mL / min (Landgraf et al., 2019). In several retrospective observational studies, sulfonylureas have been shown, alone or in

combination with other drugs, to increase cardiovascular morbidity and mortality. (Azoulay & Suissa, 2017; Madsen et al., 2019; Zhuang et al., 2018).

DPP-4 (Dipeptidyl peptidase-4) is an enzyme responsible for the degradation of GLP-1 (Glucagon-like peptide-1). DPP-4 inhibitors (eg: sitagliptin, vildagliptin and linagliptin) increase the levels of endogenous GLP-1 and have a favorable safety profile with low rates of hypoglycemia and good tolerability, which is particularly important in elderly patients. Except for linagliptin, all DPP-4 inhibitors dosage must be adapted to kidney function (K. Chen et al., 2018).

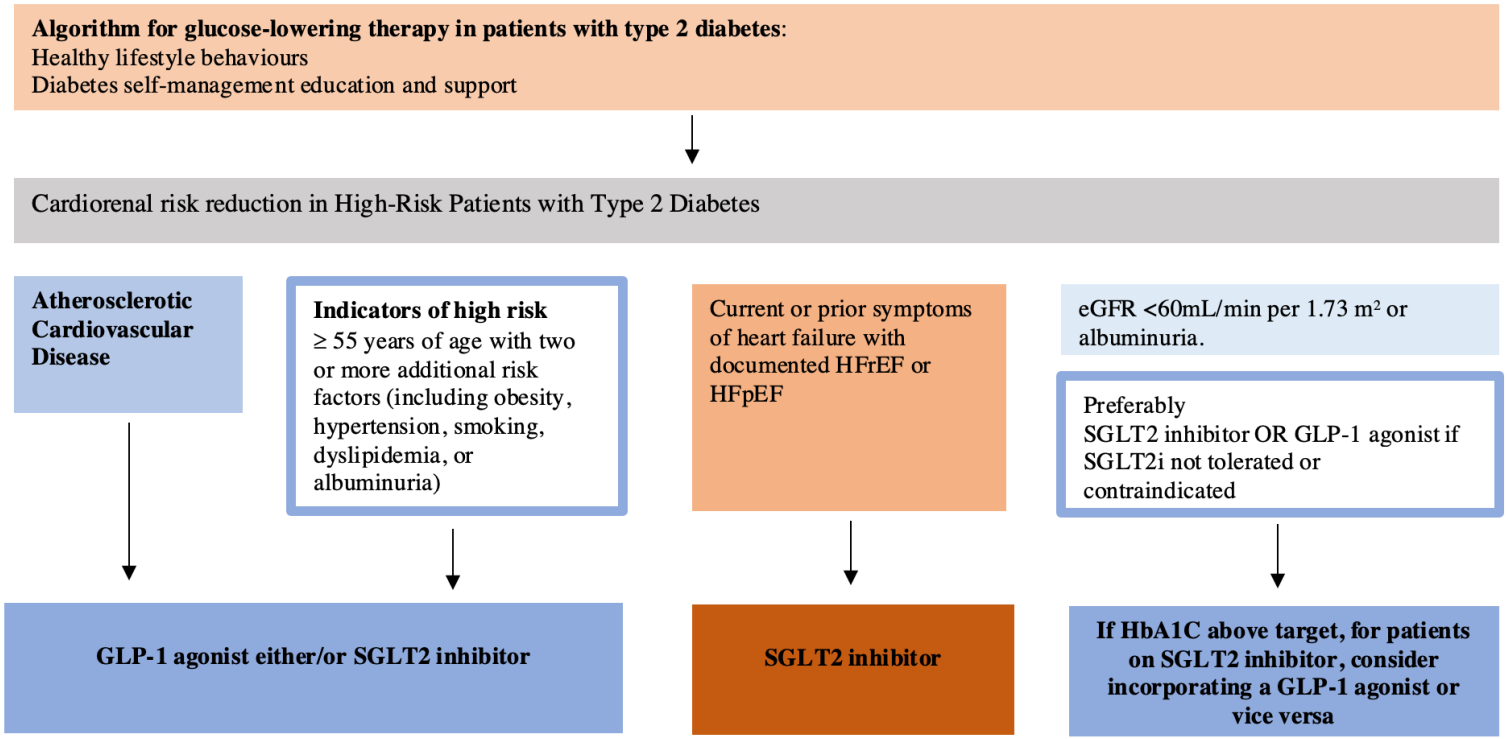
GLP-1 agonist resistant to DPP-4 degradation (eg exenatide, liraglutide, dulagutide and semaglutide) are subcutaneous drugs, associated with a greater mean reduction in plasma glucose, compared to the other non-insulin antidiabetics, presenting a low risk of hypoglycemia and also reducing systolic blood pressure and weight. (Levin et al., 2017). They can be used in combination with other antidiabetics (except DPP-4 inhibitors) and/or insulin and have specific effects of renal and cardiovascular protection (Landgraf et al., 2019). The Randomized Controlled Trial (RCT) LEAD-ER, which followed an average of 9,340 patients for 3.8 years, demonstrated that the combined primary endpoint (death by primary cardiovascular event, non-fatal AMI and non-fatal stroke) was significantly lower for liraglutide compared to placebo (13 vs. 14.9%; HR 0.87; $P < 0.001$ for non-inferiority and $P = 0.01$ for superiority). The same study demonstrated a reduction in the rate of development and progression of renal composite endpoints (development of macroalbuminuria, reduction of GFR or progression to terminal renal failure) (HR 0.78; $P = 0.03$) (Levin et al., 2017). Other clinical study with dulaglutide, randomized, double-blind, held at 371 sites in 24 countries with 9,901 participants have shown beneficial effects on the development and progression of renal and cardiovascular endpoints, although exenatide has not shown differences in cardiovascular mortality compared to the control group (Gerstein et al., 2019). In addition, GLP-1 receptor analogues delay gastric emptying, reducing food intake and promoting weight loss (Artasensi et al., 2020).

The sodium-glucose co-transporter-2 (SGLT2) is responsible for 80-90% of glucose reabsorption in the renal proximal tubules. (Artasensi et al., 2020). SGLT 2 inhibitors (ex: empagliflozin, dapagliflozin, ertugliflozin and canagliflozin) have the therapeutic objective

of inhibiting renal glucose reabsorption, facilitating the loss of about 70g of glucose per day (causing glycosuria). This new class of drugs has a favorable efficacy profile, also because the risk of hypoglycemia is low, lead to weight loss and reduced systolic blood pressure, and can be used as monotherapy or combination therapy. (Sinha & Ghosal, 2019; Zheng et al., 2018).

In addition, SGLT2 inhibitors significantly decrease cardiovascular and renal complications. The RCT EMPA-REG OUTCOME study, published in 2015, demonstrated that patients with T2D and cardiovascular disease who were taking empagliflozin had fewer cardiovascular events (10.5 vs. 12.1%; HR 0.85, P <0.04 for superiority) during an average observation period of 3.1 years compared to placebo. (Zinman et al., 2015). There was a significantly lower rate of cardiovascular mortality (3.7 vs 4.1%; HR 0.62, p <0.001), mortality from all causes (5.7 vs 8.3%; HR 0.68; p <0.001) and hospitalization for heart failure (2.7 vs 4.1%; HR 0.65; p = 0.002). Empagliflozin has also been shown to delay the onset or progression of nephropathy in patients with GFR> 30mL / min compared to standard therapy (12.7 vs. 18.8%; HR 0.61; p <0.001) (Zinman et al., 2015). In an analysis of the effects over a 164 week period with 7,020 participants, empagliflozin led to an average reduction of microalbuminuria of 22% and macroalbuminuria of 29%, regardless of the initial albuminuria level. (Cherney et al., 2017). Other clinical studies with canagliflozin and dapagliflozin have shown beneficial effects on the development and progression of renal and cardiovascular endpoints (Ferrannini et al., 2016; Sattar et al., 2016). Thus, SGLT2 inhibitors are often used in patients with established cardiovascular or kidney disease. (Landgraf et al., 2019).

