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CORSO DI LAUREA MAGISTRALE IN MEDICINA E CHIRURGIA

DIPARTIMENTO DI MEDICINA
Direttore: Prof. Roberto Vettor

TESI DI LAUREA

EFFECTS OF GLUCAGON-LIKE PEPTIDE 1 RECEPTOR AGONISTS AND SODIUM-GLUCOSE COTRANSPORTERS 2 INHIBITORS ON N-TERMINAL PROHORMONE OF BRAIN NATRIURETIC PEPTIDE CONCENTRATIONS: A META-ANALYSIS APPROACH

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Abstract

The purpose of this work is to demonstrate, through a meta-analysis of the main scientific works on the subject, the efficacy of two categories of recently introduced drugs in the therapy of diabetes - glucagon-like peptide 1 receptor agonists (GLP-1) and sodium-glucose cotransporters 2 inhibitors (SLGT-2) - with particular attention to patients with heart failure.

With worsening epidemiological trends for both the incidence and prevalence of type 2 diabetes mellitus (T2DM) and heart failure (HF) worldwide, it is critical to implement optimal prevention and treatment strategies for patients with these comorbidities, either alone or concomitantly. Several guidelines and consensus statements have recommended GLP-1 and SLGT-2 as add-ons to lifestyle interventions with or without metformin in those at high atherosclerotic cardiovascular disease risk.

In addition to the international health emergency represented by diabetes with the consequent increase in cardiovascular diseases, the scope of this thesis is to describe the mechanisms of action of the aforementioned molecules and methods used in the meta-analysis.

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1. Introduction

1.1 Diabetes Mellitus

Diabetes mellitus (DM) is considered one of the three sanitary emergencies form UN and WHO, along with malaria and tuberculosis - the only one among them not infectious. This warning comes from the significant health and social impact of this disease worldwide, linked both to the prevalence of the disease and both to the complexity and severity of its complications. [2]

The prevalence of DM worldwide continues to increase. In 2011, approximately 360 million people had DM, of whom 95% had type 2 DM (T2DM). A number is rising significantly in countries such as China and India, which are now embracing western lifestyles: it is estimated to grow to more than 600 million individuals developing T2DM in 2045, with more or less the same number developing pre-DM. In 2017, 60 million adult Europeans were thought to have T2DM—half undiagnosed. The majority of new cases of T2DM occur in the context of westernized lifestyles, such as high-fat diets and decreased exercise, leading to increasing levels of obesity, insulin resistance (IR), compensatory hyperinsulinemia, and ultimately, beta-cell failure and T2DM.

The clustering of vascular risk associated with insulin resistance, often referred to as “the metabolic syndrome”, has led to the view that the cardiovascular risk appears early, before the development of T2DM. On the other hand, the strong relationship between hyperglycemia and microvascular disease (such as retinopathy, nephropathy, neuropathy) indicates that this risk is not evident until frank hyperglycemia appears.

These concepts highlight the progressive nature of both T2DM and associated cardiovascular risk, which pose specific challenges at different stages of the individual's life with DM.

DM and cardiovascular disease (CVD) develop in concert with metabolic abnormalities mirroring and causing changes in the vasculature: more than half the mortality and a vast amount of morbidity in people with DM is related to CVD.[3]

1.2 Definition and classification

DM is a condition defined by an elevated level of blood glucose.

- **Type 1 diabetes (T1DM)** is characterized by insulin deficiency due to the destruction of pancreatic beta-cells, progressing to absolute insulin deficiency. Typically, T1DM occurs in young, slim individuals presenting with polyuria, thirst, and weight loss, with a propensity to ketosis. However, T1DM may occur at any age, sometimes with slow progression. In the latter condition, latent auto-immune DM in adults (LADA), insulin dependence develops over a few years. People with auto-antibodies to pancreatic beta-cell proteins, such as glutamic-acid-decarboxylase, protein tyrosine phosphatase, insulin, or zinc transporter protein, are likely to develop either acute-onset or slowly progressive insulin dependence. Auto-antibodies targeting pancreatic beta-cells are a marker of T1DM, although they are not detectable in all patients and decrease with age. Compared with other ethnicities and geographic regions, T1DM is more common in Caucasian individuals.

- **Type 2 diabetes (T2DM)** is characterized by a combination of insulin resistance (IR) and beta-cell failure, in association with obesity (typically with an abdominal distribution) and sedentary lifestyle (major risk factors for T2DM). Insulin resistance and an impaired first-phase insulin secretion causing post-prandial hyperglycemia mark the early stage of T2DM. This is usually followed by a deteriorating second-phase insulin response and persistent hyperglycemia in the fasting state. T2DM typically develops after middle age and comprises over 90% of adults with DM. However, with increasing obesity in the young and non-European populations, there is a trend towards a decreasing age of onset.

- **Gestational diabetes** develops during pregnancy. After delivery, most return to a euglycemic state, but they are at increased risk for overt T2DM in the future.

- **Other specific types of diabetes** include single genetic mutations that lead to rare forms of DM such as maturity-onset DM of the young, DM secondary to other pathological conditions or diseases (pancreatitis, trauma or surgery of the pancreas), and drug or chemically induced DM. Disorders of glucose metabolism, impaired fasting glucose (IFG), and IGT, often referred to as ‘pre-diabetes’, reflect the natural history of progression from normoglycemia to T2DM.

A problem when diagnosing T2DM is the lack of a unique biological marker — besides post-prandial plasma glucose (PG) — that would separate IFG,

IGT, or T2DM from normal glucose metabolism. T2DM develops following a prolonged period of euglycemic IR,

which progresses with the development of beta-cell failure to frank DM with an increased risk of vascular complications. The current definition of DM is based on the level of glucose at which retinopathy occurs, but macrovascular complications such as coronary, cerebrovascular, and peripheral artery disease (PAD) appear earlier and, using current glycemic criteria, are often present at the time when T2DM is diagnosed. Over 60% of people with T2DM develop CVD, a more severe and costly complication than retinopathy. Thus, CVD risk should be prioritized when cut-points for hyperglycemia are defined and should be re-evaluated based on the CVD risk.[4]

1.3 Epidemiology

1.3.1 Diabetes in Europe

According to WHO data, diabetes will represent the fourth leading cause of death in Europe by 2030. The prevalence of diabetes is continuously growing in all European countries. In 2014, 26.6 million European citizens were affected by diabetes, i.e., 7.1% of the population. In 9 of the 27 EU countries, more than 7% of the population had diabetes.

The prevalence of diabetes increases with age and with body mass index (BMI). In particular, the risk of diabetes is more than double for obese people compared to individuals with a normal BMI and tends to grow progressively with increasing age. Between 2008 and 2014, the average age of the

resident population in EU countries increased by 1.8 years, and the percentage of the population over 65 grew by 1.4 percentage points. Overall, the risk of having diabetes increased by 368% for individuals over 65 compared to individuals aged 15 to 64. For women, the increase in risk was 368%, and for men, 367%.

The International Diabetes Federation's global estimates for 2011 suggest that 52 million Europeans aged 20–79 years have DM and that this number will increase to over 64 million by 2030. [5]

Table I: Burden of DM in Europe in 2011 and predictions for 2030 [5]

The burden of DM in Europe in 2011 and predictions for 2030		
Variable	**2011**	**2030**
Total population (millions)	896	927
Adults (20–79 years; millions)	651	670
DM (20–79 years)		
European prevalence (%)	8.1	9.5
Number with DM (millions)	52.6	64.0
IGT (20–79 years)		
Regional prevalence (%)	9.6	10.6
Number with IGT (millions)	62.8	71.3
Type 1 DM in children (0–14 years)		
Number with type 1 DM (thousands)	115.7	–
Number newly diagnosed/year (thousands)	17.8	–
DM mortality (20–79 years)		
Number of deaths; men (thousands)	281.3	–
Number of deaths; women (thousands)	316.5	–
Healthcare expenditure due to DM (20–79 years, Europe)		
Total expenditure (billions of €)	75.1	90.2

Based on the current prevalence of diabetes and future demographic transitions, in the absence of targeted interventions, we expect the prevalence of diabetes to reach 9.3% by 2060. This equates to a 35.2% increase over the rates reported among EU 28 countries in 2014.

Comparing the trends and projections of diabetes prevalence in Europe with that in the United States, there are no signs that diabetes prevalence rates will stabilize in the foreseeable future. (Figure 1) [2]

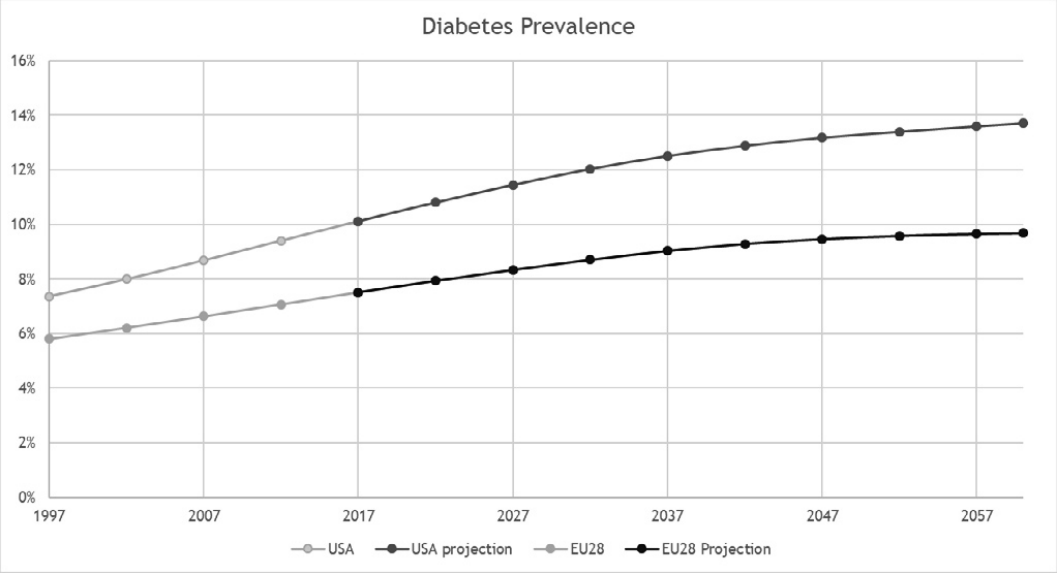


Figure 1: Diabetes Prevalence

1.3.1.1 Diabetes, obesity and aging

The prevalence of diabetes increases with age and with body mass index (BMI). In particular, the risk of diabetes is more than double for obese people compared to individuals with a normal BMI, and tends to grow progressively with increasing age. Between 2008 and 2014, the average age of the resident population in EU countries increased by 1.8 years, and the percentage of the population over 65 grew by 1.4 percentage points. Overall, the risk of having diabetes increased by 368% for individuals over

65 compared to individuals aged 15 to 64. For women, the increase in risk was 368%, and for men, 367% (Figure 2). [2]

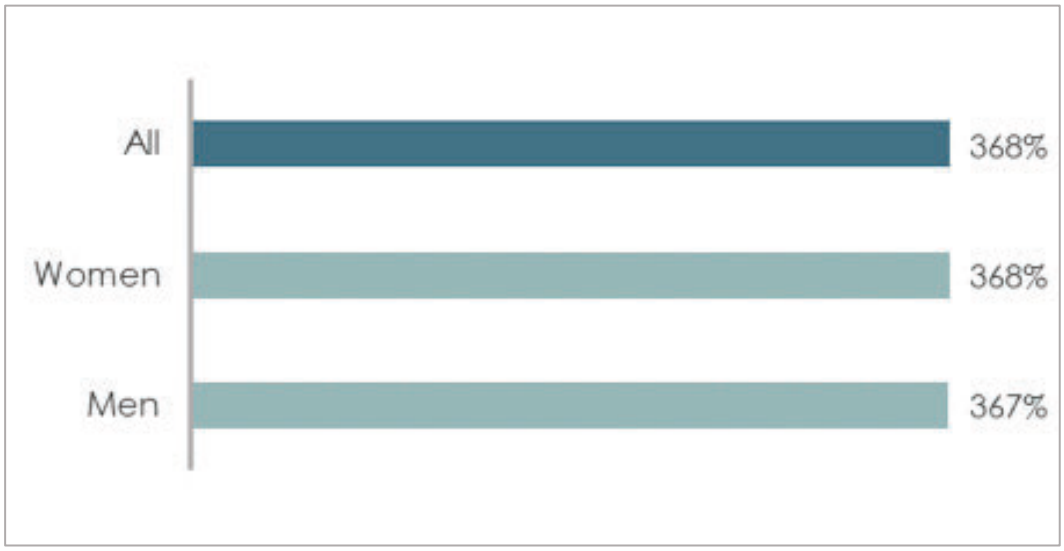


Figure 2: Prevalence of diabetes increases with age and with body mass index

1.3.1.2 Diabetes and socio-economic factors

EU27 countries have a higher prevalence of diabetes among the 20% of the population earning the lowest income compared to the wealthiest 20%. Individuals in the lowest income group have a 58% higher risk of having diabetes than individuals in the highest income group. In the risk of having diabetes, women have higher levels of income-related inequality than men. In fact, the increase in risk is 93% for women, while for men, it is 38% (Figure 3). [2]

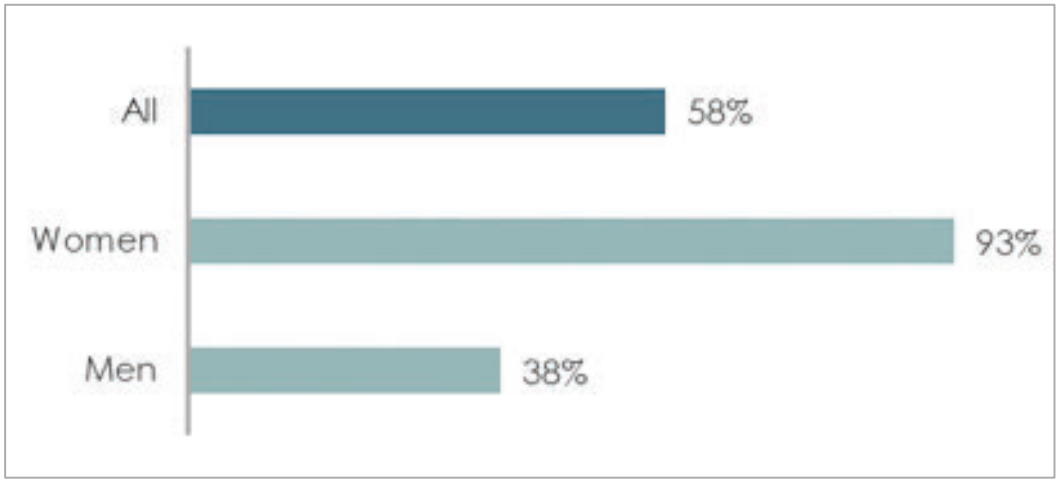


Figure 3: Prevalence of diabetes increases income-related

Disparities in diabetes prevalence between income groups vary significantly across EU27 countries. For all countries except Luxembourg and Poland, the prevalence of diabetes is higher among low-income individuals (Figure 4). [2]

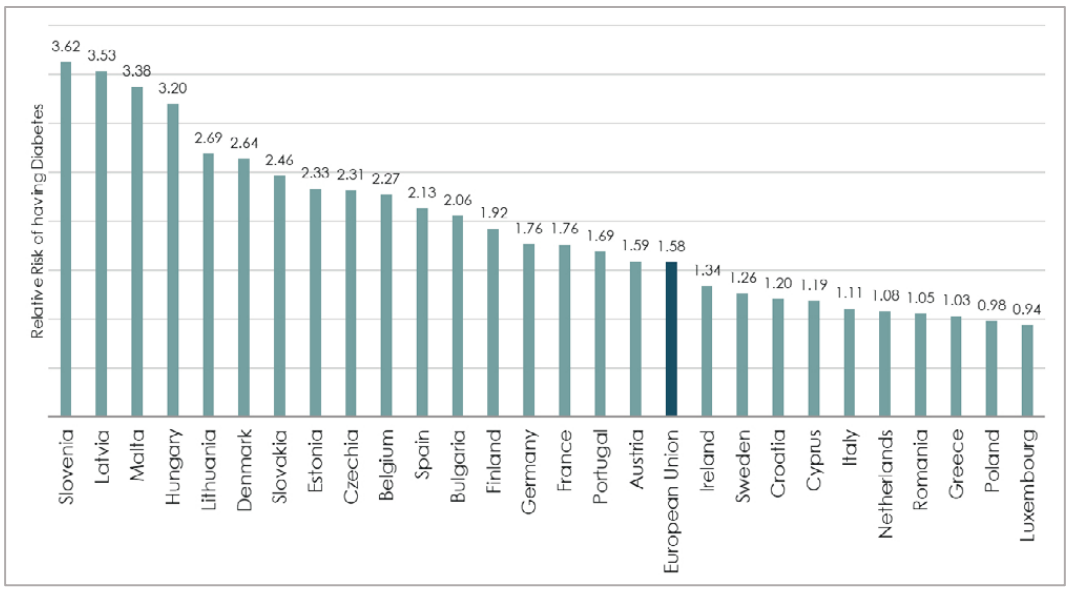


Figure 4: Prevalence of diabetes across EU27 countries income-related

The prevalence of diabetes is also associated with the level of education. In fact, in all countries, the prevalence of diabetes is higher among the less educated. On average, people who have not graduated from high school are 160% more likely to have diabetes than people with higher education. In diabetes risk, women have higher levels of education-related inequality than men. The increase in risk is 263% for women, and for men, 89% (Figure 5). [2]

