Metabolic and endocrine effects of SGLT2 inhibition

PER LUNDKVIST
Dissertation presented at Uppsala University to be publicly examined in Enghoff lecture-hall, Entrance 50, Uppsala academic hospital, Uppsala, Friday, 8 March 2019 at 13:15 for the degree of Doctor of Philosophy (Faculty of Medicine). The examination will be conducted in English. Faculty examiner: Docent Michael Alvarsson (Department of Molecular Medicine and Surgery (MMK), K1, Karolinska institute, Stockholm, Sweden).

Abstract


Obesity and type 2 diabetes (T2D) are two growing global health problems with similar comorbidity profiles. SGLT2 inhibitors (SGLT2i) improve blood glucose control and can relieve both T2D and obesity, as well as their associated health problems such as hypertension, kidney failure, and cardiovascular disease.

In paper I, 50 obese patients without diabetes were treated for 24 weeks with SGLT2i dapagliflozin + GLP-1 receptor agonist (GLP-1RA) exenatide or placebo. They were examined regarding body weight loss and body composition. The placebo-adjusted weight loss was 4.13 kg, mostly attributable to adipose tissue loss.

In paper II, 43 completers of the study in paper I entered a 28-week extension phase in which all participants received active treatment. We found major reductions in body weight, adipose tissue volume, blood pressure and prediabetes that were sustained at 52 weeks.

In paper III, 84 patients with T2D and non-alcoholic fatty liver disease underwent a 12-week treatment with dapagliflozin, omega-3 (n-3) carboxylic acids (OM-3CA), the combination of both or placebo to assess effects on liver fat content. MRI showed significant reductions of liver fat versus baseline and, for the combination, versus placebo.

In paper IV: 15 metformin-treated patients with T2D were assessed for changes in plasma glucagon levels following a single dose of dapagliflozin during experiments with stable versus falling plasma glucose. Changes in glucagon levels could largely be explained by changes in glucose levels.

In conclusion, SGLT2 inhibition can lower body weight and cardiovascular risk factors in obese patients without diabetes when combined with GLP-1RA, and it can reduce liver fat in T2D patients, in particular when given together with OM-3CA. SGLT2i effects on glucagon secretion can largely be explained by lower glucose levels rather than direct α-cell effects.

Keywords: SGLT2 inhibition, GLP-1 receptor agonism, DPP4 inhibition, NAFLD, prediabetes, type 2 diabetes, obesity, metabolic syndrome, glucagon.

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Dedicated to Hannah and Ingrid
"Life can only be understood backwards, but it must be lived forwards."

/Søren Kierkegaard
List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.


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# Contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Introduction</td>
<td>11</td>
</tr>
<tr>
<td>Obesity, type 2 diabetes and non-alcoholic fatty liver disease - definitions and prevalence</td>
<td>12</td>
</tr>
<tr>
<td>Obesity</td>
<td>12</td>
</tr>
<tr>
<td>Type 2 diabetes</td>
<td>12</td>
</tr>
<tr>
<td>Non-alcoholic fatty liver disease</td>
<td>13</td>
</tr>
<tr>
<td>Pathobiology</td>
<td>14</td>
</tr>
<tr>
<td>Obesity</td>
<td>14</td>
</tr>
<tr>
<td>Type 2 diabetes</td>
<td>15</td>
</tr>
<tr>
<td>Non-alcoholic fatty liver disease</td>
<td>15</td>
</tr>
<tr>
<td>Impact on morbidity and mortality</td>
<td>17</td>
</tr>
<tr>
<td>Obesity</td>
<td>17</td>
</tr>
<tr>
<td>Type 2 diabetes</td>
<td>17</td>
</tr>
<tr>
<td>Non-alcoholic fatty liver disease</td>
<td>18</td>
</tr>
<tr>
<td>Prevention and therapy</td>
<td>19</td>
</tr>
<tr>
<td>Lifestyle</td>
<td>19</td>
</tr>
<tr>
<td>Bariatric surgery</td>
<td>19</td>
</tr>
<tr>
<td>Obesity medication</td>
<td>20</td>
</tr>
<tr>
<td>Antidiabetic medication</td>
<td>21</td>
</tr>
<tr>
<td>Treatments options for non-alcoholic fatty liver disease</td>
<td>22</td>
</tr>
<tr>
<td>SGLT2 inhibitors</td>
<td>23</td>
</tr>
<tr>
<td>Incretin-based therapies</td>
<td>26</td>
</tr>
<tr>
<td>Omega-3 carboxylic acids</td>
<td>28</td>
</tr>
<tr>
<td>Overview of investigated treatment effects</td>
<td>29</td>
</tr>
<tr>
<td>Study aims and hypotheses</td>
<td>30</td>
</tr>
<tr>
<td>Subjects and study design</td>
<td>31</td>
</tr>
<tr>
<td>Paper I</td>
<td>31</td>
</tr>
<tr>
<td>Paper II</td>
<td>32</td>
</tr>
<tr>
<td>Paper III</td>
<td>32</td>
</tr>
</tbody>
</table>
Abbreviations

α-MSH  Alpha melanocyte stimulating hormone
AASLD  American association for the study of liver disease
AE    Adverse event
aGLP-1 active GLP-1
AgRP  Agouti-related peptide
ALT   Alanine transaminase
AST   Aspartate transaminase
AUC   Area under curve
BMI   Body mass index
CART  Cocaine amphetamine regulated transcript
CI    Confidence interval
CRP   C-reactive protein
CV (D) Cardiovascular (disease)
CVOT  Cardiovascular outcome trial
DAPA  Dapagliflozin
DHA   Docosahexaenoic acid
DPP4 (i) Dipeptidyl peptidase 4 (inhibitor)
eGFR  Estimated glomerular filtration rate
ELISA Enzyme-linked immunosorbent assay
EPA   Eicosapentaenoic acid
EXE   Exenatide
FGF21 Fibroblast growth factor 21
GBP   Gastric bypass
GCP   Good clinical practise
GLP-1 (RA) Glucagon-like peptide 1 receptor agonist
GMR   Geometric mean ratio
HbA1c Glycosylated haemoglobin
HDL   High density lipoproteins
IBT   Incretin-based therapies
IFG   Impaired fasting glucose
IGT   Impaired glucose tolerance
IL-6  Interleukin 6
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDL</td>
<td>Low density lipoproteins</td>
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<tr>
<td>LSM</td>
<td>Least square means</td>
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<tr>
<td>MA</td>
<td>Monoamine</td>
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<td>MACE</td>
<td>Major cardiovascular event</td>
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<td>MetS</td>
<td>Metabolic syndrome</td>
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<tr>
<td>MMRM</td>
<td>Mixed model of repeated measurements</td>
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<tr>
<td>MRI</td>
<td>Magnetic resonance imaging</td>
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<tr>
<td>NAFLD</td>
<td>Non alcoholic fatty liver disease</td>
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<tr>
<td>NASH</td>
<td>Non alcoholic steatohepatitis</td>
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<tr>
<td>NPY</td>
<td>Neuropeptide Y</td>
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<tr>
<td>n-3CA</td>
<td>see OM-3CA</td>
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<tr>
<td>OD</td>
<td>Once daily</td>
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<tr>
<td>OGTT</td>
<td>Oral glucose tolerance test</td>
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<td>OH</td>
<td>Hydroxyl group</td>
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<td>OM-3CA</td>
<td>Omega-3 carboxylic acids</td>
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<tr>
<td>PBO</td>
<td>Placebo</td>
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<tr>
<td>PDFF</td>
<td>Proton density fat fraction</td>
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<tr>
<td>PG</td>
<td>Plasma glucose</td>
</tr>
<tr>
<td>PNPLA3</td>
<td>Patatin-like phospholipase domain-containing protein 3</td>
</tr>
<tr>
<td>POMC</td>
<td>Proopiomelanocortin</td>
</tr>
<tr>
<td>PYY</td>
<td>Polypeptide Y</td>
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<tr>
<td>QW</td>
<td>Once a week (quantum vis)</td>
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<tr>
<td>SAXA</td>
<td>Saxagliptin</td>
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<tr>
<td>SCD-1</td>
<td>Stearoyl-CoA desaturase 1</td>
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<tr>
<td>SD</td>
<td>Standard deviation</td>
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<tr>
<td>SEM</td>
<td>Standard error of the mean</td>
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<tr>
<td>SGLT</td>
<td>Sodium glucose cotransporter</td>
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<tr>
<td>SGLT2 (i)</td>
<td>Sodium glucose cotransporter 2 (inhibitor)</td>
</tr>
<tr>
<td>SubQ</td>
<td>Subcutaneous</td>
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<td>T2D</td>
<td>Type 2 diabetes mellitus</td>
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<td>Tmax</td>
<td>Time to maximum concentration</td>
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<tr>
<td>TNF-α</td>
<td>Tumor necrosis factor-alpha</td>
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<td>TG</td>
<td>Triglycerides</td>
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<tr>
<td>Visc</td>
<td>Visceral</td>
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<tr>
<td>γ-GT</td>
<td>Gamma-glutamyl transferase</td>
</tr>
</tbody>
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Introduction

Adipose tissue or fat constitutes most of man's stored energy reserves and have no doubt been life-saving at times of famine or disease throughout history. However, following the agricultural revolution and subsequent industrialization, food supply has risen and labor-intensive work diminished. As this trend has seemingly continued, today's easy calorie access and sedentary lifestyle have skewed energy balance, arguably transforming the once life-saving fat reserve into one of today's most significant threats to public health.
Obesity, type 2 diabetes and non-alcoholic fatty liver disease - definitions and prevalence

**Obesity**

Overweight and obesity are defined as a body mass index (kg/m\(^2\); BMI) ≥ 25 and ≥ 30 respectively. According to world health organization, more than 1.9 billion of the global adult population were overweight, and 650 million were obese in 2016.\(^1\) Corresponding percentages in Sweden was 36% overweight and 15% obesity, a prevalence rising with age.\(^2\)

BMI is used as a simple and yet powerful measure of over- or underweight\(^3,4\), but it can be misleading as it does not take body composition and fat distribution into account\(^5\). Body fat amount is in itself linked to the risk of metabolic complications of obesity\(^5\). Moreover, intraabdominal or visceral adipose tissue has proven more harmful than subcutaneous fat depots\(^6,7,8,9\). Body fat amount and distribution can be estimated with bioimpedance scales or more precisely with dedicated ultrasound/magnetic resonance imaging (MRI) scans. The simple biometrics waist circumference and waist-to-hip ratio are also shown to correlate highly with radiological findings of visceral adiposity\(^10\).

