Diplomarbeit

SGLT2 Inhibitors: Redefining Antidiabetic Therapy

Examining SGLT2 inhibitors' position in modern day antidiabetic treatment

Eingereicht von

Jan Lewis

zur Erlangung des akademischen Grades:

Doktor der gesamten Heilkunde

(Dr. med. univ.)

an der

Medizinischen Universität Graz

ausgeführt am

Lehrstuhl für Pharmakologie

unter der Anleitung von

Univ. Prof. i. R. Mag. pharm. Dr. Eckhard BEUBLER

Ao.Univ.-Prof. Dr.phil. Dr.h.c. Irmgard LIPPE

Graz, am 27.03.2021

EIDESSTAATLICHE ERKLÄRUNG

Eidesstattliche Erklärung Ich erkläre ehrenwörtlich, dass ich die vorliegende Arbeit selbstständig und ohne fremde Hilfe verfasst habe, andere als die angegebenen Quellen nicht verwendet habe und die den benutzten Quellen wörtlich oder inhaltlich entnommenen Stellen als solche kenntlich gemacht habe.

Graz, am 27.03.2021 Jan Lewis eh.

Table of Contents

Table of Figures

Abbreviations

ACCORD	Action to Control Cardiovascular Risk in Diabetes
ADVANCE	Action in Diabetes and Vascular Disease
AMPK	Adenosinemonophosphate-Dependent Phosphokinases
CANVAS	Canagliflozin Cardiovascular Assessment Study
DPP-4 inhibitors	Dipeptidylpeptidase-4 inhibitors
EMPA-REG OUTCOME	Empagliflozin Cardiovascular Outcome
ESC	European Society of Cardiology
FAERS	FDA Adverse Event Reporting System
GLP-1 receptor agonists	Glucagon Like Peptide-1 receptor agonist
HbA_{1C}	Haemoglobin A1c
LDL	Low Density Lipoprotein
LEADER	Liraglutide Effect and Action in Diabetes
OEDG	Österreichische Diabetes Gesellschaft

PPARγ	Peroxisome Proliferator-activated Receptor
RCTs	Randomised Controlled Studies
SGLT2 Inhibitor	Sodium Dependent Glucose Transporter 2 Inhibitor
SUSTAIN6	Semaglutide in Subjects with Type 2 Diabetes
T2D	Type 2 Diabetes
TRPM6	Transient Receptor Potential Ion Channel 6
UKPDS	United Kingdom Prospective Diabetes Study
UTIs	Urinary Tract Infections
VADT	Veterans Affairs Diabetes Trial

Abstract

Background

SGLT2 inhibitors were developed to combat the growing epidemic of diabetes and the consequences of diabetes, e.g. chronic kidney disease and cardiovascular complications, by increasing the excretion of glucose in urine and thereby reducing blood sugar levels. These medications have shown to be effective both in lowering blood sugar as well as preventing adverse cardiac events. Why then are SGLT2 inhibitors not being used in all treatments of diabetes as the mortality rates of the two leading causes of death in diabetic patients could be lowered?

Methods

A basis of inquiry was formed by studies from the Pubmed and Cochrane library, as well as more clinical aspects in the form of lectures by the Medical University Graz or through further online sources and the guidelines issued by the OEDG (Austrian Diabetes Society). The risk benefit profiles of other antidiabetic medications were compared to SGLT2 inhibitors.

Results

Whilst the effectiveness of SGLT2 inhibitors' ability to lower HbA_{1C} values is similar to other antidiabetic agents, they have the ability to improve both cardiovascular and chronic kidney disease outcomes and lead to weight loss.

The often cited side effects of SGLT2 inhibitors were scrutinised and myths about SGLT2 inhibitors wide side effect spectrum could be dispelled.

Some side effects and risks for certain substances in the SGLT2 inhibitor group could be confirmed by studies, RCTs and meta-analyses.

Conclusion

The ability of SGLT2 inhibitors to reduce cardiac and renal causes of mortality whilst reducing blood sugar at the same time in addition to their limited side effect profile, they play a critical role in today's and the future treatment of type 2 diabetes. The fact that a few substances within the SGLT2 inhibitor group have been proven to have drastic side effects should not lead to a loss of confidence or a reduction of use in the remaining substances of the group. SGLT2 inhibitors position in the treatment scheme of the OEDG as one of the main secondary substances used is well justified.

Key Words: Antidiabetics, SGLT2 Inhibitors, Efficacy

Zusammenfassung - SGLT2 Hemmer: Antidiabetische Therapie Neu Definiert

Hintergrund

SGLT2 Hemmer wurden entwickelt um durch eine erhöhte Ausscheidung von Glukose im Harn gegen die Diabetesepidemie und die daraus resultierenden Konsequenzen, wie chronische Nierenerkrankung oder kardiovaskuläre Komplikationen, zu kämpfen. Diese Medikamente senken den Blutzucker und reduzieren kardiologische Ereignisse. Daher die Frage, weshalb SGLT2 Hemmer nicht bei jeder Diabetestherapie dabei sind, da sie die Mortalitätsrate der zwei führenden Todesursachen diabetischer Patientinnen und Patienten verringert.

Methoden

Als Grundlage wurden Studien und Fachliteratur von Pubmed und der Cochrane Library herangezogen, klinische Aspekte aus Vorlesungen der Medizinischen Universität Graz oder anhand von weiteren Online Ressourcen und die Leitlinien der Österreichischen Diabetes Gesellschaft (OEDG) untersucht. Das Risiko-/Nutzenprofil anderer Antidiabetika wurde mit dem der SGLT2 Hemmer verglichen, sodass Rückschlüsse gezogen werden konnten bezüglich des verbesserten Wirkprofils im Vergleich zu den Vorgängern der SGLT2 Hemmer.

Ergebnisse

Die Wirksamkeit der SGLT2 Hemmer auf den HbA_{1C} Spiegel ähnelt dem anderer Antidiabetika, die Fähigkeit den Verlauf von kardiovaskulären als auch chronischen Nierenerkrankungen zu verbessern und eine Gewichtsreduktion zu erlangen wurde bestätigt. Nebenwirkungen der SGLT2 Hemmer wurden untersucht und häufige Anschuldigungen konnten wiederlegt werden. Manche Nebenwirkungen und Risiken konnten für einzelne Substanzen der SGLT2 Hemmer Gruppe durch Studien, RCTs und Metaanalysen bestätigt werden.

Fazit

Die Fähigkeit der SGLT2 Hemmer das kardiale und nephrologische Risiko und gleichzeitig den Blutzucker zu senken, mit einem limitierten Nebenwirkungsprofil, berechtigt ihren Platz heute und auch in der Zukunft bei der Behandlung von Diabetes. Manche Substanzen innerhalb der SGLT2 Hemmer Gruppe lösen schwere Nebenwirkungen aus, jedoch ist das kein Grund die restlichen Substanzen zu meiden. Die Stellung der SGLT2 Hemmer in den OEDG Leitlinien als einer der meistverwendeten sekundären Substanzen ist äußerst berechtigt.

Stichworte: Antidiabetika, SGLT2 Hemmer, Wirksamkeit

Introduction

The World Health Organisation has stated that within the European region 60 million people, roughly the population of Italy, are suffering from diabetes. With 10,3% of men and 9,6% of women in Europe over the age of 25 suffering from diabetes it is already a shocking state of affairs, which is compounded in its severity by the World Health Organisations prediction that the deaths due to diabetes will double between the years 2005 and 2030 (1). Patients suffering from diabetes are most likely to die as a result of cardiovascular or end-stage renal diseases (50% and 10-20% respectively) (2). Therefore, the ideal antidiabetic agent would be one that not only combats the increased blood sugar levels due to diabetes but also reduces cardiovascular events and chronic kidney disease.

Before the new millennium began insulin, metformin and sulfonylureas were being used to treat diabetes. At the dawn of the new millennium several new products have entered the market, with DPP-4 inhibitors, GLP-1 agonists and SGLT2 inhibitors being introduced within the past 15 years (3).

Many antidiabetics have only a limited therapeutic efficacy or cause unwanted side effects such as weight gain or dangerous hypoglycaemic events whilst not reducing cardiovascular complications that can ensue, some even leading to an increase in cardiac mortality. Therefore, the research for new therapeutic agents that offer an improved benefit-risk profile has continued.

In 2012, a class of antidiabetics called SGLT2 inhibitors were introduced in Europe. Their site of action is a glucose transporter in the kidneys, inhibiting it with the aim to prevent the reuptake of glucose, thereby lowering the patient's blood sugar.

SGLT2 inhibitors have been undergoing a series of studies, RCTs and meta-analyses, leading to several promising results, such as their cardio- and renal-protective function. However, during the development and testing of SGLT2 inhibitors observations have also been made concerning certain side effects ranging from diabetic ketoacidosis to an increased risk of amputation.

In this piece of work the results of the studies, RCTs and meta-analyses on SGLT2 inhibitors will be reviewed, summarised and compared with other antidiabetic medication being used. The place of SGLT2 inhibitors in the treatment guidelines of diabetes by the Austrian Diabetes Society (OEDG) will be examined and the question answered whether the place of SGLT2 inhibitors within the treatment guidelines is

justified, whether the potential side effects are too great or whether more diabetics should benefit from this novel therapeutic agent.

An Introduction to Antidiabetics

All pharmacological agents that lead to a reduction in blood sugar to treat type 2 diabetes, save insulin, are referred to as antidiabetics. If a change of lifestyle, such as weight loss or a combination of diet or exercise does not lead to a satisfactory reduction in the HbA_{1C} value, the target being between 6,5-7,5% (48-58 mmol/mol Hb), a pharmacological approach should be considered (4).

Depending on their method of action, antidiabetic medications are divided into two major groups. These are the insulinotropic substances and non-insulinotropic substances. The important difference between the two groups being that insulinotropic substances result in an increased release of insulin from the β-cells of the pancreas, meaning a minimum remaining function of the pancreas must be present, whereas non-insulinotropic substances function independently of the pancreas and can therefore be used even after the endocrine function of the pancreas can no longer be performed.

Insulinotropic substances include sulfonylureas, glinides, glucagon-like peptide-1 receptor agonists (GLP-1 receptor agonists) and dipeptidyl-peptidase-4-inhibitors (gliptins). Non-insulinotropic substances are for instance biguanides, SGLT2 inhibitors, glitazones and alpha-glucosidase inhibitors.

Non-Insulinotropic Substances

Biguanides

Biguanides are an essential part of today's treatment of diabetes and have been for almost 70 years. The most prescribed drug used in diabetes treatment, metformin, is a biguanide. Biguanides lead to a reduction in the insulin resistance by modifying the metabolism of glucose, which results in a reduction in blood sugar. It explains why the first trade name given to a biguanide by the French physician Jean Sterne was Glucophage, the glucose eater. By reducing the activity of the mitochondria, the anaerobic metabolism of glucose is increased. This in turn leads to an activation of AMP-dependent phosphokinases (AMPK) which inhibit the liver's production of glucose and lipids, thereby reducing blood sugar, LDLs and triglycerides. Hereby, the need for insulin is lowered and the appetite reduced. It has also been shown that an increased uptake of glucose into the muscle and fat cells occurs, which in turn increases the tolerance towards glucose after periods of acute high glucose levels in the blood.

It quickly becomes clear why metformin is the most prescribed antidiabetic agent. It has one of the best effects on the glucose metabolism, reducing the HbA_{1C} between 1-2% (4), leads to weight reduction and has been proven to reduce both the long-term complications of diabetes and mortality. It's longevity in the field of antidiabetic agents also means that large amounts of data are available on it.

Metformin can lead to the dreaded side-effect of lactic acidosis but rarely leads to life-threatening hypoglycaemic incidences. It can readily be combined with other antidiabetic medications but has been shown to lead to cardiovascular events when combined with sulfonylureas. Figure 10 shows how metformin is frequently used in combination drugs with SGLT2 inhibitors. Frequent side effects such as diarrhoea and meteorism and vitamin B_{12} deficiency can occur. These side effects may not sound quite so drastic at first, apart from the cardiovascular risk when combined with sulfonylureas, however if an antidiabetic agent could provide similar benefits with less side effects this would be an improvement.

In summary, due to metformin's limited side effects, its price and the long-term effects being well known it is the ideal drug for most type-2 diabetes patients and forms the

first stage of pharmacological antidiabetic treatment for most patients suffering from diabetes.

Biguanide	
International Non-proprietary Name	**Brand Name in Austria**
Metformin	Glucophage©

Figure 1 Biguanide (5 S. 97)

Glitazones

Glitazones work by activating the transcription factor PPARγ in the cell nucleus, leading to the induction of genes that are responsible for the metabolism of glucose and lipids. This results in a higher rate of triglycerides, a reduction of free fatty acids and also a reduction in insulin resistance. More glucose is used and the hepatic production of glucose is reduced leading to a reduction in HbA_{1C} by 1-2% (4) within 3 months.

Glitazones are only used in very few cases as an antidiabetic medication, two of the three substances are no longer on the market due to cardiac or hepatic toxicity, only pioglitazone is still on the market and even here increased rates of bladder cancer were observed in studies (6). This coupled with several other side effects such as an increase in body weight, oedema, reduction in haemoglobin and an increased risk of fractures limit the use of glitazones.