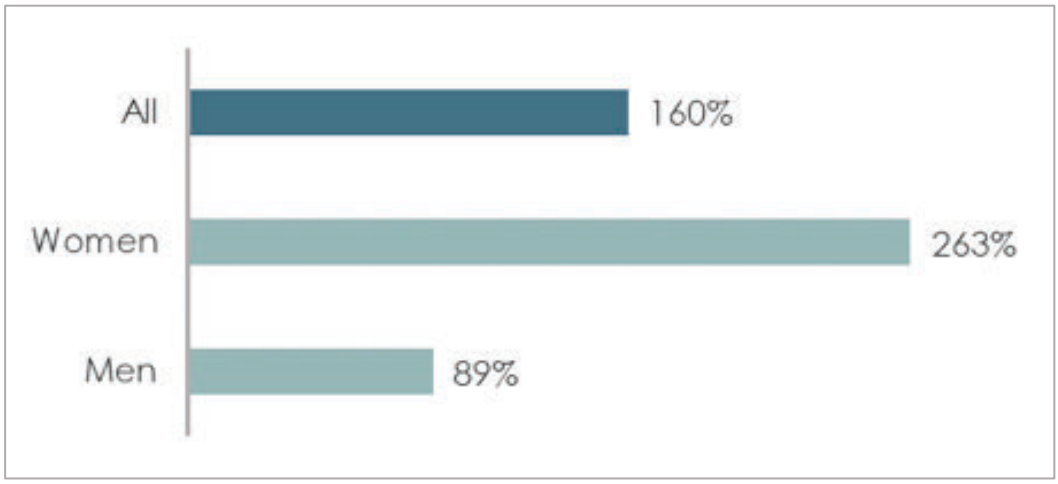


Figure 5: Prevalence of diabetes between graduated and non-graduated people

46.6% of people aged between 18 and 65 who lived in the EU in 2014 had secondary education (high school diploma). In all EU countries, individuals in this group have a 36% higher risk of having diabetes than university graduates. Again, women face higher levels of education-related inequality than men. The increased risk of diabetes is 65% for women, and for men, 19% (Figure 6). [2]

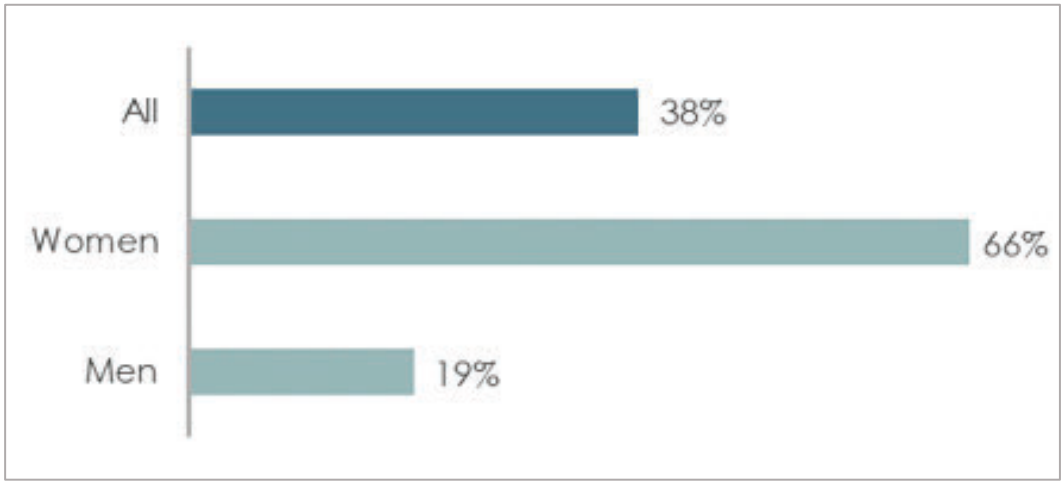


Figure 6: Prevalence of diabetes between high-school graduated and university graduated people

Taking into account the entire distribution of education levels, the overall gradient of inequality is significant in all EU countries, except for Cyprus, and is very low for Italy (3%) (Figure 7). [2]

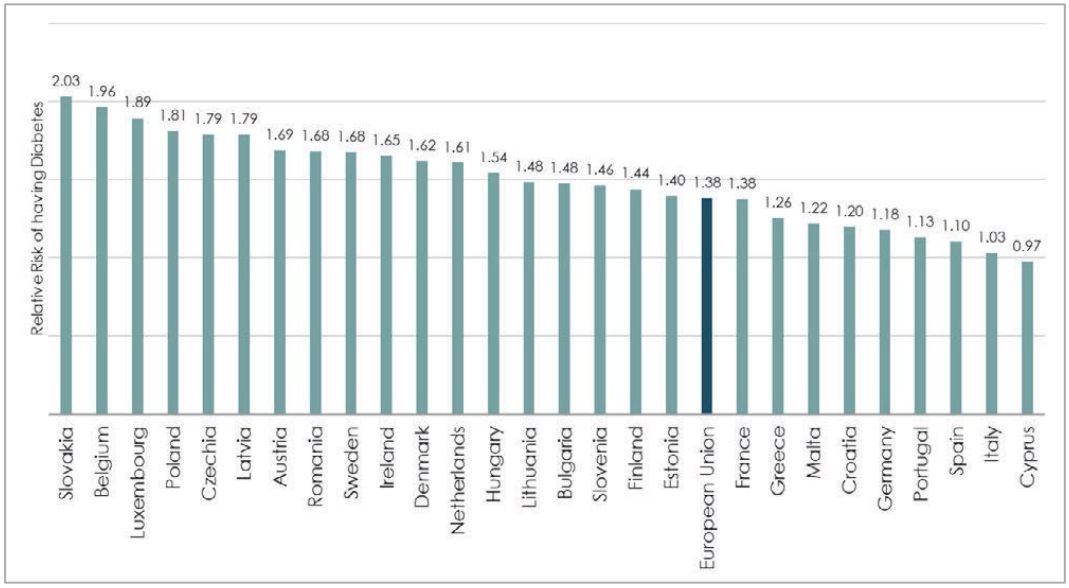


Figure 7: Prevalence of diabetes across EU27 countries schooling-related

In addition to gender disparities, various socio-demographic factors have a close relationship with the risk of diabetes, such as urbanization, divorce rates, and aging.

The prevalence of diabetes is closely associated with urbanization. Countries, where more people moved to cities from 2008 to 2014 experienced an increase in the prevalence of diabetes over the same period. A 10% increase in the proportion of the population residing in the city is associated with an 8.6% increase in the prevalence of diabetes.

Changes in marital status in the population also significantly affect diabetes prevalence: Countries where divorce rates increased from 2008 to 2014 experienced an increase in diabetes prevalence over the same period. A 1% decrease in the marriage rate is associated with an increase in the prevalence of diabetes by 0.94%.

Finally, the aging of the population determines an increase in the risk of diabetes: countries where the share of the population aged 65 or over increased from 2008 to 2014 recorded a rise in the prevalence of diabetes over the same period. A 1% increase in the share of the population over 65 is associated with a higher prevalence of diabetes of 3.4%.

1.3.2 Diabetes in Italy

1.3.2.1 Prevalence

In Italy there are over 3.5 million people who in 2019 declared to be affected by diabetes, equal to 5.8% of the entire population (source ISTAT). In comparison with 2000, it is estimated that diabetic people have increased by over 60% (+1.360.000) in our country, an increase that does not have the only explanation of the aging of the population, but a plurality of reasons, which are often combined between them. Among these, factors such as sedentary lifestyle and obesity and in general the lack of attention to healthy

lifestyles certainly contribute decisively, but also aspects that recall the continuous progress in contrasting chronic morbidities, such as the improved diagnostic skills accompanied by a precocity in the age of the diagnosis or the ability of the care system to extend the survival of people with diabetes and related complications.

Diabetes is more common among men: overall in the male population, the prevalence in 2019 is estimated at 6.2% and in the female population at 5.5%. Comparing the phenomenon without the different longevity between the two sexes, the differences increase: the age-standardized rate equals 5.9% in the former and 4.6% in the latter.

In the last 20 years, the most significant increases concern men over 70, in particular, the prevalence passes from 14.9% in 2000 to 21.2% in 2019 in the 75-79 age group and increases by almost 10 percentage points among the over the eighties (from 14.4% in 2000 to 23.9% in 2019). Similarly, for women, the increases are found in the same age groups but appear much less marked, reaching a maximum of 3 percentage points (Figure 8). [2]

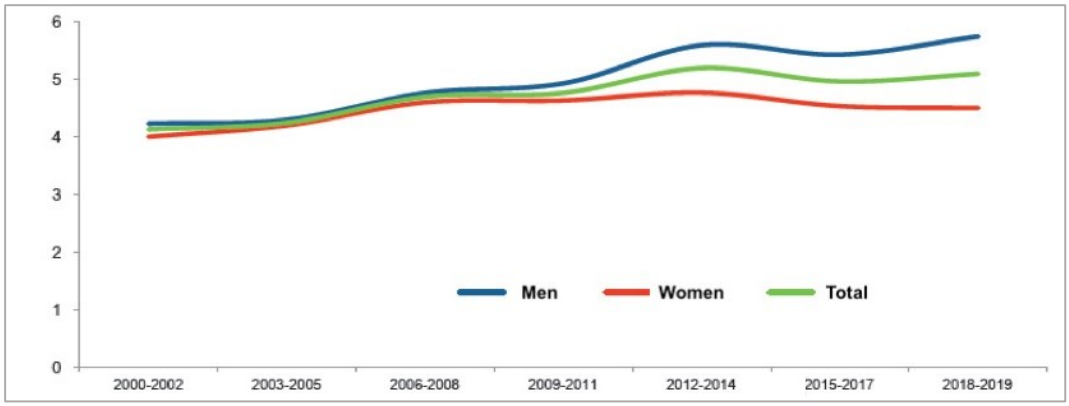


Figure 8: Population with diabetes. Years 2000-2019 [6]

At the territorial level, the clear North-South gradient is confirmed, with many regions of the South that are above the national average. The rough prevalence estimates at the regional level attest that Calabria, Molise, and Sicily are the regions with the highest values, the estimates of the total population are respectively 8%, 7.6%, and 7.3%, significantly higher than the average figure for Italy (5.8%). The overall prevalence of the diabetic disease is, instead, substantially lower than the national average for the autonomous provinces of Bolzano and Trento and for the Veneto, which with 3.4%, 4.2%, and 4.9% are the regions placed in the lowest part of the ranking. Similar regional differences are found for older people, although the distances between regions are much higher in absolute value. The prevalence of diabetes among the elderly resident in Calabria (25.4%) is 8 percentage points higher than the Italian average (17.2%), and is 3 times higher than that recorded in the Bolzano PA (8.3%).) where the minimum value is recorded. [2]

1.3.2.2 Veneto Region

In Veneto 4.9% of the population declares itself diabetic. Veneto is a region with a prevalence of childhood obesity and diabetes below the national average. Hospitalization rates for uncontrolled diabetes and diabetes with complications show worse data than the national average. The standardized mortality rate from diabetes is increasing for males and decreasing in females between 2000 and 2017, but still below the national average for both sexes. [2]

1.3.2.3 Mortality

Diabetes is reported as the initial cause in about 23,000 deaths, but it is present among the diseases that play a role in determining death (contributing cause) in about 4 times as many cases. The diseases most associated with diabetes are gangrene and other microcirculatory complications, metabolic diseases such as obesity and dyslipidemia, hypertensive and ischemic heart diseases, atherosclerosis, and liver diseases. Over time, the absolute number of deaths with an initial cause of diabetes increased from 19,677 in 2003 to 22,354 in 2017 (+ 13.6%). The rate concerning the population in the same period went from 3.43 to 3.69 deaths per 10,000 residents. However, this increase is due to the aging of the population. The age-standardized death rate decreased from 3.69 to 2.96 per 10,000 residents, albeit with an irregular trend over time: the decrease was more marked between 2009 and 2014, and in the last three years, the trend is variable. The gender gap has increased over time for a more rapid reduction of mortality rates in women, already lower than men at the beginning of the period (Figure 9). [2]

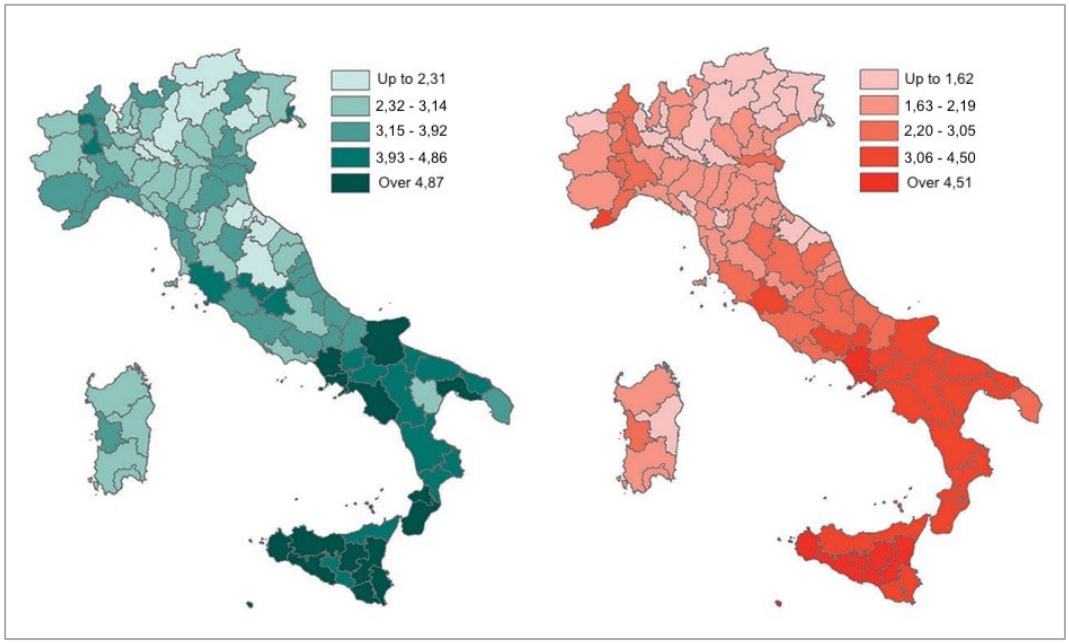


Figure 9: Mortality due to diabetes by the province of residence and sex. 2017, standardized rates per 10,000 residents

The strong association with social hardship is also confirmed for mortality. Generally, the most deprived social groups (with low education and income) have higher mortality from diabetes. Mortality from diabetes in men aged 30-89 is 1.6 times higher in the presence of low educational qualifications than in those with high education (4.9 deaths per 10,000 residents vs. 3.1). The gap rises to 2.3 times (3.5 vs. 1.5).

The territorial differences found in mortality from diabetes can find a reason in the social inequalities that characterize the different geographical areas of our country (Figure 10).

Figure 10: Mortality among 30-89 years old diabetes patients for sex, education and region. Years 2012-2014

1.3.2.4 Diabetes and quality of life

Diabetes can compromise the physical state of health and significantly affect mental well-being. This happens above all when the diabetic pathology is not well treated, as in the case of decompensated diabetes, or because diabetes is often associated with other pathologies that also worsen the overall state of mental health, with depressive syndromes or

anxiety disorders. The Mental Health Index (MH), calculated based on the SF362 questionnaire, is a psychometric tool that investigates four main dimensions: anxiety, depression, loss of behavioral/emotional control, and psychological well-being. The index shows lower and lower values among people who declare they have diabetes, indicating a worsening of the state of mental health compared to the rest of the population. Following figure shows that more than 6 average points shift down the curves of both men and women with diabetes compared to the scores found in the general population. Among the youngest, from 15 to 44 years, the most significant gap is observed, 7.5 points reduce the average score among those who declare they have diabetes compared to the total population of the same age group, both for men than for women. The differences between the average scores decrease with increasing age, more markedly among women over 75 (3.2 points less) (Figure 11). [2]

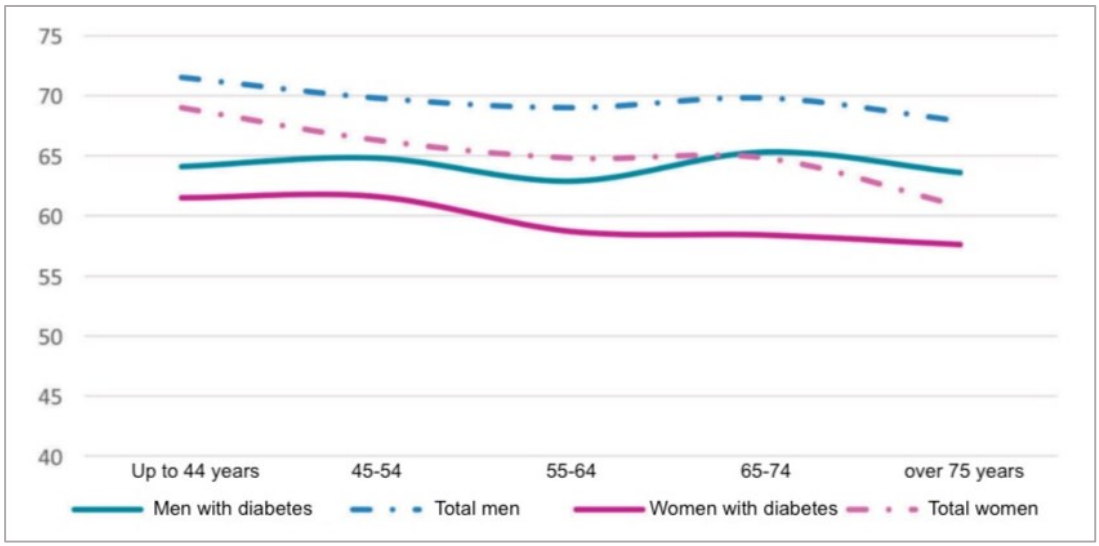


Figure 11: People aged 15 and over by Mental health index and diabetes, by gender and age group. Year 2019, an average value of the Mental Health Index

The presence of diabetes worsens many of the dimensions of quality of life. The judgment on life satisfaction shows a much more negative trend in the population with diabetes than in the total population. The share of those who declare high satisfaction in life among people with diabetes, i.e. who give a score higher than 7 on a scale from 0 to 10, is reduced by about 10 points in the population aged 14 and over (33.4% vs 43, 3%) and on the other hand, the share of those reporting a low degree of satisfaction is higher (24.7% vs 14.2%), i.e., assigning a score that does not reach 6. In the elderly population, the dynamics are similar even if the distance between the two groups is slightly attenuated. But the most severe judgment, witnessed by much higher differentials, is the level of satisfaction with one's health which among diabetic people records a lower share of more than 30 percentage points among people who consider themselves very satisfied. More than half of people with diabetes say they are poorly satisfied with their health compared to about one-fifth of the population as a whole. [2]

1.3.2.5 The economic impact of diabetes

In Italy 8% of the health budget is invested in diabetes. The average annual cost per person with diabetes is € 2,800 (Figure 12).