**Type 2 diabetes**

Type 2 diabetes mellitus (T2D) is a dysregulated metabolic state characterized by hyperglycemia due to insulin resistance together with a relative insulin deficiency\(^11\). High glucagon secretion\(^12\), increased renal glucose reabsorption\(^13\) and impaired incretin signaling\(^14,15\) have also been shown to contribute to the development of T2D.

Prediabetes often precedes T2D and is characterized by impaired fasting glucose (IFG; 5.6-6.9 mmol/l), impaired glucose tolerance at 120 min post glucose challenge (IGT; 75 grams glucose, 7.8-11.0 mmol/l) or raised glycosylated hemoglobin (HbA1c; 39-47 mmol/mol)\(^16\). Impaired fasting glucose is linked with peripheral insulin resistance (skeletal muscle and adipose tissue), while IGT is thought to reflect hepatic overproduction of glucose\(^17\). The reactive beta-cell hypertrophy and hyperinsulinemia transiently stave off T2D, but without lifestyle or medical intervention, a subsequent beta cell failure and hyperglycemia generally follows. Type 2 diabetes is diagnosed when one or more of the following criteria are met; fasting
plasma glucose (PG) ≥ 7 mmol/l, random or 2-hour OGTT PG ≥ 11.1 mmol/l, or HbA1c ≥ 48 mmol/mol. The global prevalence of T2D in 2017 was estimated to be 451 million and may reach 693 million by year 2045. In Sweden, 3-5% of the population have a T2D diagnosis and prevalence rises with age to around 9% ≥ 65 years.

**Non-alcoholic fatty liver disease**

Around 75% of people with T2D have pathological amounts of ectopic fat depositions in the liver. Liver fat is considered a disease when it exceeds 5.5% and is then called non-alcoholic fatty liver disease (NAFLD). Around 25-30% of NAFLD patients develop a more severe inflammatory liver condition called non-alcoholic steatohepatitis (NASH), accounting for 5-12% in the entire population.
Obesity

The etiology of overweight and obesity is complex and often a mix of genetic predisposition\textsuperscript{24} and the environment. Factors like high/low birthweight\textsuperscript{25,26}, neuroendocrine disorders\textsuperscript{27}, pharmaceuticals\textsuperscript{28}, activity levels\textsuperscript{29}, psychosocial\textsuperscript{30}, and socioeconomic status\textsuperscript{31} all contribute. Although reasons vary, a positive energy balance is the common denominator. Feeding behavior and energy metabolism play integral parts here and are both largely regulated via the arcuate nucleus located in the hypothalamus\textsuperscript{32}. Furthermore, the amygdala and nucleus accumbens have been associated with satiety and food reward respectively\textsuperscript{33,34}.

Genetic predisposition to obesity is important, with twin and adoptee studies indicating a heritability of 55-85 percent\textsuperscript{24}. However, the genetics are complex, heterogeneous and only explains small BMI differences overall\textsuperscript{35}. Monogenic mutations can cause morbid obesity, affecting central appetite regulation in the hypothalamus or amygdala\textsuperscript{36} (e.g., leptin signaling pathway\textsuperscript{37}). A similar loss of function due to traumatic injury, tumor or radiotherapy may also induce overeating and obesity\textsuperscript{38}.

In health, many of the arcuate nucleus’ neuroendocrine neurons contribute to balancing the energy homeostasis and are themselves governed by systemic circulation of metabolic peptide hormones. Anorexigenic peptides induce satiety and include glucagon-like peptide 1 (GLP-1)\textsuperscript{39}, leptin\textsuperscript{40}, insulin, and peptide YY (PYY)\textsuperscript{41}. They promote satiety by stimulating α-melanocyte stimulating hormone (α-MSH) release via proopiomelanocortin/cocaine-amphetamine-regulated transcript (POMC/CART) and through inhibition of neuropeptide Y/agouti-related peptide (NPY/AgRP) neurons. Ghrelin, on the other hand, is an orexigenic peptide that inhibits the POMC/CART and stimulates NPY/AgRP pathways respectively.\textsuperscript{42} Similarly, glucocorticoids stimulate appetite centrally but also induce peripheral insulin resistance and contribute towards visceral adiposity.\textsuperscript{43} While insulin has a net anabolic effect in practice and leptin supplementation only works in the genetically leptin-deficient, GLP-1 receptor agonists (GLP-1RA) has shown promise in obesity treatment clinically\textsuperscript{44,45}. 
Type 2 diabetes

The development of type 2 diabetes is dependent on genetic and environmental factors. Low birthweight, ethnicity, inactivity, disease and medications can all increase T2D risk. However, two of the most prominent T2D risk factors are obesity and large adipose tissue mass. The body fat distribution also matters, with visceral fat correlating stronger with insulin resistance and cardiovascular (CV) disease than gluteal and femoral adiposity. Furthermore, ectopic fat – lipid accumulation in non-adipose tissue – in skeletal muscle, heart, and liver seem to have a proinflammatory role and can decrease organ function and glucose disposal. Interestingly, inflammation markers C-reactive protein (CRP), interleukin 6 (IL-6) and tumor necrosis factor-α (TNF-α) correlate to the prevalence of diabetes and prediabetes.

Non-alcoholic fatty liver disease

Non-alcoholic fatty liver disease is diagnosed when ectopic liver fat deposition exceeds 5.5 %. Around 25-30% of NAFLD patients also develop NASH, a more severe condition where liver lobes become inflamed and liver cells start to balloon.
This inflammation causes subsequent liver cell damage and increased hepatocyte injury markers in circulation.

Why liver inflammation develops in some patients, but not others is incompletely known. There is however evidence that specific genes play a role in NASH susceptibility, most notable of which is single nucleotide polymorphism in I148M in gene \textit{PNPLA3}\textsuperscript{53}. Moreover, the inflammatory component seems linked to the inability of liver cells to accommodate the increased flux of fatty acids causing toxic lipid intermediates like diacylglycerol and ceramides. Mechanisms of toxicity remain largely unknown but may include endoplasmic reticulum stress and oxidative stress due to mitochondrial overload and subsequent dysfunction.\textsuperscript{54}
Impact on morbidity and mortality

Obesity

Overweight and obesity negatively affect several CV and metabolic risk factors including hypertension, coronary heart disease, stroke, sleep apnea, osteoarthritis, hyperlipidemia, insulin-resistance, NAFLD and T2D. It further increases the cancer risk of the breast, colon, prostate, endometrium, kidney, and gallbladder.\(^1\)

Although not accounting for body composition, BMI in itself is a strong predictor of all-cause mortality, both above and below an optimal ratio of 22.5-25 kg/m\(^2\). This forms a J-shaped curve showing a substantial increase in excess mortality for each 5 kg/m\(^2\) increment of BMI. Median survival is decreased by 2-4 years at BMI 30-35 kg/m\(^2\), and 8-10 years at BMI 40-45 kg/m\(^2\). When broken down into categories, this excess mortality is driven mainly by vascular disease.\(^4\) The so-called metabolically healthy obese – pertaining to unaffected blood pressure, blood lipids and glucose metabolism – also display increased CV risk and all-cause mortality over ten years.\(^55\) Hence, large fat mass per se seemingly increases the risk of mortality and cardiovascular disease (CVD) beyond these surrogate metabolic markers.

Type 2 diabetes

While lifestyle interventions can slow the progression of prediabetes into T2D\(^56\), further dysregulation is far more common. Type 2 diabetes has implications for many organ systems as it is a prominent risk factor for micro- and macrovascular disease, often affecting heart, liver, nervous system, arteries, kidney, and retina.\(^57\) While CV-risk factor reduction has intensified in recent years, benefitting people with and without diabetes alike, the relative risk to die from CVD remains two- to threefold higher in the diabetes cohort.\(^58\) More than 80 percent of T2D patients (in the U.S.) are also overweight or obese\(^59\), many of them with central or visceral adiposity. Such dysregulated metabolic states often occur together with hypertension and deranged blood lipids clustering into the so-called Metabolic Syndrome (MetS).\(^60\)
Non-alcoholic fatty liver disease

The link between NAFLD and metabolic disease with visceral adipose tissue, obesity and hepatic insulin resistance is strong, estimated to affect a majority of T2D-patients\textsuperscript{21,22}. Patients with NAFLD have an increased risk of dying from liver disease, malignancy and CVD and around one quarter of them develop NASH, raising this risk even further\textsuperscript{23}. Type 2 diabetes is associated with such disease progression, as well as an increased risk of further developing liver fibrosis and hepatocellular carcinoma\textsuperscript{23,61}. NASH has also been linked with higher risk of chronic kidney disease and CV events\textsuperscript{62}. 
Prevention and therapy

Except for lipase inhibitors and traditional hypoglycaemic agents respectively, treatment options for obesity and T2D (in Sweden) largely overlap. Lifestyle modification and bariatric surgery target both obesity and T2D and antihypertensive and lipid-lowering drugs tackle the CV risk factors they often share.\textsuperscript{60} Interestingly, the two new drug classes incretin-based therapies (IBT) and SGLT2 inhibitors (SGLT2i) have now been shown to similarly reduce body weight, CVD incidence, plasma glucose, and systolic blood pressure, but via different mechanisms of action.\textsuperscript{63,64,65,66,67}

**Lifestyle**

Lifestyle recommendations include a varied, healthy diet (e.g., mediterranean) and regular exercise most days to decrease body weight. These interventions can improve many risk factors of T2D including body weight and composition, glycemic control, lipid profile and peripheral insulin sensitivity. Lifestyle changes also lower the risk for commonly associated comorbidities such as cancer and CVD. Long term study results cannot distinguish between various hypocaloric diet fads regarding weight loss.\textsuperscript{68}

The combined diet and exercise interventions can improve cardiometabolic risk factors, increase insulin sensitivity and prevent progression into type 2 diabetes as compared to controls, especially over 60 years of age.\textsuperscript{69,70,71} However, exercise alone has little effect on body weight loss over time, probably as feeding increase in a compensatory manner.\textsuperscript{72} A negative energy balance is also accompanied by a decrease in energy expenditure while appetite for energy-dense foods increases, seemingly protecting against weight loss.\textsuperscript{73} This is perhaps a critical explanatory factor to why long term weight loss by lifestyle modification alone has proven so difficult to maintain.