In figure 2, it is possible to verify the algorithm currently used for the reduction of glycemia in T2D. SGLT-2 inhibitor or a GLP-1 agonist are the first choice in patients with kidney, cardiovascular disease or high cardiovascular risk. A review including 764 studies and 421,346 individuals, showed that SGLT-2 inhibitors and GLP-1 agonists reduced all-cause mortality, cardiovascular mortality, non-fatal AMI and renal failure, with high scientific evidence. (ElSayed, 2023; Palmer et al., 2021) . The therapeutic options must always be discussed according to the co-morbidities and preferences of each patient.


Algorithm for glucose-lowering therapy in patients with type 2 diabetes:
Healthy lifestyle behaviours
Diabetes self-management education and support
Cardiorenal risk reduction in High-Risk Patients with Type 2 Diabetes
Atherosclerotic Cardiovascular Disease
Indicators of high risk
≥ 55 years of age with two or more additional risk factors (including obesity, hypertension, smoking, dyslipidemia, or albuminuria)
Current or prior symptoms of heart failure with documented HFrEF or HFpEF
eGFR <60mL/min per 1.73 m² or albuminuria.
Preferably
SGLT2 inhibitor OR GLP-1 agonist if SGLT2i not tolerated or contraindicated
GLP-1 agonist either/or SGLT2 inhibitor
SGLT2 inhibitor
If HbA1C above target, for patients on SGLT2 inhibitor, consider incorporating a GLP-1 agonist or vice versa

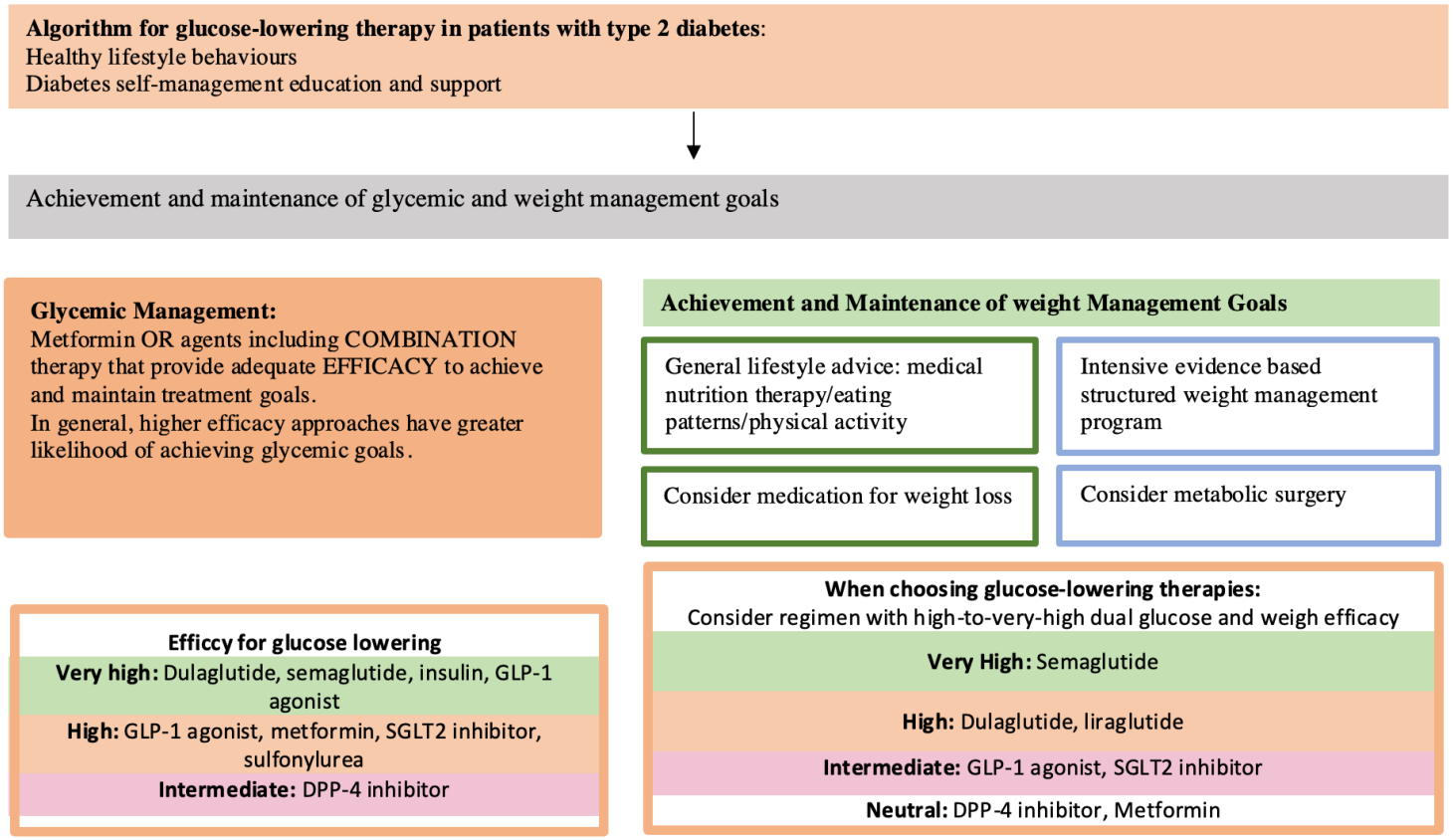


Figure 2. Algorithm of glucose-lowering therapy in patients with T2D. Adapted from ElSayed et al., 2023. HFrEF – Heart Failure with Reduced Ejection Fraction; HFpEF – Heart Failure with Preserved Ejection Fraction

4. INSULIN

4.1. Insulin therapy in type 2 diabetes

Insulin is an anabolic polypeptide hormone, produced in pancreatic β-cells through the processing of its precursor, proinsulin (Bahman et al., 2019). It is responsible for the uptake of glucose from the blood to the main organs of the human body, namely, the liver, skeletal muscle and adipose tissue. It also facilitates the conversion of glucose into glycogen, as a form of storage in the liver (Wong et al., 2016). The action of insulin in transporting glucose into cells is mediated by some transporters, the most well-known being glucose transporter type 4 (GLUT-4). Under normal conditions, this transporter is at the cytoplasmic level, but the binding of insulin to the cell membrane receptor stimulates its translocation to the membrane, which allows glucose to enter the cell (Wong et al., 2016).