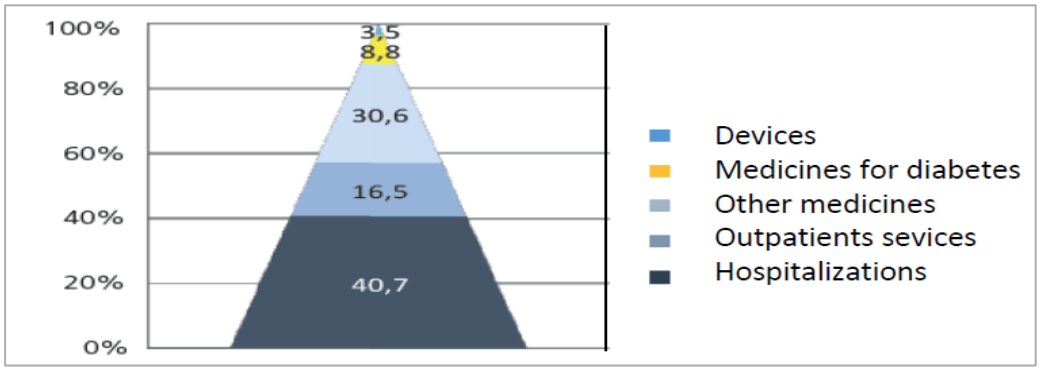


Figure 12: Factors that contribute to direct healthcare costs for diabetes [7]

Most of the costs are attributable to hospital admissions, while drugs for diabetes account for 8.8%.[2]

1.4 Diagnosis

Fasting blood sugar:

• Normal: <100 mg / dL or <5.6 mmol / L

• IFG (impaired fasting blood glucose): between 100-125 mg / dl or between 5.6-6.9 mmol / l

• Diabetes: ≥ 126 mg / dL or ≥ 7.0 mmol / L

Oral glucose load curve (OGTT):

• Normal: <140 mg / dl p <7.8 mmol / l

• IGT (impaired glucose tolerance): between 140-199 mg / dl or between 7.8-11.0 mmol / l

• Diabetes: ≥ 200 mg / dL or ≥ 11.1 mmol / L

In 2010, another important element was added for the diagnosis of diabetes: the values of glycated hemoglobin.

The American Diabetes Association has established that the glycated hemoglobin threshold indicative of diabetes is HbA_{1c} ≥ 6.5%. The novelty was not only the introduction of a new marker for the diagnosis of diabetes but above all, a marker for disease monitoring.

Ultimately we have 4 ways to diagnose diabetes:

• Fasting blood glucose ≥ 126 mg / dl, which must necessarily be repeated on plasma blood.

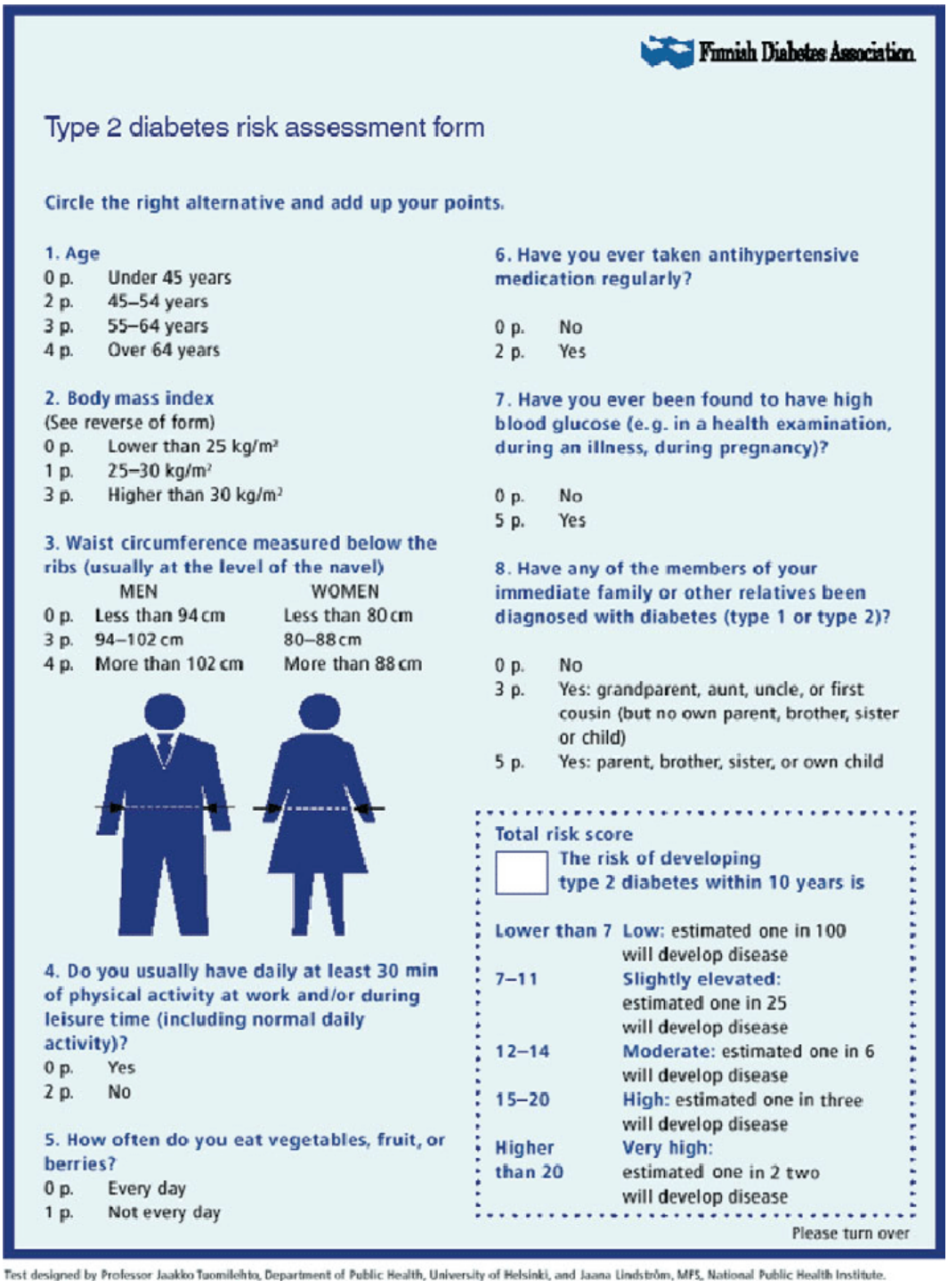
• Random blood glucose ≥ 200 mg / dl, which must be repeated if there are no symptoms, but not necessarily repeat it if this blood sugar level is accompanied by typical symptoms of diabetes (polyuria, polydipsia, polyphagia, weight loss, infections ..).

• Glycated hemoglobin HbA_{1c} ≥ 6.5%, must be repeated with the standardized method.

• Oral glucose load curve with blood glucose after 2 h ≥ 200 mg / dl. There is no need to repeat it.

1.4.1 Diabetes risk score

Based on a Finnish study, it was developed the Diabetes risk score, a questionnaire that investigates the risk of developing diabetes (Figure 13).



Finnish Diabetes Association

Type 2 diabetes risk assessment form

Circle the right alternative and add up your points.

1. Age
0 p. Under 45 years
2 p. 45–54 years
3 p. 55–64 years
4 p. Over 64 years

2. Body mass index
(See reverse of form)
0 p. Lower than 25 kg/m²
1 p. 25–30 kg/m²
3 p. Higher than 30 kg/m²

3. Waist circumference measured below the ribs (usually at the level of the navel)

	MEN	WOMEN
0 p.	Less than 94 cm	Less than 80 cm
3 p.	94–102 cm	80–88 cm
4 p.	More than 102 cm	More than 88 cm

4. Do you usually have daily at least 30 min of physical activity at work and/or during leisure time (including normal daily activity)?
0 p. Yes
2 p. No

5. How often do you eat vegetables, fruit, or berries?
0 p. Every day
1 p. Not every day

6. Have you ever taken antihypertensive medication regularly?
0 p. No
2 p. Yes

7. Have you ever been found to have high blood glucose (e.g. in a health examination, during an illness, during pregnancy)?
0 p. No
5 p. Yes

8. Have any of the members of your immediate family or other relatives been diagnosed with diabetes (type 1 or type 2)?
0 p. No
3 p. Yes: grandparent, aunt, uncle, or first cousin (but no own parent, brother, sister or child)
5 p. Yes: parent, brother, sister, or own child

Total risk score
The risk of developing type 2 diabetes within 10 years is

Lower than 7	**Low:** estimated one in 100 will develop disease
7–11	**Slightly elevated:** estimated one in 25 will develop disease
12–14	**Moderate:** estimated one in 6 will develop disease
15–20	**High:** estimated one in three will develop disease
Higher than 20	**Very high:** estimated one in 2 two will develop disease

Please turn over

Test designed by Professor Jaakko Tuomilehto, Department of Public Health, University of Helsinki, and Jaana Lindström, MPS, National Public Health Institute.

Figure 13: TDM2 risk assessment form

The Diabetes risk score takes into account various risk factors:

• Age (the risk increases with age);

• BMI (the risk increases greatly in obese people - BMI ≥ 30);

• Waist circumference;

• Physical activity;

• Diet, more or less rich in fruit and vegetables;

• Hypertension;

• Occasional glycemic alteration;

• Family history of diabetes.

By answering these 8 questions, a score is obtained that informs about the individual's risk of developing diabetes in the following 10 years:

• score ≤7 low risks;

• score between 7 and 11, slightly high risk;

• score between 12 and 14, moderate risk;

• score between 15 and 20, high risk;

• score ≥ 20, very high risk.

1.5 Type 2 Diabetes Therapy

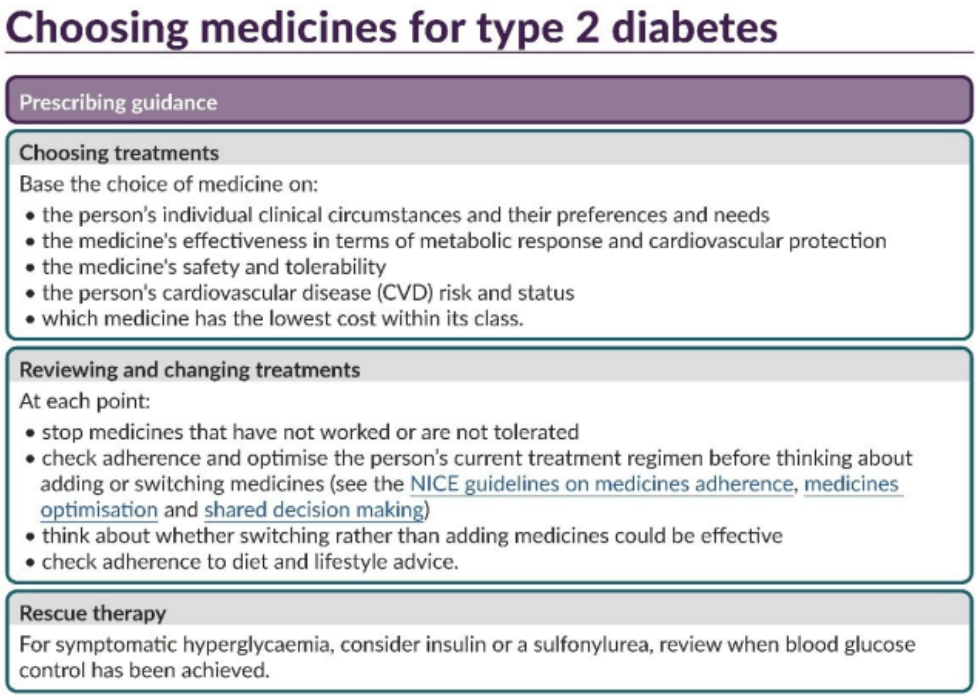


Figure 14: Prescribing guidance, NICE

The main drugs for therapy are:

- **Sulfonylureas and glinides**: the first to be used, they act mainly at the pancreatic level, where stimulate insulin secretion by β cells. However, they do have some side effects, since in the long run, they contribute to the destruction of the cell itself, thus being effective but not optimal. Sulfonylureas (e.g., glyburide, glipizide, glimepiride) are insulin secretagogues. The lower plasma glucose by stimulating pancreatic beta-cell insulin secretion may secondarily improve peripheral and hepatic insulin sensitivity by reducing glucose toxicity. First-generation sulfonylureas (acetohexamide, chlorpropamide, tolazamide, tolbutamide) are more likely to cause adverse effects and are used infrequently. All sulfonylureas promote hyperinsulinemia and weight gain of 2 to 5 kg, which may potentiate insulin resistance and limit their usefulness over time. All also can cause hypoglycemia. Risk factors include age > 65, use of long-acting drugs (especially chlorpropamide, glyburide, or glipizide), erratic eating and exercise, and renal or hepatic insufficiency. Short-acting insulin secretagogues (repaglinide, nateglinide) stimulate insulin secretion like sulfonylureas. However, they are faster acting and may stimulate insulin secretion more during meals than at other times. Thus, they may be especially effective for reducing postprandial hyperglycaemia and appear to have a lower risk of hypoglycaemia. There may be some weight gain, although less than with sulfonylureas. Patients who have not responded to other oral

drugs (e.g., sulfonylureas, Metformin) are not likely to respond to these drugs.

- **Biguanides**: among these, Metformin stands out, the only one still on the market today. This acts on the liver and muscles, and although its action is not entirely unknown in this area, it reduces insulin resistance in the intestine. It still turns out to be the drug of choice in the treatment of DMT2. Biguanides (Metformin) lower plasma glucose by decreasing hepatic glucose production (gluconeogenesis and glycogenolysis). They are considered peripheral insulin sensitizers, but their stimulation of peripheral glucose uptake may simply result from reductions in glucose from their hepatic effects. Biguanides also lower lipid levels and may also decrease gastrointestinal nutrient absorption, increase beta-cell sensitivity to circulating glucose, and decrease levels of plasminogen activator inhibitor 1, thereby exerting an antithrombotic effect. Metformin is the only biguanide commercially available in the US. It is as effective as sulfonylureas in reducing plasma glucose rarely causing hypoglycemia and can be safely used with other drugs and insulin. In addition, Metformin does not cause weight gain and may even promote weight loss by suppressing appetite. However, the drug commonly causes gastrointestinal adverse effects (e.g., dyspepsia, diarrhea), which for most people recede with time. Less commonly, Metformin causes vitamin B12 malabsorption, but clinically significant anemia is rare.

The contribution of Metformin to life-threatening lactic acidosis is scarce. Still, the drug is contraindicated in patients at risk of acidemia (including those with significant renal insufficiency, hypoxia, severe respiratory disease, alcohol use disorder, other forms of metabolic acidosis, or dehydration). The drug should be withheld during surgery, administration of IV contrast, and any serious illness. Many people receiving metformin monotherapy eventually require additional medication.

- **Thiazolidinediones (TZD)**: similar to Metformin, they act on insulin resistance but are abandoned after initial enthusiasm for them due to the side effects they present. Thiazolidinediones (TZDs—pioglitazone, rosiglitazone) decrease peripheral insulin resistance (insulin sensitizers), but their specific mechanisms of action are not well understood. The drugs bind a nuclear receptor primarily present in fat cells (peroxisome-proliferator-activated receptor-gamma [PPAR-γ]) involved in transcription genes that regulate glucose and lipid metabolism. TZDs also increases high-density lipoprotein (HDL) levels, lower triglycerides, and have anti-inflammatory and anti-atherosclerotic effects. TZDs are as effective as sulfonylureas and Metformin in reducing hemoglobin A1C. TZDs may be beneficial in treating nonalcoholic fatty liver disease (NAFLD).

  Though one TZD (troglitazone) caused acute liver failure, currently available drugs have not proven hepatotoxic. Nevertheless, periodic monitoring of liver function is recommended. TZDs may cause peripheral edema, especially in patients taking insulin, and may

worsen heart failure in susceptible patients. Weight gain due to fluid retention and increased adipose tissue mass is typical and may be substantial (> 10 kg) in some patients. Rosiglitazone may increase the risk of heart failure, angina, myocardial infarction, stroke, and fracture. Pioglitazone may increase the risk of bladder cancer (although data are conflicting), heart failure, and fractures.

- **Intestinal α-glucosidase inhibitors**: although less effective, they act only locally, reducing intestinal absorption of glucose, but they cause side effects in this area and have lower power because they only reduce post-prandial blood sugar. Alpha-glucosidase inhibitors (acarbose, miglitol) competitively inhibit intestinal enzymes that hydrolyze dietary carbohydrates; carbohydrates are digested and absorbed more slowly, thereby lowering post-prandial plasma glucose. Alpha-glucosidase inhibitors are less effective than other oral drugs in reducing plasma glucose, and patients often stop the drugs because they may cause dyspepsia, flatulence, and diarrhea. But the drugs are otherwise safe and can be used in combination with all other oral drugs and with insulin.