**Bariatric surgery**

Bariatric surgery has become a prevalent method to reduce body weight, with surgical procedures varying with body weight (at least BMI $\geq 35$ kg/m$^2$) and patient preference. The so-called Roux-en-Y gastric bypass (GBP) is, however, the most common variant in Sweden today. In GBP, a new and shorter alimentary canal is
formed by a small stomach pouch that is attached directly to the jejunum and thereby bypassing the remaining ventricle and duodenum. The pouch mechanically only accommodates 15-30 ml at a time, forcing the patient to take small meals several times a day. The bypass itself reduces energy uptake from the intestine, lowering body weight but often also causing a deficient uptake of vitamin B12, iron, protein and folate. This malnutrition leads to the need for lifelong high protein diet and vitamin supplementation.74

Following surgery, a weight loss of 0.5-1 kg per week is to be expected. Most of the weight reduction occurs during the first 6-12 months, with a plateau often reached after two years. Twelve years after surgery, average weight loss was 35 kg and T2D remission 51 % in those with the disease at baseline75. Complications of GBP include leakage or strictures of the GBP anastomosis, internal herniation, dumping, and gallstones.

Obesity medication

The majority of obesity drugs to date suppress appetite through upregulation of monoamine turnover (MA; serotonin, noradrenaline, dopamine) in the CNS. These compounds induce satiety by either MA release, reuptake inhibition or in some cases via MA receptor agonism. Treatment efficacy after one year versus placebo range from approximately 2.5 – 9 kg weight loss but is frequently accompanied by high attrition rates and partial weight rebound in the second year. Side effects are often GI or nervous system related, and percent responders (~30-40 %) are generally comparable between substances.76,77

Bupropion/naltrexone (Contrave, Mysimba) promote satiety via noradrenaline- dopamine reuptake inhibition while opioid receptor antagonist naltrexone increases its POMC/CART signal frequency. The result is a 1-year weight loss of ~5 kg versus placebo, even so, systolic blood pressure and pulse may increase. Common adverse events include nausea, vomiting, constipation, dry mouth, headache, dizziness, insomnia and anxiety.76,77

Sibutramine (Reductil) is a serotonin and noradrenaline reuptake inhibitor that was withdrawn from the market in 2010 following the CVOT study showing an increased risk of major adverse cardiovascular events (3-point MACE; including nonfatal stroke, nonfatal myocardial infarction, and CV death).76,77,78

Phentermine/topiramate (Qsymia) combines the noradrenaline release in central neurons by phentermine with anticonvulsant topiramate's weight reducing side effects. One years’ treatment yields a weight loss of 8.8 kg versus placebo as well as moderately improved glycemia, TGs, and systolic blood pressure. Common side effects include GI disturbance, cognitive and nervous system symptoms.76,77
Lorcaserin (Belviq) is a selective HT52c agonist inducing satiety through the POMC/CART pathway. It lowers body weight by 3.2 kg versus placebo after one year but is associated with a rebound in body weight the second year despite ongoing treatment. Commonly reported adverse effects are mainly attributed to the GI tract and nervous system. The safety trial CAMELLIA recently showed improved CV risk factors without significantly reducing MACE.76,77,79

Rimonabant (Acomplia, Zimulti) is an inverse agonist of cannabinoid receptor 1 (CB-1) inhibiting feeding signals in the hypothalamus. It lowered body weight 4.7 kg versus placebo with one-year treatment but was withdrawn in 2008 due to severe psychiatric side effects including anxiety, depression, and suicidal behavior.80

Orlistat (Xenical, Alli) is a pancreatic and gastric lipase inhibitor that reduces degradation and uptake of fat in the intestine. It lowers body weight moderately (~2.6 kg), can reduce prediabetes and plasma lipids. However, discontinuation of treatment is common due to unfavorable side effects such as soiling, fecal urgency, and incontinence as well as loss of efficacy over time.76,81

**Antidiabetic medication**

Before the advent of IBTs and SGLT2is, antidiabetics consisted mainly of insulin, glucose-independent insulin secretagogues, and insulin sensitizers. As such, the price of glucose lowering has often been increased body weight as well as a higher risk of hypoglycemia.

Insulin effectively decreases hyperglycemia while simultaneously increasing body weight and hypoglycemia frequency.82 Although newer insulin analogs (e.g., detemir, glargine) have flatter profiles and (possibly explaining) fewer hypoglycaemias as well as less weight gain, exogenous insulins have yet to show a significant decrease in CV disease and mortality83.

Sulfonylurea (SU; Mindiab) is an insulin secretagogue, inducing insulin secretion by closing KATP channels in pancreatic β cells. The depolarization causes glucose-independent insulin release, in turn increasing body weight and the risk of hypoglycemia.84 No dedicated outcome study has shown CV benefits of SU treatment.

Metformin (Glucophage) is a biguanide that lowers hepatic glucose production and peripheral insulin resistance. It is the first-line medication for T2D, is body weight neutral, unsuitable with renal or hepatic impairment and has GI side effects. Metformin was the first antidiabetic agent to reduce CV complications as seen with the pivotal UKPDS study85. However, the evidence is relatively weak and only valid for overweight patients and has since then been called into question86.
Thiazolidinediones (TZD; Actos, Avandia) alter gene transcriptions through PPARγ activation and thereby sensitize adipose tissue to insulin. TZDs increase adipogenesis and body weight but may also redistribute fat from visceral to subcutaneous depots. Common side effects include water retention and reduced bone mineral density while reported CV effects are contradictory.

**Treatments options for non-alcoholic fatty liver disease**

Currently, weight loss and exercise is the recommended treatment regime of NAFLD; there are no drugs approved with this indication. However, according to the American Association for the study of liver disease (AASLD) guidelines 2018, there are a couple of compounds that may be considered in biopsy-proven NASH. One such drug is TZD pioglitazone (Actos) that can reduce liver fat and improve liver histology but is associated with certain cancers, bone fractures, weight gain, and fluid retention. Another one is vitamin E, which may be considered in patients without diabetes or cirrhosis. There are however several concerns about the long-term safety of vitamin E, including the risk of hemorrhagic stroke, overall mortality, and prostate cancer. Obeticholic acid (OCA), a type of synthetic bile acid that activates the farnesoid X receptor, have also proven histologically visible NASH benefits versus placebo. OCA is currently not recommended for NASH and is associated with pruritus and increased LDL.

Other agents in the treatment pipeline include omega-3 carboxylic acids (OM-3CA) that may be considered in NAFLD with hypertriglyceridemia. Newer antidiabetic treatment modalities have also shown promise regarding NAFLD treatment. Although uncertain if and how GLP-1RA have any direct effects in the liver, several agents have shown positive effects on NASH. For instance, both exenatide and liraglutide have shown reduced liver fat, hepatocyte injury markers, and improved fibrosis or fibrosis score. SGLT2i has shown promising results on liver health in obese rodents as well as lowering of liver fat, hepatocyte injury markers and fibrosis score humans. However, the overall knowledge of SGLT2is effect on NAFLD and NASH remain limited.
SGLT2 inhibitors

The kidneys contribute to glucose homeostasis via gluconeogenesis (15-55 g/day) and glucose reabsorption through symports in the proximal convoluted tubule (180 g/day). Sodium glucose cotransporters 1 (SGLT1 high-affinity low capacity) and 2 (SGLT2 low-affinity high capacity) utilize the electrochemical sodium gradient to transport glucose against concentration gradients. SGLT2 is located primarily in segment S1 followed by SGLT1 in S3 of the proximal renal tubule. Ninety percent of filtered glucose is absorbed via SGLT2 with residuals escaping urinary excretion via SGLT1. Healthy kidneys reabsorb glucose linearly up to a concentration threshold around 11 mmol/l and after that succumb to glucosuria. However, SGLT2 gene SLC5A2 is overexpressed in chronic hyperglycaemia (e.g., prediabetes, T2D), raising the reabsorptive threshold and furthering hyperglycemia as well as promoting renal hyperfiltration. Conversely, there are rare hereditary mutations of SLC5A2 (e.g., familial renal glucosuria) that cause varying loss of the reabsorptive function of SGLT2. The mutations result in glucosuria, polyuria and sometimes urogenital infections but seldom hypoglycaemia.

In the 19th century, similar hypoglycemic effects as with SLC5A2-mutations were achieved with administration of apple tree bark isolate phlorizin, an unspecific SGLT inhibitor. Its use as a therapeutic glucoside was however eventually abandoned due to GI-related side effects, largely owing to the high density of SGLT1 in the small intestine. More recently, SGLT interest was rekindled, and first selective SGLT2 inhibitor dapagliflozin was produced in 2008.