Glitazone	
International Non-proprietary Name	**Brand Name in Austria**
Pioglitazone	Actos©

Figure 2 Glitazone (5 S. 97)

Alpha-glucosidase Inhibitors

Alpha-glucosidase inhibitors work by inhibiting the glucose resorption in the intestine. They perform this action by preventing the splitting of disaccharides through alpha-glucosidase in the villi of the epithelial cells of the intestine, thereby preventing a raise in blood sugar. This can lead to a reduction of HbA_{1C} by 0,5-1% (4) within 3 months.
Even though few hypoglycaemic events occur when taking alpha-glucosidase inhibitors due to the comparably low change in HbA_{1C} and the severe gastrointestinal side effects alpha-glucosidase inhibitors are rarely used. These gastrointestinal side effects occur due to a reduced digestion of carbohydrates. These carbohydrates are not digested by normal means but are digested by microbiological intestinal bacteria which leads to the gastrointestinal side effects, with patients complaining especially about the discomforting meteorism.

Alpha-glucosidase Inhibitors	
International Non-proprietary Name	**Brand Name in Austria**
Acarbose	Glucobay©

Figure 3 Alpha-glucosidase Inhibitors (5 S. 98)

Insulinotropic Substances

Sulfonylureas

Sulfonylureas are part of a group of insulinotropic substances that act independently to the levels of glucose in the blood. They stimulate the secretion of insulin by blocking the potassium channels of the beta-cells in the pancreas. This happens because the cell membrane of the pancreas is depolarised, calcium flows into the cells which results in an increase in insulin secretion. Sulfonylureas also have extrapancreatic effects. These are the reduction hepatic gluconeogenesis and an increased insulin sensibility.
Sulfonylureas can be used as an alternative monotherapy or in combination with metformin and can lead to a reduction in the HbA_{1C} of 1-2% (4) within 3 months. They do however present substantial risks. Sulfonylureas can lead to life-threatening

hypoglycaemic events, and due to their long half-life (10 hours) this can occur over a very long period. By patients over 60 sulfonylureas are not recommended due to the high risks that accompany their use and the risk of additional medications such as beta-blockers obscuring potential warning signs of hypoglycaemia. Studies by the UKPDS have also shown that their use in combination with metformin can lead to a higher rate of cardiovascular events.

The main advantage of sulfonylureas is their potency on the HbA_{1C}, achieving great reductions. This advantage however is outweighed by the risk of hypoglycaemic events coupled with the recommendation to not prescribe sulfonylureas to patients over 60 drastically limits their use. When used in combination with other antidiabetics, sulfonylureas retain these risks of hypoglycaemic events.

Sulfonylureas	
International Non-proprietary Name	**Brand Name in Austria**
Glibenclamid	Glucobene©
Glimepirid	Amaryl©
Gliclazid	Diamicron©
Gliquidon	Glurenorm©
Glipizid	Minidiab©

Figure 4 Sulfonylureas (5 S. 95)

Glinides

Glinides lead to an increase in insulin secretion by the same method of action as sulfonylureas. The difference being their chemical structure and the receptor site at which they bind. Their effect in reducing HbA_{1C} values is slightly reduced though, only lowering it by 1-2% (4).

Due to their similarities, the same side effects can occur as with sulfonylureas, the most important being life-threatening hypoglycaemic events. These are however rarer as glinides have a shorter half-life than sulfonylureas, leading to less accumulation and therefore reducing the risk of hypoglycaemia. Glinides have very few end point studies which means the long-term risks and effects are yet unknown. They are also

more expensive and cannot be prescribed freely to patients under the current health act laws.

Glinides	
International Non-proprietary Name	**Brand Name in Austria**
Repaglinid	Novonorm©
Nateglinid	Starlix©

Figure 5 Glinides (5 S. 96)

Dipeptidylpeptidase-4 Inhibitors and GLP-1 Receptor Agonists

Both these substances achieve their increased insulin secretion by a similar method of action. Their effects are increased when a higher level of glucose is present in the blood and their method of action is based on the incretin effect. The incretin effect occurs when food is consumed. This leads to the activation of endocrine cells in the gastrointestinal tract, which release the glucagon-like peptide 1 (GLP-1). GLP-1 stimulates the release of insulin from the beta-cells of the pancreas, thereby inhibiting the secretion of glucagon, slowing down the emptying of the stomach. This has the positive effect that patients feel satiated for longer and weight reduction is made easier. GLP-1 is then broken down by the enzyme dipeptidylpeptidase-4 thereby putting an end to the incretin effect.

The different ways of manipulating the incretin effect explains, how these substances achieve their antidiabetic effect. Dipeptidylpeptidase-4 inhibitors increase the incretin effect indirectly by reducing the enzymatic breakdown of GLP-1 which results in a higher insulin secretion, whereas GLP-1 receptor agonists produce a direct increase in the incretin effect by binding to the GLP-receptors and thereby eliciting a higher insulin secretion.

Dipeptidylpeptidase-4 inhibitors are used either in monotherapy or in combination with metformin, sulfonylureas or glitazones. The advantage of their use is they do not cause hypoglycaemic events and they are cardiovascularly neutral, as was shown in the TECOS-study (7). They can reduce the HbA_{1C} by 0,5-1%, putting their potency below

sulfonylureas and glinides but in the same range as SGLT2 inhibitors or alpha-glucosidase inhibitors (4). There have been concerns that due to the increased risk of pancreatitis dipeptidylpeptidase-4 inhibitors may also be the cause of a higher risk of pancreatic cancer. This risk needs to be considered when prescribing DPP4 inhibitors. GLP-1 receptor agonists have a similar risk benefit profile as dipeptidylpeptidase-4 inhibitors. Their ability to reduce the HbA_{1C} is the same, as it lies at 0,5-1% (4) within 3 months. Higher rates of pancreatitis and pancreatic cancer have been reported with the use of GLP-1 receptor agonists, as well as the gastrointestinal side-effects being higher compared with dipeptidypeptidase-4 inhibitors. These side effects are higher because as explained above the inhibition of GLP-1's enzymatic breakdown does not cause as great of a response as the stimulation of GLP-1 receptors, thereby also leading to greater side effects. It has however been shown that GLP-1 agonists lead to a reduction in cardiovascular events (8). This is the reason that within the treatment scheme laid out by the Austrian Diabetes Society (OEDG), GLP-1 agonists are recommended in patients with a history of cardiovascular disease at the same level as SGLT2 inhibitors.

Dipeptidylpeptidase-4 Inhibitors	
International Non-proprietary Name	**Brand Name in Austria**
Sitagliptin	Januvia©
Vildagliptin	Galvus©
Saxagliptin	Onglyza©
Alogliptin	Vipidia©
Linagliptin	Trojenta©
GLP-1 Receptor Agonists	
International Non-proprietary Name	**Brand Name in Austria**
Exanatide	Byetta©
Liraglutid	-
Dulaglutid	Trulicity©
Albiglutid	Eperzan©

Lixisenatid	Lyxumia©

Figure 6 DPP4-Inhibitors & GLP-1 Receptor Agonists (5 S. 96-97)

Treatment Scheme

Figure 1 briefly outlines and summarises the OEDG's treatment scheme of type 2 diabetes. After first diagnosing type 2 diabetes, and if the HbA_{1C} is below 9,0%, the patient should first be advised to attempt lifestyle changes. These include weight reduction through sport and a change in diet in order to reduce blood sugar levels. If this does not suffice or the disease progresses metformin should be prescribed as a basic therapy if no contraindications exist. Metformin being the primary antidiabetic substance of choice due to its risk benefit profile, its price and the knowledge of its long-term effects through numerous studies that have been conducted.

The following escalating step in the treatment scheme is dependent on whether the patient has a history of either cardiovascular disease or chronic kidney disease. If the patient has a history of either, then GLP-1 agonists or SGLT2 inhibitors should be introduced. It should be noted, as was mentioned above, that GLP-1 agonists have been shown to increase the risk of both pancreatitis and pancreatic cancer and can lead to severe gastrointestinal side effects, which poses the question why GLP-1 agonists are being recommended on the same level as SGLT2 inhibitors.

If the patient does not have a history of either cardiovascular or chronic kidney disease the main aim of the escalating therapy after metformin is to reduce the risk of hypoglycaemic events. The agents that target the incretin effect (GLP-1 agonists and DPP4 inhibitors) may reduce the risk of hypoglycaemic events but their gastrointestinal side effects and increased pancreatitis risk should be considered, whereas other recommended substances such as pioglitazone carry cancer risks of their own. On this basis SGLT2 inhibitors may be the choice with the fewest dangerous side effects. The benefit risk profile of SGLT2 inhibitors will be discussed in the following section and will lead to a re-evaluation of the current suggested treatment scheme.

<table>
<tr><td colspan="2">Lifestyle changes → weight reduction, change in diet</td></tr>
<tr><td colspan="2">Metformin as a basic therapy if no contraindications exist</td></tr>
<tr><td>Cardiovascular illness or chronic kidney disease in the patients' history
↓
GLP-1 agonist or SGLT2 inhibitors

HbA_{1C} above the target
↓
<u>Antidiabetic with cardiovascular safety</u>
GLP-1 agonists,
SGLT2 inhibitor,
DPP4 inhibitors,
Insulin,
Pioglitazone,
Sulfonylureas</td><td>No known cardiovascular risk or chronic kidney disease in the patients' history

Minimising the risk of hypoglycaemic events
↓
DPP4- inhibitors,
GLP-1 agonists,
SGLT2 inhibitors,
Pioglitazone

HbA_{1C} above the target
↓
Further antidiabetic substance with a different method of action</td></tr>
</table>

Figure 7 Treatment scheme for T2D by the OEDG (4)

SGLT2 Inhibitors Introduction

SGLT2-inhibitors are one of the latest antidiabetic medications that aim to reduce both the mortality and improve the quality of life for patients with diabetes. They work on the principle of blocking the reuptake of glucose in the proximal tubule of the kidneys leading to glucose, salt and water excretion. The consequences are a lowering of the HbA_{1C} without an increased risk of hypoglycaemia, a reduction of blood pressure, weight loss and benefits both to cardiovascular and renal outcomes.

There have however been findings that suggest that a wide range of side effects can occur in patients taking SGLT2 inhibitors, ranging from urinary tract infections and skin reactions to diabetic ketoacidosis and amputations.

Both the benefits and the risks of SGLT2 inhibitors will be examined in this next section, and through the use of data from studies, RCTs and meta-analyses conclusions will be drawn as to how they shape up compared to other antidiabetic agents.

A Brief History of SGLT Inhibition

To fully appreciate the development of SGLT inhibitors the history and fortuitous discovery of their predecessor should be briefly examined. The roots of SGLT inhibition lie in a Belgian apple orchard and with two young medical graduates, Laurent-Guillaume de Koninck and Jean Servais Stas (9). These two men both graduated from Louvain's medical university, but were far more fascinated by the field of chemistry. They both became assistants to their former professor of chemistry, Jean-Baptiste Van Mons. Monsieur Van Mons was a renowned expert in the field of pomology, the study of apples not lungs, and was in possession of an apple orchard that needed to be moved. In this undertaking he employed his assistants and their reward was a large supply of fresh apple tree roots. From these roots, or, to be more precise from the bark of these roots, the two assistants isolated a crystalline glycoside, which at first they named phlorizine, and which later was christened phlorizin (10). De Koninck and Stas were credited for the discovery of phlorizin as early as May 1835 (11).

After the discovery, de Koninck began experimenting with the substance and recorded "some success" in the treatment of fever through the use of phlorizin (12). This would prove to be a very important observation, as it led to Prof. Freiherr Josef von Mering, precisely the man needed to establish the connection to diabetes, developing an interest in the substance about half a century later. Von Mering was an expert in diabetes research and decided to administer phlorizin to dogs. He discovered that after oral as well as subcutaneous application glycosuria could be detected in the dogs (13). In 1886, von Mering postulated that the decrease of glycaemia in dogs through phlorizin was due to the fact that the substance "may induce glycosuria by changing something in the kidney" (14). This was an important hypothesis, as before it was thought that phlorizin merely induced a so-called "phlorizin-diabetes", von Mering's observations however, led him to believe that "phlorizin-diabetes" and the resulting glycosuria was very different from the glycosuria that occurs in diabetes when the blood sugar level is highly elevated. He observed that through the use of phlorizin blood sugar levels sank, whereas in genuine diabetes the blood sugar levels remained the same in spite of the glycosuria. This realisation led to him attempting to use phlorizin on people, with the result that 15-20g of phlorizin led to a glycosuria of 6-8% (15).

The proof of the theory that phlorizin acted upon the kidneys was provided by Oskar Minkowski, in a straightforward experiment. He removed the kidneys of healthy, pancreatectomised dogs and observed that the glucosuretic of phlorizin no longer occurred (16), thereby proving its effect on the kidneys.

Almost 130 years after von Mering's original discovery, the first clinical trial based upon this mechanism was undertaken.

Method of Action

This begs the question why if the glycosuretic and the therefore blood sugar lowering effects of phlorizin have been known for over a century have we only very recently begun using this mechanism when treating diabetes? In order to answer this, an explanation of the method of action of phlorizin and the more modern SGLT1 and SGLT2 inhibitors is necessary.