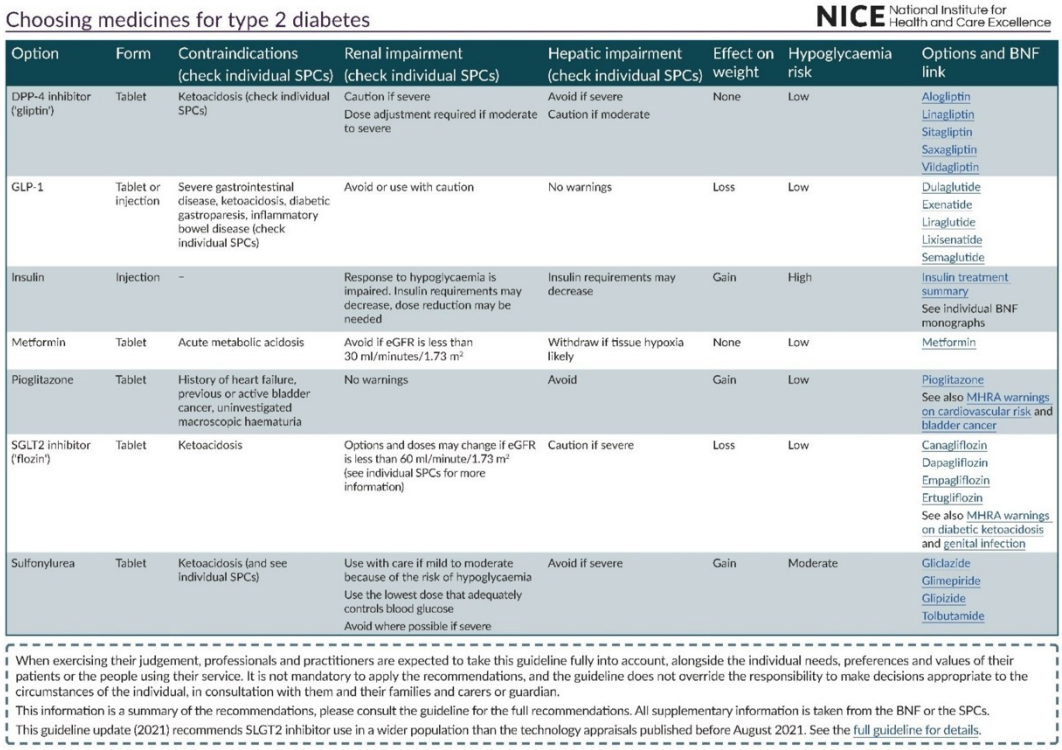
- **GLP-1 analogs and DPP-4 inhibitors**. Dipeptidyl peptidase-4 inhibitors (e.g., alogliptin, linagliptin, saxagliptin, sitagliptin) prolong the action of endogenous glucagon-like peptide-1 (GLP-1) by inhibiting the enzyme dipeptidyl peptidase-4 (DPP-4), which is involved in the breakdown of GLP-1. GLP-1 is a peptide made in the small intestine that stimulates insulin secretion and inhibits glucagon

secretion, prolonging its action, thereby lowering plasma glucose. There is a slight increase in risk for pancreatitis with DPP-4 inhibitors, but they are otherwise considered safe and well-tolerated. The hemoglobin A1C decrease is modest with DPP-4 inhibitors. GLP-1 receptor agonists mimic the effects of GLP-1, a peptide made in the small intestine that enhances glucose-dependent insulin secretion and slows gastric emptying. GLP-1 agonists may also reduce appetite, promote weight loss, and stimulate beta-cell proliferation. Examples include exenatide (an incretin hormone), lixisenatide, liraglutide, dulaglutide, albiglutide, and semaglutide. Formulations are available for dosing twice a day, once a day, and weekly. The most common adverse effects of GLP-1 agonists are gastrointestinal, especially nausea and vomiting. GLP-1 agonists also cause a slight increase in the risk of pancreatitis. They are contraindicated in patients with a family history of medullary thyroid cancer because an increased risk of this cancer has occurred in tested rodents.

- **SGLT2 inhibitors**. Sodium-glucose co-transporter 2 (SGLT2) inhibitors (canagliflozin, dapagliflozin, empagliflozin) inhibit SGLT2 in the proximal tubule of the kidney block glucose reabsorption, thus causing glycosuria and transient natriuresis. SGLT2 inhibitors may also cause modest weight loss and lowering of blood pressure. SGLT-2 inhibitors have recently been shown to decrease mortality, major adverse cardiovascular events, and heart failure hospitalizations in patients with an increased risk for cardiovascular

disease. In addition, SGLT-2 inhibitors have been shown to prevent the progression of chronic kidney disease in patients with diabetes and reduced glomerular filtration rate or albuminuria.

The most common side effects are genitourinary infections, especially mycotic infections. Orthostatic symptoms can also occur. SGLT-2 inhibitors have been implicated in causing diabetic ketoacidosis (DKA) in type 1 and type 2 diabetes patients. Ketoacidosis may occur at lower blood glucose levels than in other causes of DKA. One large study showed an increase in lower limb amputation with canagliflozin.



Choosing medicines for type 2 diabetes

NICE National Institute for Health and Care Excellence

Option	Form	Contraindications (check individual SPCs)	Renal impairment (check individual SPCs)	Hepatic impairment (check individual SPCs)	Effect on weight	Hypoglycaemia risk	Options and BNF link
DPP-4 inhibitor ('gliptin')	Tablet	Ketoacidosis (check individual SPCs)	Caution if severe Dose adjustment required if moderate to severe	Avoid if severe Caution if moderate	None	Low	Alogliptin Linagliptin Sitagliptin Saxagliptin Vildagliptin
GLP-1	Tablet or injection	Severe gastrointestinal disease, ketoacidosis, diabetic gastroparesis, inflammatory bowel disease (check individual SPCs)	Avoid or use with caution	No warnings	Loss	Low	Dulaglutide Exenatide Liraglutide Lixisenatide Semaglutide
Insulin	Injection	–	Response to hypoglycaemia is impaired. Insulin requirements may decrease, dose reduction may be needed	Insulin requirements may decrease	Gain	High	Insulin treatment summary See individual BNF monographs
Metformin	Tablet	Acute metabolic acidosis	Avoid if eGFR is less than 30 ml/minutes/1.73 m^2	Withdraw if tissue hypoxia likely	None	Low	Metformin
Pioglitazone	Tablet	History of heart failure, previous or active bladder cancer, uninvestigated macroscopic haematuria	No warnings	Avoid	Gain	Low	Pioglitazone See also MHRA warnings on cardiovascular risk and bladder cancer
SGLT2 inhibitor ('flozin')	Tablet	Ketoacidosis	Options and doses may change if eGFR is less than 60 ml/minute/1.73 m^2 (see individual SPCs for more information)	Caution if severe	Loss	Low	Canagliflozin Dapagliflozin Empagliflozin Ertugliflozin See also MHRA warnings on diabetic ketoacidosis and genital infection
Sulfonylurea	Tablet	Ketoacidosis (and see individual SPCs)	Use with care if mild to moderate because of the risk of hypoglycaemia Use the lowest dose that adequately controls blood glucose Avoid where possible if severe	Avoid if severe	Gain	Moderate	Gliclazide Glimepiride Glipizide Tolbutamide

When exercising their judgement, professionals and practitioners are expected to take this guideline fully into account, alongside the individual needs, preferences and values of their patients or the people using their service. It is not mandatory to apply the recommendations, and the guideline does not override the responsibility to make decisions appropriate to the circumstances of the individual, in consultation with them and their families and carers or guardian.

This information is a summary of the recommendations, please consult the guideline for the full recommendations. All supplementary information is taken from the BNF or the SPCs.

This guideline update (2021) recommends SLGT2 inhibitor use in a wider population than the technology appraisals published before August 2021. See the full guideline for details.

Figure 15: Main drugs for therapy

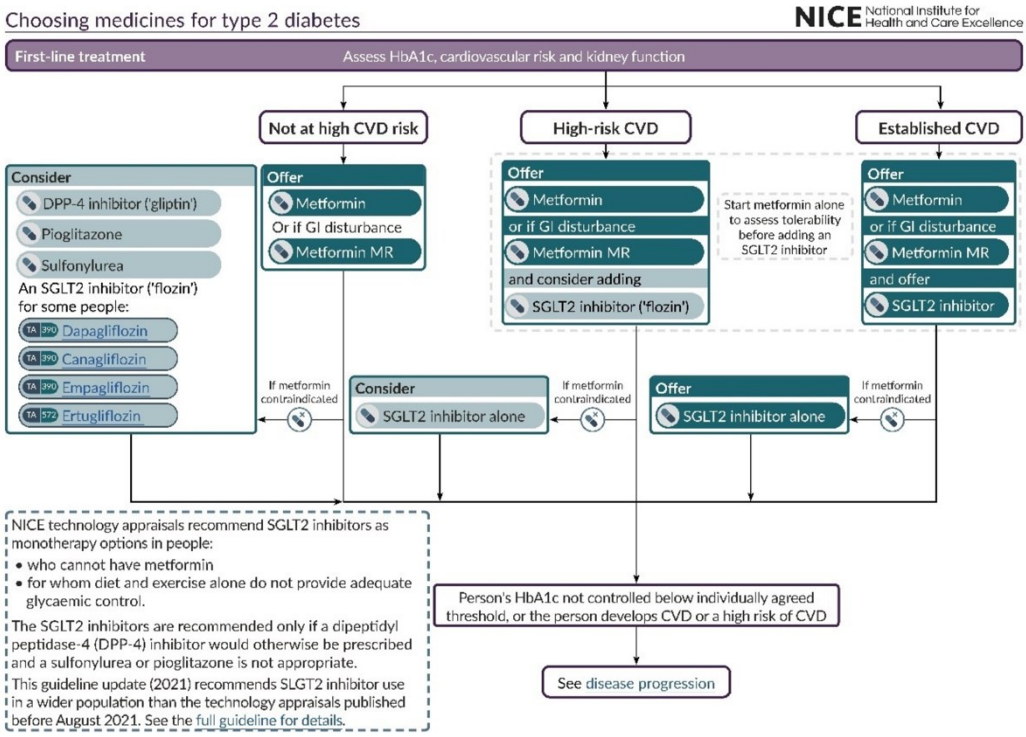


Figure 16: First-line treatment

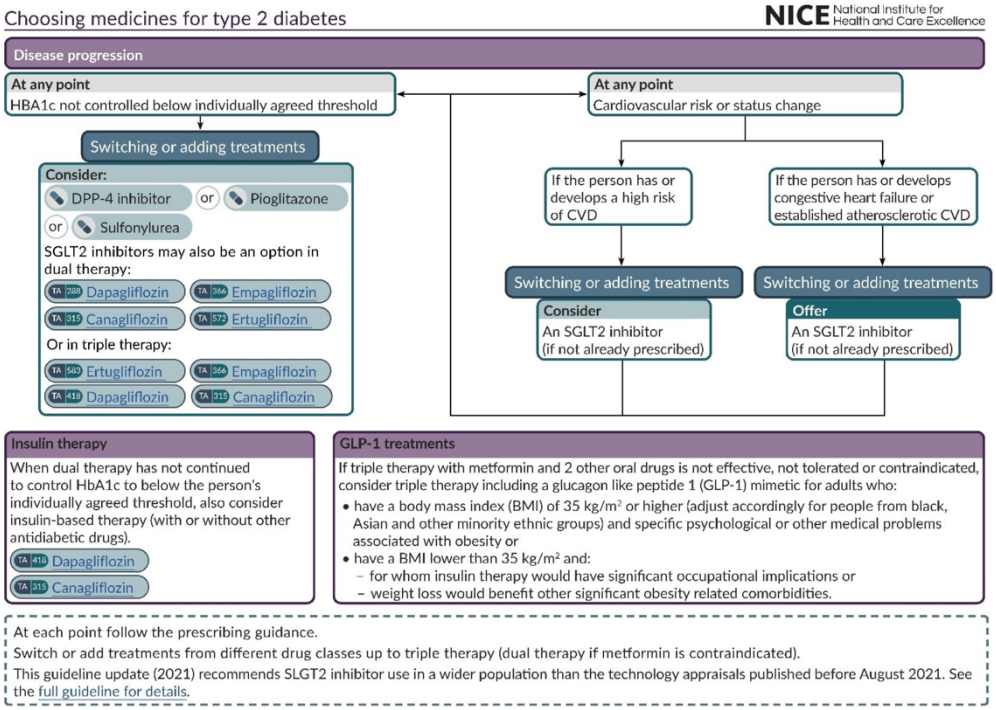


Figure 17: Disease progression

1.6 Complications of Diabetes Mellitus

Diabetes can lead to several serious complications that typically occur after a few years. The onset of complications is particularly relevant in the case of an untimely diagnosis of diabetes and inadequate treatments. Because of these complications, people with diabetes have nearly twice the risk of premature death than people without diabetes.

They are:

- cardiovascular disease (angina, heart attack, stroke, peripheral arterial disease, and congestive heart failure) is the most common cause of death among people with diabetes;
- nephropathy in people with diabetes is one of the leading causes of end-stage renal failure requiring transplantation or dialysis;
- eye disease (retinopathy) can cause low vision or even blindness;
- damage to the nerves (neuropathies) can lead to numbness, ulcers, infections, and even amputations.

Complications, particularly cardiovascular complications, generate a heavy burden related to type 2 diabetes. However, intensive long-term interventions targeting multiple risk factors in people with type 2 diabetes and early-stage nephropathy (microalbuminuria) can reduce the risk of cardiovascular and microvascular events by approximately 50%. Furthermore, a 1% decrease in HbA_{1c} values is associated with a 21% decrease in the risk of developing a complication. The intensive intervention combined with drug polytherapy and behavioral modification can

permanently benefit vascular complications and mortality from all causes, including cardiovascular ones.

In patients with diabetes mellitus, years of poorly controlled hyperglycemia lead to multiple, primarily vascular, complications that affect small vessels (microvascular), large vessels (macrovascular), or both.

The mechanisms by which vascular disease develops include

- Glycosylation of serum and tissue proteins with the formation of advanced glycation end products
- Superoxide production
- Activation of protein kinase C, a signaling molecule that increases vascular permeability and causes endothelial dysfunction
- Accelerated hexosamine biosynthetic and polyol pathways leading to sorbitol accumulation within tissues
- Hypertension and dyslipidemias that commonly accompany diabetes mellitus
- Arterial microthromboses
- Proinflammatory and prothrombotic effects of hyperglycemia and hyperinsulinemia that impair vascular autoregulation

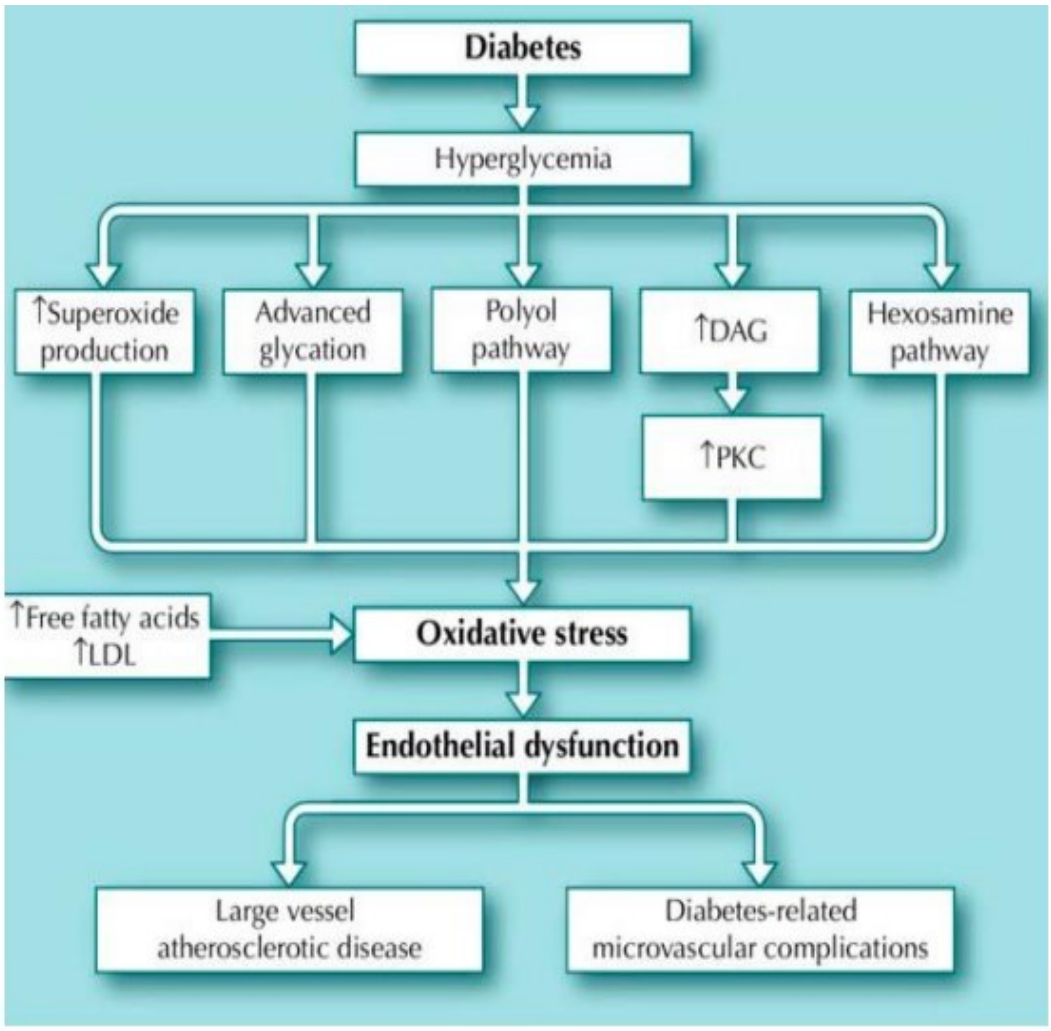


Figure 18: Main diabetes complications

Microvascular disease underlies 3 common manifestations of diabetes mellitus:

- Retinopathy
- Nephropathy
- Neuropathy

The microvascular disease may also impair skin healing so that even minor breaks in skin integrity can develop into deeper ulcers and quickly become infected, particularly in the lower extremities. Intensive control of plasma glucose can prevent or delay many of these complications but may not reverse them once established. [9]

The macrovascular disease involves atherosclerosis of large vessels, which can lead to

- Angina pectoris and myocardial infarction
- Transient ischemic attacks and strokes
- Peripheral arterial disease. [9]

Immune dysfunction is another major complication and develops from the direct effects of hyperglycemia on cellular immunity. Diabetic patients are particularly susceptible to bacterial and fungal infections. [9]

1.6.1.1 Cardiovascular complications

Cardiovascular diseases represent approx 80% of chronic diabetes complications. About two-thirds of people with diabetes die of cardiovascular disease, 50% ischemic heart disease.