The first in class highly selective SGLT2 inhibitor (SGLT2i), dapagliflozin (Forxiga 10 mg) was introduced to the market in 2012 for the T2D indication and was soon followed by empagliflozin (Jardiance 25 mg) and canagliflozin (Invocana 100/300 mg). A fourth compound, ertugliflozin (Steglatro 5/15 mg) recently came to market in the US and EU. The four US/EU approved substances induce up to ~80 grams of glucosuria and a mild osmotic diuresis/natriuresis. Results show improved glycemia and insulin sensitivity, reduced systolic/diastolic blood pressure 3-5/2 mmHg as well as slowed progression of kidney disease. Furthermore, a consistent and maintained body weight loss around 2-3 kg may be mostly due to decreased fat mass. Actual weight loss is however substantially smaller than the approximate -11 kg expected reduction with unchanged caloric intake.
Sodium glucose cotransporter 2 (SGLT2) reabsorbs 90% of filtered glucose. Inhibition of SGLT2 blocks glucose reabsorption in renal proximal tubule. This inhibition results in urinary glucose excretion ~60-80 grams/day which in turn lowers plasma glucose, blood pressure and body weight. Na+, sodium.

SGLT2i-induced glucosuria lower HbA1c 6-13 mmol/mol compared with placebo, can raise LDL moderately and increases the frequency of genital mycotic infections. The osmotic diuresis increases blood viscosity and can exacerbate volume depletion, raising the risk of serious adverse events such as venous thromboembolism as well as hypotension and acute kidney damage respectively. Other serious adverse events reported include euglycemic diabetic ketoacidosis (euDKA), lower limb amputations, bone fractures, and acute pancreatitis. In particular euDKAs have been debated following findings that SGLT2i can increase glucagon and lipid oxidation and can have a direct effect on pancreatic α-cell glucagon release.

Two cardiovascular outcome studies (CVOT) with SGLT2i have shown reduced MACE to date. These studies are EMPA-REG (empagliflozin) and CANVAS (canagliflozin), where the former also lowered CV death. Dapagliflozin CVOT (DECLARE-TIMI 58) displayed significantly lowered hospitalization for heart failure but not reduced MACE or CV-death. Furthermore, the CVD-REAL observational study indicates that the SGLT2i class decreased risk for premature death.
Mechanisms that are responsible for the apparently fast working CV and kidney-sparing properties of SGLT2i are incompletely known. A prevailing hypothesis focus on fluid volume changes, causing interstitial congestion relief through osmotic diuresis and a lower blood pressure\textsuperscript{121}. Natriuresis is thought to contribute through increased tubuloglomerular feedback thereby alleviating hyperfiltration and glomerular pressure\textsuperscript{122}. A shift towards cardiac substrate utilization towards energy efficient ketone bodies and an organ-sparing inhibition of $\text{Na}^+/$H$^+$ exchanger 1 and 3 of the heart and kidney respectively are two other current theories\textsuperscript{117,123,124}. 
Incretin-based therapies

Incretins are a small group of metabolic hormones with hypoglycemic properties and include GLP-1 and glucose-dependent insulinotropic peptide (GIP). GLP-1 and GIP mainly originate from enteroendocrine L and K cells respectively and are secreted from the intestinal mucosa within minutes of feeding. These peptides act by delaying GI passage, stimulating glucose-dependent β-cell insulin release, downregulating gluconeogenesis by lowering glucagon as well as suppressing appetite. \(^{125}\) GLP-1 is lower in T2D and obesity, and dipeptidyl peptidase-4 (DPP4) upregulated in T2D according to some studies \(^{126,127,128}\).

Native GLP-1 is a 30 amino acid neuropeptide cleaved from proglucagon, have a half-life of 1 - 3 minutes and is broken down primarily by DPP-4 before it is excreted via the kidneys. \(^{125}\) GLP-1 receptors (GLP-1R) have been found in the pancreas, GI-tract, kidneys, brain \(^{129}\), peripheral nervous system. GLP-1 also seem to have direct cardiovascular effects \(^{130,131,132}\).

Incretin-based therapies to date include injectable GLP-1 receptor agonists (GLP-1RAs) and DPP4 inhibitors (DPP4i) taken orally, although an oral preparation of GLP-1RA semaglutide is currently in phase III \(^{133}\). GLP-1RA mimics endogenous GLP-1 while DPP4i prevents the enzymatic degradation of endogenous GLP-1 and GIP, thereby increasing their concentrations post-prandially \(^{134}\).

Marketed GLP-1RAs are most often human GLP-1 molecules with altered amino acid sequence and added protein or lipid moieties for increased stability and half-life (90-98 % homology). Synthetic GLP-1 mimetics exenatide and lixisenatide are based on exendin 4, a peptide in the Gila monster saliva (50-53 % homology) and have similar GLP-1R-agonistic properties to human GLP-1 \(^{135,136,137}\).

GLP-1RA and DPP4i similarly promote glycaemic control by stimulating insulin release, inhibiting glucagon secretion and lowering glucose levels in the post-prandial state \(^{138,139}\). Generally, GLP-1RAs are more potent than DPP4is but also have more side effects \(^{140}\), most notably increased GI discomfort and nausea \(^{141}\).

GLP-1RAs further reduce systolic blood pressure by ~2-3 mmHg, increase heart rate by 2-3 bpm \(^{130}\), lower kidney disease \(^65\), TGs and LDL moderately and reduce body weight by 2-4 kg \(^{132,142}\). Also, studies with increased GLP-1RA dosage and treatment time have shown further weight loss potential. Liraglutide 3.0 (Saxenda)
reduced body weight by 4.3% after three years of treatment after placebo-adjustment as well as improved glycemia and lipid profile\textsuperscript{63}. Semaglutide 1 mg (Ozempic) weekly preparation show similar weight loss properties\textsuperscript{45}. Although knowledge of central GLP-1 signaling is incomplete, weight loss effects have been linked with inhibition of NPY/AgRP\textsuperscript{39}. The combined effect of central appetite suppression and gastric emptying is likely to explain the weight loss with GLP-1RA treatment.

In addition, GLP-1RA CVOTs have now demonstrated a reduced incidence of MACE when added to standard of care in patients with T2D. Four human GLP-1 RAs (albiglutide, dulaglutide, liraglutide, semaglutide) have displayed superiority versus placebo for 3-point MACE. Liraglutide also reduced all-cause mortality and CV death and semaglutide also decreased nonfatal strokes\textsuperscript{64,65,143,144}. Incretin mimetics exenatide and lixisenatide have shown non-inferiority to placebo\textsuperscript{145,146}. 
Omega-3 carboxylic acids

Omega-3, or n-3, carboxylic acids, consist of fish oil derivatives eicosapentaenoic (EPA) and docosahexaenoic (DHA) fatty acids. OM-3CA can reduce triglycerides and liver fat without simultaneous body weight loss and is a treatment option in patients with both hypertriglyceridemia and NAFLD. Underlying mechanisms of action are incompletely understood, but increased basal metabolic rate and lipid oxidation seen with OM-3CA may matter. On the flipside, DHA has also been associated with raised levels of LDL, an acknowledged risk factor of coronary heart disease.

There are no OM-3CA formulations for prescription in Sweden, meanwhile there are half a dozen of them available in the U.S. These include EPA/DHA mixtures Epanova, Omtryg, Lovaza (and two generics thereof) as well as EPA-only formulation Vascepa which recently showed ~25 % reduction MACE in a population with high CV-risk (REDUCE-IT). Although, no EPA/DHA-formulation has shown positive CV-outcomes to date, a study of cardiovascular benefit with Epanova (STRENGTH) is currently ongoing. OM-3CA side effects include GI-disturbances like nausea, burping, diarrhea, and abdominal pain.
Overview of investigated treatment effects

Investigated treatments have different mechanisms of action, potentially increasing their effects when combined. Hence, giving SGLT2i together with incretin-based therapies (GLP-1RA or DPP4i) or OM-3CA are interesting therapy options for lowering of cardiometabolic risk factors and liver fat respectively.

![Diagram](image)

**Figure 3: Effects of investigated treatment modalities.** Pharmacotherapy effects of interest for one or more of the treatments employed. Less established research questions in *italics*. SGLT2i = sodium glucose co-transporter 2 inhibitor; omega-3CA = omega-3 carboxylic acids; GLP-1RA = glucagon-like peptide-1 receptor agonist; DPP4i = dipeptidyl peptidase 4 inhibitor; systolic BP = systolic blood pressure; MACE = major adverse cardiovascular events.
Study aims and hypotheses

The overall aim of this thesis is to improve understanding of how SGLT2 inhibition affect glucose, lipid and energy metabolism as well as to elucidate underlying hormonal and metabolic mechanisms. To this aim, the following four tasks are performed:

The first task is to evaluate the effects of SGLT2i with GLP-1RA on body weight and body composition in obese nondiabetic subjects compared with placebo. The hypothesis is that the combination treatment yields positive additive or synergistic effects on body weight and body composition.

The second task is to evaluate the maintenance of body weight loss, body fat reduction and decreased prediabetes, lipids and blood pressure with continuing SGLT2i and GLP-1RA treatment for 52 weeks. The hypothesis is that reductions in these outcome variables are maintained following one year of combination treatment.

The third task is to evaluate how SGLT2i and OM-3CA, alone and in combination affect liver fat content in T2D patients with NAFLD compared with placebo. The hypothesis is that combination treatment can yield additive effects on liver fat deposition.

The fourth task is to assess T2D patients for changes in glucagon levels, following a single dose of SGLT2i during experiments with stable versus falling plasma glucose. The hypothesis is that changes in glucagon levels could largely be explained by changes in glucose levels.
Subjects and study design

Paper I

A phase IIa, 24-week, double-blind, parallel group, placebo-controlled study of body weight changes in 50 obese subjects without diabetes (prediabetes, defined as either or both IFG and IGT, was 73.5 %) randomized to oral dapagliflozin 10 mg (DAPA) daily plus subcutaneous long-acting exenatide 2 mg once weekly (EXE) or placebo. Participants were instructed to follow a balanced diet and exercise moderately. Visits included screening, 1-2 weeks before randomization (week 0) with follow-up visits at weeks 4, 8, 12 and 24 as well as an optional 28-week open-label extension. Exploratory measures included body composition (by MRI), waist and hip circumference as well as hormones and substrates in fasting and during oral glucose tolerance tests (OGTT).