The human body evolved to best survive in an environment of scarce energy resources; all sources of energy had to be conserved as best they could. It is a very recent development that mankind has an overabundance of external energy resources, and our bodies have not yet evolved to accommodate this. Our body still preserves all the sugar it can by reabsorbing approximately 180g/day in the kidney's proximal tubule (17). This reabsorption takes place due to the active basolateral Na^+ removal by Na^+/K^+- ATPase, which generates an electrochemical driving force so that glucose can enter apically through Na^+-driven sodium-glucose cotransporters (18). Then, following its concentration gradient, glucose leaves the cells via so-called GLUT2 and re-enters the blood stream, so that this valuable energy source is not lost to the body (19). This mechanism by our body, developed to survive the harsh conditions that existed even in recent centuries, is not ideal in a patient suffering from diabetes; it simply worsens their condition. Through multiple studies it has been demonstrated that SGLTs are responsible for all of the reabsorption of glucose and that SGLT2s, expressed at the beginning of the proximal tubule, are responsible for roughly 97% of the kidney's total glucose reabsorption (20). By comparison the SGLT1 which occurs in the more distal parts of the proximal tubule accounts for only a mere 3% of the total reabsorption of glucose, when the SGLT2 are intact and normoglycaemia prevails (21). Therefore, under physiological conditions, the SGLT2 and the SGLT1 together regulate the entire renal glucose reabsorption (22). This makes it a superb target for antidiabetic medications in order to lower blood sugar.

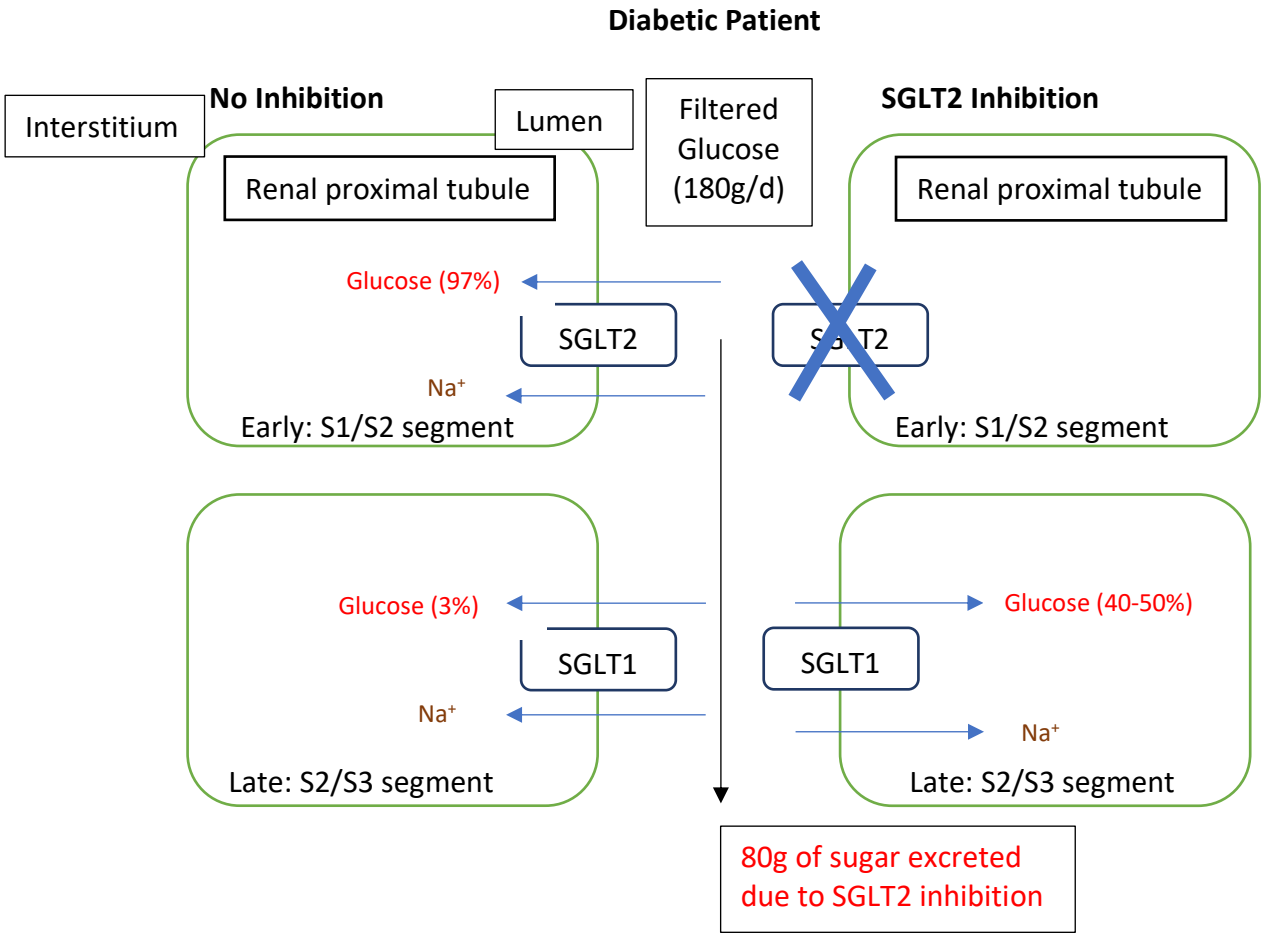

Figure 8 The Role of SGLT1 & SGLT2 in Glucose Reabsorption (23)

Figure 9 depicts the role of the SGLT1 and the SGLT2 in glucose reabsorption in the kidneys, both physiologically and when inhibition of SGLT2 takes place. As aforementioned physiologically the kidney reabsorbs glucose independent of blood sugar levels. This means that in most diabetics, glycosuria won't occur in spite of rising hyperglycaemia. Through the use of SGLT2 inhibitors however sugar can be excreted by the kidneys. As SGLT2s absorb 97% of the glucose in the kidneys inhibiting their function leads to glycosuria. Inhibiting SGLT2 unmasks SGLT1's ability for glucose reabsorption. As can be seen in figure 1, when SGLT2 is inhibited in its function SGLT1 can increase its glucose reabsorption to 40-50% in normoglycaemic and hyperglycaemic conditions (17). Notwithstanding the efforts of the SGLT1, the inhibition of SGLT2 can lead to the excretion of approximately 80g of sugar per day. This does however raise the question why there are not more dual SGLT1 and SGLT2 inhibitors on the market in order to achieve an even greater amount of glycosuria. As

opposed to SGLT2, the SGLT1 occur not only in the kidney but also in the intestine, where the transporters also mediate the transport of glucose. The side effects can therefore be considerable. The additional inhibition of SGLT1 transporters bears such risks as, due to its additive effects with the kidneys, it can lead to diarrhoea, further volume depletion and therefore an increased risk of hypoglycaemia, hypotension, pre-renal failure and euglycaemic diabetic ketoacidosis is increased (24). There are however several studies into whether the dual inhibition of SGLT1 and SGLT2 can be used effectively and safely.

Different Substances

The beginnings of SGLT1 and SGLT2 inhibition lie, as aforementioned, in phlorizin. Phlorizin was discovered in the bark of apple trees over 150 years ago and the connection was soon made that it led to an increase in urinary glucose excretion (25). Studies proved that the application of subcutaneous phlorizin in diabetic rats (diabetic as a result of a partial pancreatectomy) normalised blood glucose levels as well as insulin sensitivity (26). Phlorizin did have a few major drawbacks though: its poor oral bioavailability, the almost insolubility in water and the unselective nature of its SGLT1 and SGLT2 inhibition meant that phlorizin was not an ideal substance to be used as medication.

The substances known as T-1095 and T-1095A, synthetic agents that are derived from phlorizin, were supposed to address some of the issues with phlorizin. They possessed the advantage that, as opposed to phlorizin, they could be absorbed into the circulation after oral intake and were metabolised in active form (27). These new substances, as well as phlorizin, however were all *O*-glucosides. Real progress in the field of SGLT inhibition was made when a novel *C*-glucoside was discovered.

The discovery of the meta *C*-glycosylated diarylmethane pharmacophore was ground-breaking. The selective SGLT2 inhibitors dapagliflozin, canagliflozin, empagliflozin and ertugliflozin are all based on this *C*-glucoside. The advantage of this *C*-glycosylation compared to *O*-glycosylation is that it makes the molecules resistant to hydrolysis by β-glucosidases, thereby increasing their half-life (28).

The main difference between the different SGLT inhibitors lies in their affinity for either SGLT2 or SGLT1; here there are differences ranging between 260:1 for canagliflozin

and 2700:1 for empagliflozin (29). Sotagliflozin displays a very minor favour towards the SGLT2, 20:1, and is considered a dual SGLT inhibitor (30).

As previously mentioned the reason to take care with the dual inhibition of SGLT1 and SGLT2 is the potential side effects of the cumulative SGLT inhibition as well as the additional side effects caused by SGLT1 in the gastrointestinal system.

It should be mentioned though that SGLT inhibitors with a tendency towards the SGLT1 are being developed and used in order to treat diseases such as chronic constipation, e.g. Mizagliflozin (31), or obesity and polycystic ovary syndrome, e.g. LIK066.

The following table shows the international non-proprietary names of different SGLT2 inhibitor substances and their brand names in Austria, as well as their selectivity bias towards SGLT2 vs SGLT1. Additionally, showing the ease with which SGLT2 inhibitors' can be combined with other antidiabetics, the combination drugs including SGLT2 inhibitors have also been listed.

International Non-proprietary Name (Brand Names in Austria)	**Selectivity SGLT2 vs SGLT1**
Sotagliflozin (-)	20-fold
Canagliflozin (Invokana©)	250-fold
Dapagliflozin (Forxiga©)	1200-fold
Ertugliflozin (Steglatro©)	2000-fold
Empagliflozin (Jardiance©)	2500-fold
Phlorizin (-)	1,5-fold

Figure 9 Selectivity of different SGLT inhibitors (3) (5 S. 98)

International Non-proprietary Name	**Brand Name in Austria**
Canagliflozin + Metformin	Vokanamet©
Dapagliflozin + Saxagliptin	Qtern©
Dapagliflozin + Metformin	Xigduo©
Ertugliflozin + Metformin	Segluromet©

Empagliflozin + Linagliptin	Glyxambi©
Empagliflozin + Metformin	Synjardy©

Figure 10 Combination Drugs including SGLT2 Inhibitors

Benefits of SGLT2 Inhibition

In the past, most of the efforts in fighting diabetes were geared towards reducing blood glucose levels to a normal range, with the belief that this would lead to beneficial effects in on both the micro- and the macrovascular scale. This logical assumption has, however, in recent times, been challenged by large short- and midterm cardiovascular outcome trial. Among these trials were the Action to Control Cardiovascular Risk in Diabetes (ACCORD), which concluded that the intensified therapy to achieve normal glycated haemoglobin levels increased mortality and did not significantly reduce major cardiovascular events (32), the Action in Diabetes and Vascular Disease (ADVANCE) trial revealed a 10% relative reduction in both major micro- and macrovascular events, attributing these though to the 21% relative reduction in nephropathy (33) and the Veterans Affairs Diabetes Trial (VADT), which could show no significant reduction in macrovascular events in patients with a long history of type 2 diabetes.

These, almost counterintuitive observations, which also suggest that certain therapeutic strategies may lead to patients being at a greater risk of clinically adverse events, has led to the ESC (European Society of Cardiology) to declare in its guidelines that blood sugar targets should be individualised according to the patient's age, duration of diabetes and the presence of a cardiovascular disease or comorbidity. This is where SGLT2 inhibitors take the stage; the trials mentioned above were conducted using older antidiabetic medication. An exploratory analysis called the Empagliflozin Cardiovascular Outcome Event Trial in Type 2 Diabetes mellitus Patients (EMPA-REG OUTCOME) pitted empagliflozin against a placebo. Changes in the HbA_{1C} were observed, but it was suggested that this only had a modest mediatory effect on the hazard ratio for cardiovascular death; it could therefore be assumed that the effects were not only due to the lowering of the glucose level (34). The Canagliflozin Cardiovascular Assessment Study (CANVAS) also reported a significant reduction in the incidence of adverse cardiovascular events (35). It was hypothesised

that this reduction in cardiovascular and heart-failure events was due to mechanisms that improved the haemodynamic and metabolic situation of the patients. Further studies with other antidiabetic substances supported this hypothesis. Clinical trials such as the Liraglutide Effect and Action in Diabetes (LEADER) and the trial to Evaluate Cardiovascular and Other Long-term Outcomes with Semaglutide in Subjects with Type 2 Diabetes (SUSTAIN6) also showed significant reductions in adverse cardiovascular events that were independent of their glucose-lowering properties (36).
The question by which mechanisms SGLT2 inhibitors could help prevent glucose-independent, adverse cardiovascular events, and how this could be taken advantage of in antidiabetic therapy and maybe even further afield, had been asked.

Apart from the resulting excretion of 80g of sugar per day in an individual with a healthy kidney, further benefits may be derived from the use of SGLT2 inhibitors, such as the treatment of heart failure, kidney disease progression and fatty liver disease. In this section, the additional benefits and studies thereof will be analysed and summarised.