In T2D, insulin should be administered when it is not possible to obtain good glycemic control, despite diet, physical activity and therapy with non-insulin antidiabetics for 3 to 6 months, when the initial HbA1c is very high and in the presence of symptoms of insufficient insulin levels, such as weight loss or after acute decompensation (ketoacidosis, hyperosmolar state), or when non-insulin antidiabetic drugs are contraindicated (Aschner, 2020; Russell-Jones et al., 2018). According to the guidelines of the American College of Endocrinology (ACE), insulin can be administered in combination with metformin as initial therapy when the initial HbA1c is greater than 9% or in combination with two non-insulin antidiabetic drugs when the HbA1c is greater than 8% (Thrasher, 2017).

It should be noted that, although there is still some controversy as to when to start and intensify insulin therapy, when it is necessary, its introduction should not be delayed. (Aschner, 2020; Russell-Jones et al., 2018). Adequate glycemic control reduces micro and macrovascular complications of T2D, however, there is some inertia in the introduction of insulin in individuals with these plasma glucose levels above the therapeutic goal for years, who have never taken it, due to the fear of causing hypoglycemia or weight gain, or because

the patient does not adhere to therapy (Aschner, 2020; Evans et al., 2021; Russell-Jones et al., 2018). A retrospective study of more than 80,000 people in the United States showed that the mean time of intensification of insulin treatment was 7.1, 6.1 or 6.0 years, respectively, for patients taking 1, 2 or 3 pharmacological treatments orally (Thrasher, 2017). The longer the T2D follow-up time, the more difficult it is to control hyperglycemia, so many patients need to intensify their insulin treatment in order to prolong their lifetime (Krzymien & Ladyzynski, 2019).

The need for insulin in T2D is variable, being reduced by physical activity (which reduces insulin resistance) and by renal failure (which reduces insulin elimination). On the other hand, it is increased by sedentary lifestyle and stress (which induces the secretion of diabetogenic hormones, such as cortisol) (Wong et al., 2016).

Insulin therapy can be easily combined with other antidiabetics, including GLP-1 agonists. The large number of types of insulin facilitates the individualization of therapy (table 1) (Landgraf et al., 2019; Wong et al., 2016).

Basal insulin analogs (detemir, glargine and degludec) are administered once daily, reaching a slight peak after 2 to 3 hours after administration, with duration of action ranging between 16 and 42 hours, thus being associated with postprandial hyperglycemia, when used alone (ASCHNER, 2020). They cause less hypoglycemia than intermediate-acting insulin NPH (Neutral Protamine Hagedorn) (duration of action 10 to 18h). NPH is administered once or twice daily to cover basal requirements (Landgraf et al., 2019).

The combination of long-acting insulin with GLP1 agonists has advantages over intensive therapy with basal and prandial insulin, in terms of adherence to therapy, hypoglycemia rate and weight gain (Landgraf et al., 2019).

The rapid-acting insulin analogs (Lispro, Aspart and Glulisine) show an onset of action from 15 to 30 minutes after administration, a peak of action between 30 and 90 minutes and an action duration of 2 to 5 hours. (Aschner, 2020; Bahman et al., 2019; Landgraf et al., 2019).

Ultrafast-acting insulin aspart (faster insulin aspart – FiAsp®) and ultrafast-acting insulin lispro (Lyumjev®) are a new formulations of these insulins (Davis et al., 2019; Kruger & Novak, 2019).

Short-acting insulins, such as regular/neutral insulin, have an onset of action between 30 to 60 minutes and action duration of 6 to 8 hours, with a peak between 2 to 4 hours (Bahman et al., 2019).

Table 1. Human insulin analogs on the market. Adapted from Landgraf et al., 2019 and Wong et al., 2016.

Insulin Formulation	Onset (min-hrs)	Duration (hrs)	Chemical Structure
Long-acting Insulin			
Detemir	2h	16-42 h	LysB29 (Nε-tetradecanoyl) des(B30)
Glargine			Gly A21, Arg B31, Arg B32
Degludec			$C_{274}H_{411}N_{65}O_{81}S_6$
Intermediate-acting Insulin			
Neutral Protamine Hagedorn	1-2 h	10-18 h	Proline at position 29 in the B-chain is replaced by lysine
Rapid-acting Insulin			
Lispro	15-30 min	2-5 h	28(B)-L-lysine-29(B)-L-proline-human insulin
Aspart			AspB28
Glulisine			3(B)-Lys, 29(B)-Glu-human insulin
Short-acting Insulin			
Regular insulin	0.5-1 h	6-8 h	51 amino acids arranged in two chains, an A chain (21 amino acids) and B chain (30 amino acids) that are linked by two disulfide bonds

In T2D, insulin therapy is usually directed to the control of fasting plasma glucose, which has a greater influence on the control of HBA1c than post-prandial plasma glucose. According to the American Diabetes Association (ADA), it starts with basal insulin (glargine, detemir or degludec) in a dose of 10 U or 0.1-0.2 U/kg of body weight. Subsequently, the insulin dose is adjusted based on fasting plasma glucose, which may be increased once or twice a week by 10 to 15% or 2 to 4U or reduced by 4 U or 10 to 20%. When the therapeutic objective is not reached, rapid-acting insulin can be administered before the main meal, in a dose of 4U or 0.1 U/kg or 10% of the basal dose insulin. (Evans et al., 2021; Krzymien & Ladyzynski, 2019). Figure 3 summarizes the ADA guidelines for the introduction of insulin in T2D (Howard-Thompson et al., 2018).

In the Yki et al. study (2006) performed on 110 patients with T2D with a mean HbA1c value of 9.5%, treated with one or two non-insulin antidiabetics, it was found that after 36 weeks administration of insulin glargine and metformin, there was a significant improvement metabolic control reducing fasting plasma glucose, and in the last 12 weeks the average HbA1c value was 7.14% (Yki-Järvinen et al., 2006).

The subcutaneous administration of insulin has been widely used in the last 9 decades to control diabetes, due to its high bioavailability, and it is currently the preferred method (Bahman et al., 2019; Harjoh et al., 2020). The traditional administration of insulin is through multiple subcutaneous injections (usually 3 to 4 daily injections, for those patients in intensive therapy, with eventual dose adjustment along with self-monitoring of blood glucose, often leading to an improvement in metabolic control), but with a higher risk of hypoglycemia, peripheral hyperinsulinemia and weight gain, implying subcutaneous injections that can be painful, inconvenient and uncomfortable, which make patient compliance difficult and can cause erythema, pruritus, lipodystrophy associated with the insulin administration site, risk of injuries associated with the use of needles and skin infection by *Staphylococcus aureus* and *Mycobacterium chelonae* (Harjoh et al., 2020; Wong et al., 2018; Zhou et al., 2020). In addition, some patients have fear or psychological stress associated with the use of needles (Wong et al., 2016).