Table 1: Studies I and II participants clinical characteristics

<table>
<thead>
<tr>
<th></th>
<th>SGLT2i + GLP-1RA (n=25)</th>
<th>Placebo (n=24)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>53 (13)</td>
<td>50 (12)</td>
</tr>
<tr>
<td>Male sex, n (%)</td>
<td>10 (40)</td>
<td>9 (38)</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>106.4 (15.6)</td>
<td>102.7 (17.3)</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>35.8 (2.9)</td>
<td>35.0 (3.7)</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>117.6 (11.3)</td>
<td>114.4 (12.6)</td>
</tr>
<tr>
<td>Total adipose tissue (l)</td>
<td>57.1 (9.7)</td>
<td>53.4 (7.0)</td>
</tr>
<tr>
<td>Liver fat (%)</td>
<td>10.9 (10.6)</td>
<td>10.0 (8.5)</td>
</tr>
<tr>
<td>HbA1c (mmol/mol)</td>
<td>37.2 (3.9)</td>
<td>38.1 (3.3)</td>
</tr>
<tr>
<td>Impaired fasting glucose, a n (%)</td>
<td>16 (64)</td>
<td>17 (71)</td>
</tr>
<tr>
<td>Impaired glucose tolerance, b n (%)</td>
<td>12 (48)</td>
<td>8 (33)</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>75.8 (10.6)</td>
<td>77.0 (12.6)</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>133.7 (12.7)</td>
<td>133.8 (17.5)</td>
</tr>
</tbody>
</table>

Data are N or mean (SD). eGFR, estimated glomerular filtration rate; HbA1c, glycated hemoglobin; MDRD, modification of diet in renal disease formula; n, number of randomized subjects. a fasting plasma glucose 5.6-6.9 mmol/l b 120-min plasma glucose 7.8-11 mmol/l.
**Paper II**

A 28-week open-label extension study, in which all participants received active treatment, evaluating how changes from baseline in body weight, adipose tissue volume, glycemia, and systolic blood pressure was maintained in the continuing active treatment group. Outcome measures in the group switching from placebo to active treatment were evaluated from a confirmatory perspective. Participants were 38 of the study completers in paper I.

![Design of studies I and II](image)

*Figure 4: Design of studies I and II. Participants were randomized to 24 weeks of treatment with either SGLT2i dapagliflozin 10 mg once daily plus GLP-1RA exenatide 2 mg once weekly or placebo; QD, once daily; QW, once weekly. Open-label extension from week 24-52 in which all participants receive active treatment.*

**Paper III**

A randomized, four group, placebo-controlled double-blind, double-dummy, multicentre study of liver fat changes (measured as proton density fat fraction (PDFF) by MRI) in 84 subjects with T2D (HbA1c 58.9 mmol/mol) and NAFLD (PDFF 18 %) following 12-weeks of daily dosing of dapagliflozin 10 mg and OM-3CA Epamova 4 g alone and in combination compared with placebo. Exploratory outcome measures included changes in body weight, glycemic control, hepatocyte injury markers, oxidative stress biomarkers, and fatty acid metabolism.
Table 2: Study III participants clinical characteristics

<table>
<thead>
<tr>
<th></th>
<th>Placebo (n = 21)</th>
<th>OM-3 CA (n = 20)</th>
<th>SGLT2i (n = 21)</th>
<th>OM-3CA+ SGLT2i (n = 22)</th>
<th>Total (N = 84)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>65.6 (6.1)</td>
<td>66.2 (5.9)</td>
<td>65.0 (6.5)</td>
<td>65.0 (5.4)</td>
<td>65.5 (5.9)</td>
</tr>
<tr>
<td>Sex (male/female)</td>
<td>17/4</td>
<td>11/9</td>
<td>16/5</td>
<td>15/7</td>
<td>59/25</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>93.0 (12.2)</td>
<td>95.6 (13.7)</td>
<td>90.2 (8.7)</td>
<td>91.7 (12.9)</td>
<td>92.6 (12.0)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>30.3 (3.1)</td>
<td>33.0 (4.1)</td>
<td>30.5 (2.8)</td>
<td>31.1 (3.6)</td>
<td>31.2 (3.5)</td>
</tr>
<tr>
<td>Overweight/obese³</td>
<td>10/11</td>
<td>5/15</td>
<td>11/10</td>
<td>10/12</td>
<td>36/48</td>
</tr>
<tr>
<td>T2D dur (years)</td>
<td>6.5 (4.2)</td>
<td>6.3 (5.1)</td>
<td>6.7 (6.0)</td>
<td>8.5 (4.5)</td>
<td>7.0 (5.0)</td>
</tr>
</tbody>
</table>

Data are reported as mean (SD), unless otherwise stated. Overweight, BMI 25–30 kg/m²; obese, BMI > 30 kg/m². OM-3 CA, omega-3 carboxylic acids; SGLT2i, SGLT2 inhibition; BMI, body mass index; T2D dur, type 2 diabetes duration.

Figure 5: Design of study III. A multi-center randomised placebo-controlled double-blind parallel-group study. Participants with type 2 diabetes and NAFLD were randomly assigned 1:1:1:1 to treatments with SGLT2i dapagliflozin 10 mg and/or OM-3CA epanova 4 g or placebo. The primary endpoint was liver fat content assessed by MRI (proton density fat fraction) and biomarkers associated with T2D and NAFLD.

Paper IV

A phase IV, randomized 3-treatment crossover, open-label study of glucagon changes following a single-dose of dapagliflozin 10 mg with 5-h isoglycemic clamp or saline infusion (experiment DG and D respectively). The third experiment was with saline infusion, exploring the effects of adding DPP4i saxagliptin 5 mg to SGLT2 inhibition (experiment DS). All experiments were immediately followed by 2-h OGTTs. The 15 participants had T2D and were treated with metformin. Exploratory outcomes included changes in lipid energy substrates and additional hormones like insulin and aGLP-1.
Table 3: Study IV participants clinical characteristics

<table>
<thead>
<tr>
<th>Baseline characteristics</th>
<th>n=15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender, male/female (n)</td>
<td>12/3</td>
</tr>
<tr>
<td>Age (years)</td>
<td>67 (6)</td>
</tr>
<tr>
<td>Type 2 diabetes duration (years)</td>
<td>7.4 (4.2)</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>27.1 (2.9)</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>87 (12)</td>
</tr>
<tr>
<td>Lean mass (kg)</td>
<td>62 (11)</td>
</tr>
<tr>
<td>Waist/hip ratio</td>
<td>1.0 (0.1)</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>104 (7)</td>
</tr>
<tr>
<td>Hip (cm)</td>
<td>105 (6)</td>
</tr>
<tr>
<td>HbA1c (mmol/mol)</td>
<td>56.5 (5.8)</td>
</tr>
<tr>
<td>Fasting plasma glucose (mmol/L)</td>
<td>9.6 (1.1)</td>
</tr>
<tr>
<td>Metformin dose (mg)</td>
<td>1933 (594)</td>
</tr>
<tr>
<td>eGFR MDRD (mL/min/1.73m²)</td>
<td>97 (18)</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>69 (9)</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>83 (9)</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>142 (11)</td>
</tr>
</tbody>
</table>

Data are N or mean (SD). eGFR, estimated glomerular filtration rate; HbA1c, glycated hemoglobin; MDRD, modification of diet in renal disease formula; n, number of subjects.

Figure 6: Design of study IV. At the three visits participants received a single-dose of SGLT2i dapagliflozin 10mg accompanied by either of the following in randomized order (R): isoglycemic clamp (experiment DG); saline infusion (experiment D); or DPP4i saxagliptin 5mg plus saline infusion (experiment DS). Directly after 5-h infusion periods, a 2-h oral glucose tolerance test (OGTT) was performed.
Methods

Body weight
Body weight changes were measured with an electronic scale (TANITA) at baseline (I-IV) and 4, 8, 12 (III), 24, 38 and 52 weeks (I, II) with participants in light indoor clothing without shoes.

Magnetic Resonance Imaging
In paper I-III, changes in body composition were measured by MRI using a 1.5T Achieva scanner (Philips Healthcare, Best, The Netherlands). All data was collected and analyzed at a single center under blinded conditions.

Liver volume and fat content were both quantified by dedicated whole liver single breath-hold scans. Liver volume was assessed by a trained professional following semi-automated segmentation. Liver fat content was quantified as the median PDFF after manual segmentation of axial image slices.

In paper I and II, a semi-automated segmented whole body water-fat MRI scan was used to assess total adipose and lean tissue respectively as well as subcutaneous and visceral abdominal adipose tissue depots.

In paper III, abdominal fat content was assessed from 21-slice, 8 mm slice thickness of a single breath-hold axial scan at L4/L5. Subcutaneous and visceral adipose tissue were then separated and quantified by an automated technique.

Oral Glucose Tolerance Test
Oral glucose tolerance testing was performed before and after study treatments in paper I, II (0, 15, 30, 60, 90, 120 and 180 min) and III (−15, 0, 30, 60 and 120 min) with blood sampling for glycemic, metabolic and exploratory labs to assess glucose tolerance, insulin sensitivity and energy substrate utilization.
Biochemical analyses

Analyses of blood samples were performed in accordance with the clinical routine at the Department of clinical chemistry at Uppsala academic hospital or the Covance Laboratories (Madison, WI, USA). The in-house analysis included glucagon (ELISA, Mercodia AB, Uppsala, Sweden), aGLP-1 (electrochemiluminescence kit, MesoScale Discovery, Rockville, MD, USA) as well as substrate analyses glycerol (free glycerol reagent, Sigma Chemical Co, St. Louise, MO, USA) and nonesterified fatty acids (NEFA; fluorometric assay kit, Cayman Chemical, Ann Arbor, MI, USA).