Heart failure

An often overlooked and underestimated comorbidity, when it comes to individuals suffering from diabetes, is heart failure. The analysis of epidemiological data shows that men with type 2 diabetes have double the risk of heart failure and women three times the risk (37). The data in recent years have clearly indicates that not only macrovascular complications, such as heart attacks and strokes, have led to a higher mortality in diabetes patients, but that heart failure is also a large contributor (38). Gregg, Wang et al were able to show in an in depth study that over the past three decades a reduction in macrovascular events, such as heart attacks and strokes, has been achieved through new diabetic treatments. However, their analysis highlighted that when it comes to the morbidity and mortality of heart failure in diabetes patients, similar reductions have not yet been achieved (39). This is especially true, when it comes to patients with heart failure with a preserved ejection fracture. As this type of heart failure is prevalent amongst diabetics, it is of vital importance that any antidiabetic medication does not have a negative effect on the heart, and if possible can ameliorate heart failure. The previously mentioned EMPA-REG OUTCOME and CANVAS studies have proved the beneficial effect SGLT2 inhibitors can have on heart-failure-associated endpoints (40). How SGLT2 inhibitors achieve these beneficial effects on a molecular level is still poorly understood. One of the reasons hypothesised is the haemodynamic effect SGLT2 inhibitors have; the glycosuria, the diuretic and the natriuretic effects inevitably lead to a reduction in blood volume and therefore alleviates stress placed on the heart. Hallow et al. claimed that this osmotic diuresis leads to a greater electrolyte-free water clearance, which has the consequence that more fluid is cleared from the interstitial fluid space than from the circulation. This results in the relief of congestion whilst only impacting the blood volume, organ perfusion and arterial filling minimally (41).

Another theory is the change in cardiac substrate utilisation. It has been observed that in patients receiving SGLT2 inhibitors an elevated number of ketone bodies are present, such as in the post hoc analysis from an open-label Japanese study with canagliflozin (42). These ketone bodies have been referred to as “an energy-efficient super fuel”, which improves the efficiency and the function of the myocardium. It is postulated that this small beneficial change in the energetics of the heart adds up to a

great overall difference in energy efficiency, which leads to an improved cardiac outcome (43).

A further theory states that a change in the function of the mitochondria and the kidney-heart interaction by SGLT2 inhibitors leads to this improved outcome. SGLT2 inhibitors could be responsible for the blocking of Na^+/H^+ exchangers (NHE1) and NHE3 thereby reducing intraglomerular pressure benefitting the patient in the long-term (44).

Even though the exact mechanism is not understood exactly, the results of studies conducted so far into the efficacy of SGLT2 inhibitors have shown that clear and significant benefit to a diabetes patient's cardiac health, which is the reason why the OEGD suggests the use of SGLT2 inhibitors as a second-line therapy in patients with a history of cardiovascular disease.

Diabetic and non-diabetic chronic kidney disease

Through large placebo-controlled trials it has been discovered that SGLT2 inhibitors not only reduce hyperglycaemia but also reduce the risk of renal disease progression in patients with diabetes and cardiac comorbities, even when it comes to chronic kidney disease (45). Similar to the observed improvement in cardiovascular outcomes, the reason why SGLT2 inhibition improves renal outcomes is unclear. The reduction in sodium and water, the improvement in blood pressure and the weight loss must however all be associated with the improved renal outcomes in patients with type 2 diabetes (46). SGLT2 inhibitors reduce the intraglomerular pressure, this may play a vital role in the protection of the kidneys, as evidence suggests that thereby renal tubuloglomerular feedback is activated by the increased delivery of sodium into the macula densa, which leads to the restoration of adenosine production. This adenosine production then results in adenosine-mediated vasoconstriction of the afferent renal arterioles, resulting in a reduction of the intraglomerular pressure and albuminuria and a suppression of the hyperfiltration. This is an important method of action by the SGLT2 inhibitors as the progression of chronic kidney disease is based on intraglomerular hypertension and hyperfiltration. Additionally, this mechanism complements the inhibition of the renin-angiotensin system (47).

The Canagliflozin and Renal Endpoints in Diabetes with Established Nephropathy Clinical Evaluation (CREDENCE) study has confirmed that these methods of action by the SGLT2 inhibitors improve renal outcomes (48).

As type 2 diabetes and chronic kidney disease are both associated with an increased cardiovascular risk, an antidiabetic that can improve a patient's blood sugar levels as well as the progression of chronic kidney disease would be ideal. The OEDG therefore recommends the use of SGLT2 inhibitors as a second-line therapy if a patient has a history of chronic kidney disease. Ongoing studies are even looking into the possibility as to whether SGLT2 inhibitors should be added to the treatment scheme of all patients with chronic kidney disease, both with and without type 2 diabetes. (49).

Non-alcoholic fatty liver disease

A further field for the potential application of SGLT2 inhibitors is non-alcoholic fatty liver disease (NAFLD). NAFLD is the most prevalent liver disease without an approved and agreed upon pharmacotherapy. It covers a spectrum of disorders that lead to the liver accumulating excessive fat, fibrosis and inflammation can be consequences. In addition to damaging the liver, NAFLD is associated with a higher rate of cardiovascular events (50). This group of disorders is strongly associated with type 2 diabetes, and higher rates of mortality, which is why SGLT2 inhibitors are under close scrutiny when it comes to their effects on the liver. Numerous studies with SGLT2 inhibitors have documented a significant in body weight, alanine aminotransferase levels and the fatty liver index in patients with NAFLD (51). In Japan especially empagliflozin and its effect on the hepatocellular lipid content, liver energy metabolism and body composition in newly diagnosed type 2 diabetes patients has been examined. The clinical data shows that the inhibition of SGLT2 leads to an improvement of body composition and liver tests, a decrease in insulin concentrations and blood sugar and a reduction in visceral fat (52).

Figure 3 summarises the different effects and benefits SGLT2 inhibitors can have on different organ systems, showing the cardiac, the renal and the hepatic benefits of SGLT2 inhibitors.

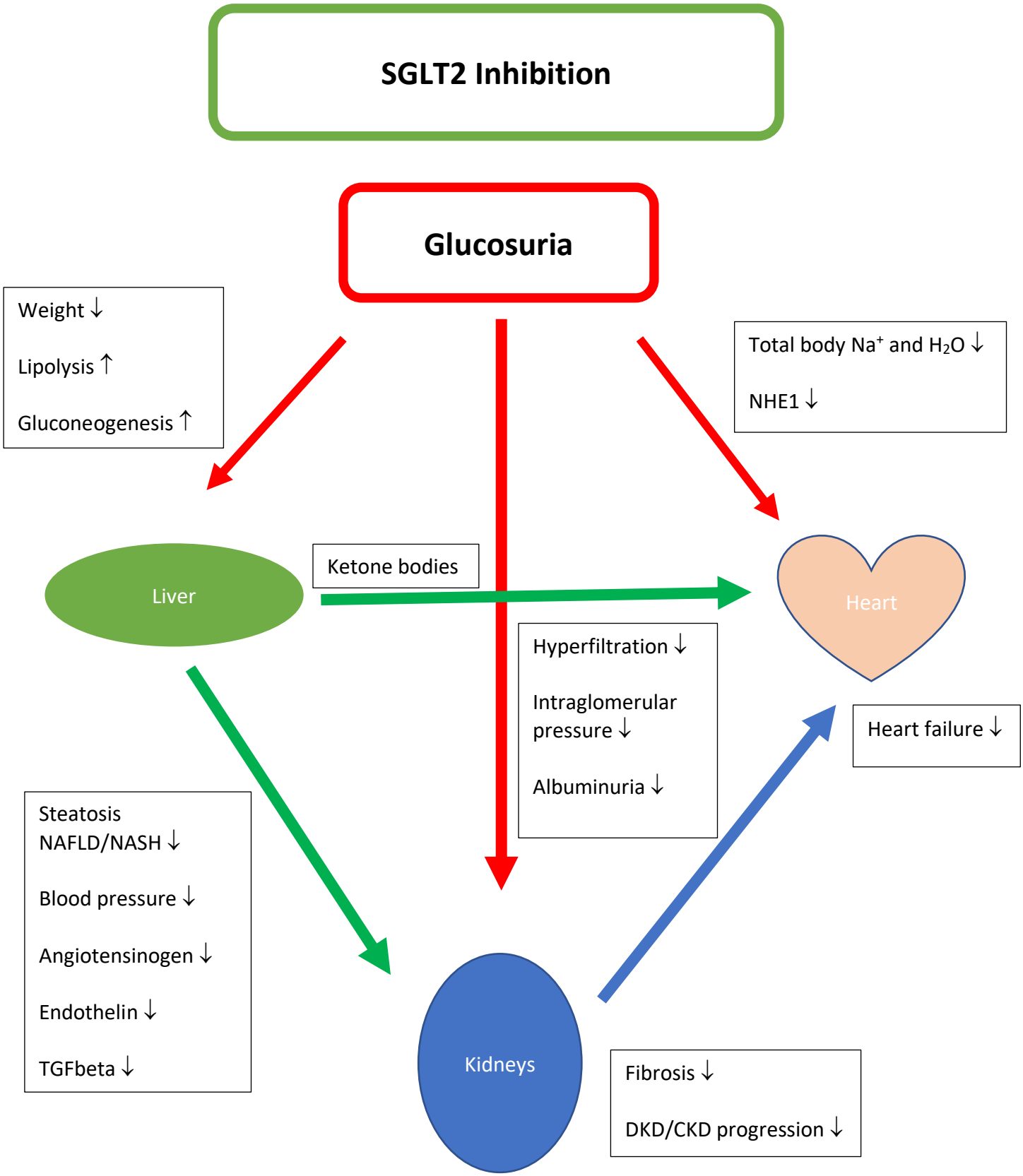

Figure 11 Summary of the Effects of SGLT2 Inhibitors (128)

Risks of SGLT2 Inhibition

All new drugs on the market must go through a rigorous vetting process, and SGLT2 inhibitors are no exception. Numerous studies were conducted to evaluate the safety and the effectiveness of these new substances. During these studies, the risks and side effects that had been clinically detected and observed, ranging from an increased risk of cancer to urinary tract infections, were analysed and statistically evaluated in order to see if they were significant in the treatment of diabetes with SGLT2 inhibitors.

Genitourinary infections

Urinary tract infections (UTIs) are an ideal place to start, as these are the most common complication when dealing with SGLT2 inhibitors. It should be kept in mind that all type 2 diabetes patients already present a higher risk of UTIs. This is due to numerous factors, such as glycosuria, increased oestrogen levels, the increased ability of bacteria to adhere to the uroepithelium and the generalised reduction of a diabetic patient's immune system (53). Type 2 diabetes patients are more prone to genital fungal infections as well; balantitis-balanposthitis in men and vulvovaginitis caused by *Candida* species in women (54).

As SGLT2 inhibitors increase the amount of glucose in urine it can be expected that a higher number of UTIs will occur. It has been shown that the SGLT2 inhibitors canagliflozin, empagliflozin and dapagliflozin increase the likelihood of UTIs (55).

The earlier mentioned CANVAS study observed an increase in male genital infections and female mycotic infections in the group that received canagliflozin. Here 34.9 events per 1000 patient-years were recorded in contrast to 10.8 events per 1000 patient-years, p was < 0.001 for male infections and 68.8 events per 1000 patient-years in contrast to 17.5 events per 1000 patient- years, $p < 0.001$ for female infections. Interestingly no increase in UTIs was observed in this study, only 40 events per 1000 patient-years with canagliflozin when by comparison 37 events per 1000 patient-years occurred in the patients taking a placebo, p was therefore 0.38 (56).

The EMPA-REG OUTCOME trial showed similar results. It concluded that genital infections, which were mainly observed in women, increased in the empagliflozin group (6,4%) when compared with the placebo group (1,8%). Similar to the results in

the CANVAS study the EMPA-REG OUTCOME trial also observed that the amount of UTIs when comparing the empagliflozin group and the placebo group were much the same (18,1% in the placebo group and 18% in the empagliflozin group). Even when it came to complicated UTIs both groups were similar (placebo 1,8% vs 1,7% empagliflozin) (57). Both these large double-blind randomly controlled trials therefore concluded that SGLT2 inhibitors increase the rate of genital infections not however UTIs.