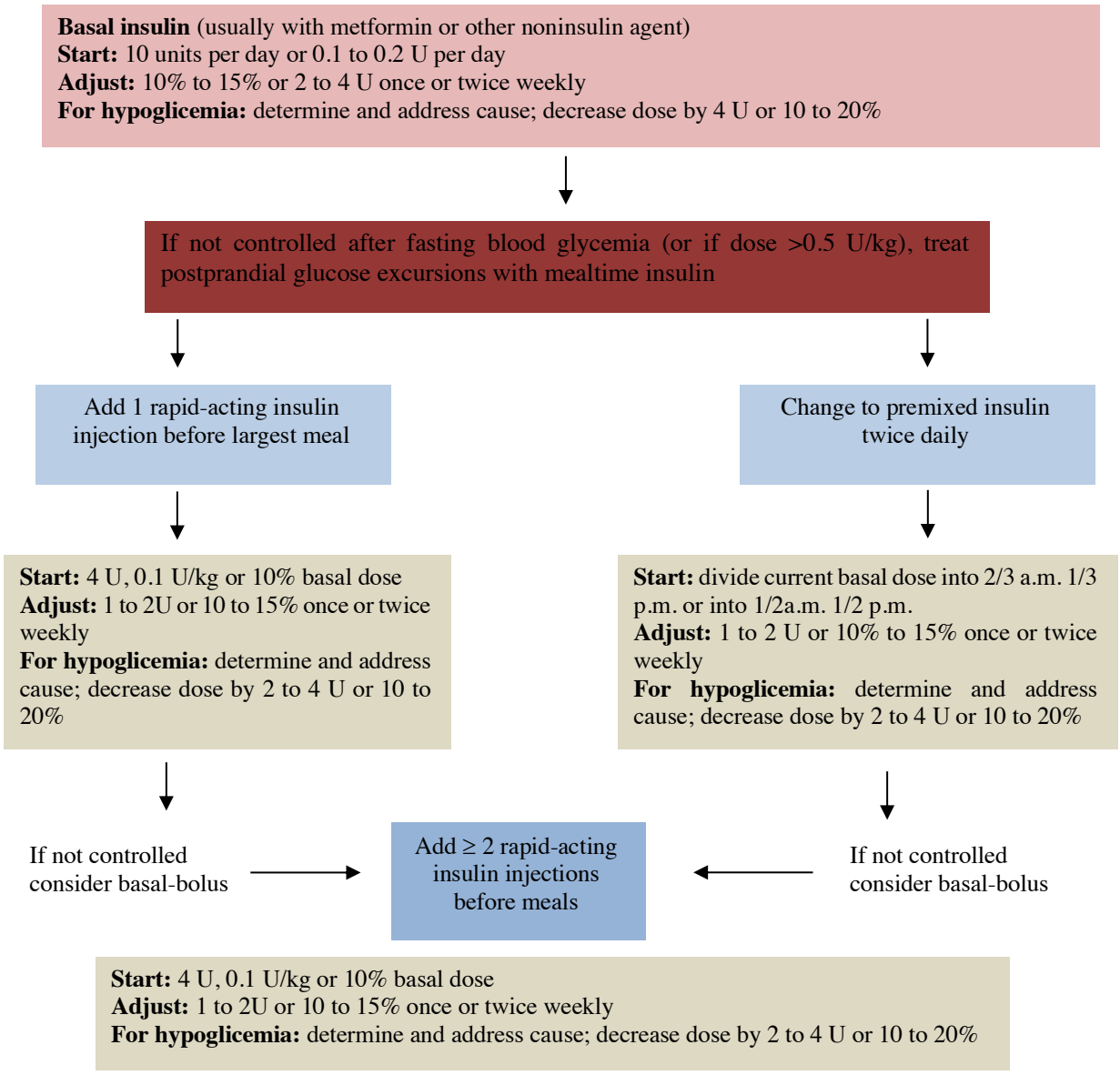


Figure 3. Starting insulin in patients with T2D based on ADA.

Insulin can also be administered through a subcutaneous continuous infusion pump (CSII), with an external device connected to a catheter/cannula that delivers a continuous basal insulin dose and bolus insulin doses after at meals (Bahman et al., 2019). The devices that integrate with continuous glucose monitoring have predictive suspension of insulin release before hypoglycemia. Some authors report that better glycemic control can be achieved by CSII pumps alone (Wong et al., 2016). Moreover, it was associated with a reduction in the number and severity of hypoglycemia, especially at night, as well as an increase in the

quality of life of the patients (Zhou et al., 2020). However, it has higher costs compared to daily subcutaneous injections and if the infusion ends by mechanical failure, it may be associated with ketoacidosis (Bahman et al., 2019). Nevertheless, in developed countries, eventually related to education delivered to patients using CSII, less ketoacidosis episodes were observed in patients using CSII compared to those using multiple daily injections (Figueiredo et al., 2022). Currently, physicians should strongly consider the use of CSII systems in patients with diabetes who would benefit from this technological option, given the associated improvements in glycemic control and measures of quality of life. Additionally, studies suggest long-term cost savings for healthcare systems that use these systems (Phillip et al., 2022). Although rare, it can also occur skin infections, erythema and abscesses due to colonization with microorganisms, such as *Staphylococcus* or *Streptococcus*, due to the susceptibility of microorganisms proliferation in the catheter (Wong et al., 2016).

Thus, the insulin administration route should facilitate the adherence to the long-term therapy and allow to adjust the insulin release according to the concentration of glucose in the blood, thus being able to reduce the multiple side effects of diabetes. (Zhou et al., 2020).

4.2. Endogenous insulin and drug mimicry

It should be noted that endogenous insulin directly suppresses the hepatic production of glucose, due to its binding to its hepatic receptor, but it can also act indirectly on non-hepatic tissues, for example, through the lipolysis suppression (Edgerton et al., 2019).

The liver is the main source of circulating glucose during fasting and, after a meal, the main storage place for glucose. However, in individuals with diabetes, the peripheral hyperinsulinemia that results from the subcutaneous injection of insulin leads to pharmacokinetics and pharmacodynamics that do not replicate the endogenous release, being associated with metabolic defects that include abnormal glucose metabolism, at the hepatic and muscular levels, which can lead to weight gain, hypertension, peripheral edema and cardiovascular diseases. Peripheral hyperinsulinemia can cause insulin

resistance, hypersensitivity immunogenicity and hypoglycemia (Edgerton et al., 2019; Wong et al., 2016). This happens because, in the subcutaneous administration of insulin, only 20% of it is released into the portal hepatic circulation, while the remainder is diffused into the peripheral circulation, compromising the mimicry of the physiological action of insulin in the liver. A large part of this insulin reach to the kidneys, which are able to remove 50% of peripheral insulin (Wong et al., 2016).

Alternatives under development are hepato-preferential insulin analogues, which aim to mimic the effects of endogenous insulin secretion, leading to advantages of the portal vein absorption pathway (Edgerton et al., 2019).

In a study by Edgerton *et al* (2019) in canines, due to the similarity in insulin-regulated glucose metabolism between humans and canines, basal insulin levels in hepatic sinusoids were 2-3 times higher than arterial levels, due to endogenous insulin secretion. In conditions of postprandial hyperglycemia, when insulin was administered through the hepatic portal vein, glucose uptake was divided between liver and muscle similarly to basal insulin values, with levels increasing to 400±41 pM and 125±14 pM, respectively, within two hours after glucose administration as a meal simulator. Thus, glucose uptake was evenly distributed across liver and muscle (44±9% vs 46±9%, respectively) (Edgerton et al., 2019).

In contrast, when the same dose of insulin was administered into a peripheral leg vein, the percentage of glucose taken up by the muscle was 2 times greater (276±9pM) than that by the liver, with glucose uptake by the liver being about half that when insulin was administered by the hepatic portal vein (226±7pM). Thus, when insulin was administered peripherally, muscle absorbed 3 to 4 times more glucose than liver (73%±5% vs. 21%±4%, muscle vs. liver), overexposing muscle and other non-hepatic tissues to the action of insulin (Edgerton et al., 2019).