Experimental visit design

Paper IV was a 3-visit crossover study with the following experimental visit design. Participants fasted overnight until baseline measurements at 8-9 am and immediately after that received a single-dose of dapagliflozin 10 mg accompanied by either of the following in randomized order: isoglycemic clamp controlled with variable glucose (10 %) infusion based on bedside measurements every 5-10 min. The amount of glucose infused was noted every 20 min during the 5-h glucose infusion for calculation of glucose infusion rate. (experiment DG); isotonic saline infusion (experiment D); or saxagliptin 5 mg plus isotonic saline infusion (experiment DS). Directly after 5-h infusion periods, a 2-h OGTT was performed.

Concentrations of glucagon, insulin, aGLP-1, glucose, glycerol, and NEFA were measured at 0, 60, 120, 180, 300, 305 (and 315 for hormones and glucose), 330, 360, and 420 min. β-hydroxy (OH)-butyrate were measured at 0, 120, 300, and 420 min. Urine was collected, volume and glucose concentrations measured for infusion and OGTT periods respectively.

Statistical analyses

All statistical analyses were performed with software SAS version 9.4 (SAS Institute Inc., Cary, North Carolina) or SPSS version 24 (IBM Corp, Armonk, New York). 95% confidence intervals (CI) or standard error of the mean (SEM) were used and p-values < 0.05 were considered significant.

Primary outcome measures were defined as change from baseline (II) and as compared to placebo (I, III, IV). Safety, full and per protocol (I, II only) analyses sets were analyzed (I-IV). Non-normally distributed data according to Shapiro-Wilks test was log transformed unless otherwise stated. Analyses were unadjusted for multiple comparisons (I, II, IV) or adjusted with Tukey’s method (III).
In paper I and II, least-squares mean (LSM) changes using a mixed model for repeated measures with baseline value (continuous), and treatment (I only), week, treatment-by-week interaction and sex (categorical) as covariates. Similar model terms were used in analyses of covariance of MRI, fasting plasma glucose and ketones measurements. An unstructured matrix for the within-participant error variance-covariance was used. Proportional changes in prediabetes within and between groups were tested with paired McNemar (within the group) and Cochran–Mantel–Haenszel test (adjusted for treatment between groups) respectively.

In paper III, log-transformed, 12-week LSM changes were analyzed in a mixed linear model with baseline value as a covariate, treatment as fixed effect and study center as a random effect. Dunnett’s test was used for pairwise comparisons versus placebo. Spearman’s rank correlation test was used to analyze pairwise correlations of the changes in outcome variables from baseline to week 12. The interaction between PNPLA3 genotype, baseline values, treatment (exploratory covariates), study site (random effect covariate) and the primary outcome variables were investigated using a mixed model.

In paper IV, a paired Student's t-test was used to assess relative changes in AUC from baseline (calculated with the trapezoidal rule) in experiment DG and DS as compared with experiment D. A forward stepwise multiple regression analysis of pooled data from the two main visits (D and DG), included sex, subject, experiment type, change in plasma glucose and insulin to analyse their relative contribution to change in glucagon.
Results

**Paper I**
Following 24 weeks of combination treatment, participants decreased body weight by 4.13 kg, adipose tissue volume by 4.09 l and systolic blood pressure by 6.7 mmHg when adjusted for placebo. Percent of participants achieving weight loss ≥ 5 % and 10 % was 36 % and 12 % with active treatment compared with 4 % and 0 % with placebo respectively. The proportion of prediabetes improved significantly from 68% to 34.8% with active treatment.

**Paper II**
In the continuing active treatment group, reductions at 24 weeks were sustained at 52 weeks, for body weight (−4.5 and −5.7 kg), total adipose tissue volume (−3.8 and −5.3 l), proportion with prediabetes (34.8 % and 35.3 %), and systolic blood pressure (−9.8 and −12.0 mmHg) respectively. Additionally, waist circumference, triglycerides and LDL were significantly lowered from baseline with active treatment. The placebo-group switching to active treatment attained similar results to those of the active treatment group in paper I.
Figure 7: Body weight (kg), 1-year change (studies I and II). Active treatment reduced body weight 5.69 kg from baseline, the placebo-group switching to active treatment showed similar body weight decreases -4.15 kg compared with main study outcome with active treatment. DAPA+ExQW, dapagliflozin 10 mg + exenatide 2 mg; PBO, placebo; PBO→DAPA+ExQW, placebo-group switching to active treatment dapagliflozin 10 mg + exenatide 2 mg at extension study start.

Paper III

Following 12 weeks treatment, liver fat content was significantly reduced from baseline in all active treatment groups. Combination treatment (-21 %, adjusted p <0.05) but not monotherapy dapagliflozin (-13 %) or OM-3CA (-15 %) reduced liver fat versus placebo (-3 %). Presence of G-allele in the PNPLA3 was associated with larger liver fat reductions with combination therapy, and smaller ones with monotherapies.

SGLT2 inhibitors alone decreased liver cell injury markers including aminotransferases, fibroblast growth factor 21 (FGF21) and γ-glutamyl transferase (γ-GT). Dapagliflozin-groups generally also lowered abdominal adipose tissue, waist circumference, and body weight.
### Table 4: Treatment effects on body weight, abdominal adipose tissue, liver fat and liver damage markers (study III)

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>OM-3CA</th>
<th>SGLT2i</th>
<th>OM-3CA + SGLT2i</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Body weight, kg</strong></td>
<td>B 92.9 (12.16)</td>
<td>95.6 (13.68)</td>
<td>90.3 (9.04)</td>
<td>91.6 (12.84)</td>
</tr>
<tr>
<td></td>
<td>C −0.27 (1.79)</td>
<td>−0.16 (1.02)</td>
<td>−2.44 (2.14)*</td>
<td>−2.16 (1.30)*</td>
</tr>
<tr>
<td><strong>Liver PDFF, %</strong></td>
<td>B 15.1 (6.5)</td>
<td>22.2 (11.0)</td>
<td>17.3 (9.1)</td>
<td>17.8 (9.2)</td>
</tr>
<tr>
<td></td>
<td>C −0.59 (1.86)</td>
<td>−3.15 (2.88)</td>
<td>−2.23 (3.30)</td>
<td>−3.15 (3.49)*</td>
</tr>
<tr>
<td><strong>SubQ adipose tissue, l</strong></td>
<td>B 3.91 (1.59)</td>
<td>4.93 (2.03)</td>
<td>3.84 (1.40)</td>
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Data are reported as: B, mean at baseline (SD); C, mean change at 12 weeks treatment (SD); *p<0.05 as significant difference in descriptive geometric mean ratio (95% CI) versus placebo in a mixed model analysis. SubQ, subcutaneous. Visc, visceral. OM-3CA, omega-3 carboxylic acids, PDFF, proton-derived fat fraction.

### Paper IV

During 5-h infusions, levels of glucagon (-20 and -9 %), glucagon/insulin ratio (-25 and 37 %), glycerol, NEFA, and beta-OH-butyrate were lower when plasma glucose (3 versus -17 %) was stable versus allowed to fall. Insulin changed significantly from baseline only as glucose fell (-31%). Change in glucose level was the main predictor of change in glucagon level according to multivariate analyses (r²
0.23). Urinary glucose excretion was numerically higher with stable versus falling glucose. With add-on DPP4i saxagliptin, glucagon and aGLP-1 were higher during infusions, and glucose rose less and glucagon fell more in subsequent OGTTs.

Figure 8 A-B: Glucose and glucagon changes from baseline (study IV). Data are means ± SEM. N=14 completers for dapagliflozin + isoglycemic clamp and saline, N=13 completers for dapagliflozin + saxagliptin. ΔAUC, area under the curve for change in concentration from time 0 to 300 mins (infusion) or from time 300 to 420 min (OGTT); DAPA, dapagliflozin; h, hour; mins, minute; NS, non-significant; OGTT, oral glucose tolerance test; SAXA, saxagliptin; SEM, standard error of mean.
Discussion

Obesity and type 2 diabetes often go with cardiovascular risk factors dyslipidemia, hypertension, and NAFLD. As disease prevalence grows, so does the need for efficacious and safe therapy options. SGLT2 inhibitors and GLP-1 receptor agonists are potentially game-changing in this respect, with proven pleiotropic positive effects on body weight, blood pressure, MACE and promising liver fat results\(^\text{63–67, 90,92–95,97–99,106–109,154}\). OM-3CA is also shown to reduce liver fat and lower MACE\(^\text{147,150}\).

These drug classes have fundamentally different mechanisms of action, and combining them may produce additive or even synergistic effects. Hence, SGLT2 inhibitors with incretin-based therapies and OM-3CA are interesting therapy options for lowering CV risk and liver fat respectively.