Diabetes involves an increased cardiovascular risk because it involves the onset of a condition of insulin resistance, which is common to all the conditions associated with atherosclerosis, such as:

- Obesity;
- Hypertension;
- Hyperinsulinemia;
- Frankish diabetes;
- Hypertriglyceridemia;
- small and dense LDLs;
- Low level of HDL;
- Hypercoagulability.

1.6.1.1.1 Cardiovascular disease screening

Very often, already at the time of the diagnosis of diabetes, the cardiovascular condition of the patient is already impaired and he already has:

- Abnormal ECG or pulse deficiency found during the visit medical
- Heart attack
- Stroke / TIA
- Intermittent claudication
- Acute lower limb ischemia

This is because macrovascular involvement is already present in the condition of pre-diabetes (i.e., of reduced tolerance to carbohydrates) and worsens as the disease progresses.

Conversely, the microvascular consequences, being linked to glucose toxicity, occur only in the phase of frank diabetes.

Therefore, it is essential to refer these patients, as early as at the time of diagnosis, to a cardiovascular screening. This consists of:

- Vascular semeiological examination: the wrists are evaluated peripheral and the possible presence of vascular murmurs;
- Baseline ECG
- Determination of the Winsor index or pressure index ankle-arm or ankle-brachial index (ABI).

The latter is considered the gold standard for diagnosis of certainty of peripheral arterial disease because it is susceptible (95%) and specific (100%): at the time of diagnosis, this parameter is altered in 21% of patients while alterations of vascular semeiotics are found in only 8% of cases. ABI

is important in diagnosing peripheral vascular disease and is also a predictor of cardiovascular mortality and total mortality: they are 3-4 times higher in a patient with ABI <0.6 compared to a patient with normal ABI.

1.6.1.1.2 Ischemic heart disease

The algorithm for the diagnostic screening of ischemic heart disease in diabetes essentially divides patients into two groups according to the ECG result: normal or abnormal. In the asymptomatic with normal ECG, a periodic check-up is required while the asymptomatic with abnormal EGC is a candidate for a deepening employing an exercise ECG and an echocolordoppler which, in case they are positive, will require a stress myocardial scintigraphy with dipyridamole or coronary angiography and therapy optimal medical.

The high risk of silent coronary heart disease regardless of ECG or ECO is given by:

- Diabetic macroangiopathy, a tendency to develop atherosclerosis earlier and more intensely than what occurs in the population mean, it can be both symptomatic (previous events atherothrombotic or reperfusion surgery) or asymptomatic (a peripheral arterial disease with ABI <0.9, asymptomatic carotid stenosis> 50% and aortic aneurysm). Has a coronary risk score (UKPDS)> 30% at 10 years.
- One of the following risk factors: GFR <30 ml / min x 1.73 m2, cardiac autonomic neuropathy, erectile dysfunction, first-degree family history of ischemic heart disease at a young age <65 years in

females / 55 years in males. These diseases have a coronary risk score (UKPDS) > 20% at 10 years.

- Albuminuria and retinopathy together have a coronary risk score (UKPDS)> 20% at 10 years.

If the normal ECG is positive, but the patient is symptomatic, secondary examinations should not be performed, but it is immediately a candidate for coronary reperfusion surgery.

1.6.1.1.3 Heart failure

DM does affect the cardiac perfusion and the muscle component of the heart. The more frequent cause of hospitalization in a diabetic patient is heart failure. It should also be noted that there is a vicious circle between insulin resistance and heart failure, both risk and consequence factors of the other.

Patients with diabetes mellitus have over twice the risk of developing HF than patients without diabetes mellitus.[10] The Framingham Heart Study suggests that diabetes mellitus independently increases the risk of HF up to 2-fold in men and 5-fold in women compared with age-matched controls,[11] highlighting a sex discrepancy that is incompletely understood. The increased incidence of HF in diabetic patients persists even after adjusting for other risk factors such as age, hypertension, hypercholesterolemia, and coronary artery disease. Thus, the term diabetic cardiomyopathy was coined over 40 years ago and was initially used to describe ventricular dysfunction in the absence of coronary artery disease and hypertension in diabetic patients.[12] However, its use has been

broadened to describe the increased vulnerability of the myocardium to dysfunction that characterizes individuals with diabetes mellitus. While 10% to 15% of the general population have diabetes, a recent study suggested that 44% of patients hospitalized for HF have diabetes mellitus.[13] The coexistence of comorbidities poses unique clinical challenges.[14] While the association between mortality and HbA_{1c} in diabetes mellitus patients with HF appears to be U-shaped, with the lowest risk of death in patients with HbA_{1c} levels of ≈7.1%,[15] other studies suggest that diabetes mellitus is independently associated with greater risk of death and rehospitalization compared with nondiabetics with HF.[14] Additionally, observational data suggests a higher HbA_{1c} level was associated with increased incidence of HF.[16] Therefore, an important question to address is whether improved glycemic control improves HF outcomes.

2.3.6.1.4. HF risk and glycemic control

Many landmark clinical trials have addressed the relationship between tight glycemic control and cardiovascular endpoints. The ADVANCE trial (Action in Diabetes and Vascular Disease: Preterax and Diamicron MR Controlled Evaluation) showed that intensive glucose control, which lowered HbA_{1c} to 6.5% in type 2 diabetics, showed no evidence of a reduction in macrovascular events with no increase in mortality.[17] In contrast, the ACCORD trial (Action to Control Cardiovascular Risk in Diabetes), which targeted HbA_{1c} to 6% in the intensive therapy group, had increased mortality of 22%, suggesting a potentially unexpected increased risk of intensive glucose lowering in high-risk patients with T2DM. The finding of higher

mortality resulted in this arm of the trial being terminated.[18] These findings were further supported by intensive glycemic control in a veteran cohort over a 7.5-year period. They, too reported intensive glycemic control in patients with poorly controlled T2DM (baseline HbA_{1c} of 9.4%) had no significant effect on major cardiovascular events or death rates.[19] Similarly, the UKPDS (United Kingdom Prospective Diabetes Study) successfully reduced HbA_{1c} by 11% over a 10-year follow-up but did not substantially reduce diabetes mellitus related mortality or myocardial infarction (MI).[20] Together these studies suggested that despite the efficacy of diabetes mellitus therapies in achieving lower HbA_{1c}, these therapies were not necessarily advantageous from a cardiovascular standpoint. Some studies even showed an increase in cardiovascular events. These findings underscore the important conundrum that normalization of glycemia might not restore cardiovascular disease (CVD) risk to the nondiabetic baseline. Although HF was not a primary endpoint of these studies, post hoc analyses also suggested that intensive glucose lowering did not reduce and, in some cases, increased the risk for HF or HF hospitalization.[21] In summary, pharmacological agents that may benefit cardiovascular outcomes include Metformin, SGLT2i (sodium-glucose cotransporter 2 inhibitor) and certain GLP1RA (glucagon-like peptide 1 receptor agonist). However, others such as sulfonylureas (SUs), thiazolidinediones (TZDs), insulin, some GLP1RAs and some DPP4i (dipeptidyl peptidase 4 inhibitors) might exacerbate or increase the risk for HF.[14]

The observations that blood glucose-lowering might not be sufficient to prevent increased hospitalization and mortality from HF, reinforce the

possibility that additional factors beyond glycemia might contribute to the increased HF risk in diabetes mellitus or that independent mechanisms might exist linking antihyperglycemic therapies and left ventricle (LV) remodeling. Beyond the structural and functional changes that occur with diabetic cardiomyopathy, a complex underlying and interrelated pathophysiology exists and may contribute to HF in the context of diabetes mellitus, some of which may be amenable to pharmacological therapy. A consistently reported finding in the diabetic myocardium is cardiac hypertrophy, characterized by increased LV mass and wall thickness. Population studies have reported an independent association between diabetes mellitus, cardiac hypertrophy, and systolic dysfunction.[14] The ARIC study (Atherosclerosis Risk in Communities) provided evidence for subclinical myocardial damage in subjects with pre-diabetes and T2DM as measured by subclinical circulating concentrations of TnT (troponin T), using a highly sensitive assay. Subclinical myocardial damage increased linearly across the glycemic spectrum from no diabetes mellitus to pre-diabetes and diabetes mellitus. This correlated with increased risk for cardiovascular events, HF, or death, highest in those with T2DM.[22] A correlation between microvascular complications of diabetes mellitus and HF has long been established.[23] More recently, direct evidence of microvascular dysfunction and impaired myocardial perfusion reserve has been demonstrated implicating tissue hypoxia as another mechanism contributing to accelerated ventricular remodeling in diabetes mellitus. [24] Although the correlation between glycemia and myocardial injury could represent cause and effect, it could also reflect the existence of additional risk factors for the myocardial injury that track with glycemia. Therefore, any

analysis of the relationship between antihyperglycemic therapies and HF risk must also account for the impact of these agents on other potential mechanisms that could lead to cardiac injury. Thus, direct or indirect mechanisms that could link current antihyperglycemic therapies with LV remodeling and myocardial injury independent of their blood glucose-lowering effects may exist. The remainder of this review will examine current antihyperglycemic therapies and discuss potential mechanisms that could influence their efficacy in terms of modulating HF risk, and then will review additional pathophysiological targets implicated in diabetic cardiomyopathy that could be amenable to therapeutic manipulation. [14]

2.3.6.2. Effects of Diabetes Mellitus Treatments on Risk of Heart Failure

Table II: Summary of Effects of Diabetes Mellitus Treatments on Risk of Heart Failure [14]

DM Therapy	**Effects of Diabetes Mellitus Treatments on Risk of HF**
Biguanide (Metformin)	Associated with better short-term and long-term prognosis in patients with HF
	Associated with reduced mortality in HF patients
	Reduces cardiac hypertrophy by AMPK-mediated repression of mTOR and, as a consequence, protein synthesis
	AMPK activation by Metformin can stimulate cardiac glucose uptake
Sulfonylureas (SU)	Thought initially to increase mortality
	No definitive CV outcome trial to evaluate CV safety of SUs vs placebo or other diabetic agents
	Meta-analysis reports no increased CV risk with SU treatment vs metformin
	A retrospective cohort study reported an increased CV risk in patients on SU vs Metformin or DPP4 inhibitor
	No definitive CV outcome trials examining SUs in HF have been conducted

Thiazolidinediones (TZDs)	Reports on effects of TZDs on CV safety are conflicting.
	Beneficial effects were anticipated given improvements in glycemic control, inflammatory biomarkers, BP, TG levels and HDL
	PROactive trial showed no reduction in CV outcomes in patients on pioglitazone
	A meta-analysis reported an increased risk of MI with rosiglitazone
	IRIS trial reported a lower risk of stroke and MI in patients on pioglitazone vs placebo
	Occurrence of fluid retention and weight gain is a reproducible side-effect of TZD therapy, which precludes its use in NYHA III and IV HF
Glucagon-like peptide-I (GLP-I) receptor agonist	Meta-analysis reports no increased risk in HF or hospitalization for HF among type 2 diabetics
	A meta-analysis revealed a modest improvement in ejection fraction in HF patients
	Trial of GLP-1 agonist in advanced HF revealed a trend toward increased hospitalization in diabetes mellitus subgroup
Dipeptidyl peptidase 4 (DPP4) Inhibitors	SAVOR-TIMI-53 trial reported a significant increase in hospitalization for HF in patients on saxagliptin vs placebo
	EXAMINE, and TECOS trials do not reveal increased HF risk
	Experimental studies in humans and animals showed improvements in cardiac function when GLP-1 was activated by DPP4 inhibitor
	DPP4 knock out mice showed induction of cardioprotective gene signature post-MI
Sodium-glucose cotransporters 1 and 2 (SGLT1 and 2) Inhibitors	SGLT2 improves CV risk factors (weight reduction, reduction in SBP and improved lipid profile)
	EMPA-REG OUTCOME trial reported a reduction in CV mortality and hospitalization from HF using empagliflozin
	CANVAS trial reported similar results for canagliflozin
	Meta-analysis of CV events in people with type 2 diabetes on dapagliflozin reported no increased risk for CV events
Insulin	Some observational trials have suggested a relationship between insulin use and HF risk
	CVOT with long-acting insulin analogs do not demonstrate increased CV event rate or HF

AMPK indicates AMP-activated protein kinase; BP, blood pressure; CV, cardiovascular; CVOT, cardiovascular outcome trial; HDL, high-density lipoprotein; HF, heart failure; MI, myocardial infarction; mTOR, mechanistic target of rapamycin; NYHA, New York Heart Association; and TG, triglycerides.

2.3.6.2.1 Glucagon-like peptide-1 receptor agonist

Glucagon-like peptide-1 receptor agonists, also known as GLP-1 receptor agonists or incretin mimetics, are agonists of the GLP-1 receptor. This class of medications is used for the treatment of type 2 diabetes.[25] One of their advantages over older insulin secretagogues, such as sulfonylureas or meglitinides, is that they have a lower risk of causing hypoglycemia.[26] GLP-1 has a short duration of action, so to overcome this limitation several modifications either in the drug or the formulations are being developed.[27] The glucagon-like peptide-1 receptor (GLP1R) is a receptor protein expressed in various organs such as the heart, kidney, and pancreas (beta cells) and have important systemic effects as well. It is involved in the control of blood sugar level by enhancing insulin secretion. In humans it is synthesised by the gene GLP1R, which is present on chromosome 6.[28] It is a member of the glucagon receptor family of G protein-coupled receptors.[29] GLP1R is composed of two domains, one extracellular (ECD) that binds the C-terminal helix of GLP-1 [30] and one transmembrane (TMD) domain that binds the N-terminal region of GLP-1. In the TMD domain there is a fulcrum of polar residues that regulates the biased signaling of the receptor while the transmembrane helical boundaries and extracellular surface are a trigger for biased agonism.[31]

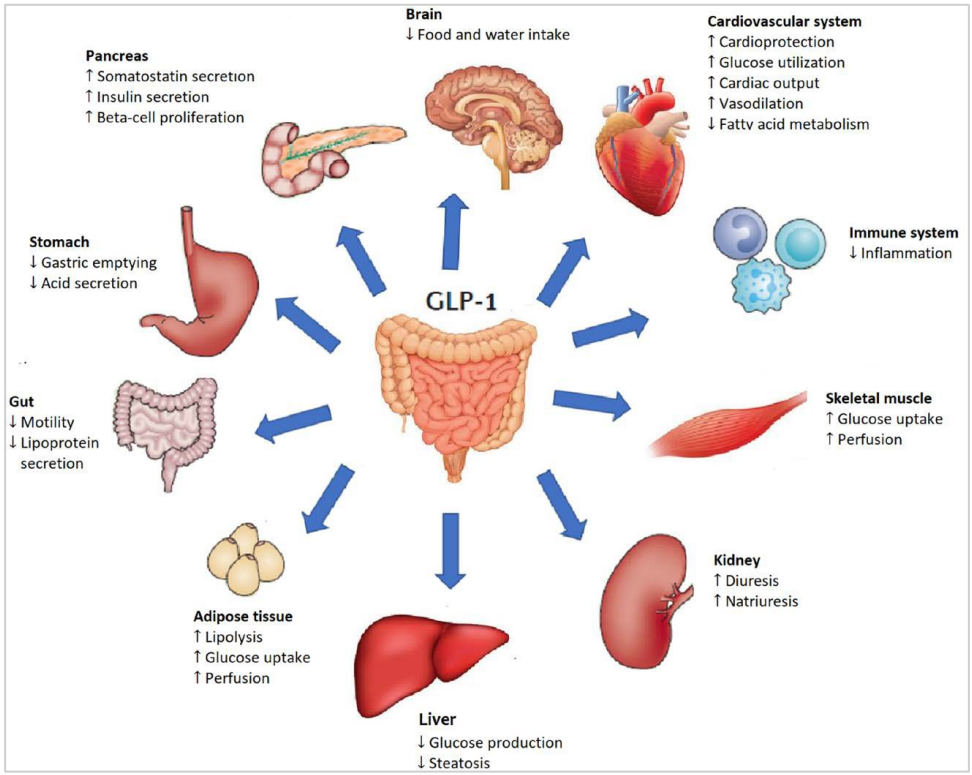


Figure 19: Systemic effects of GLP-1 RA

More in specifically, GLP-1 Ras activate the cAMP-protein kinase A pathway in the kidneys that reduces the production of reactive oxygen species and thus potentially protects the kidney from oxidative injury. GLP-1 RAs reduce markers of renin-angiotensin-aldosterone system activation and thereby may contribute to blood pressure lowering and protect the kidneys.[32] GLP-1 RAs reduce the generation of reactive oxygen species in the endothelial cells and macrophages and reduce systemic inflammation.[33] Some data show that GLP-1 RAs may improve diastolic function by reducing diastolic filling pressures and unloading the ventricle.[1]