Study	Type	SGLT2 Inhibitor Used	Results
CANVAS	RCT (n=10 142)	Canagliflozin	No increase in UTIs
EMPA-REG OUTCOME	RCT (n=7 020)	Empagliflozin	No increase in UTIs
Liu et al. (58)	Meta-analysis (n=50 820)	All SGLT2 inhibitors	No increase in UTIs, only dapagliflozin lead to increase in UTI risk
Zaccardi et al. (59)	Meta-analysis (n=23 997)	All SGLT2 inhibitors	Only dapagliflozin lead to increase in UTI risk
Vasilakou et al. (60)	Meta-analysis (n=16 407)	All SGLT2 inhibitors	UTIs more frequently associated with SGLT2 inhibitors
Kawalec et al. (61)	Meta-analysis (n=1 150)	Dapagliflozin and canagliflozin	No increase in UTIs
Li et al. (62)	Meta-analysis (n=36 689)	All SGLT2 inhibitors	No increase in UTIs only with dapagliflozin
Yang et al. (63)	Meta-analysis (n=3 669)	All SGLT2 inhibitors	No increase in UTIs

Figure 12 Summary of SGLT2 inhibitors and the risk of UTIs

Figure 4 summarises the risks associated with SGLT2 inhibitors and UTIs discovered in randomised controlled trials as well as meta-analyses by different authors. Kawalec et al. and Yang et al. through meta-analysis came to the same conclusion as the CANVAS and the EMPA-REG OUTCOME studies, that no increase of UTIs in patients being treated with SGLT2 inhibitors could be observed. Vasilakou et al. however found results in their meta-analysis that indicated that UTIs were more common in patients

receiving SGLT2 inhibitors, when they were compared to a placebo group or an active comparator group.

Liu et al. conducted a meta-analysis of 77 randomised controlled trials and concluded that these did not show a statistically significant difference in the incidence of UTIs, they did however show that the rate of genital infections increased. The same meta-analysis showed that dapagliflozin was responsible for an increased risk of UTIs. This finding by Liu et al. was corroborated by Zaccardi et al. and Yang et al., they were also able to show that dapagliflozin was associated with a higher risk of UTIs when compared with a placebo.

It is important to point out though that, all the studies mentioned above reported that the majority of UTIs occurred within the first 24-26 weeks of treatment, and that the rate of incidence decreased after this point in time.

Cancer

Numerous studies have suggested that type 2 diabetes is associated with a higher incidence of cancer. The mechanisms and the pathophysiology remains unknown, but studies such as the one conducted by Giovannucci et al. concluded that type 2 diabetes is associated with a higher risk of liver, pancreas, endometrium, colon, rectum, breast and bladder cancer. It was assumed that the link between diabetes and cancer may be due to hyperinsulinaemia, hyperglycaemia and inflammation. The evidence between drugs used to treat diabetes and cancer were deemed limited, however it was shown that metformin may be able to reduce the rate of cancer in type 2 diabetic patients. (64)

Bearing these already higher cancer rates in mind it is important to vet all new antidiabetic medication. Alarms were first raised, with regard to SGLT2 inhibitors, when in 2011 the FDA suspected a higher risk of breast and bladder cancer associated with dapagliflozin (65).

By 2013, 10 cases of bladder cancer were detected in a group of 6 045 patients (0,17%) taking dapagliflozin. Only 1 case of bladder cancer occurred in the placebo group that consisted of 3 512 patients (0,03%). The 10 cases of bladder cancer whilst taking dapagliflozin were all reported within 2 years of beginning treatment and all, save one, displayed haematuria within the first 6 months. At first this sounds rather

damning, on closer inspection however a biological heterogeneity of the bladder cancer could be proven. This heterogeneity ranged from low grad to high grade and from non-invasive to highly metastatic. This wide range indicates that the cancer did not stem from a single triggering cause (66).

Dapagliflozin was also accused of being responsible for an increase of breast cancer cases when compared with a placebo, 12 vs 3. Similar to the findings with bladder cancer these doubts around dapagliflozin were also dispelled as tumour type, patient age, stage, progesterone and oestrogen receptor status and HER2/neu status were all different. This once again suggests that the cause of cancer is no one triggering factor (66). A recent analysis of 21 clinical trials backed up this assumption that dapagliflozin does not increase the risk of cancer (67).

Not only humans were observed to see if the use of SGLT2 inhibitors leads to a higher rate of cancer. A group of Sprague-Dawley rats, outbred, multipurpose albino rats, was exposed to up to the 186-fold human dose of dapagliflozin for 90 weeks in males and 105 weeks in females. This study showed no increase in breast cancer in either the female or the male rats (68).

A meta-analysis of 8 canagliflozin trials also showed no signs of an increased risk of breast bladder and renal cancer (69). The two large randomly controlled trials referred to already in the text also analysed the occurrence and risk of cancer and the use of SGLT2 inhibitors. The CANVAS study concluded that the incidence of bladder, breast and renal cancer did not increase under the use of canagliflozin (70). The EMPA-REG OUTCOME study did however show that the rate of bladder cancer was significantly increased in the group of patients treated with empagliflozin. Nine cases of cancer were detected in contrast to no cases in the placebo group (71). Tang et al. also identified an increase in bladder cancer incidence with the use of empagliflozin (72). It could be argued however that these case numbers are too low to draw definite conclusions from them.

Taking all the available data from randomly controlled trials it can be concluded that it indicates no clear relationship between SGLT2 inhibitors and the risk of cancer. However, the findings of the EMPA-REG OUTCOME trials and other analyses with regard to empagliflozin do indicate the need for further research. It should be noted that there is fascinating research taking place in the field of cancer prevention through the use of especially SGLT1 inhibitors in order to prevent from certain cancers through the inhibition of glucose uptake into the cancerous cells.

Diabetic Ketoacidosis

Diabetic Ketoacidosis is a medical emergency that is more commonly linked with type 1 diabetes. The reason it can occur are a lack of insulin or stressful conditions which require a higher dosage of insulin e. g. reduced food or fluid intake, surgery, trauma, infection, myocardial infarction or alcohol abuse. The classic criteria for diabetic ketoacidosis are a serum bicarbonate level that is less than 15mmol/l, a serum anion gap of more than 12 mmol/l, an arterial pH of less than 7,3 and the presence of ketones.

The reason diabetic ketoacidosis is interesting in patients taking SGLT2 inhibitors is because it does not present as it typically would in diabetic patients. An increased anion gap metabolic acidosis with ketonuria is observed, as in typical diabetic ketoacidosis, however it is accompanied with a blood sugar level of less than 200 mg/dl and therefore is defined as euglycaemic diabetic ketoacidosis (73). The process that leads to this is due to the SGLT2 inhibitors creating an imbalance between the serum glucagon and insulin levels. The reduction of serum insulin levels causes increased lipolysis, which with the combined reduction in blood sugar induces an increase in fatty acid oxidation which produces acetyl-CoA that are then converted into ketones as a result of the glucagon to insulin ratio. The renal effects of SGLT2 inhibitors may also play a role, as the inhibition of SGLT2 increases the renal tubule sodium concentration, which leads to the increased reabsorption of both sodium and ketoacids in the collecting tubules (74).

These mechanisms explain the increase in ketones whilst taking SGLT2 inhibitors but how do they bear out when examined and analysed? The FDA Adverse Event Reporting System (FAERS) recorded 680 cases of diabetic ketoacidosis in a group of 5 694 individuals (11,9%) that were taking dapagliflozin, between the years of 2014-2016. Similar groups were observed for canagliflozin and empagliflozin where 9,6% and 13,1% of the patients respectively were affected by diabetic ketoacidosis. In contrast a meta-analysis of 10 randomised controlled trials (n=13 134) reported a mere 14 diabetic ketoacidosis cases and concluded therefore that no additional risk is incurred by the use of SGLT2 inhibitors (75). It should be mentioned however that randomised clinical trials often include a carefully selected group that may already have a lower risk of diabetic ketoacidosis.

Further study is definitely needed as the results from the randomised controlled study and the data from the FDA shows. What has also been hypothesised and previously mentioned in this dissertation is that the ketone bodies produced could very well be the reason that SGLT2 inhibitors have such a cardioprotective function as they increase energy efficiency and result in an improvement of cardiac contractility and the oxygenation of the kidney (76).

Amputation Risk

During the CANVAS study an increased risk of amputation was detected. This risk concerned the toes, feet and legs of the patients being treated with canagliflozin, 6,3 vs 3,4 per 1000 patient years with the hazard ratio being 1,97. The risk was determined to be more prevalent in patients with a previous history of vascular disease or amputation (77).

In stark contrast the EMPA-REG OUTCOME study showed no increase in the risk of amputation in the empagliflozin group, their rate of amputation was almost the same as the placebo group, 1,9% to 1,8% respectively (78). This finding was backed up by a pooled analysis of clinical trials, which also concluded that empagliflozin did not increase the rate of amputations (79).

It can therefore be assumed that the increased risk of amputation is confined to canagliflozin and not the entire class of SGLT2 inhibitors.

Electrolyte Disorders

SGLT2 inhibitors have through meta-analyses and post hoc analyses been observed to cause disruptions in the body's electrolytes. These findings will be discussed and their potential origins further explained.

Magnesium

Tang et al. conducted a meta-analysis of 18 randomised controlled trials, which included more than 15 000 patients and discovered that an increase of serum magnesium levels of 0,1-0,2 mEq/l in patients that were not suffering from chronic kidney disease occurred. These changes were observed in patients taking canagliflozin, empagliflozin, dapagliflozin and ipragliflozin (80).

The post hoc analysis of randomised controlled trials by Gilbert et al. found similar increases in serum magnesium levels in patients taking either 100 or 300mg of canagliflozin. In this case, the conclusion was that the magnesium levels were only returned to normal, however it must be born in mind that magnesium levels tend to be lower in patients suffering from diabetes (81).

One of the potential mechanisms by which the increase in serum magnesium can be explained is the fact that a reduction of magnesium excretion takes place through the transient receptor potential ion channel 6 (TRPM6). The assumption this mechanism is based on is because in obese diabetic rats the downregulation of TRPM6 led to hypermagnesiuric hypomagnesemia (82). Inversely this could mean that an improvement in a patient's insulin resistance through the use of SGLT2 inhibitors may lead to an increased expression of the TRPM6 which results in a decreased excretion of magnesium and therefore an increase in serum magnesium levels.

Further suggestions have been made concerning the effect of SGLT2 inhibitors on the serum magnesium concentration. Tang et al. (80) suggested that the increase in serum magnesium may be due to a change in haeomoconcentration. The fact that SGLT2 inhibitors can lead to higher glucagon levels has been put forward as a potential cause, as higher glucagon levels lead to a greater absorption of magnesium in the distal convoluted tubules (83). The decrease of insulin has also been sighted as a reason, due to the fact that the decrease in insulin, which would normally help magnesium move into the intracellular space, leads to magnesium shifting from the intracellular space into the extracellular space (84).

These small shifts in magnesium levels may first appear inconsequential, they should however be taken into consideration when treating patients with chronic kidney failure. This magnesium-increasing effect may also be one of the reasons that SGLT2 inhibitors have cardiovascular benefits.

Potassium

In the CANVAS study, it was shown that patients being treated with canagliflozin suffered 6,9 events of hyperkalaemia per 1 000 patient years whilst the placebo group only suffered 4,4 events. The patients that were affected the most were those that had a reduced renal function and those that were taking additional drugs that affected the potassium homeostasis, e.g. inhibitors of the renin angiotensin aldosterone system (RAAS).

This side effect could not be observed in patients taking empagliflozin. The EMPA-REG OUTCOME study showed that no increase in potassium levels occurred (85). Dapagliflozin was even shown to not be associated with increased potassium levels in patients that a reduced estimated glomerular filtration rate (<60ml/min) (86).

The possible reason for the increases in serum potassium could either be through haemoconcentration or due to decreased insulin. As with the magnesium concentration, the reduction in insulin leads to potassium leaving the intracellular space and moving to the extracellular space.

The studies and analyses conducted indicate that canagliflozin causes a small increase in serum potassium levels, which should be kept in mind especially when treating patients that are predisposed to hyperkalaemia.

Phosphate

Clinical trials have reported that small increases in serum phosphate levels have occurred in patients after beginning the use of SGLT2 inhibitors. It has been hypothesised that the main reason for this is the reduction in proximal sodium reabsorption due to the inhibition of SGLT2 which leads to a higher availability of sodium which then is reabsorbed alongside phosphate in the Na^{+}-$3PO_4^{3-}$ cotransporters in the proximal tubules in order to restore the extracellular volume.

Fracture Risk

SGLT2 inhibitors have been suspected of upsetting the body's bone homeostasis. They have been thought to cause a slight increase in phosphate levels, as previously discussed, leading to an increase in the serum's parathormone and fibroblast growth

factor 23, the latter has been thought to lead to a decrease in the body's vitamin D concentration, resulting in decreased absorption of calcium. This combination of increased parathormone and a reduction in the absorption of calcium may be the reason SGLT2 inhibitors cause reduced bone mineral density and therefore lead to a greater risk of fractures (87).

During the CANVAS study, it was observed that patients receiving canagliflozin had a higher rate of all fractures than the patients receiving the placebo (15,4 vs 11,9 fractures per 1 000 patient years) (88). This increase was detected in patients within a few weeks after the onset of the treatment. The patients in which the fractures occurred most frequently were a subset; the elderly, those with a lower baseline eGFR, those with a higher cardiovascular risk and those with a higher baseline use of diuretics compared to the remaining study population.

An analysis that encompassed eight non-CANVAS studies with canagliflozin, by contrast, indicated that the incidence of fractures in patients receiving canagliflozin was similar to those receiving non-canagliflozin treatment (1,7% vs 1,5%) (89).

The reason why the fracture risk increases under canagliflozin may have been explained by Watts et al.. They showed that over a 104-week period under 100mg canagliflozin the total hip bone mineral density decreased by 0,9% and by 1,2% if the patients were give 300mg of canagliflozin. It should be noted that this decrease in bone density could only be observed in the hip though (90).