Cardiometabolic risk factors

Body weight and body composition

In studies I and II, obese non-diabetic participants given SGLT2i and GLP-1RA achieved ~ 4 kg placebo-adjusted weight loss at 24 weeks, a weight reduction that was maintained at 52 weeks. A comparable body weight reduction was observed in the placebo group switching to active treatment in study II. Participants receiving SGLT2i with and without OM-3CA in study III lost ~ 2 kg body weight, mirroring monotherapy results with SGLT2i in T2D-studies\(^\text{155}\). However, the extrapolated weight change from calorie loss with SGLT2i is around -11 kg, most of which remain unrealized.\(^\text{110}\) The calorie loss is seemingly compensated at a new and lower equilibrium, often attributed to an increased appetite.\(^\text{156}\)

Weight loss in studies I and II were more substantial than previously reported with monotherapy SGLT2i.\(^\text{108}\) This is probably the effect of combining SGLT2i with GLP-1RA, and that these drugs have different and complementary mechanisms of action. Our weight loss results are in line with additive effects of each drug.\(^\text{108}\)

In study I, a majority of weight loss occurred in the first 12 weeks of treatment. This pattern was mirrored by the placebo group switching to active treatment in study II, and is in line with previous weight loss results with SGLT2i.\(^\text{157}\)
Body weight effects of SGLT2i can probably be attributed to losses in both calories and body water. Early effects are likely to mirror urinary fluid loss through natriuresis and osmotic diuresis, as reflected by the higher hematocrit previously reported.\textsuperscript{158} The concurrent glucosuria is shown to lower plasma glucose and insulin levels while sometimes raising glucagon.\textsuperscript{116} The resulting rise in glucagon/insulin ratio would stimulate glycogenolysis, potentially lowering mean hepatic glycogen stores, glycogen-bound water and hence reduce body weight marginally. A rise in glucagon/insulin ratio with SGLT2i is also seen to stimulate lipid mobilization, β-oxidation, and ketone body formation.\textsuperscript{116}

Increased utilization of fatty acid energy substrates with SGLT2i was reflected in our results in study IV. Comparing effects of SGLT2i with falling versus stabilized glucose, results showed increased glucagon/insulin ratio and rising glycerol, free fatty acids and ketone bodies. A partial shift towards lipid mobilization and oxidation is likely to be a primary contributor to long-term weight loss. Our findings in study IV reinforce previous results on SGLT2i and body composition\textsuperscript{107,109} and is confirmed by MRI changes in visceral and subcutaneous adipose tissue in studies I and II, as well as abdominal and liver fat lowering in study III.

A large part of weight loss in studies I and II was due to decreased adipose tissue volume according to MRI. As lipids have more than twice the energy density of glucose, this can partly explain sustained energy requirements inspite of only moderate weight loss. Additionally, oxidation of ketone bodies is shown to be relatively energy efficient in terms of oxygen consumption, at least in the heart.\textsuperscript{117} In study IV, plasma concentrations of ketone body increased with SGLT2i and glucose lowering, most likely reflecting intracellular substrate availability. If aerobic fuel efficiency of ketones can be extrapolated to other ketone-oxidizing tissues, energy expenditure could decrease overall and in part also contribute to an early tapering of weight loss.

Body weight was maintained following the first 12 weeks of study I (-4.1 kg) and throughout study II (-4.5 to -5.7 kg). The placebo group switching to active treatment achieved a weight loss similar to that in study I, consolidating these findings. In comparison, a recent meta-analysis of approved obesity pharmacotherapies showed that placebo-adjusted one year weight loss was 2.6 - 8.8 kg (23-49 % ≥5 %).\textsuperscript{76} Varying designs and populations make cross-study comparisons inherently difficult. For instance, our study differed by lack of formal lifestyle intervention and a continuing placebo-group. That said, a continuing pattern from study I would yield 1-year weight loss of -5.3 kg (40 % ≥5 %) versus placebo. When the same drug combination was given to mostly obese T2D-patients for one year, results were slightly lower than ours (DURATION-8: combined -3.3 kg, GLP-1RA - 1.5, SGLT2i -2.3 kg).\textsuperscript{159}
It seems possible that weight loss effects in studies I and II could be augmented by a formal lifestyle intervention. Raising the dose of the GLP-1RA component may also increase weight loss, as seen with high-dose liraglutide 3.0 mg\textsuperscript{160}.

Individual weight loss varied in studies I and II, potentially due to differing GLP-1RA response, which in part may be genetically determined\textsuperscript{161}. This suggests that patient genetics can help to predict GLP-1RA treatment response.

**Liver fat**

Non-alcoholic fatty liver disease is strongly associated with visceral adiposity, T2D, and hepatic insulin resistance and is in itself a CV risk factor.\textsuperscript{62} SGLT2 inhibition can contribute towards reduced liver fat by urinary calorie loss and a negative energy balance. Although mechanisms remain unclear, both OM-3CA and GLP-1RA can also reduce liver fat, and GLP-1RA can also lower liver cell damage markers.\textsuperscript{92,93}

Change in liver fat were exploratory endpoints in studies I and II. However, no significant changes were detected, as have previously been reported in preclinical studies with SGLT2i and GLP-1RA monotherapies\textsuperscript{92,94–97}. These findings may be partly due to lack of power and an unselected material regarding hepatic steatosis. However, since these drugs have partly contrary effects on the glucagon/insulin ratio – an established regulator hepatic glucose and lipid metabolism – GLP-1RA may impair the shift towards increased lipid oxidation seen with monotherapy SGLT2i. Lower hepatic insulin resistance at baseline in this non-diabetic population may also contribute to smaller liver fat reductions than in T2D patients.

In study III, we investigated liver fat changes with mono- and dual therapy SGLT2i and OM-3CA versus placebo. Participants had T2D and NAFLD at baseline and reduced liver fat percent with both monotherapies versus the starting value and in combination versus placebo. SGLT2i also reduced liver cell damage markers, not seen with dual treatment, indicating complex drug interaction effects on liver cells.

Monotherapy SGLT2i decreases plasma glucose concentrations, leading to a rise in glucagon/insulin ratio, as seen in study IV. This hormonal response, in turn, is well documented to mobilize stored energy through glycogenolysis, gluconeogenesis, lipolysis and lipid oxidation.\textsuperscript{162} OM-3CA can similarly shift the fatty acid balance from synthesis towards oxidation but without concurrent weight loss.\textsuperscript{116,117,147} These effects together can explain the numerically larger liver fat reduction seen with combination therapy SGLT2i and OM-3CA versus monotherapy arms in study III.

The largely improved hepatic biomarker profile with SGLT2i in study III is in line with decreased lipotoxicity and oxidative stress, as supported by reduced $\gamma$-GT
levels. The same effect was not seen when both SGLT2i and OM-3CA were given. While OM-3CA can upregulate transaminase transcription and thereby dampen SGLT2is transaminase reduction, this does not explain other ameliorated positive results seen with monotherapy SGLT2i. FGF21 was also reduced with monotherapy SGLT2i. High FGF21 levels have been associated with NASH and mitochondrial dysfunction. As such, lowered FGF21 indicates NASH resolution and alleviated metabolic stress.\textsuperscript{163,164}

Liver fat treatment response in study III differed with \textit{PNPLA3 I148M} genotype, an association seen previously\textsuperscript{53}. In our study, G allele-carriers responded more than others to combined treatment but less to monotherapy OM-3CA. Hence, \textit{PNPLA3} genetic analyses are warranted in future NAFLD studies.

\textit{Glucose}

SGLT2 inhibitors lower the threshold for urinary glucose excretion from -11 to -4 mmol/L, thereby decreasing plasma glucose. As the mechanism is dependent on the glucose and sodium concentration gradients, monotherapy SGLT2i is associated with little-added risk of hypoglycemia but have been reported in some studies to increase glucagon\textsuperscript{118,153}.

A majority of participants in studies I and II had prediabetes at inclusion, and those receiving active treatment had improved glycemic measures after 24 and 52 weeks. Participants in study III had T2D, and subgroups receiving SGLT2i showed significant HbA1c reductions after twelve weeks. These results were expected and in line with the established glucosuric effects of SGLT2i. While OM-3CA had no apparent effect on glycemia, it notably decreased stearoyl-CoA desaturase 1 (SCD-1) and \(\delta\)-6 desaturase and increased \(\delta\)-5 desaturase indices, an enzyme activity pattern associated with lowered propensity to T2D development\textsuperscript{165}.

In study IV, single dose SGLT2i was given to participants with T2D to examine effects on glucagon, other hormones, and energy substrates. In these experiments, plasma glucose was the controlled factor. Following SGLT2i dosing, glucagon levels decreased. This drop was more pronounced with stabilized versus falling plasma glucose. Concomitantly, glucagon/insulin ratio decreased in isoglycemia and increased as glucose fell.

During the isoglycemic experiment in study IV, 45-50 grams of intravenous glucose was required to keep glucose at baseline levels. The concurrent urinary glucose excretion (UGE) was significantly lower, averaging 17 grams. The apparent difference between administered and excreted glucose may be due to sustained glucose availability and oxidation. This discrepancy is reflected by the simultaneous decrease in glucagon/insulin ratio, likely to move the balance away from net
endogenous glucose production towards energy storing glycogenesis and lipogenesis.

In comparison, UGE during saline infusion was 13 grams, less than with the glucose clamp. This difference probably reflects ongoing glucose oxidation and excretion, lowering glucose concentration gradient over time (plasma glucose decreased 2.8 mmol/l). As moderate hyperglycemia was maintained even with saline infusion, this may explain the lack of glucagon stimulation. Findings on glucagon baseline values and trajectories with SGLT2i have differed between studies, and in comparison our baseline values were rather high. Indeed, glucagon level and outcome is shown to vary both with the participants metabolic control and with the choice of glucagon assay, both of which may influence these results. Although some uncertainty remains, recently reported results support our findings on glucagon changes with SGLT2i. Changes in insulin and c-peptide largely mirrored glucose trajectories as expected.

In study IV, plasma glucose was the controlled variable by experimental design, and glucagon was seen to fall significantly more with stable versus falling glucose. In multivariate analyses, glucose level was the most important factor in explaining glucagon changes. Taken together, this suggests that glucose levels rather than SGLT2 inhibition per se mattered most to glucagon secretion.

**Lipids**

A blood lipid profile with high triglycerides and LDL is associated with an increased risk of cardiovascular disease. In T2D patients, monotherapy SGLT2i generally lower triglycerides but can raise LDL whereas GLP-1RAs lower them both. In studies I and II, triglycerides and LDL decreased significantly from baseline to 52 weeks. This lowering could reflect a dominating GLP-1RA effect on lipids, and the glucagon-lowering effect of GLP-1RA may also dampen SGLT2is lipid mobilization. A lower insulin-resistance in this nondiabetic cohort and a stabilized body weight after one year may also contribute to the improved lipid profile.