On the other hand, under non-ischemic conditions, cardiac myocytes readily use both fatty acids and glucose to efficiently generate ATP via oxidative phosphorylation. Fatty acids are the predominant substrate under normal

conditions. In chronically overloaded hearts, altered expression of key metabolic genes reduces fatty acid uptake and metabolism. As heart failure progresses, cardiac myocyte insulin resistance develops and leads to reductions in glucose uptake via the transporters GLUT1 and GLUT4. Together, these changes impair the uptake and utilization of two chief substrates for ATP generation via oxidative phosphorylation. Either naturally occurring GLP-1 or degradation-resistant GLP-1 agonists reduce insulin resistance and increase cardiac myocyte glucose uptake via signaling through the GLP-1R receptor that induces phosphorylation (activation) of AMP-activated protein kinase (AMPK). [34] (Figure 20)

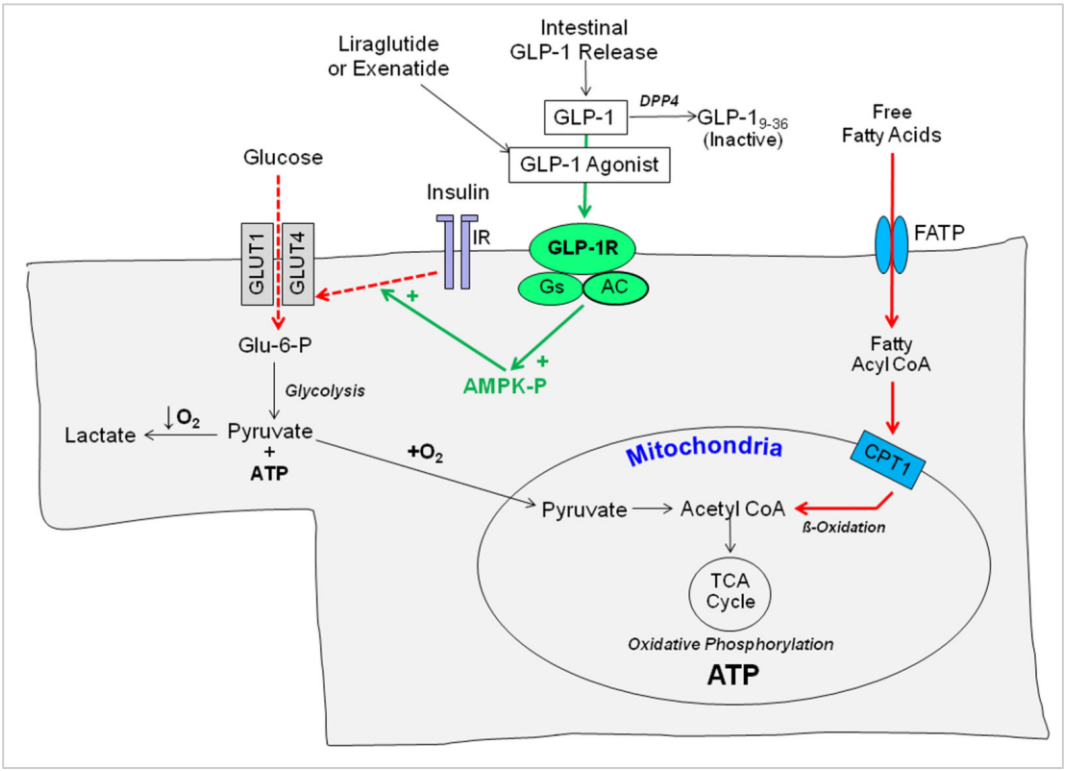


Figure 20: GLP-1 pathway [1]

2.3.6.2.2 SGLT2 inhibitor

SGLT2 inhibitors, also called gliflozins, are a class of medications that alter essential physiology of the nephron, they act by inhibiting sodium-glucose transport protein 2 (SGLT2). The foremost metabolic effect appears to show that this pharmaceutical class inhibits reabsorption of glucose in the kidney and therefore lower blood sugar.[35] SGLT2 inhibitors are used especially in the treatment of type II diabetes mellitus (T2DM). Apart from blood sugar control, gliflozins have been shown to provide significant cardiovascular benefit in T2DM patients.[36] Several medications of this class have been approved or are currently under development.[37] In studies on canagliflozin, a member of this class, the medication was found to enhance blood sugar control as well as reduce body weight and systolic and diastolic blood pressure.[38]

Sodium Glucose cotransporters (SGLTs) are proteins that occur primarily in the kidneys and play an important role in maintaining glucose balance in the blood.[39] SGLT1 and SGLT2 are the two most known SGLTs of this family. The SGLT2 is a protein that in humans is encoded by the SLC5A2 (solute carrier family 5 (sodium/glucose cotransporter)) gene [38] and it is the major transport protein and promotes reabsorption from the glomerular filtration glucose back into circulation: is responsible for approximately 80-90% of the kidney's glucose reabsorption.[35] SGLT2 is mainly expressed in the kidneys on the epithelial cells lining the first segment of the proximal convoluted tubule. By inhibiting SGLT2, gliflozins prevent the kidneys' reuptake of glucose from the glomerular filtrate and subsequently lower the glucose level in the blood and promote the excretion of glucose in the urine

(glucosuria). Most of the remaining glucose absorption is by sodium/glucose cotransporter 1 (SGLT1) in more distal sections of the proximal tubule. [38] Sodium and glucose are co-transported by the SGLT-2 protein into the tubular epithelial cells across the brush-border membrane of the proximal convoluted tubule. This happens because of the sodium gradient between the tubule and the cell and therefore provides a secondary active transport of glucose. Glucose is later reabsorbed by passive transfer of endothelial cells into the interstitial glucose transporter protein.[40]

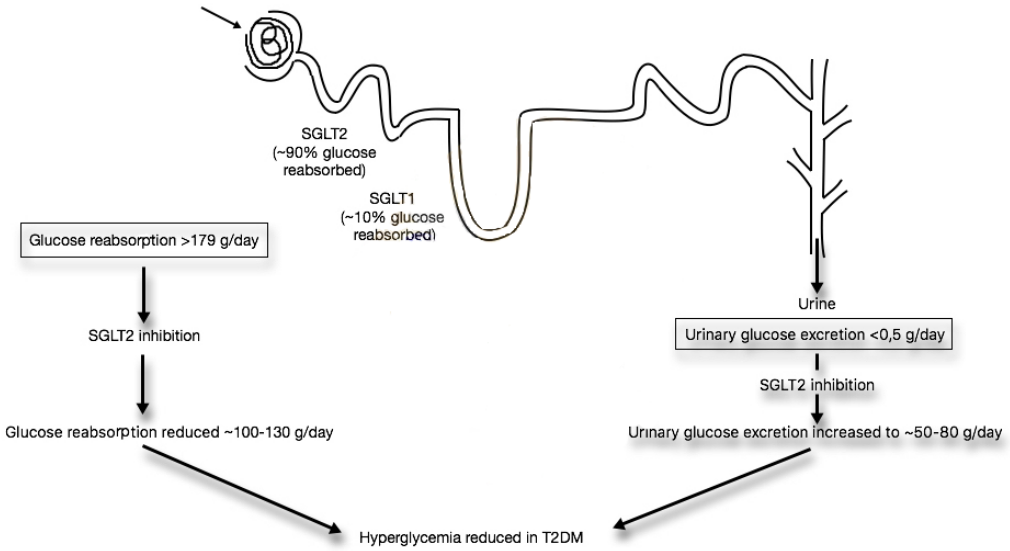


Figure 21: Reabsorption of glucose in the nephron[41]

Dapagliflozin is an example of an SGLT-2 inhibitor, it is a competitive, highly selective inhibitor of SGLT. It acts via selective and potent inhibition of SGLT-2, and its activity is based on each patient's underlying blood sugar control and kidney function. The results are decreased kidney reabsorption of glucose, glucosuria effect increases with higher level of glucose in the blood circulation. Therefore, dapagliflozin reduces the blood glucose concentration with a mechanism that is independent of insulin secretion and

sensitivity, unlike many other antidiabetic medications. Functional pancreatic β-cells are not necessary for the activity of the medication, so it is convenient for patients with diminished β-cell function.[40], [41]

2. The effect of glucagon-like peptide 1 receptor agonists and sodium-glucose cotransporters 2 inhibitors on N-terminal pro-brain natriuretic peptide

2.1 Introduction

Sodium-glucose cotransporters 2 inhibitors (SGLT2i) significantly reduce the hospitalization for heart failure (hHF), in patients with or without diabetes, irrespective of previous cardiovascular events[42]. This observation has been consistent in patients with heart failure (HF) with reduced ejection fraction (HFrEF), with a 32% decrease in risk in hHF, and reported in patients with HF with preserved ejection fraction (HFpEF) as well[43].

The European Society of Cardiology states that natriuretic peptides oppose the vasoconstriction, sodium retention, and anti-diuretic effects of the activated renin-angiotensin-aldosterone and sympathetic nervous systems observed in patients with HF[44]. For these reasons, they have good diagnostic accuracy in discriminating HF from other causes of dyspnoea.

Natriuretic peptides are also of value for the screening of the general population since they may be elevated early in the course of the disease process, and the efficacy of treatment of HF can be reliably detected by their

circulating concentrations[45]. Among natriuretic peptides, the N-terminal pro-BNP has been identifying as a reliable marker to guide treatment in a population of diabetic patients without a history of cardiac disease (PONTIAC) study[46]. N-terminal pro-BNP may also help to identify patients with left ventricular dysfunction[47].

Yet, the surprising results of sodium-glucose co-transporter 2 inhibitors (SGLT2i) on hHF have been observed without an apparent effect of these drugs on natriuretic peptides[48]. Conversely, in the PARADIGM-HF, sacubitril–valsartan reduced hHF by 21% with a concomitant reduction of plasma concentrations of N-terminal proBNP in patients randomized to the sacubitril-valsartan group at both 30 days and 8 months[49]. These results are in line with the ability of this drug combination to block the action of neprilysin, thus preventing the breakdown of natriuretic peptides, which thus exert a positive biological effect on the kidney, vasculature, and nervous system[50]. To complicate this further, the increase in pro-inflammatory cytokines observed in patients with HF is linked to a concomitant increase in plasma levels of natriuretic peptides[51]. SGLT2i can significantly inhibit inflammasome, beyond their ability to reduce plasma glucose, yet this effect did not resound into a consistent effect on natriuretic peptides[52].

The majority of patients with type 2 diabetes are affected by obesity, which is associated with reducing concentrations of natriuretic peptides. With excess fat mass, an increased expression of neprilysin leads to the degradation of natriuretic peptides[53]. An essential role for the reduced concentration of natriuretic peptides is also exerted by chronically elevated

insulin levels, possibly due to insulin resistance[54]. The relationship between obesity and the reduced concentration of natriuretic peptides might explain the HF benefits of interventions associated with drugs that induce weight loss. In particular, glucagon-like peptide 1 receptor agonists (GLP-1RA), thanks to their ability to cause a remarkable weight loss may decrease the risk of hHF[55], [56].

Therefore, 1. it is unclear whether a significant effect on natriuretic peptides mediates the decreased risk of SGLT2i, and 2. whether the ability of GLP-1RA to induce weight loss can be reflected in amelioration of natriuretic peptide, and hence in their potential to decrease the risk of hHF. Thus, this scoping review was conducted to systematically map the effects of both SGLT2i and GLP-1RA on N-terminal pro-BNP. Furthermore, we wished to assess the potential effect of age, body weight, and metabolic control.

2.2 Material and methods

This protocol was drafted according to the PRISMA 2020 explanation and elaboration. The protocol was registered on the International prospective register of systematic reviews (PROSPERO CRD42021252536: to enable PROSPERO to focus on COVID-19 registrations during the 2020 pandemic, this registration record was automatically published exactly as submitted).

2.2.1 Eligibility criteria

Studies were included in the analysis if they met all the following conditions:

a. to be written in English;

b. to be performed in humans;
c. to be designed as clinical trials;
d. to report N-terminal proBNP concentration both a baseline and after either GLP-1RA or SGLT2i treatment;
e. to include a control group.

Although both BNP and N-terminal pro-BNP has recognized indices of severity of HF[57], this analysis was restricted only to N-terminal pro-BNP, the N-terminal, inactive counterpart of BNP. This restriction allowed a better data standardization since most of the papers report the N-terminal pro-BNP as the reference natriuretic peptide. Moreover, BNP and N-terminal pro-BNP can significantly differ in clinical prediction[58]. Furthermore, immunoassays may detect different BNP degradation products and, therefore, the performance of each assay might vary [59], [60].

2.2.2 Search strategy

A Pubmed and EMBASE search without period restriction was performed using the search string reported in Supplementary Materials. Article extract was based on the PICO approach as follows. *Patient, Population or Problem* In patients with or without type 2 diabetes with or without HF; *Intervention* do long-term treatment of GLP-1RA and SGLT2i; *Comparison* and if so, to which extent they differ either to one each other or as compared to placebo; *Outcome* in modifying the circulating levels of N-terminal proBNP.

The searches and the selection of studies were independently performed by two authors, and conflicts were resolved by a third investigator. For each article, a JADAD score to assess the methodological quality of the trial was performed. For each article, relevant data for the present meta-analysis,

such as the number of patients included, age, body mass index, gender, the degree of metabolic control, the prevalence of both HF and diabetes were charted.

2.2.3 Data extraction

ENDNOTE X9 literature management software was used to manage the literature search records.

Studies that met the inclusion criteria were subjected to full-text evaluation are reported in Table III.

The following parameters/information were extracted: first author, publication year, Clinical Trial number (NCT) or other registration identifiers/acronyms, types of treatment, sample size, duration of the trial, age, randomization procedure, description of withdrawal from trial, duration of diabetes, as well as baseline and endpoints.

Table III: Detailed presentation of the individual trial group's patient characteristics from studies included in the present meta-analysis comparing the efficacy and safety of GLP-1 receptor agonists and sodium glucose cotransporters 2 inhibitors

Study/ publication	Number of patients		Age [Years]		(% Male)		BMI [kg/m^2]		Duration of diabetes [Years]		HbA1c (%)		Heart failure (%)		Diabetes%	
	Drug	Placebo	Drug	Placebo	Drug	Placebo	Drug	Placebo	Drug	Placebo	Drug	Placebo	Drug	Placebo	Drug	Placebo
Glucagon-like peptide 1 receptor agonists																
Arturi et al. 2016	26	10	56±9	61±10	70	60	33.2±2	30.9±2.8	NA	NA	8.2±1	8.3±1	100	100	100	100
Bizino et al. 2019	23	26	60±6	59±7	61	58	32.6±4.4	31.6±3.4	11±6	11±7	8.4±1.1	8.2±1.0	0	0	100	100
Jin Ying Zhang et al. 2017	28	26	59±12	59±11	77	73	25.3±3.4	24.8±3.8	NA	NA	5.4±0.6	5.3±0.4	100	100	19	27
Jorsal et al. 2017	122	119	65±9	65±11	89	90	28.0±3.8	29.8±4.6	NA	NA	5.9±0.7	6.0±0.8	100	100	32	29
Lambadiari et al. 2018	30	30	51±10	50±12	67	67	32.9±5	27.7±2	NA	NA	8.6±2	8.4±1.2	0	0	100	100
Lovshin et al. 2015	18	18	62±NA	62±NA	100	100	29.5±NA	29.5±NA	NA	NA	NA	NA	0	0	0	0
Margules 2016	154	146	62 (52-68)	61 (51- 67)	80	77	31 (26–36)	33 (25–38)	NA	NA	6 6 (6.0-7.6)	6.7 (5.9-7.9)	100	100	59	60
Nielsen et al. 2020	115	116	65 ± 9	65 ± 11	90	90	33.8 ± 5.8	33.1 ± 6.2	NA	NA	5.9±0.7	6.0±0.8	100	100	30	28
Wei-Ren Chen 2016	45	45	58±11	59±12	76	71	NA	NA	NA	NA	5.3±0.3	5.4±0.5	100	100	30	28
Mean±SD	60±54	60±52	60±4	60±4	79±12	76±14	31.6±11.3	30.1±2.8			6.8±1.4	6.8±1.3				
Sodium-glucose co-transporters 2 inhibitors																
Brown et al. 2020	52	32	64±7	67±7	63	53	32.3±4.7	32.6±4.2	9 (5-15)	10 (8- 15)	7.8±1.2	7.6±1.1	0	0	100	100
Ejiri et al. 2020	86	83	71±8	75±8	66	59	25.4±4.3	25.3±4.4	6 (2–11)	6 (3–12)	7.0±0.7	6.9±0.8	100	100	100	100
Jannuzzi et al. 2017	450	216	64±6	63±6	55	62	31.4±4.5	31.9±4.8	10 (6-16)	10 (6-15)	7.7±0.8	7.8±0.8	0	0	100	100
Kario et al. 2018	41	37	70±10	68 ±10	66	51	25.5 ± 3.3	26.3 ± 4.1	NA	NA	7.4±0.7	7.2±0.6	0	0	100	100
Latva-Rasku et al. 2019	15	16	62±8	60±7	87	75	32.1±3.7	31.7±5.0	8±4	7±4	7.0±0.6	6.8±0.5	0	0	100	100
Petrie et al. 2021 (w/oDM)	1075	1064	66±12	66±12	75	76	27.3±5.9	27.1±5.6	NA	NA	5.7±0.4	5.8±0.4	100	100	0	0
Petrie et al. 2021 (with DM)	1298	1307	66±10	67±10	78	78	29.3±5.9	29.4±6.1	NA	NA	7.4±1.5	7.4±1.6	100	100	100	100
Phrommintikul et al. 2019	25	24	63±8	64±7	50	56	25.6±3.0	24.9±3.2	NA	NA	8.2±1.4	8.2±1.1	20	29	100	100
Verma et al. 2019	49	48	64 (57-69)	64 (56-72)	90	96	26.7 (24.5-30.2)	26.6 (24.4-29.3)	10 (4-15)	10 (5-15)	7.9 (7.5-8.4)	7.9 (7.3-8.7)	4	8	100	100
Mean±SD	314±501	343±500	65±3	66±4	70±14	67±15	28.4±2.9	28.4±3.0			7.3±0.7	7.3±0.7	0.8905	0.0002	0.0001	0.0001
P GLP-1RA vs. SGLT2I	P=0.099	P=0.067	0.005	0.004	0.221	0.186	0.058	0.251			0.385	0.291				

2.2.4 Primary outcome

The primary outcome of the present meta-analysis was the difference between N-terminal pro-BNP concentrations before and after treatment either with GLP-1RA and SGLT2i.