After insulin administration trough the portal vein, it rapidly and completely suppressed endogenous glucose production (50% in 30 minutes and 100% in 90 minutes), compared to the delayed effect when insulin was administered into a leg vein (50% in 60 minutes and more than 120 minutes to get complete suppression) (Edgerton et al., 2019).

This study also quantified the effect of a peripherally administered insulin prototype, insulin-327 (a compound that is acylated with a 22-carbon fatty acid to promote a strong but reversible binding to plasma albumin), designed to be hepato-preferential, because the ability of insulin-327 to cross the muscles rigid endothelial capillary barrier is limited, whereas it can cross the fenestrated hepatic sinusoids (Edgerton et al., 2019).

In order to verify the distribution and uptake of glucose by the liver and muscle, under simulated meal conditions, with glucose infusion in the portal vein and administration of insulin-327 peripherally at a variable rate, it was found that arterial and hepatic insulin-327 levels were similar, as well as glucagon levels. Insulin-327 suppressed endogenous glucose production similarly to insulin administered into the portal vein. Glucose uptake was uniform between liver and muscle (45%±6% vs. 44%±6%) (Edgerton et al., 2019). However, one of the concerns is related to the possible lack of suppression of lipolysis in adipose tissue, which could lead to an accumulation of lipids in the liver (Edgerton et al., 2019).

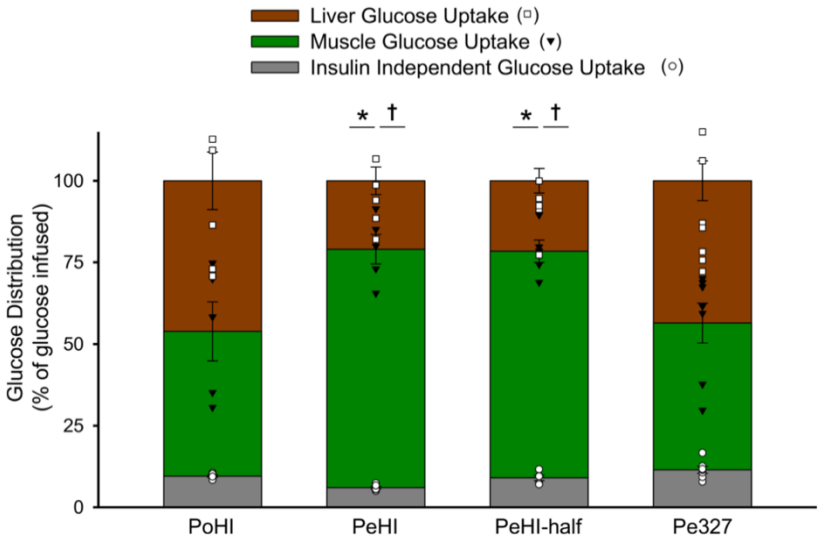


Figure 4. Glucose distribution during the experimental period in overningt-fasted conscious dogs in the portal vein insulin (PoHI), peripheral vein insulin (PeHI), peripheral vein insulin half dose (PeHI-Half), and peripheral vein insulin-327 (Pe327). Adapted from EDGERTON; SCOTT; FARMER; WILLIAMS *et al.*, 2019.

Thus, this study indicates that when regular insulin is administered peripherally, it is not possible to normalize the distribution of insulin between the liver and muscle, since when administering a larger amount of peripheral insulin to increase its liver availability, it significantly increases the risk of hypoglycaemia, as well as being a risk factor for insulin resistance, weight gain, atherosclerosis, hypertension, and long-term micro- and

macrovascular complications. On the other hand, hepatic insulin deficiency results in excessive production of glucose, the main contributor to hyperglycemia in individuals with T2D (Edgerton et al., 2019).

4.2.1. Other routes of insulin administration

There is a need to explore the non-invasive insulin delivery system in diabetes therapy. Several strategies have emerged, such as the transdermal route, the inhalation route, the intranasal route and the oral route (Zhou et al., 2020). However, several of these strategies were not successful, due to only a small portion of the insulin achieving the liver to have a physiological effect, in addition to the potential cilia-eliminating effect, undetermined biological safety and inconvenient management (Wong et al., 2016).

In the inhalation route, patients with diabetes have been shown to have reduced lung function with lower Forced Expiratory Volume in the First Second (FEV1) and Forced Vital Capacity (FVC). Compared with the oral insulin administration route, the inhalation route has a relatively faster onset of action due to the systemic absorption of the drug by the pulmonary alveolus, which are well perfused (Bahman et al., 2019; Wong et al., 2016). In 2006, a recombinant human insulin inhalation powder (Exubera®) was approved by the Food and Drug Administration (FDA) for adults and children over 6 years of age, with onset of action within 10 to 15 minutes. However, this inhaler was withdrawn from the market 1 year later, due to its high price, being very bulky and because the absorption of inhaled insulin varied significantly, especially in obese patients, smokers, patients with asthma and Chronic Obstructive Pulmonary Disease (COPD), with alveolar surface area reduced and altered functional structure (Skyler et al., 2008).

Another insulin human inhalation powder (Afrezza®) was approved by FDA in 2014, being a dry powder formulation of recombinant human insulin adsorbed on microparticles of fumaryl diketopiperazine, which are excreted in the urine (Hoogwerf et al., 2021). It has the advantages of being less bulky than the previous one and insulin absorption is not altered in patients who smoke or have COPD. However, it is dependent on correct inhalation technique for optimal effect. This product is intended to be administered before a meal, with an onset of action of 15 minutes, being absorbed more quickly than fast acting

subcutaneous analogues, mitigating postprandial hyperglycemia, and preventing late hypoglycemia (Neumiller et al., 2010; Rendell, 2014). A study by Hoogwerf *et al.* for 24 weeks in 309 subjects with T2D, of which 150 patients were treated with insulin human inhalation powder (Afrezza®) and 154 patients were treated with insulin Aspart, with the HbA1c decreasing to 7.9% (1.3%) and to 7.7% (1.1%), respectively, with insulin human inhalation powder (Afrezza®) and Aspart insulin. Individuals who were treated with insulin human inhalation powder (Afrezza®) insulin decreased by an average of 0.78 kg and those who were treated with Aspart insulin increased by 0.23 kg. The incidence of mild/moderate hypoglycemia was lower in subjects who were treated with insulin human inhalation powder (Afrezza®), although the difference was not statistically significant. These data may support the treatment with insulin human inhalation powder (Afrezza®) in individuals with T2D (Hoogwerf et al., 2021).

Non-invasive transdermal route is an alternative with several advantages, such as the high body surface area for drug absorption, ease of application, allowing repositioning, removal and replacement whenever necessary, and the enzymatic degradation of insulin in the gastrointestinal tract is minimized (Harjoh et al., 2020).

Passive skin penetration methods have chemical absorption promoters that require the penetrating agent to have an octanol:water partition coefficient of 1 to 3 and a molecular weight of less than 500Da, and the drug absorption time may be hours. Moreover, this approach is not possible for high molecular weight drugs, such as insulin. The physical modulation of the skin barrier, through active technology, such as microneedles, iontophoresis, ultrasound and electroporation allows the permeation of hydrophilic and macromolecular drugs and a fast action. Disadvantages are related to the high cost of disposable microneedles, the increased current intensity in iontophoresis which can lead to skin burns, necrosis, blisters and erythema. Electroporation, ultrasound and laser cause an increase in local temperature, presenting risks of skin burns. Electroporation causes muscle contraction and itching, tingling and pain. Multiple administrations of photomechanical waves can cause cell damage, which is not suitable for a frequently administered drug, such as insulin (Harjoh et al., 2020).