The EMPA-REG OUTCOME trial documented no increased risk of fractures in patients taking empagliflozin. Here the rate of bone fractures were 3,9% in the placebo group vs 3,8% in the empagliflozin group. Further pooled analyses show the same results, in these the placebo group had a fracture rate of 1,7%, the empagliflozin 10mg a rate of 1,6% and the empagliflozin 25mg group a rate of 1,4% per 100 patient years (91).

Additionally, a meta-analysis of 38 randomised controlled trials using all kinds of SGLT2 inhibitors concluded that none were associated with a higher rate of fractures. They found that the rate was 1,59% in the SGLT2 inhibitor compared to 1,56% in the placebo group (92). This meta-analysis even showed that the event rates were similar in all SGLT2 inhibitors when compared with the placebo group (odds ratios: canagliflozin 0,93; dapagliflozin 0,63; empagliflozin 0,57). These are fascinating results as they contradict the findings of the CANVAS study and cast doubt on whether SGLT2 inhibitors do cause a higher rate of fractures in patients.

Study	Type	SGLT2 inhibitor used	Results
CANVAS (88)	RCT	Canagliflozin	Increased fracture rate when compared with placebo
EMPA-REG OUTCOME (93)	RCT	Empagliflozin	No increased fracture rate
Watts et al. (92)	Pooled analysis	Canagliflozin	Increased fracture rate
Kohler et al. (94)	Pooled analysis	Empagliflozin	No increased fracture rate
Tang et al. (95)	Meta-analysis	All SGLT2 inhibitors	No increased fracture rate with SGLT2 inhibitors; all SGLT2 inhibitors with similar event rates
Ruanpeng et al. (96)	Meta-analysis	All SGLT2 inhibitors	No increased fracture rate with SGLT2 inhibitors; all SGLT2 inhibitors with similar event rates

Figure 13 Summary of SGLT2 inhibitors and Fracture Risk

Lipid Metabolism

SGLT2 inhibitors have been accused of increasing the amount of low-density lipoprotein (LDL) cholesterol. A study observed that patients that had been treated with canagliflozin over a period of 26 weeks experienced an increase in LDL cholesterol, high-density lipoprotein cholesterol and a decrease in triglycerides. The study also showed that a higher dose in canagliflozin led to a higher increase in LDL cholesterol, 4,5% when treated with 100mg and 8% when being treated with 300mg (97). The CANVAS study corroborated these findings, stating that the patients being

treated with canagliflozin experienced a 5-6 mg/dl increase in serum LDL cholesterol (4-5%) (98).

The EMPA-REG OUTCOME trial and a meta-analysis showed that empagliflozin leads to a similar 4,5-6,5% increase in serum LDL cholesterol (99). Dapagliflozin has also been scrutinised and a meta-analysis of 13 trials has shown that it also results in an increase of 2-9% of LDL cholesterol (100). A systematic meta-analysis and review concluded that canagliflozin was linked with larger changes in the LDL cholesterol than either empagliflozin or dapagliflozin (101).

As with other side effects due to SGLT2 inhibitors it was first suspected that the resulting increase in LDL cholesterol and triglycerides was due to haemoconcentration, an experimental study suggested that SGLT2 inhibitors lead to a decrease in LDL receptor activity by increasing liver cholesterol synthesis as a result of an increase in the levels of hepatic fatty acid (102).

The reason this side effect is such an important one is because LDL cholesterol is a known cardiovascular risk factor. If SGLT2 inhibitors were responsible for the increase of LDL cholesterol, this may cancel out any other positive cardiovascular effects they may have. It should once again be noted that this did not bear out in either the EMPA-REG OUTCOME or the CANVAS trial.

Hypoglycaemia

There are several reasons why SGLT2 inhibitors are not predisposed to cause hypoglycaemia, the main reason being the physiology of the SGLT1 inhibitor. As previously discussed in a normal kidney 97% of the sugar in urine is reabsorbed by the SGLT2 and only 3% by the SGLT1, in a situation in which the SGLT2 is not functioning, or has been deliberately inhibited, the SGLT1 can up its workload and reabsorb more sugar. This means that when SGLT2 is inhibited SGLT1 prevents excessive glycosuria and thereby hypoglycaemia.

SGLT2 inhibitors have also been shown to improve insulin resistance, thereby causing a decrease in the insulin secretion of the β-cells of the pancreas. This coupled with their ability to increase the secretion of glucagon in the α-cells of the pancreas, as a result of both lower glucose levels and SGLT2 inhibitors ability to influence the

pancreatic α-cells, leads to fewer events of hypoglycaemia, because of an increase in the hepatic glucose production (103).

What has been discovered is that the risk of a hypoglycaemic event does increase when SGLT2 inhibitors are given in combination with sulfonylureas, this risk however does not increase when either metformin or insulin is given in combination with SGLT2 inhibitors (104). A meta-analysis showed that especially canagliflozin was responsible for an increased risk of hypoglycaemia when it is compared with either a placebo or other SGLT2 inhibitors, such as dapagliflozin and empagliflozin (105). This observation seemed to be backed up by, and even put further SGLT2 inhibitors in doubt, a meta-analysis of 33 randomised controlled trials that showed that SGLT2 inhibitors led to a significantly higher risk of hypoglycaemia when compared to placebos. However, the subgroup analysis of the meta-analysis confirmed what was discovered by Zaccardi et al. (105), that only canagliflozin caused a higher risk of hypoglycaemia, but not dapagliflozin or empagliflozin. It also demonstrated that fewer hypoglycaemic events occurred in the SGLT2 inhibitor group than in the sulfonylurea group. Metformin, DPP-4 inhibitors and SGLT2 inhibitors showed the same rate of hypoglycaemia (106).

Skin Reactions

There have been studies in which a link has been suspected between SGLT2 inhibitors and skin reactions in the patient. Yabe et al. found that skin reactions were most likely to occur within the first two weeks of SGLT2 inhibitor use. The side effects which were observed included urticarial, erythema, eczema and a generalised rash. Interestingly ipragliflozin showed a higher rate of skin reactions compared to other SGLT2 inhibitors, this could be because in animal studies ipragliflozin and its metabolites have been detected more often in the skin than other SGLT2 inhibitors (107).

Haemoconcentration and Strokes

As SGLT2 inhibitors cause an increase in haematocrit due to greater haemoconcentration and lead to increased erythropoietin, which both result in the blood being more viscous, it has been proposed that SGLT2 inhibitors may lead to a greater stroke risk (108).

This assumption was first made due to the results of the EMPA-REG OUTCOME trial which showed that empagliflozin led to a higher risk of both fatal and non-fatal strokes (109). Interestingly the trial showed that the patients that had the largest increase in their haematocrit were not the ones with the higher stroke risk (110). A different meta-analysis of 57 studies indicated that a clear increase in non-fatal strokes were observed with SGLT2 inhibitors (111).

This increase in the stroke risk could not be confirmed by the CANVAS study, it showed no increase in the stroke risk. The same finding was made by a meta-analysis of 21 dapagliflozin trials (112).

The conclusion to be drawn from these data is that, whilst SGLT2 inhibitors increase the haematocrit and lead to a greater viscosity of the blood, it is inconclusive if this leads to a higher stroke risk in all patients.

Adverse Event	Discoveries
Cancer risk	- No increased cancer risk in most trials - Caution with dapagliflozin and empagliflozin in patients with haematuria or history of bladder cancer
UTIs	- UTIs not increased in larger RCTs - Mixed results in meta-analyses
Genital Infections	- Increased risk of fungal infections (10% risk in women : 5% risk in men)
Amputations	- Canagliflozin led to an increased risk of amputation
Fractures	- Canagliflozin may have decreased the hip's bone mineral density leading to a higher fracture rate
Electrolyte disorders	- Serum potassium, phosphate & magnesium minimally increased
Hypoglycaemia	- Caution when combined with sulfonylureas, however no
DKA	- Atypical presentation (Euglycaemic DKA) - FAERS reported DKA in patients taking dapagliflozin, canagliflozin and empagliflozin (11,9%, 9,6% & 13,1% respectively of the observed group) - No higher rate of DKA observed in large RCTs (possible due to preselection process in patient group)
Skin Reactions	- Urticaria - Erythema - Eczema - Generalised rash

Figure 14 Summary of Adverse Events and Discoveries of SGLT2 Inhibitors

Conclusion

Having examined both the benefits and the drawbacks of SGLT2 inhibitors and compared them to other commonly used antidiabetics, what conclusions can be drawn? Are they safer and more effective? Is their place justified in the current treatment scheme laid out by the OEDG?

The extensive research of SGLT2 inhibitors since their introduction in 2012 has proven in studies as well as in RCTs that they can achieve positive therapeutic goals beyond merely lowering the patients' glucose levels. The EMPA-REG OUTCOME and CANVAS studies have shown that that SGLT2 inhibitors have a positive effect on heart-failure endpoints. This is outstanding news as 50% of diabetics die due to cardiovascular disease. This justifies the OEDG's position that SGLT2 inhibitors should be prescribed when presented with a patient that with cardiovascular disease or a heart attack in their patient history, as other antidiabetic are at best cardiovascularly neutral or even have a negative effect on cardiovascular mortality. GLP-1 agonists should be mentioned here as they also have been shown to have a positive effect on cardiovascular endpoints, however these are accompanied by a higher degree of side effects, the gastrointestinal side effects that are common in both DPP4-inhibitors and GLP-1 agonists, and also the increased risk of both pancreatitis and pancreatic cancer. It should therefore be considered that SGLT2 inhibitors are preferred to GLP-1 agonists when treating patients with both diabetes and a higher cardiovascular risk profile.

Not only have SGLT2 inhibitors improved the mortality rate when it comes to the number 1 cause of death in diabetics, the CREDENCE study also exposed SGLT2 inhibitors' ability to significantly improve chronic kidney disease in patients with type 2 diabetes. This justifies SGLT's position as the agent of choice when it comes to treating diabetics with a history of chronic kidney disease.

The effects that SGLT2 inhibitors (e.g. empagliflozin) can have on the patients' liver must also be mentioned. Their effect on the liver's energy metabolism and thereby a reduction in visceral fat is key to the treatment of type 2 diabetics as many suffer not only from their insensitivity to insulin but also from obesity.

When compared to other antidiabetic agents in their facility of use and their ability both to reduce HbA_{1C} values whilst not threatening to cause hypoglycaemic events SGLT2 inhibitors also prove their superiority. The taking of one pill a day is a far easier task

than multiple tablets distributed throughout the day or the need for subcutaneous injections. Their ability to reduce the HbA_{1C} values in patients by 0,5-1% puts them within the same category as other antidiabetic agents, whilst unlike sulfonylureas and glinides causing no life-threatening hypoglycaemic events.

Commonly assumed risks when using SGLT2 inhibitors were also examined and studies, RCTs and meta-analyses have shown these assumptions often do not bear out statistically. The most frequently described risk when prescribing SGLT2 inhibitors, an increase in urinary tract infections, could not be confirmed by two of the largest studies of SGLT2 inhibitors (EMPA-REG and CANVAS). Certain meta-analyses claimed that all or specific SGLT2 inhibitors lead to a statistically relevant increase in urinary tract infections. This information should lead to the conclusion that SGLT2 inhibitors should not be discarded as a potential treatment but patients with a history of multiple urinary tract infections should be closely monitored.

What must be mentioned is the fact that certain SGLT2 inhibitor substances have been found to carry individual risks. Canagliflozin has been shown to cause an increased risk of amputation and fractures. This is the reason why canagliflozin is not used in Europe or America. Dapagliflozin and empagliflozin have also been shown to carry an elevated risk of bladder cancer. These substances should be treated with greater scrutiny, it should however not lead to the discarding out of hand of the whole substance group.

SGLT2 inhibitors prove that novel antidiabetic agents can play a vital role in the treatment of type 2 diabetes, reducing risks of treatment and even going beyond the treating of only high blood sugar. Further studies are still necessary to fully understand how all the mechanisms work that lead to a reduction in cardiovascular mortality rates and the improvement of chronic kidney disease, however due to the research that has already been conducted it can be concluded that SGLT2 inhibitors are safe for use and reduce a patients cardiac and renal health, whilst at the same time helping them to control their HbA_{1C}, a claim that cannot be made by all antidiabetic medications and therefore should place SGLT2 inhibitors at the forefront of current treatments of diabetes.