Secondary outcomes were: 1. The impact of age; 2. The effect of body mass index; 3. The effect of metabolic control.

2.3 Statistics

The effect of GLP-1RA treatment on the N-terminal pro-BNP means delta outcome, compared to the placebo arm, has been considered for the analysis. The effect of SGLT2I on the difference in change score concerning the placebo arm has also been considered a secondary endpoint.

2.4 Data pre-processing

The N-terminal pro-BNP mean and standard deviation (SD) collected at baseline (pre), follow-up (post) together with delta (post-pre) data are the measure considered to summarize the continuous endpoints in the meta-analysis. Any alternative reporting measures of the study endpoints were converted to mean and SD; for example:

- Whenever the study reported the median m instead of the mean $\bar{X}$, the mean has been calculated as $\bar{X} \approx \frac{q_1+m+q_3}{3}$ where $q_1$and q_3 are the first and third quartiles[61].

- Whenever the study reports the interquartile range in place of the SD, then the whole is converted to SD using the formula proposed in the literature $SD \approx \frac{q_3 - q_1}{1.35}$ [62].
- If the studies report the confidence interval lower and upper limits, the information is converted into SD using the inverse formula $\mathrm{SD} = (\sqrt{\mathrm{n}}$ (upper limit - lower limit))$/3.92[63]$.The sample size in the previous relationship is n.

The available information (baseline and follow-up) has been combined to obtain a complete outcome assessed on the delta scale. Specifically, the following procedures have been performed:

1) The missing $SD_{t,post}$ for studies reporting the delta data have been computed by considering the following formula as indicated in the literature:

$$\mathrm{SD}^2_{t,\,post} = \mathrm{SD}^2_{t,\,pre} - \mathrm{SD}^2_{t,\,delta} + 2 \times SD_{t,\,pre} \times SD_{t,\,delta}$$

$$SD_{t,post} = \sqrt{\mathrm{SD}^2_{t,\,post}}$$

In the notation $SD_{t,\,delta}$ indicates the SD of delta outcome and $SD_{t,\,pre}$ or $SD_{t,\,post}$ denotes respectively the SD of baseline and follow-up information$[62]$.

2) The remaining $SD_{t,post}$ data have been computed conservatively, as suggested in the literature, imputing the missing SD with the maximum SD across the studies reporting the outcome in the same scale of measure[63].

2.4.1 Delta outcome calculation

The primary outcome has been reported on the change scale. Studies report the endpoint on different N-terminal pro-BNP units of measure; to make trial results comparable across studies, we have computed the standardized effect size. The method proposed by Becker has been considered for the calculation[64]. The mean change score for treatment (t=T) and Placebo arm (t=P) has been calculated as:

$$d_T = c(n_T - 1)\frac{\bar{x}_{T,post} - \bar{x}_{T,pre}}{SD_{T,pre}}$$

$$d_P = c(n_P - 1)\frac{\bar{x}_{P,post} - \bar{x}_{P,pre}}{SD_{P,pre}}$$

The n_T and n_P are the sample size in the treatment (T) and Placebo (P) arm; $\bar{x}_{T,post}$ and $\bar{x}_{P,post}$ are the post-treatment NT means and the $\bar{x}_{T,pre}$ and $\bar{x}_{P,pre}$ are the baseline mean NT values. In the equations reported below $c(m)$ is the correction factor $\sqrt{\frac{2}{(m-1)}}\frac{\Gamma[(m)/2]}{\Gamma[(m-1)/2]}$ where Γ denotes the Gamma function[65].

The variance of the d_T and d_P effect size id calculated as:

$$\mathrm{var}(d_T) = \frac{2(1-r_T)}{n_T} + \frac{(d_T)^2}{2n_T};\ \mathrm{var}(d_P) = \frac{2(1-r_P)}{n_P} + \frac{(d_P)^2}{2n_P}$$

The values of $r_T\ and\ r_P$ are the pre-post study correlation values.

The difference in the two standardized mean change values in the treatment becomes $d = d_T - d_P$. The variance of the difference in mean change scores between treatment and placebo becomes $\mathrm{var(d)} = \mathrm{var}(d_T) + \mathrm{var}(d_P)$[64].

2.4.2 Computation of the pre-post treatment correlations

For both the treatment arms the pre-post correlation r_T or r_P has been computed for the studies reporting the complete information concerning the baseline, post, and delta values. The computation has been performed by using the procedure suggested in the literature[62]:

$$r_T = \frac{\mathrm{SD}^2_{T,\,pre} + \mathrm{SD}^2_{T,\,post} - \mathrm{SD}^2_{T,\,delta}}{2 \times SD_{T,\,pre} \times SD_{T,\,delta}} \; ; \; r_P = \frac{\mathrm{SD}^2_{P,\,pre} + \mathrm{SD}^2_{P,\,post} - \mathrm{SD}^2_{P,\,delta}}{2 \times SD_{P,\,pre} \times SD_{p,\,delta}}$$

Whenever the information required to calculate the pre-post correlation was not available, then values were imputed by performing a sensitivity analysis by considering a pre-post-treatment correlation of 0.5; 0.8, and 0.7 correlation. These correlation values are widely used to impute the pre-post study correlation values also in other research settings[66].

2.5 Meta-analysis

2.5.1 Funnel plot

The publication bias assessment has been performed by considering the funnel plot representation. The study-specific standardized mean change scores have been represented on the x-axis and the study-specific standard error on the y-axis. The represented confidence bounds report the confidence limit around the mean value. Specifically, this means that any observation plotted within the limits will have a confidence interval that includes the average value. The study-specific confidence represented outside the funnel limits would not include the mean value and could denote a possible source of publication bias and warrant further investigation[67].

2.5.2 Random effect Meta-Analysis

A random-effect meta-analysis has been carried out on the difference in standardized mean change score between treatment and Placebo arms. The standard errors of the study estimates are adjusted by including a measure of the amount of variation, or heterogeneity, among the study treatment effects. The heterogeneity is estimated from the intervention effects and standard errors of the studies included in the meta-analysis via Der Simonian and Laird Estimator[68].

The I^2 measure has been considered to quantify the heterogeneity. The measure expresses the percentage of between-study variability that is related to heterogeneity rather than chance[62]. The Cochran's Q-test has been computed to identify a significant source of heterogeneity among the studies[68].

The study-specific estimates with the 95% confidence intervals have been reported in a forest plot representation together with the pooled meta-analytical estimate.

Several univariable meta-regression models have been computed to assess the possibility that the study characteristics may act as effect modifiers on the final meta-analysis estimate. The considered moderators are:

1) Low versus High (>= 3) quality study assessed on Jadad score;
2) Mean age of study participant;
3) Mean of the Body Mass Index of the study sample;
4) HbA1c mean level of the study participants.

The comparison of GLP-1RA versus SGLT2I has been performed in a metaregression random effect moderator meta-analysis.

The analyses have been performed with the R[69] system and metafor[70] package.

2.6 Results

2.6.1 Trial characteristics

The trial flow summary is reported in Fig. 22.

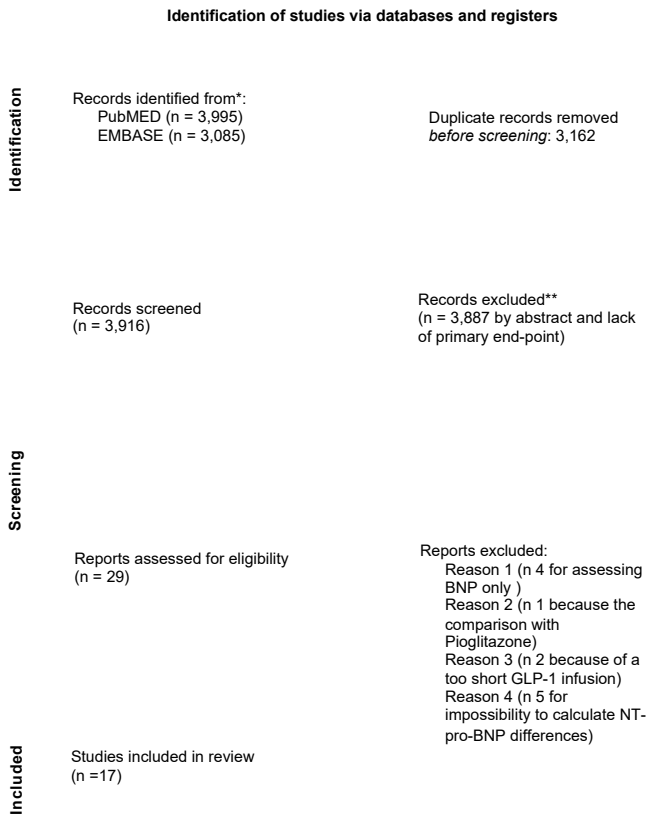


Figure 22: PRISMA flow for study selection

Nine trials, including a total of 543 patients in active treatment and 536 patients in placebo, were eligible to test the effect of GLP-1RA on N-terminal pro-BNP [71-79]. Eight trials, including 2827 patients in active treatment and 3091 in placebo, were eligible for SGLT2i [80-87]. The Cohen's parameter,

which reports the effect size, was 0.641 for trials testing GLP-1RA (medium effect size) and 0.102 for trials testing SGLT2i (small effect size).

The studies are reported in Table III. A statistical difference between those enrolled in GLP-1RA and those enrolled in SGLT2i trials was observed for age, the prevalence of heart failure, and diabetes. The mean duration of follow-up was 20±15 weeks for GLP-1RA and 41±34 for SGLT2i trials, respectively (p=0.114).

As shown in Table IV, the 'Jadad Score' estimating the study quality of the trials included in the present meta-analysis comparing the effect of GLP-1RA and SGLT2i on N-terminal pro-BNP levels, reveals overall comparability between trials with the 2 different classes of drug (2.67±1.46 for GLP-1RA vs. 3.38±0.72; p=0.246) except for the description of withdrawals and dropouts, which was significantly better for SGLT2i trials.

Table IV: 'Jadad Score' estimating the study quality of the trials included in the present meta-analysis comparing the effect of GLP-1RA and SGLT2i on N-terminal pro-BNP levels

Study/publication	Clinical trials registration number	Study described as randomized	Study described as double blind	Withdrawals and dropouts described	Appropriate description of details regarding randomization and double blinding	Either randomization or double blinding inappropriate (- 1 point)	Sum
Glucagon-like peptide-1 receptor agonists studies							
Arturi et al. 2016	N.A.	1	0	0	0	-1	0
Bizino et al. 2019	NCT01761318	1	1	1	1	0	4
Jin Ying Zhang et al. 2017	NCT02490176	1	1	0	0	0	2
Jorsal et al. 2017	NCT01472640	1	1	1	1	0	4
Lambadiari et al. 2018	NCT03010683	1	0	0	0	0	1
Lovshin et al. 2015	NCT01755572	1	1	1	0	0	3
Margules 2016	NCT01800968	1	1	1	1	0	4
Nielsen et al. 2020	NCT01472640	1	1	0	0	0	2
Wei-Ren Chen 2016	N.A.	1	1	1	1	0	4
GLP-1 RA (Mean ± SEM[a])		1±0	0.8 ±0.4	0.6±0.5	0.4 ±0.5	-0.1±0.3	2.7 ±1.4
Sodium-glucose cotransporters 2 inhibitors							
Brown et al. 2020	NCT02956811	1	1	1	1	0	4
Ejiri et al. 2020	UMIN000018395	1	0	1	1	0	3
Jannuzzi et al. 2017	NCT01106651	1	1	1	1	0	4
Kario et al. 2018	UMIN000023487)	1	0	1	0	0	2
Latva-Rasku et al. 2019	NCT02426541	1	1	1	1	0	4
Petrie et al. 2021	NCT03036124	1	1	1	1	0	4
Phrommintikul et al. 2019	NCT03178591	1	1	1	0	0	3
Verma et al. 2019	NCT02998970	1	1	1	0	0	3
SGLT2i (Mean ± SEM[a])		1±0	0.8±0.5	1±0	0.6±0.5	0±0	3.4±0.7

2.6.2 GLP-1RA effect on N-terminal pro-BNP

The funnel plot representation indicates that the study-specific standardized mean change score lies in confidence bounds indicating no evidence of publication bias among GLP-1RA trials. (Figure 23)

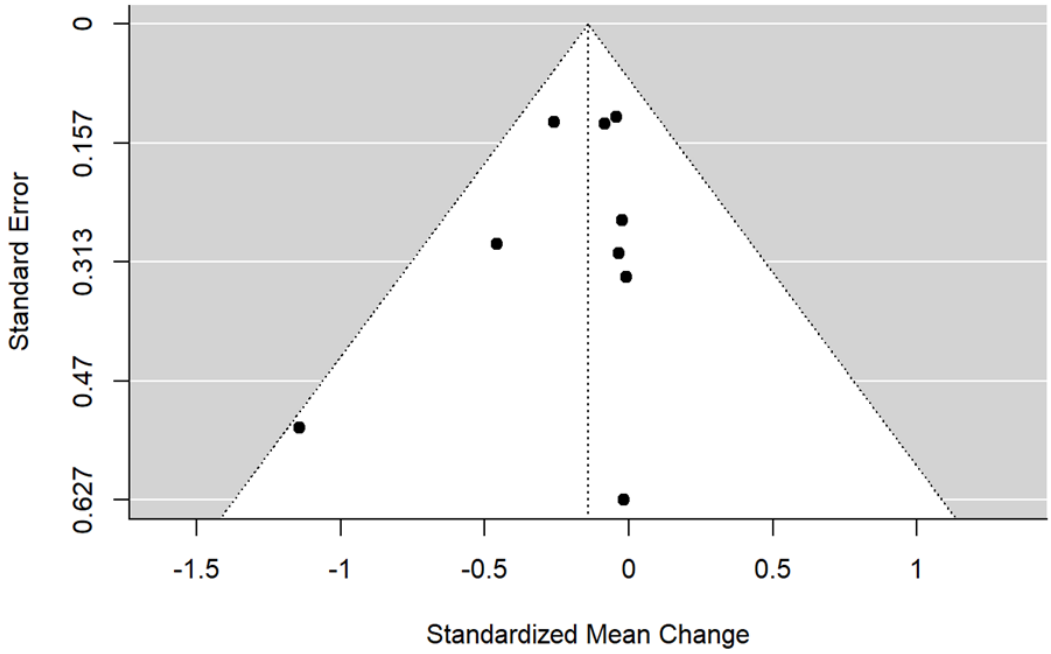


Figure 23: Funnel Plot of GLP-1RA trials; assumed 0.5 pre-post correlation

The Forest plot (Figure 24) reports a significant N-terminal pro-BNP reduction in comparison with Placebo for the GLP-1RA trial corresponding to a reduction of -0.14 [95% CI = -0.27 ; -0.01] (P-value=0.03) standard deviations.

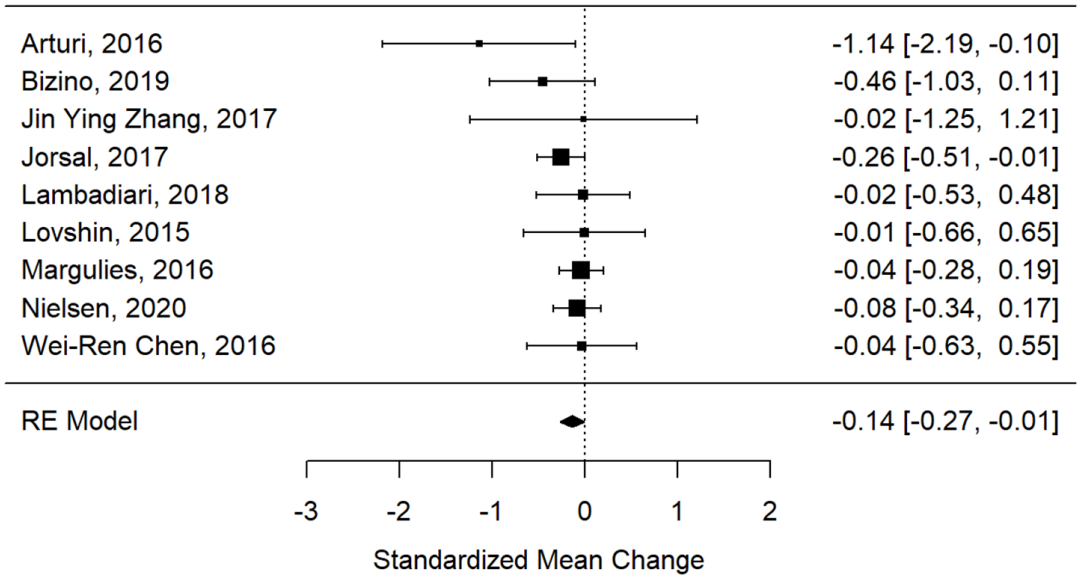


Figure 24: Forest plot of GLP-1RA trials; assumed 0.5 pre-post correlation. The Cochran's Q-test identified no significant source of correlation; I2=0.01

The single study outcome is significant only for the Arturi 2016 trial. The moderator meta-regression results (Table V) evidenced the absence of any significant effect modifier for age, BMI, HbA1c, and high-quality studies (Jadad ≥ 3).

Table V: Random Effect moderator meta-regression results, GLP-1RA trials. The model estimate with the 95% Confidence Intervals (CI) and P-value has been reported

Moderator	**Estimate [95% CI]**	**P-value**
Age	-0.01 [-0.04 ; 0.03]	0.71
BMI	-0.04 [-0.12 ; 0.04]	0.32
HbA1c	-0.05 [-0.27 ; 0.17]	0.67
Jadad ≥ 3	0.04 [-0.23 ; 0.31]	0.79

The results have also been confirmed by assuming a pre-post study correlation of 0.7 and 0.8 (Figure 20, 25); only Artury's study of moves slightly outside the confidence bands in the funnel plot for both the correlation scenarios (Figure 26).