As for the administration of insulin intranasally, the advantages are related to the fact that the nasal cavity presents a mucosa with a high surface area for absorption, very vascularized, which can promote the absorption of insulin directly into the systemic circulation, bypassing thus the effect of the first hepatic pass. Disadvantages are that intranasal insulin absorption may be delayed by the difficulty of insulin penetration through mucus and by its enzymatic degradation. The use of insulin-loaded liposomes has been shown to potentiate intranasal insulin absorption in rabbits, with blood glucose levels being reduced for 8h, with insulin bioavailability greater than 13% (Bahman et al., 2019).

As for the buccal route of administration, oral insulin spray (Oral Lyn®) is an insulin spray for absorption from the oral mucosa, which has been approved in Ecuador and Lebanon, but which is still in phase III clinical trials in Europe, Canada and the USA (Easa et al., 2019; Heinemann & Jacques, 2009).

The oral administration of insulin can have several advantages, due to its greater simplicity and convenience, absence of pain and skin infections, increasing adherence to therapy, improving insulin levels in the portal vein and avoiding side effects of subcutaneous insulin such as increased weight, hypoglycemia and peripheral hyperinsulinemia. In addition, the oral route would potentially promote the initiation of treatment with insulin earlier in patients with severe hyperglycemia at an early stage, with good adherence to therapy and managing to delay the progression of the disease (Bahman et al., 2019; Wong et al., 2018; Wong et al., 2016).

However, some obstacles in this route of administration must be overcome before bioactive peptides and proteins can be administered via this route, such as product stability, gastrointestinal stability and adequate absorption (Zhou et al., 2020).

The structure and conformation of insulin must be kept intact in the gastrointestinal tract, until reaching the systemic circulation and the insulin transporter must have a good adhesion capacity, in order to prolong the time in the intestine (Wong et al., 2016; Zhou et al., 2020). However, insulin is a protein drug that has low oral bioavailability, due to its susceptibility to enzymatic proteolysis by gastrointestinal enzymes (the main ones being pepsin in the stomach and trypsin, chymotrypsin and carboxypeptidase in the intestine), to its high weight molecular weight (from 5800 daltons), and its low rate of diffusion through

the mucosa of the gastrointestinal tract, which is hampered by the passage of hydrophilic insulin through the outer hydrophobic and hydrophilic inner membrane of the mucosa of the gastrointestinal tract. Transcellular uptake in the gastrointestinal tract can be mediated by simple diffusion or by transport mediated by transporter proteins. In the case of insulin, its absorption depends on the paracellular pathway, which is restricted by *thight junctions* (Wong et al., 2018; Wong et al., 2016). Another barrier is the mucus layer secreted by caliciform cells to capture pathogens and other particles, protecting the exposed epithelial surface (Wong et al., 2016).

As for the absorption site, the enzymatic activity of trypsin and chymotrypsin is reduced from the duodenum to the jejunum and by only 1/3 in the ileum, which may be the ideal absorption site. Colonic absorption has the advantage of longer retention time near the epithelial surface, reduced expression of P-glycoprotein, better response to absorption promoters and reduced concentration of proteolytic enzymes, compared to the jejunum and ileum, in addition to potentially prevent erratic absorption and bioavailability in the small intestine (Wong et al., 2016).

5. STRATEGIES FOR ORAL INSULIN ADMINISTRATION

5.1. Strategies to improve the absorption of oral proteins

Calcitonin is a polypeptide hormone secreted by the thyroid gland to reduce blood calcium levels, inhibiting bone resorption, and increasing urinary calcium excretion. Oral administration has been achieved by formulating an acid-resistant enteric coating that prevents dissolution in the stomach. Citric acid is added to the tablet to inhibit intestinal proteases and increase paracellular transport. This type of approach can also facilitate oral insulin absorption (Wong et al., 2016).

Table 2. Strategies to improve the absorption of oral proteins and peptide drugs.

	Examples	Advantage	Limitation	References
Enzyme inhibitors	Sodium glycocholate, camostat mesylate, bacitracin, soybean trypsin inhibitors and aprotinin duck and chicken ovomucoid carboxymethylcellulose elastin inhibitor (CMC-ELA)	Increases insulin stability, protecting it against proteases	Protein malabsorption	(Bahman et al., 2019; Meneguin et al., 2021; Wong et al., 2016) (Agarwal & Khan, 2001) (Bahman et al., 2019)
Absorption enhancers	Bile salts, surfactants, calcium ion chelators	Increased permeability across the	Systemic toxicity and impairment of	(Lee et al., 2016;

	and fatty acids (palmitic acid) Chitosan and thiolated polymers ZOT	intestinal membrane	the integrity of the intestinal mucosa	Meneguin et al., 2018) (Ahn et al., 2013; Li et al., 2013) (Lee et al., 2016)
Chemical modification	Conjugation with transferrin Polymer P(MAA-g-EG)	Binders that facilitate insulin absorption	Delayed hypoglycemic effect	(Xia et al., 2000) (Kavimandan et al., 2006; Shofner et al., 2010)
Mucoadhesive polymers	Chitosan and PLGA Thiolated groups	Closer contact with the intestinal mucosa for a prolonged time	Insulin more susceptible to enzymatic degradation	(Chopra et al., 2006; Wong et al., 2016) (Zhou et al., 2020)

ZOT - Toxin secreted by vibrio cholera

PLGA - poly lactic co-glycolic acid

In order to improve the absorption of oral proteins and peptide drugs, strategies including chemical structure modification, mucoadhesive polymers, addition of an absorption promoters and enzyme inhibitors have been widely used (table 2) (Zhou et al., 2020).

Enzyme inhibitors increase insulin stability in the stomach and small intestine. In the study by Yamamoto *et al.,* in healthy rats, absorption in the colon was improved by enzyme inhibitors sodium glycocholate, camostat mesylate, bacitracin, soybean trypsin

inhibitors and aprotinin (Bahman et al., 2019; Meneguin et al., 2021; Wong et al., 2016). Another study showed that ovomucoid, which is isolated from duck and chicken egg whites, demonstrated 100% *in vitro* insulin protection against trypsin and alpha-chymotrypsin. With a gastrointestinal enzyme ovomucoid ratio of 1:2, it was shown that insulin degradation was delayed 1h (Agarwal & Khan, 2001). The carboxymethylcellulose elastin inhibitor (CMC-Ela) has been shown to protect insulin from elastase, with almost 33% of insulin remaining stable after 4h of incubation. However, the high concentration of enzyme inhibitors can cause deficient levels of gastrointestinal enzymes, with consequent protein malabsorption (Bahman et al., 2019). It is important to mention that systemic absorption of enzyme inhibitors can lead to systemic toxicity (Rekha & Sharma, 2013).