In study III, the balance of plasma lipids changed in participants receiving OM-3CA whereas SGLT2i showed no such apparent effects on fatty acid composition. However, butyrylcarnitine levels increased in the SGLT2i treatment group possibly reflecting increased fatty acid metabolism.

In study IV, single dose SGLT2i was accompanied by stable versus falling glucose by study design. Fatty acid energy substrates were generally lower with stable compared with falling plasma glucose. This difference can be accounted for by higher carbohydrate availability and thereby more glucose oxidation in isoglyce-
mia. Conversely, relatively higher levels of fatty acid substrates with falling glucose and SGLT2i are signs of a shift towards lipid mobilization.

These changes are probably mediated by the concurrent differences in insulin levels and glucagon/insulin ratio. Our findings are in line with previously published results\textsuperscript{172,116} and support that increased lipid mobilization and oxidation is secondary to SGLT2i’s glucose lowering effect.

**Blood pressure**

Blood pressure was an exploratory outcome variable in studies I and II, in which SGLT2i and GLP-1RA were combined for one year of treatment. Previous results show reductions of 3-5 and 2-5 mmHg for SGLT2i and GLP-1RA respectively\textsuperscript{106,142}. In studies I and II, systolic but not diastolic blood pressure was significantly lowered at 24 weeks and maintained at 52 weeks. Changes were more substantial than seen previously with monotherapies. The seemingly increased efficacy in blood pressure lowering can be explained by their differing and seemingly complementary mechanisms of action. SGLT2 inhibitors reduce blood pressure through induced natriuresis and osmotic diuresis. Although incompletely known, mechanisms behind GLP1-RAs blood pressure lowering effects may involve natriuresis\textsuperscript{173} but also a complementary vasodilation\textsuperscript{174}.

**Cardiometabolic benefits**

In studies I and II, long-term cardiometabolic risk factors were generally improved, lowering body weight, lipid profile, HbA1c and systolic blood pressure. However, swiftly attained positive results in CV-outcome trials suggest that protective mechanisms involve other pathways. For SGLT2i this may include fluid congestion relief\textsuperscript{121} and increased cardiac ketone body utilization\textsuperscript{117}, while GLP-1RA seemingly has direct effects on heart and vasculature function\textsuperscript{132}.

In study III, reduced liver fat and inflammation may also contribute towards CV-protection long-term. However, opinion and research outcomes regarding cardiovascular benefits with OM-3CAs currently diverge. While EPA-only formulation Vascepa recently announced significant MACE reductions in the US\textsuperscript{150}, the European medical agency have reported that they no longer consider any OM-3CAs effective in secondary CVD-prevention\textsuperscript{175}.

**Incretins**

Feeding stimulates GLP-1 secretion, which generally lowers glucagon and raises insulin levels. DPP4 inhibitors hinder GLP-1 breakdown. In study IV, active GLP-1 levels decreased irrespective of plasma glucose changes while rising with the addition of DPP4i. Unexpectedly, glucagon concomitantly rose with active GLP-1,
differing from results in previous longer-term studies. Varying choices of gluca-
gon assay may impact these differing outcomes. It is also possible that physiologi-
cal adaptations to chronic dosing per se have an impact on glucagon trajectories,
partly explaining differences.

The changes in hormones and energy substrates during 2-h OGTT largely reflect
different starting values and should be interpreted with caution. Notably, active
GLP-1 rose in these conditions, while glucose rose numerically less with DPP4i
added. At the same time, insulin and lipid energy substrates did not change signifi-
cantly. These results are generally in line with previous results seen with DDP4i
although the lack of insulin increase appears surprising.

**Safety**

Pharmacological weight loss therapies have been associated with safety issues\(^\text{76}\). In contrast, SGLT2i and GLP-1RA can both reduce MACE-incidence and have
otherwise good safety and tolerability profiles\(^\text{64,66,67,120}\). No new or unexpected
safety issues were identified in any of these four studies. Notably, no hypoglyce-
mic episodes were noted with active treatment in studies I or II, despite giving two
antihyperglycemic agents to a non-diabetic study population. However as ex-
pected, more nausea and injection site issues were reported with GLP-1RA treat-
ment. AE frequency and related discontinuations were generally as previously
reported.
Figure 9: Proposed metabolic and endocrine effects of SGLT2 inhibition and coadministered drugs. SGLT2 inhibition (SGLT2i) induces urinary glucose excretion and lowers plasma glucose, which in turn raises the glucagon/insulin ratio. The resulting energy deficit stimulates appetite to replenish energy substrate stores. This is partly counteracted by decreased appetite and slowed gastric emptying through GLP-1 receptor agonism (GLP-1RA). Raised glucagon/insulin ratio also stimulates hepatic glucose production and causes a partial shift towards lipid mobilization, in turn lowering lipids stored in adipose tissue and the liver. Both omega-3 carboxylic acids (OM-3CA) and GLP-1RA seem to have liver fat lowering qualities, possibly via increased energy expenditure and indirect effects respectively, but mechanisms remain unknown.
In papers I and II, SGLT2 inhibitor dapagliflozin and GLP-1 receptor agonist exenatide reduced body weight, prediabetes and systolic blood pressure in an obese non-diabetic population without new safety concerns. Lipid profile was also improved from baseline after one year of treatment. This was a first-ever placebo-controlled study of this drug combination in a prediabetic population and suggests a potential role in T2D and CVD prevention. A dose-finding study for GLP-1 receptor agonism together with SGLT2 inhibition could yield further weight loss. Cardiovascular outcome studies of this drug combination would be of interest for both obesity- and T2D-cohorts.

In paper III, dapagliflozin and omega-3 (n-3) carboxylic acids lowered liver fat content in overweight T2D patients with NAFLD, in particular when given together. Dapagliflozin also lowered hepatocyte injury biomarkers, a promising finding for NASH prevention and treatment. Further studies with liver biopsies in a NASH population are needed to validate these findings.

In paper IV, with single-dose dapagliflozin, changes in glucose can explain changes in glucagon levels. The impact of SGLT2 inhibition on glucagon and fatty acid mobilization seems to be mainly in response to glucose lowering via urinary excretion rather than through direct drug effects on pancreatic α-cells. This finding indicates largely intact counter-regulatory mechanisms in the maintenance of glucose homeostasis with an SGLT2 inhibitor present. Future crossover studies including a placebo arm and at varying glucose levels could further elucidate SGLT2 inhibitors glucagon effects.

Artikel I-II: I dessa två studier undersökt den kombinerade effekten av 24 veckors behandling med SGLT2-hämmaren dapagliflozin och GLP-1 receptor agonisten exenatid på första hand kroppsvikt, men även kroppssammansättning, blodglukos och systoliskt blodtryck hos 50 obesa vuxna utan diabetes. Behandlingen tolererades väl och skillnaden i kroppsvikt var -4,1 kg efter 24 veckor, mängden fettväv minskade nästan lika mycket enligt magnetkameraundersökning. Även prediabetiska (mättligt förhöjda) blodsockervärden och systoliskt blodtryck minskade signifikant. 38 av dessa deltagare gick in i den efterföljande 28 veckor långa studieförlängningen, alla deltagare fick nu aktiv behandling. Förlängningsstudiens resultat visade bibehållet minskad kroppsvikt (-5.7 kg), volym fettväv, blodsocker- och blodtrycksnivå efter 52 veckors behandling. Även blodfettsprofilen förbättrades från studiestart med ett års behandling.

Ovanstående resultat pekar mot att läkemedelskombinationen SGLT2-hämmare och GLP-1-receptor agonist kan förebygga T2D och hjärtkärlssjukdom. Detta är extra intressant eftersom man har påvisat minskad hjärtkärlsjuklighet hos T2D-patienter med båda läkemedelstyperna var för sig.

Artikel III: I denna 12-veckorsstudie utvärderades effekten av dapagliflozin, fria omega-3 (n-3) karboxylysyror var för sig och tillsammans på leverns fettinnehåll jämfört med placebo. 84 personer med T2D, övervikt/fetma och fettlever deltog. Precent leverfett minskade med kombinationsbehandlingens jämfört med placebo och gentemot startvärdet med vardera preparat enskilt. Vidare minskade leverska-demarkörer med dapagliflozin ensamt. Resultaten är sammantaget lovande och motiverar mer forskning avseende preparatens roll i framtida fettleverbehandling.
Artikel IV: Här studerades effekten av endos dapagliflozin på glukagon (blodssockerhöjande hormon), andra hormoner samt energislag, vid stabilt respektive fallande blodssocker. 15 patienter med T2D deltog. Glukagon sjönk från startnivån både vid stabilt och fallande blodssocker, men sjönk mindre när glukos sjönk. Vid sjunkande blodssocker ökade mängden fettsyror i blodet. Tillägg av en DPP4-hämmaren saxagliptin höjde oväntat glukagon-nivån i fasta.

Effekterna av SGLT2-hämning på glukagon och fettsyramobilisering framstår som en fysiologisk reaktion på glukosminskning via urinutsöndring snarare än genom direkta läkemedelseffekter på buksبوتكورتهνς α-celler. Resultatet tycks visa i stort sett intakta motreglerande mekanismer vid upprätthållandet av glukosbalansen i närvaro av en SGLT2-hämmare.

Sammanfattningsvis kan SGLT2-hämmare sänka kroppsvikt och kardiovaskulära riskfaktorer hos obesa patienter utan diabetes när de kombineras med en GLP-1 receptor agonist, samt minska leverfett hos T2D-patienter, framförallt när de ges tillsammans med fria omega-3-karboxylsyror. Effekten av SGLT2-hämmare på glukagonfrisättning kan till stor del förklaras av glukosförändringar.
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A doctoral dissertation from the Faculty of Medicine, Uppsala University, is usually a summary of a number of papers. A few copies of the complete dissertation are kept at major Swedish research libraries, while the summary alone is distributed internationally through the series Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine. (Prior to January, 2005, the series was published under the title “Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine”.)