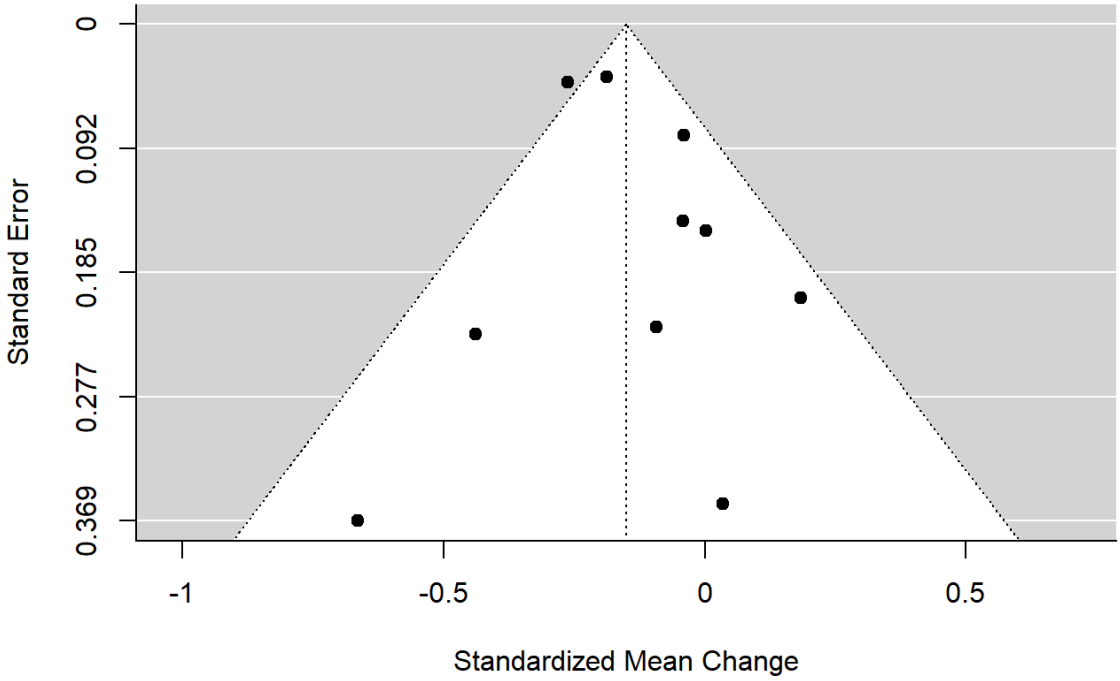


Figure 25:Funnel Plot of SGLT2I trials; assumed 0.5 pre-post correlation

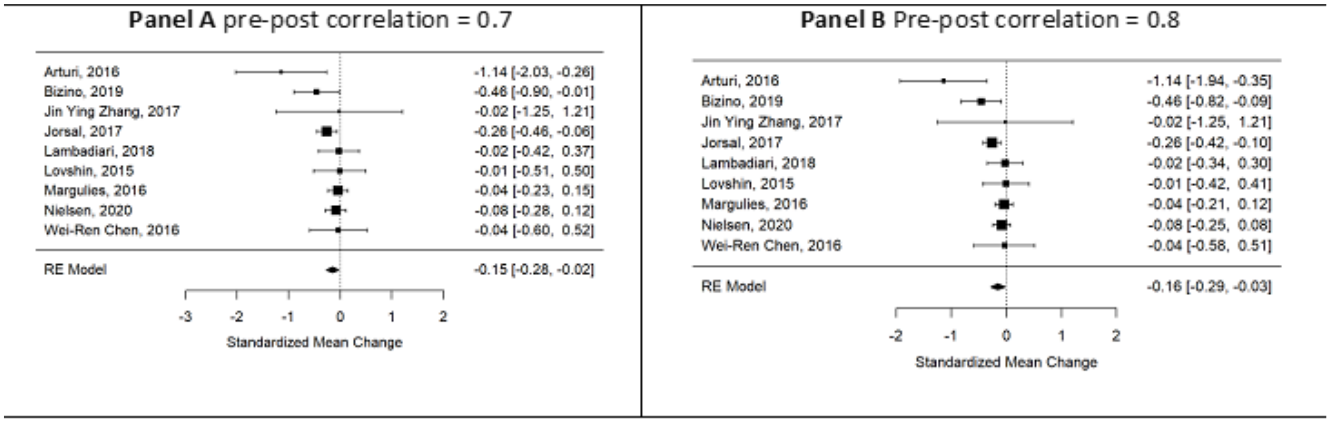


Figure 26:Forest Plot of GLP-1RA trials; assumed 0.7 (Panel A) and 0.8 (Panel B) pre-post correlation

The funnel plot indicates no evidence of publication bias among SGLT2I trials; only the Petrie 2021 study stands outside the confidence limits (Figure 27).

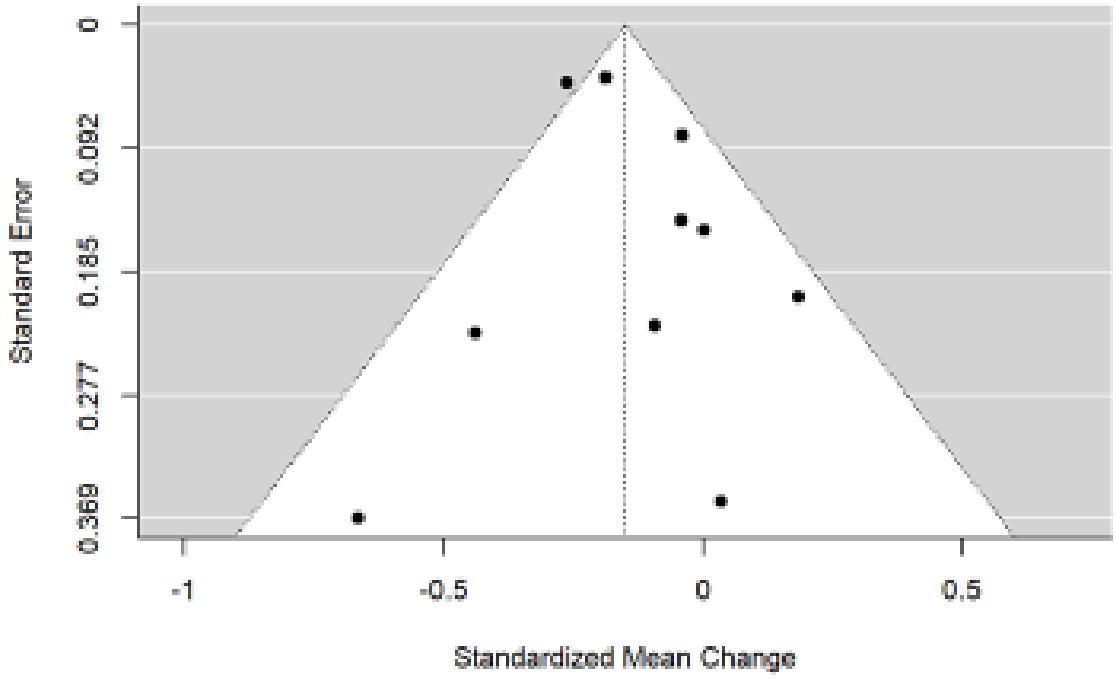


Figure 27: Funnel Plot of SGLT2I trials; assumed 0.5 pre-post correlation

The forest plot (Figure 28) indicates an NT reduction, for the SGLT2I treatment of 0.15 [95% CI = -0.24; -0.06] standard deviations. The single study outcome is significant for the two Petrie 2021 studies included in the meta-analysis.

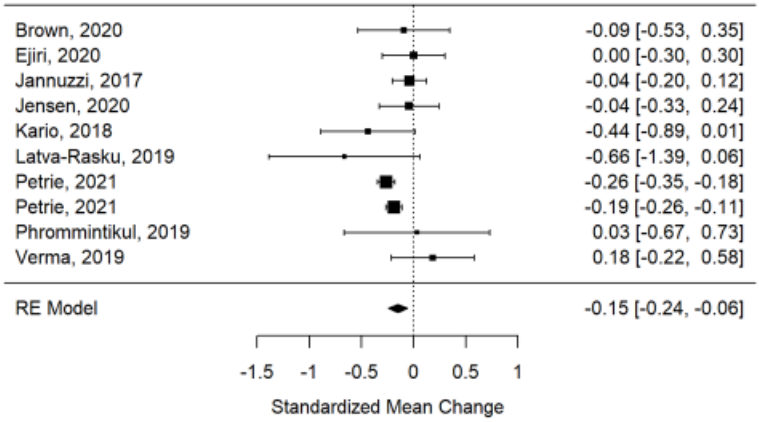


Figure 28: Funnel Plot of SGLT2I trials; assumed 0.5 pre-post correlation. The Cochran's Q-test identified no significant source of correlation; I2=0.43

The moderator meta-regression results (Table VI) evidenced the absence of any effect for age, BMI, HbA1c, and high-quality studies (Jadad >= 3).

Table VI: Random Effect moderator meta-regression results, SGLT2I trials. The model estimate with the 95% Confidence Intervals (CI) and P-value has been reported

Moderator	Estimate [95% CI]	P-value
Age	-0.01 [-0.05 ; 0.03]	0.58
BMI	-0.01 [-0.05 ; 0.04]	0.84
HbA1c	0.03 [-0.10 ; 0.16]	0.66
Jadad ≥ 3	-0.3 [-0.78 ; 0.19]	0.23

The results are similar by assuming a pre-post study correlation of 0.7 and 0.8 (Figure 20, 25); the only difference is in the funnel plot representation

(Figure 29) where it is evidenced that, for an imputed correlation of 0.8, four studies are slightly out of the confidence bands.

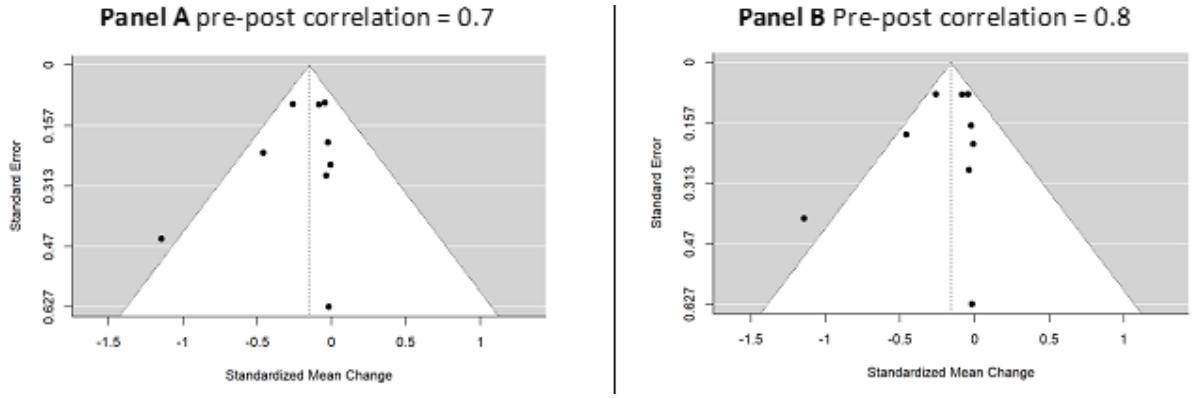


Figure 29: Funnel Plot of GLP-1RA trials; assumed 0.7 (Panel A) and 0.8 (Panel B) pre-post correlation

2.7 Discussion

The present meta-analysis provides the following findings:

1. SGLT2i induce a slight, albeit significant decrement in N-terminal pro-BNP concentrations;

2. Likewise, GLP-1RA produce a slightly more consistent reduction;

3. We found no interaction between the responses to these two different classes of drugs and age, body mass index, and metabolic control.

SGLT2i safely reduce a composite endpoint of cardiovascular death or hospitalization for heart failure in patients with both HFrEF and HFpEF, regardless of diabetes mellitus. Interestingly, subgroup analyses of available trials suggest that the effects of SGLT2i are additive to those of ARNI[80]. The treatment with sacubitril/valsartan significantly reduces N-

terminal pro-BNP levels after approximately four weeks from the initiation of the treatment, possibly reflecting an improvement in cardiac wall stress[81]. In contrast, all the trials demonstrating a positive cardiovascular effect of SGLT2i did not show a clear impact of these drugs on natriuretic peptides. Many hypotheses have been proposed to explain the action of SGLT2i on the reduction in the risk of hospitalization for HF: increased natriuresis, Inhibition of cardiac Na-H exchanger, reduction in adrenergic nervous system activation, reduction in myocardial oxygen supply-demand mismatch[82]. Whatever the cause, the results on N-terminal pro-BNP have not been consistent. By analyzing 8 trials with coherent data before and after the treatment of SGLT2i, this meta-analysis reports that they can reduce N-terminal pro-BNP by almost 30% after a mean follow-up of 41 weeks. Unfortunately, we could not include in the present meta-analysis relevant trials in which this peptide was assayed before and after SGLT2i challenge due to the impossibility of derived outcome measures compared with those in the other papers. Despite this limitation, the reported reductions in N-terminal pro-BNP are significantly smaller than those observed after sacubitril/valsartan in three large trials [83-85]. The relative decreases in N-terminal pro-BNP were 37%, 60%, and 35%, respectively. Not all the trials included in our meta-analysis reported the type of HF and, probably, several patients had also by HFpEF: this potentially relevant since, in the PARAGON-HF, in which the effect of ARNI was tested in patients with HFpEF, the decrease in N-terminal pro-BNP was only of 19%[86]. It would be relevant to gain further data on the effect of SGLT2i on N-terminal pro-BNP in patients with HFpEF.

SGLT2i seem to exert their positive effects mainly, but not exclusively, on hemodynamic factors; conversely, GLP-1RA are thought to act mainly on the cellular component of the atherothrombotic process[87]. However, GLP-1RA also reduce several important risk factors for HF other than hyperglycemia, such as blood pressure and body weight. Meta-analyses of clinical trials have shown that GLP-1RA can slightly reduce the risk of hospitalization for HF at least in patients with compensated HF[55], [56]. In patients with diabetes at high cardiovascular risk, the treatment with agonists of the GLP-1 receptor reduces the incidence of major coronary events, which play a crucial role in the pathogenesis of HF[88]. The reduction of hospitalizations for HF effect can also be attributable, at least partly, to their ability to induce weight loss, which is beneficial in decreasing the risk for HF[89]. Notably, obesity is associated with a depressed concentration of natriuretic peptides due to suppression of the *bnp* gene by factors produced by adipose tissue. In patients with severe obesity natriuretic peptides may be less reliable prognostic index[90]. Based on these findings, the assessment of the effects of GLP-1RA non natriuretic peptides could add further insight. Our results show that GLP-1RA can significantly decrease N-terminal pro-BP, even to a greater extent than SGLT-2i (24%). Interestingly, the effect of GLP-1RA on N-terminal-pro-BNP appears to be independent of baseline body weight, as observed for SGLT2i. Unfortunately, we could not correlate the entity of body weight loss to the effect on N-terminal-pro-BNP, but it would be interesting to explore this relationship in the future.

Elevated HbA1c levels are associated with a higher risk of heart failure[91]: compared with HbA1C ≤6.1%, patients with HbA1C >7.3% are more likely

to have a longer length of hospital stay. However, it has been no differences in BNP, both on admission and at discharge, were observed between those with HbA1c <6.3% and those with HbA1c>7.3%[92]. In our meta-regression, both for GLP-1RA and SGLT2i, we could not find any effect of actual metabolic control in the response of N-terminal pro-BNP to the drugs.

N-terminal pro-BNP concentrations also increase with age, and its concentration might be not only an initial sign of abnormal cardiac function [93] but also an incremental prognostic value above and beyond the GRACE risk score and traditional biomarkers after acute myocardial infarction[94]. Moreover, elderly patients, especially those affected by diabetes, frequently have chronic kidney disease, further increasing N-terminal pro-BNP concentration. Our meta-regression also suggests that the effects of both SGLT2i and GLP-1RA on N-terminal pro-BNP are independent of age, although the age of patients treated with GLP-1RA was significantly lower than those treated with SGLT2i.

CVOTs trials have consistently shown a striking reduction in hHF in patients on SGLT2i treatment; unexpectedly, we found that their effect on N-terminal pro-BNP is comparable to that observed, although in subjects with different characteristics, after GLP-1RA treatment. As reported in table 1, there are significant differences between the populations treated with GLP-1RA and those treated with SGLT2i: therefore, a direct comparison between the two can be misleading. Unfortunately, N-terminal pro-BNP was not determined in CVOTs testing the safety of GLP-1RA: we are keenly weighting for additional studies (NCT01800968 and NCT04535960) to gain more detailed information on this effect in patients with HF.

2.8 Conclusions

Some limitations of the present meta-analysis should be recognized. N-terminal pro-BNP levels were not available in many trials, even when heart failure was listed among pre-defined outcomes. Consequently, the analysis could be performed on a small number of trials, with relatively small sample size and limited duration of observation. Notably, the mean length of included studies was substantially lower than that of CVOT with the SGLT-2i and GL-1RA in patients with diabetes, and that of trials specifically designed for HF with SGLT-2i. The relatively small number of trials limits the reliability of the assessment of publication bias, which can more easily remain undetected when the number of studies is relatively low. The number of trials also limits the reliability of meta-regression analyses, which should be considered purely exploratory.

In conclusion, both GLP-1RA or SGLT2i appear to decrease the levels of this natriuretic peptide. From our data, it is impossible to draw any conclusion about the mechanisms underlying this effect: however, it seemed to be independent of age, body weight, and metabolic control. Our observation is consistent with the hypothesis that N-terminal pro-BNP does not mediate the protective effect of SGLT2i.

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