Bibliography

1. Organisation, World Health. euro.who.int. who.int. [Online] [Cited: 14 01 2021.] https://www.euro.who.int/en/health-topics/noncommunicable-diseases/diabetes/data-and-statistics.
2. 33:442–449, Laakso M (2010) Cardiovascular disease in type 2 diabetes from population to man to mechanisms: the Kelly West Award Lecture 2008. Diabetes Care.
3. Sourij, Assoz. Prof. Priv.-Doz. Dr.med.univ. Harald. PPT Diabetes mellitus Typ 2: Die Rolle der SGLT-2 Hemmer: https://inneremedizinonline.at/wp-content/uploads/2019/01/sglt2_final.pdf. Graz : s.n.
4. OEDG. OEDG Pocket Guide 2019-07.
5. Beubler, Eckhard. Kompendium der Pharmakologie - Gebräuchliche Arzneimittel in der Praxis 4. Auflage. s.l. : Springer-Verlag GmbH Deutschland, 2018. 978-3-662-54558-4.
6. Tang H, Shi W, Fu S, Wang T, Zhai S, Song Y, Han J. Pioglitazone and bladder cancer risk: a systematic review and meta-analysis. Cancer Med. 2018 Apr, 29476615, 7(4):1070-1080. doi: 10.1002/cam4.1354. Epub 2018 Feb 24. PMID: and PMC5911601., PMCID:.
7. Cornel JH, Bakris GL, Stevens SR, Alvarsson M, Bax WA, Chuang LM, Engel SS, Lopes RD, McGuire DK, Riefflin A, Rodbard HW, Sinay I, Tankova T, Wainstein J, Peterson ED, Holman RR, Dec, TECOS Study Group. Effect of Sitagliptin on Kidney Function and Respective Cardiovascular Outcomes in Type 2 Diabetes: Outcomes From TECOS. Diabetes Care. 2016 and 27742728., 39(12):2304-2310. doi: 10.2337/dc16-1415. Epub 2016 Oct 14. PMID:.
8. Kristensen SL, Rørth R, Jhund PS, Docherty KF, Sattar N, Preiss D, Køber L, Petrie MC, McMurray JJV. Cardiovascular, mortality, and kidney outcomes with GLP-1 receptor agonists in patients with type 2 diabetes: a systematic review and meta-analysis of cardiovascular outcome trials. Lancet Diabetes Endocrinol. 2019 Oct, Mar, 7(10):776-785. doi: 10.1016/S2213-8587(19)30249-9. Epub 2019 Aug 14. Erratum in: Lancet Diabetes Endocrinol. 2020 and 31422062., 8(3):e2. PMID:.
9. Delhez R (1968) Jean-Servais Stas 1813-1891. In: Florilège des Sciences en Belgique pendant le XIXe siècle et le début du XXe. Brussel, pp285-321.
10. The roots of SGLT inhibition: Laurent-Guillaume de Koninck, Jean Servais Stas and Freiherr Josef von Mering. Jörgens, Viktor. 56, s.l. : Springer-Verlag Italia S.r.l., part of Springer Nature 2018, 2019, Acta Diabetologica (2019), pp. 29-31.
11. Phloridzin von Konnink und Stas (1835) Pharmaceutisches Central-Blatt, short announcements, Leipzig, Leopold Voss vol 1, 25 6.1835, p 398.
12. Über das Ploridzin (Phlorrhizin) Annalen der Pharmacie. Koninck, LG De. Heidelberg : s.n., 1835.
13. Über künstlichen Diabetes. J, Von Mering. 31, 1886, Centralblatt für die medizinische Wissenschaft, Vol. 22.
14. Diabetes, Von Mering J (1886) Über experimentellen and Verhandlungen des V Congresses für Innere Medizin in Wiesbaden. J F Bergmann, Wiesbaden, pp 185–189.
15. mellitus, Von Mering J (1887) Über Diabetes and Verhandlungen des VI Congresses für Innere Medizin in Wiesbaden. J. F. Bergmann, Wiesbaden, pp 349–358.
16. 29:90–93, Minkowski O (1892) Weitere Mittheilungen über den Diabetes mellitus nach Exstirpation des Pankreas. Berliner Klin Wochenschr.

17. Rieg, T., Vallon, V. Development of SGLT1 and SGLT2 inhibitors. Diabetologia 61, 2079–2086 (2018). https://doi.org/10.1007/s00125-018-4654-7.
18. Dennis VW, Brazy PC (1978) Phosphate and glucose transport in the proximal convoluted tubule: mutual dependency on sodium. Adv Exp Med Biol 103:79–80.
19. Cramer SC, Pardridge WM, Hirayama BA, Wright EM (1992) Colocalization of GLUT2 glucose transporter, sodium/glucose cotransporter, and gamma-glutamyl transpeptidase in rat kidney with double-peroxidase immunocytochemistry. Diabetes 41:766–770.
20. Vallon V, Platt KA, Cunard R et al (2011) SGLT2 mediates glucose reabsorption in the early proximal tubule. J Am Soc Nephrol 22:104–112.
21. Gorboulev V, Schurmann A, Vallon V et al (2012) Na+-D-glucose cotransporter SGLT1 is pivotal for intestinal glucose absorption and glucose-dependent incretin secretion. Diabetes 61:187–196.
22. Rieg T, Masuda T, Gerasimova M et al (2014) Increase in SGLT1-mediated transport explains renal glucose reabsorption during genetic and pharmacological SGLT2 inhibition in euglycemia. Am J Physiol Ren Physiol 306:F188–F193.
23. Song P, Onishi A, Koepsell H, Vallon V (2016) Sodium glucose cotransporter SGLT1 as a therapeutic target in diabetes mellitus. Expert Opin Ther Targets 20:1109–1125.
24. Vasilis Tsimihodimos, Sebastien Filippas-Ntekouan, Moses Elisaf, SGLT1 inhibition: Pros and cons, European Journal of Pharmacology, Volume 838, 2018, Pages 153-156, ISSN 0014-2999.
25. Chasis H, Jolliffe N, Smith HW (1933) The action of phlorizin on the excretion of glucose, xylose, sucrose, creatinine and urea by man. J Clin Invest 12:1083–1090.
26. Rossetti L, Smith D, Shulman GI, Papachristou D, DeFronzo RA (1987) Correction of hyperglycemia with phlorizin normalizes tissue sensitivity to insulin in diabetic rats. J Clin Invest 79:1510–1515.
27. T-1095, an inhibitor of renal Na+-glucose cotransporters, may provide a novel approach to treating diabetes. A Oku, K Ueta, K Arakawa, T Ishihara, M Nawano, Y Kuronuma, M Matsumoto, A Saito, K Tsujihara, M Anai, T Asano, Y Kanai and H Endou Diabetes 1999 Sep and 1794-1800, 48(9):.
28. S14–S19, Isaji M (2011) SGLT2 inhibitors: molecular design and potential differences in effect. Kidney Int 79 (Suppl 120):.
29. Grempler R, Thomas L, Eckhardt M et al (2012) Empagliflozin, a novel selective sodium glucose cotransporter-2 (SGLT-2) inhibitor: characterisation and comparison with other SGLT-2 inhibitors. Diabetes Obes Metab 14:83–90.
30. Lapuerta P, Zambrowicz B, Strumph P, Sands A (2015) Development of sotagliflozin, a dual sodium-dependent glucose transporter 1/2 inhibitor. Diab Vasc Dis Res 12:101–110.
31. Inoue T, Takemura M, Fushimi N et al (2017) Mizagliflozin, a novel selective SGLT1 inhibitor, exhibits potential in the amelioration of chronic constipation. Eur J Pharmacol 806:25–31.
32. Gerstein HC, Miller ME, Byington RP et al (2008) Action to Control Cardiovascular Risk in Diabetes Study Group. Effects of intensive glucose lowering in type 2 diabetes. N Engl J Med 358:2545–2559.
33. The ADVANCE Collaborative Group, Patel A, MacMahon S et al (2008) Intensive blood glucose control and vascular outcomes in patients with type 2 diabetes. N Engl J Med 358:2560–2572.

34. Inzucchi SE, Zinman B, Fitchett D et al (2018) How does empagliflozin reduce cardiovascular mortality? Insights from a mediation analysis of the EMPA-REG OUTCOME trial. Diabetes Care 41:356–363.
35. Neal B, Perkovic V, Mahaffey KW et al (2017) Canagliflozin and cardiovascular and renal events in type 2 diabetes. N Engl J Med 377:644–657.
36. Marso SP, Daniels GH, Brown-Frandsen K et al (2016) Liraglutide and cardiovascular outcomes in type 2 diabetes. N Engl J Med 375:311–322.
37. Bell DS. Heart failure: the frequent, forgotten, and often fatal complication of diabetes. Diabetes Care 2003 and 26:2433–2441pmid:12882875.
38. Lehrke M, Marx N (2017) Diabetes mellitus and heart failure. Am J Cardiol 120:S37–S47.
39. Gregg EW, Li Y, Wang J et al (2014) Changes in diabetes-related complications in the United States. 1990-2010. N Engl J Med 370:1514–1523.
40. Radholm K, Figtree G, Perkovic V et al (2018) Canagliflozin and heart failure in type 2 diabetes mellitus: results from the CANVAS Program (Canagliflozin Cardiovascular Assessment Study). Circulation 137.
41. Hallow KM, Helmlinger G, Greasley PJ, McMurray JJV, Boulton DW (2018) Why do SGLT2 inhibitors reduce heart failure hospitalization? A differential volume regulation hypothesis. Diabetes Obes Metab 20:479–487.
42. Inagaki N, Goda M, Yokota S, Maruyama N, Iijima H. Safety and efficacy of canagliflozin in Japanese patients with type 2 diabetes mellitus: post hoc subgroup analyses according to body mass index in a 52-week open-label study. Expert Opin Pharmacother 2015 and 16:1577–1591pmid:26104600.
43. Can a Shift in Fuel Energetics Explain the Beneficial Cardiorenal Outcomes in the EMPA-REG OUTCOME Study? A Unifying Hypothesis Sunder Mudalia, Sindura Alloju and Robert R. Henry Corresponding author: Sunder Mudaliar, Diabetes Care 2016 Jul and 1115-1122, 39(7):.
44. Pessoa TD, Campos LC, Carraro-Lacroix L, Girardi AC, Malnic G (2014) Functional role of glucose metabolism, osmotic stress, and sodium-glucose cotransporter isoform-mediated transport on Na+/H+ exchanger isoform 3 activity in the renal proximal tubule. J Am Soc Nephrol 25:2028–2039.
45. Wanner C, Inzucchi SE, Lachin JM et al (2016) Empagliflozin and progression of kidney disease in type 2 diabetes. N Engl J Med 375:324–334.
46. Heerspink HJ, Perkins BA, Fitchett DH et al (2016) Sodium glucose cotransporter 2 inhibitors in the treatment of diabetes mellitus: cardiovascular and kidney effects, potential mechanisms, and clinical applications. Circulation 134:752–772.
47. Cherney DZI, Zinman B, Inzucchi SE et al (2017) Effects of empagliflozin on the urinary albumin-to-creatinine ratio in patients with type 2 diabetes and established cardiovascular disease: an exploratory analysis from the EMPA-REG OUTCOME randomised, placebo-controlled trial. Lancet Diabetes Endocrinol 5:610–621.
48. Kluger AY, Tecson KM, Lee AY, Lerma EV, Rangaswami J, Lepor NE, Cobble ME, McCullough PA. Class effects of SGLT2 inhibitors on cardiorenal outcomes. Cardiovasc Diabetol. 2019 Aug 5, 31382965, 18(1):99. doi: 10.1186/s12933-019-0903-4. PMID: and PMC6683461., PMCID:.
49. Kelly MS, Lewis J, Huntsberry AM, Dea L, Portillo I. Efficacy and renal outcomes of SGLT2 inhibitors in patients with type 2 diabetes and chronic kidney disease. Postgrad Med. 2019 Jan;131(1):31-42. doi: 10.1080/00325481.2019.1549459. Epub 2018 Nov 30. PMID: 30449220. [Online] https://pubmed.ncbi.nlm.nih.gov/30449220/.