Some examples of absorption enhancers are bile salts, surfactants, calcium ion chelators and fatty acids (palmitic acid). They can modulate the structure of the cell membrane (transcellular absorption) or alter the *tight junctions* between cells of the intestinal epithelium, which restrict the paracellular absorption of hydrophilic macromolecules (Lee et al., 2016; Meneguin et al., 2018; Meneguin et al., 2021). Chitosan and thiolated polymers act on actin filaments and have the ability to transiently enlarge *tight junctions*, increasing the permeability of macromolecules such as proteins, also presenting mucoadhesive properties, increasing the residence time in the intestine (Ahn et al., 2013). Li *et al.* demonstrated increased relative bioavailability in oral insulin administration (50 IU/kg) using nanoparticles with chitosan cores, with good protein-trapping capacity and favorable epithelial permeability (Li et al., 2013). ZOT (toxin secreted by vibrio cholera) demonstrated a 10-fold increase in oral insulin absorption in the ileum and jejunum, without compromising the integrity of the gastrointestinal tract or causing hypoglycemia (Wong et al., 2016).

In the study by Lee *et al.* a nanocarrier with active dual ligand of chitosan and a peptide derived from ZOT (PEP-Chi-NC) was developed, having shown a synergistic effect *in vivo* in diabetic rats, with blood glucose levels decreasing up to 9 hours after oral administration of PEP -Chi-NC and remained stable until 12 hours. After 24 hours of oral administration of PEP-Chi-NC, blood glucose levels were 80% of the initial blood glucose.

In contrast, when chitosan and the ZOT-derived peptide were used alone, the blood glucose reduction was only 20% during 3h after oral administration, followed by a gradual increase in blood glucose (Lee et al., 2016).

As for the limitations of this approach, the integrity of membrane cells and *tight junctions* can be compromised by the absorption promoter, which can cause infection due to the passage of toxins and intestinal flora to the systemic circulation (Meneguin et al., 2021). Bile salts, surfactants, sodium dodecyl sulfate and lysolecithin showed acute local damage to the intestinal wall, compromising its viability. Thus, most absorption enhancers are only suitable for short-term oral administration, and their long-term administration can cause irreversible damage to the gastrointestinal tract (Wong et al., 2016; Zhou et al., 2020).

Another technique consists of chemical modification through the conjugation of insulin with ligands that facilitate its absorption, such as transferrin, over a disulfide bond. Transport of the insulin-transferrin conjugate is accomplished by enterocyte-like Caco-2 cells by receptor-mediated transcytosis. Transferrin has been shown to be resistant to digestion by trypsin and chymotrypsin (Xia et al., 2000). Kavimandan *et al.,* developed a system for the administration of insulin-transferrin conjugates through hydrogels complexation, and these microparticles, namely the polymer P(MAA-g-EG), increased the permeability of the conjugate in vitro, probably by additionally induce dilations in *tight junctions* (Kavimandan et al., 2006). The study of Shofner *et al.*, performed *in vitro*, found that insulin transport was increased 7-fold when conjugated with transferrin and 14-fold when P(MAA-g-EG) microparticles were present, concluding that the presence of the microparticles in solution improves the transport of the conjugate by almost 100%, with little or no change in the integrity of the cell monolayer (Shofner et al., 2010). However, after modifying the protein's structure, its biological activity may be reduced and/or its pharmacokinetics may be altered (Zhou et al., 2020).

Mucoadhesive polymers can be used to encapsulate insulin, adhering to gastrointestinal mucus. Some nanoparticle formulations used chitosan and poly lactic co-glycolic acid (PLGA) to protect insulin from enzymatic attack by trypsin, chymotrypsin and elastase (Chopra et al., 2006; Wong et al., 2016). Other nanoparticle formulations used a

mucoadhesive polymer (thiol groups) and sulfhydryl-modified nanoparticles have the ability to prevent enzymatic degradation, as well as promote carrier adhesion (Zhou et al., 2020).

5.2. Insulin micro and/or nanoencapsulation

Insulin micro and/or nanoencapsulation is a strategy to increase the oral bioavailability of insulin, which can protect insulin against premature enzymatic degradation and against acidic Ph, improve permeability in the intestinal mucosa, control release kinetics and stabilize insulin slightly soluble (Ansari, 2015; Bahman et al., 2019; Meneguin et al., 2021).

Nanoparticles are nanospheres in which insulin is evenly distributed within the polymer matrix or nanocapsules in which insulin is found within the membrane polymers. (Bahman et al., 2019). Table 3 shows different types of nanoparticles developed for the oral administration of insulin (Bahman et al., 2019).

Table 3. Different types of nanoparticles developed for the oral administration of insulin. Adapted from BAHMAN; GREISH; TAURIN, 2019.

Nano-Carrier	**Components**	**Size (nm)**	**Route of Administration**	**Dose (I.U./kg) (min/max)**
Chitosan-insulin	Carboxylated chitosan + Methyl Methacrylate	251 to 319	Oral	15 (min)-100 (max)
	Polysaccharide chitosan nanoparticles (CS-NPs)	265-3 ± 34 to 387.4 ± 35.6	Oral	21 (max)
	Chitosan + oleic acid + Plurol oleique + Labrasol	108	Oral	50
	Chitosan + Alginate	748	Oral	25 (min)
				100 (max)
	Chitosan + Dextran sulfate	527	Oral	25 (min)
				100 (max)

	Chitosan + TPP (pentasodium tripolyphosphate) + Polexamer 188	250 to 400	Oral	7 (min)
				21 (max)
	Chitosan + TPP (pentasodium tripolyphosphate)	269 to 688	Oral	50 (max)
				100 (max)
	Chitosan + Poly(ç-glutamic acid)	110 to 150	Oral	15 (min)
				30 (max)
	Chitosan + Alginate + Calcium chloride + Labrafac CC + Phospholipid + Span 80 + Cremorphor EL	488	Oral	25 (min)
				50 (max)
	Chitosan + γ-PGA	185.1 to 198.4	Oral	30
PLGA- Insulin	PLGA + Phospholipid + PVA	102 to 428	Oral	20
	PLGA + Hp55	169	Oral	20
	PLGA + Chitosan + Pluronic 188	134.4	Oral	15
	PLGA + Pluronic 188	121.3	Oral	15
	PLGA + Sodium oleate + PVA	161	Oral	20
	PLGA + PEG + Folate	~260	Oral	50
PCL-Insulin	PCL and Eudragit® RS	331	Oral	25 (min)
				100 (max)
Dextran- Insulin	Dextran + Epichlorohydrin + Vitamin B(12)	160 to 250	Oral	20
	Dextran + Alginate + Poloxamer + Chitosan + BSA	396	Oral	50

	Dextran + Alginate + Chitosan + PEG + BSA	>1842 (90%)	Oral	25(min)
		>812 (50%)		100 (max)
Polyalhylcyanoacrylate-Insulin	Isopropyl myristate + Labrosol + Plurol Oleique + butyl cyanoacrylate	200 to 400	Oral	100
	Polybutylcyanoacrylate + Tween 20	78	Oral	50
	Polybutylcyanoacrylate + Tween 20 + Soyabean oil + Vitamin E	67	Oral	50
	Isobutyl cyanoacrylate (IBCA) + Insulin	145	Oral	100
Solid Lipid-Insulin	Lecithin + Stearic acid + Ploxamer + Wheat germ agglutinin-N-glutamyl-phosphatidyl-ethanolamine	75.3	Oral	50
	Witepsol 85E	243 $\pm$ 10	Oral	25
	Witepsol 85E + Chitosan	470 $\pm$ 32	Oral	25
Targeted Insulin Nanoparticle	N-trimethyl chitosan chloride + CSKSSDYQC peptide	342	Oral	50
	PLA-PEG + Human polyclonal IgG Fc	63	Oral	1.1.

Natural polymers include polysaccharides (chitosan, dextran and alginate) and proteins (casein and gelatin) which are hydrophilic, non-toxic, biocompatible and biodegradable (Bahman et al., 2019).

The use of polysaccharides has main advantages like its low cost, reduced toxicity, biodegradability and known physicochemical properties, which can be adjusted by chemical, physical and enzymatic modifications (McClements, 2020).

Chitosan is a cationic polysaccharide, with strong electrostatic affinity for mucin, leading to the opening of tight junctions, which can act as a mucoadhesive polymer as it has a high affinity for negatively charged mucus (Souza et al., 2020; Zhao et al., 2014). The addition of alkyl groups to the amine groups of chitosan increases its solubility, increasing insulin permeability by widening *tight junctions* (Meneguin et al., 2021). Nanoparticles with chitosan and other constituents, such as poly γ-glutamic acid, poly methyl methacrylate and oleic acid, have been shown to significantly reduce blood glucose levels in diabetic rats (Cui et al., 2009; Amani Elsayed et al., 2009; Lin et al., 2007).

Alginate is obtained from seaweed, being soluble in cold water and at high pH values, but insoluble at low pH values (Meneguin et al., 2021). It allows the formation of nanoparticles with insulin from its ionotropic pre-gelation with calcium chloride, followed by its encapsulation with chitosan (due to its low encapsulation efficiency), demonstrating a reduction in glycemia in diabetic rats (Bahman et al., 2019; Mozafari & Chauhan, 2019; Sarmento et al., 2006).

Dextran sulfate is an exocellular bacterial polysaccharide, obtained by fermentation of sucrose by lactic bacteria, which is chemically coupled to insulin, protecting it against gastrointestinal tract proteases (Sharma et al., 2015). Reis *et al.* created an oral insulin nanoparticle based on alginate-dextran sulfate core, encapsulated with a shell of albumin, chitosan and polyethylene glycol, having reduced blood glucose up to 70% and, in diabetic rats, with a maximum effect after 14 hours and a duration of 24 hours (Reis et al., 2008).

The synthetic polymers poly ε-coprolactone (PCL), PLGA and polylactides (PLA) have good biocompatibility and biodegradability for oral insulin administration. Insulin encapsulation with PLGA induced a rapid fall in glycemia up to 24 hours after its administration, showing a controlled and prolonged insulin release (Malathi et al., 2015; Yang et al., 2012). Zhang *et al.* formulated a mucoadhesive PLGA particle coated with chitosan (Eudragit®), through a solvent evaporation technique to form a double emulsion (water/oil/water), having the pharmacological bioavailability of 50 IU/Kg being 9.2% in diabetic rats (Wang et al., 2022; Wang & Zhang, 2012; Wu et al., 2012).

In PLGA and PLA nanoparticles, insulin efficiency increased up to 90%, with insulin being encapsulated with soy phosphatidylcholine to improve lipid solubility and oral bioavailability (Cui et al., 2006). The PCL polymer has the advantage of having viscoelastic properties superior to PLGA and generates an environment with less acidity during degradation (Bock et al., 2012).

Polyalkylcyanoacrylate-insulin nanoparticles have high stability and are biodegradable, protecting insulin from proteolytic degradation (Graf et al., 2009; Sharma et al., 2015).

The advantages of solid lipid-insulin (SLN) nanoparticles are that their physiological lipid components present a reduced risk of toxicity and protect insulin from degradation by gastrointestinal enzymes. Fonte *et al.* developed SLN-coated chitosan nanoparticles to encapsulate insulin, lowering blood glucose up to 24 hours (Fonte et al., 2011; Mehnert & Mäder, 2012). Xu *et al.* developed an insulin encapsulated SLN, with oral administration of 50 Ul/kg decreasing glycemia in diabetic rats 35% after 3h and 20% after 12h. It is composed of mono, di and triglycerides, as well as mono and diesters of fatty acids and Polyethylene Glycol (PEG) and free PEG-8 (Labrasol®) (Xu et al., 2018). Elsayed *et al.*, prepared insulin-chitosan complexed with oleic acid, plurol oleique as cosurfactant and labrasol as a surfactant and showed a significant hypoglycemic effect in diabetic rats after oral insulin dose of 50 IU/kg throughout 24 h (A. Elsayed et al., 2009).

The formulation of oral insulin spray (Oral Lyn®), for buccal administration, described above, consists of micelles loaded with insulin and a surfactant to increase the absorption area (Bahman et al., 2019). Each spray corresponds to 10 units of regular insulin. It has been shown that with the use of oral insulin spray (Oral Lyn®), insulin levels can be detected within 5 minutes after administration and peak insulin levels are reached within 30 minutes, and by 150 min, insulin has almost reached baseline levels. Thus, oral insulin spray (Oral Lyn®) reflects a normal insulin response to meal intake, taking less time to peak and having a shorter duration of action. Its low bioavailability may, however, be a limitation for this drug to be approved worldwide, the absorption of insulin from one spray is only 10% when compared to subcutaneous insulin injection, which is 20 e 40% (Easa et al., 2019; El Maalouf et al., 2022).

Were developed insulin Tregopil (formerly referred to as IN-105), currently in phase II clinical trial, which is a modified insulin conjugated through a spacer to a small polyethylene glycol molecule which impairs its stability (J. Chen et al., 2018).

The insulin-loaded hepatocyte-directed vesicle (HDV-I), assessed in several clinical trials in phase II and one in phase III for T1D and T2D harboring on its surface a biotin-phosphatidylethanolamine to target the liver. It has a diameter below 150nm and a small amount of insulin in each capsule (5U), reducing the risk of insulin overdose (Li et al., 2012).

An oral liquid insulin delivery system developed, was assessed in phase I clinical trial, that was in which insulin-loaded chitosan nanoparticles are dispersed in an oily vehicle and demonstrated promising bioavailability (Badwan et al., 2009).

Were developed several insulin formulations utilizing Gastrointestinal Permeation Enhancement Technology (GIPET®), tested in phase I clinical trials for patients with T2D, consisting of a lipid mixture or lipid microemulsion with a surfactant in an enteric coated gel capsule (Walsh et al., 2011).

6. CONCLUSIONS

The fact that insulin currently in Europe is administered through subcutaneous injections constitutes a limitation to its use, as it becomes inconvenient and painful for many patients. Furthermore, even when correctly administered, subcutaneous insulin is associated with side effects such as hypoglycaemia and weight gain.

Thus, there has been a lot of research to find alternatives to this route of administration, which facilitates adherence of patients to treatment, contributing to optimize disease treatment, thereby improving overall prognosis, and decreasing comorbidities risk.

Nonetheless, despite more than 6 decades of speculation that inhibition of insulin degradation, increasing its bioavailability, could lead to the development of oral insulins for the treatment of type 2 diabetes mellitus, this concept has not yet been developed into a therapeutic strategy.

More recently, loaded nanoparticles have been developed showing good bioadhesion and encapsulation efficiency *in vitro,* also showing good pH stability for oral insulin administration, presenting a hypoglycemic effect *in vivo* in diabetic rats, which is very promising.

Few oral insulin formulations developed by pharmaceutical companies are now being evaluated in clinical trials. Further rigorous studies are needed to explore potential alternative routes of administration to subcutaneous insulin administration in patients with diabetes. For oral insulin pills to become a reality, the long-term safety and efficacy profile must be established through sufficient studies.

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