50. Targher G, Byrne CD, Lonardo A, Zoppini G, Barbui C (2016) Non-alcoholic fatty liver disease and risk of incident cardiovascular disease: a meta-analysis. J Hepatol 65:589–600.
51. Seko Y, Sumida Y, Tanaka S et al (2017) Effect of sodium glucose cotransporter-2 inhibitor on liver function tests in Japanese patients with non-alcoholic fatty liver disease and type 2 diabetes mellitus. Hepatol Res 47:1072–1078.
52. Tobita H, Sato S, Miyake T, Ishihara S, Kinoshita Y (2017) Effects of dapagliflozin on body composition and liver tests in patients with nonalcoholic steatohepatitis associated with type 2 diabetes mellitus: a prospective, open-label uncontrolled study. Curr Ther Res Clin Exp 87:13–19.
53. Benfield T, Jensen JS, Nordestgaard BG. Influence of diabetes and hyperglycaemia on infectious disease hospitalisation and outcome. Diabetologia. 2007 and 50:549–554.
54. Geerlings S, Fonseca V, Castro-Diaz D, et al. Genital and urinary tract infections in diabetes: impact of pharmacologically-induced glucosuria. Diabetes Res Clin Pract. 2014 and 103:373–381.
55. Bode B, Stenlof K, Harris S, et al. Long-term efficacy and safety of canagliflozin over 104 weeks in patients aged 55-80 years with type 2 diabetes. Diabetes Obes Metab. 2015 and 17:294–303.
56. Neal B, Perkovic V, Mahaffey KW, et al. Canagliflozin and cardiovas- cular and renal events in type 2 diabetes. N Engl J Med. 2017.
57. Zinman B, Wanner C, Lachin JM, et al. Empagliflozin, cardiovascular outcomes, and mortality in type 2 diabetes. N Engl J Med. 2015 and 373:2117–2128.
58. Liu J, Li L, Li S. Effects of SGLT2 inhibitors on UTIs and genital infections in type 2 diabetes mellitus: a systematic review and meta-analysis. Sci Rep. 2017 and 7:2824.
59. Zaccardi F, Webb DR, Htike ZZ, et al. Efficacy and safety of sodium- glucose co-transporter-2 inhibitors in type 2 diabetes mellitus: systematic review and network meta-analysis. Diabetes Obes Metab. 2016 and 18:783–794.
60. Vasilakou D, Karagiannis T, Athanasiadou E, et al. Sodium-glucose cotransporter 2 inhibitors for type 2 diabetes: a systematic review and meta-analysis. Ann Intern Med. 2013 and 159:262–274.
61. Kawalec P, Mikrut A, Lopuch S. The safety of dipeptidyl peptidase-4 (DPP-4) inhibitors or sodium-glucose cotransporter 2 (SGLT-2) inhi- bitors added to metformin background therapy in patients with type 2 diabetes mellitus: a systematic review and meta-analysis. Diabetes Metab Res Rev. 2014 and 30:269–283.
62. Li D, Wang T, Shen S, et al. Urinary tract and genital infections in patients with type 2 diabetes treated with sodium-glucose co- transporter 2 inhibitors: a meta-analysis of randomized controlled trials. Diabetes Obes Metab. 2017 and 19:348–355.
63. Yang Y, Chen S, Pan H, et al. Safety and efficiency of SGLT2 inhibitor combining with insulin in subjects with diabetes: systema- tic review and meta-analysis of randomized controlled trials. Medicine (Baltimore). 2017 and 96:e6944.
64. Giovannucci E, Harlan DM, Archer MC, et al. Diabetes and cancer: a consensus report. Diabetes Care. 2010 and doi:10.2337/dc10-0666, 33(7):1674-1685.
65. U.S. Food and Drug Administration. FDA briefing document, NDA 202293 dapagliflozin tablets, 5 and 10 mg. Advisory committee meeting. 2011. http://www.fda.gov/downloads/advisorycommit tees/committeesmeetingmaterials/drugs/endocrinologicandmeta bolicdrugsadvisorycommittee/ucm262994.pdf.

66. U.S. Food and Drug Administration. FDA background document, BMS-512148 NDA 202293 dapagliflozin. Proceedings of the endo- crinologic & metabolic drug advisory committee meeting. 2013.
http://www.fda.gov/downloads/drugs/endocrinologicandmetabo licdrugsadvisorycommittee/ucm378079.pdf.
67. Ptaszynska A, Cohen SM, Messing EM, et al. Assessing bladder cancer risk in type 2 diabetes clinical trials: the dapagliflozin drug develop- ment program as a 'case study'. Diabetes Ther. 2015 and 6:357–375.
68. Reilly TP, Graziano MJ, Janovitz EB, et al. Carcinogenicity risk assessment supports the chronic safety of dapagliflozin, an inhibi- tor of sodium-glucose co-transporter 2, in the treatment of type 2 diabetes mellitus. Diabetes Ther. 2014 and 5:73–96.
69. Lin HW, Tseng CH. A review on the relationship between SGLT2 inhibitors and cancer. Int J Endocrinol. 2014 and 2014:719578.
70. Neal B, Perkovic V, Mahaffey KW, et al. Canagliflozin and cardiovas- cular and renal events in type 2 diabetes. N Engl J Med. 2017.
71. Zinman B, Wanner C, Lachin JM, et al. Empagliflozin, cardiovascular outcomes, and mortality in type 2 diabetes. N Engl J Med. 2015 and 373:2117–2128.
72. Tang H, Dai Q, Shi W, et al. SGLT2 inhibitors and risk of cancer in type 2 diabetes: a systematic review and meta-analysis of rando- mised controlled trials. Diabetologia. 2017.
73. Taylor SI, Blau JE, Rother KI. SGLT2 inhibitors may predispose to ketoacidosis. J Clin Endocrinol Metab. 2015 and 100:2849–2852.
74. Abdul-Ghani MA, Norton L, DeFronzo RA. Renal sodium-glucose cotransporter inhibition in the management of type 2 diabetes mellitus. Am J Physiol Renal Physiol. 2015 and 309:F889–900.
75. Wang Y, Desai M, Ryan PB, et al. Incidence of diabetic ketoacidosis among patients with type 2 diabetes mellitus treated with SGLT2 inhibitors and other antihyperglycemic agents. Diabetes Res Clin Pract. 2017 and 128:83–90.
76. Ferrannini E, Mark M, Mayoux E. CV protection in the EMPA-REG OUTCOME trial: a "thrifty substrate" hypothesis. Diabetes Care. 2016 and 39:1108–1114.
77. Neal B, Perkovic V, Mahaffey KW, et al. Canagliflozin and cardiovas- cular and renal events in type 2 diabetes. N Engl J Med. 2017.
78. Zinman B, Wanner C, Lachin JM, et al. Empagliflozin, cardiovascular outcomes, and mortality in type 2 diabetes. N Engl J Med. 2015 and 373:2117–2128.
79. Kohler S, Zeller C, Iliev H, et al. Safety and tolerability of empagli- flozin in patients with type 2 diabetes: pooled analysis of phase I-III clinical trials. Adv Ther. 2017 and 34:1707–1726.
80. Tang H, Zhang X, Zhang J, et al. Elevated serum magnesium associated with SGLT2 inhibitor use in type 2 diabetes patients: a meta-analysis of randomised controlled trials. Diabetologia. 2016 and 59:2546–2551.
81. Gilbert RE, Mende C, Vijapurkar U, et al. Effects of canagliflozin on serum magnesium in patients with type 2 diabetes mellitus: a post hoc analysis of randomized controlled trials. Diabetes Ther. 2017 and 8:451–458.
82. Takayanagi K, Shimizu T, Tayama Y, et al. Downregulation of tran- sient receptor potential M6 channels as a cause of hypermagne- siuric hypomagnesemia in obese type 2 diabetic rats. Am J Physiol Renal Physiol. 2015 and 308:F1386–97.
83. Bankir L, Bouby N, Blondeau B, et al. Glucagon actions on the kidney revisited: possible role in potassium homeostasis. Am J Physiol Renal Physiol. 2016 and 311:F469–86.

84. Xu LH, Maalouf NM. Effect of acute hyperinsulinemia on magne- sium homeostasis in humans. Diabetes Metab Res Rev. 2017 and e2844., 33:.
85. Zinman B, Wanner C, Lachin JM, et al. Empagliflozin, cardiovascular outcomes, and mortality in type 2 diabetes. N Engl J Med. 2015 and 373:2117–2128.
86. Yavin Y, Mansfield TA, Ptaszynska A, et al. Effect of the SGLT2 inhibitor dapagliflozin on potassium levels in patients with type 2 diabetes mellitus: a pooled analysis. Diabetes Ther. 2016 and 7:125–137.
87. Taylor SI, Blau JE, Rother KI. Possible adverse effects of SGLT2 inhibitors on bone. Lancet Diabetes Endocrinol. 2015 and 3:8–10.
88. Neal B, Perkovic V, Mahaffey KW, et al. Canagliflozin and cardiovas- cular and renal events in type 2 diabetes. N Engl J Med. 2017.
89. Tang HL, Li DD, Zhang JJ, et al. Lack of evidence for a harmful effect of sodium-glucose co-transporter 2 (SGLT2) inhibitors on fracture risk among type 2 diabetes patients: a network and cumu- lative meta-analysis of randomized controlled trials. Diabetes Obes Metab. 2016 and 18:1199–1206.
90. Watts NB, Bilezikian JP, Usiskin K, et al. Effects of canagliflozin on fracture risk in patients with type 2 diabetes mellitus. J Clin Endocrinol Metab. 2016 and 101:157–166.
91. Kohler S, Zeller C, Iliev H, et al. Safety and tolerability of empagli- flozin in patients with type 2 diabetes: pooled analysis of phase I-III clinical trials. Adv Ther. 2017 and 34:1707–1726.
92. Tang HL, Li DD, Zhang JJ, et al. Lack of evidence for a harmful effect of sodium-glucose co-transporter 2 (SGLT2) inhibitors on fracture risk among type 2 diabetes patients: a network and cumu- lative meta-analysis of randomized controlled trials. Diabetes Obes Metab. 2016 and 18:1199–1206.
93. Kohler S, Zeller C, Iliev H, et al. Safety and tolerability of empagli- flozin in patients with type 2 diabetes: pooled analysis of phase I-III clinical trials. Adv Ther. 2017 and 34:1707–1726.
94. Kohler S, Zeller C, Iliev H, et al. Safety and tolerability of empagli- flozin in patients with type 2 diabetes: pooled analysis of phase I-III clinical trials. Adv Ther. 2017; 34:1707–1726..
95. Tang HL, Li DD, Zhang JJ, et al. Lack of evidence for a harmful effect of sodium-glucose co-transporter 2 (SGLT2) inhibitors on fracture risk among type 2 diabetes patients: a network and cumu- lative meta-analysis of randomized controlled trials. Diabetes Obes Metab. 2016 and 18:1199–1206.
96. Ruanpeng D, Ungprasert P, Sangtian J, et al. Sodium-glucose cotran- sporter 2 (SGLT2) inhibitors and fracture risk in patients with type 2 diabetes mellitus: a meta-analysis. Diabetes Metab Res Rev. 2017 and e2903., 33:.
97. Bode B, Stenlof K, Harris S, et al. Long-term efficacy and safety of canagliflozin over 104 weeks in patients aged 55-80 years with type 2 diabetes. Diabetes Obes Metab. 2015 and 17:294–303.
98. Zinman B, Wanner C, Lachin JM, et al. Empagliflozin, cardiovascular outcomes, and mortality in type 2 diabetes. N Engl J Med. 2015 and 373:2117–2128.
99. Liakos A, Karagiannis T, Athanasiadou E, et al. Efficacy and safety of empagliflozin for type 2 diabetes: a systematic review and meta- analysis. Diabetes Obes Metab. 2014 and 16:984–993.
100. Cha SA, Park YM, Yun JS, et al. A comparison of effects of DPP-4 inhibitor and SGLT2 inhibitor on lipid profile in patients with type 2 diabetes. Lipids Health Dis. 2017 and 16:58.

101. Zaccardi F, Webb DR, Htike ZZ, et al. Efficacy and safety of sodium- glucose co-transporter-2 inhibitors in type 2 diabetes mellitus: systematic review and network meta-analysis. Diabetes Obes Metab. 2016 and 18:783–794.
102. Briand F, Mayoux E, Brousseau E, et al. Empagliflozin, via switching metabolism toward lipid utilization, moderately increases LDL cho- lesterol levels through reduced LDL catabolism. Diabetes. 2016 and 65:2032–2038. [Online]
103. Ferrannini E, Muscelli E, Frascerra S, et al. Metabolic response to sodium-glucose cotransporter 2 inhibition in type 2 diabetic patients. J Clin Invest. 2014 and 124:499–508.
104. Wu JH, Foote C, Blomster J, et al. Effects of sodium-glucose cotran- sporter-2 inhibitors on cardiovascular events, death, and major safety outcomes in adults with type 2 diabetes: a systematic review and meta-analysis. Lancet Diabetes Endocrinol. 2016 and 4:411–419.
105. Zaccardi F, Webb DR, Htike ZZ, et al. Efficacy and safety of sodium- glucose co-transporter-2 inhibitors in type 2 diabetes mellitus: systematic review and network meta-analysis. Diabetes Obes Metab. 2016 and 18:783–794.
106. Storgaard H, Gluud LL, Bennett C, et al. Benefits and harms of sodium-glucose co-transporter 2 inhibitors in patients with type 2 diabetes: a systematic review and meta-analysis. PLoS One. 2016 and 11:e0166125.
107. Yabe D, Nishikino R, Kaneko M, et al. Short-term impacts of sodium/glucose co-transporter 2 inhibitors in Japanese clinical practice: considerations for their appropriate use to avoid serious adverse events. Expert Opin Drug Saf. 2015 and 14:795–800.
108. Sano M, Takei M, Shiraishi Y, et al. Increased hematocrit during sodium-glucose cotransporter 2 inhibitor therapy indicates recov- ery of tubulointerstitial function in diabetic kidneys. J Clin Med Res. 2016 and 8:844–847.
109. Zinman B, Wanner C, Lachin JM, et al. Empagliflozin, cardiovascular outcomes, and mortality in type 2 diabetes. N Engl J Med. 2015 and 373:2117–2128.
110. Zinman B, Inzucchi SE, Lachin JM, et al. Empagliflozin and cerebro- vascular events in patients with type 2 diabetes mellitus at high cardiovascular risk. Stroke. 2017 and 48:1218–1225.
111. Wu JH, Foote C, Blomster J, et al. Effects of sodium-glucose cotran- sporter-2 inhibitors on cardiovascular events, death, and major safety outcomes in adults with type 2 diabetes: a systematic review and meta-analysis. Lancet Diabetes Endocrinol. 2016 and 4:411–419.
112. Sonesson C, Johansson PA, Johnsson E, et al. Cardiovascular effects of dapagliflozin in patients with type 2 diabetes and different risk categories: a meta-analysis. Cardiovasc Diabetol. 2016.